Applicability of Pigment Compounds for Reducing Light Stress in Bentgrass

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DEDICATION

To my family and all those that have given me the opportunity to do something bigger than myself. Thank you.

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I would like to acknowledge Dr. Brandon Horvath, who saw in me something I did not see in myself, and inspired in me something I did not know I was capable of. My ability to perform this research would not have been possible without first an opportunity to do so.

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My primary contributions to this paper include (i) Conducting the experiments, (ii) processing, analyzing and interpreting data, (iii) reading literature, (iv) writing the manuscript.

ABSTRACT

Chlorinated copper phthalocyanine (Signature) and pulverized cells of *Chlorella vulgaris* (*Chlorella*) were evaluated in a controlled environment for their ability to act as photoprotectants under supraoptimal levels of ultraviolet (UV) and photosynthetically active radiation (PAR) when applied to plant leaves. Plant pigment changes were documented using High Performance Liquid Chromatography following 1 week of exposure to supraoptimal light in two separate experiments incorporating UV (106.6 μmol m^{-2} s^{-1}) and PAR (760.6 μmol m^{-2} s^{-1}) over a 12h photoperiod. Supraoptimal levels of UV and PAR light were found to cause significant reductions in *Agrostis palustris* chlorophyll and carotenoid leaf pigment levels. In both experiments, high light coincided with increases in zeaxanthin and antheraxanthin and decreases in violaxanthin across all treatments, suggesting that plants experienced a stress response regardless of pigment application. Under high PAR light, the levels of total carotenoid pigment degradation were significantly higher in untreated *Agrostis palustris* controls than in *Chlorella* and Signature treated plants. However, only *Chlorella* demonstrated the ability to significantly reduce instances of chlorophyll degradation in bentgrass plants under high UV light.

Spectral imaging of light following transmission through treatments demonstrated how *Chlorella* was successful in limiting the absorbance of wavelengths in regions of UV (300 to 400 nm) and PAR (480 and 580 nm). Photon flux measurements of transmitted light showed a

significant decrease in both treatments when compared to controls; the greatest reduction in light levels occurred with *Chlorella* applications under both UV and PAR light. Results of these experiments demonstrate how this interception of light may limit chlorophyll and carotenoid degradation under these conditions, suggesting that they may be used to successfully act as photoprotectants. This holds particular value in golf course maintenance, where bentgrasses are cultivated at low mowing heights in regions where supraoptimal light conditions persist throughout the growing season.

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LITERATURE REVIEW

CREEPING BENTGRASS

Creeping bentgrass (*Agrostis palustris*) is a fine textured cool-season turfgrass used on golf course greens due to its ability to tolerate low-mowing heights (Warnke, 2003). The aesthetic and performance properties of creeping bentgrass have led to its use in supraoptimal temperature climates, where quality may decline in summer months (Carrow, 1996). For cool-season turfgrasses, ambient air temperatures above 24 °C and soil temperatures above 18 °C are considered to be supraoptimal for shoot and root growth, respectively (Beard, 1973). Hot and humid conditions in the southern United States can produce temperatures above these optimal levels, resulting in a range of symptoms characterized as Summer Bentgrass Decline (SBD) (Carrow, 1996). High air and soil temperatures lead to an increased respiration rate in bentgrass roots, and subsequent dieback upon carbohydrate depletion (Xu and Huang, 2000; Xu and Huang, 2001). In particular, supraoptimal soil temperatures are detrimental to creeping bentgrass root and shoot growth and nutrient uptake (Xu and Huang, 2000). Heat can also disrupt plant cellular membranes, severely affecting plant cellular function (Larkindale and Huang, 2004).

Evaporation and transpiration release heat energy through latent heat of vaporization, which describes the transfer of energy in the state change of water from a liquid to a gas (Tarara, 2000). Conversely, the same amount of energy is released when water condenses from vapor to liquid form. In other words, evaporation will cool a surface, while condensation will warm it (Tarara, 2000). Differences between water vapor concentration in and outside the plant will determine rates of transpiration (Rawson et al., 1977). Under conditions of increased humidity, transpirational cooling will decrease, due to reductions in the water vapor concentration gradient between the plant and the air (Rawson et al., 1977). However, an increased surface temperature will cause an exponential increase in water vapor concentration at the surface, raising this

gradient, which leads to greater transpirational flow of water vapor from the plant (Gates, 1965; Pallardy and Kozlowski, 2008). Consequently, very small changes in temperature can trigger large fluxes in the rate of transpiration (Gates, 1965; Pallardy and Kozlowski, 2008).

Solar radiation is the largest contributor to increasing soil temperatures (Huang, 2002; Larkindale and Huang, 2004). Golf course greens constructed according to United States Golf Association (USGA) specifications incorporate high amounts of sand near the surface of the soil (Moore, 2004). Among various soil-types, Abu-Hamdeh and Reeder (2000) observed the highest thermal conductivity in sandy soils. The temperatures of the various soils were positively correlated with bulk density. Research has shown that the addition of organic matter will decrease bulk density (Hummel, 1993). Creeping bentgrass cultivars used on golf course greens exhibit high rates of organic matter accumulation at the soil surface (Carrow, 2004; Hudson and Shane, 1994). This accumulation of organic matter will reduce bulk density, and thus thermal conductivity (Hummel, 1993). However, organic matter can also increase water retention, which dramatically increases thermal conductivity with increases in temperature (Abu-Hamdeh and Reeder, 2000; Campbell et al., 1994). Bare portions of the turf will also expose surface organic matter, which will absorb higher amounts of incident light energy due to its darker color (Bristow and Horton, 1996; Loughrin and Kasperbauer, 2003). Heat will either move upward to the surface of soil or downward from warmer to cooler layers (Tarara, 2000).

Increases in leaf lipid saturation levels have been observed following heat treatment to creeping bentgrass plants (Larkindale and Huang, 2004). The thylakoid membrane contains a large proportion of non-bilayer forming lipids, which are thought to be required for the stabilization of the contained photosystem-II complex (Thomas et al., 1986). Increases in lipid saturation within chloroplast structures increase temperature tolerance by reducing phase-

separation of non-bilayer forming lipids from the chloroplast membrane (Gounaris et al., 1983). However, no changes in lipid composition were observed in the roots of creeping bentgrass (Larkindale and Huang, 2004). Incidences of heat damage have been shown to be light-mediated, supporting the idea that any subsequent damage could occur as a result of light induced damage to the photosynthetic apparatus (Larkindale and Knight, 2002). This idea is supported in creeping bentgrass species, where photosynthetic acclimation has been shown to be essential for increases in tolerance to severe heat stress, due to maintenance of light-harvesting capacity and carbon fixation throughout the heat stress period (Liu and Huang, 2008). The specific mechanisms relating to light induced inhibition of the photosynthetic apparatus will be further discussed later.

LIGHT AND PLANT PIGMENTS

Visible light (400 to 700 nm) accounts for 43% of the energy in the global solar irradiance spectrum (300 to 2500 nm) for North America. The remainder of this energy arrives as 52% near-infrared (NIR; 700 to 2500 nm) and 5% ultraviolet (UV; 300 to 400 nm) (Levinson et al., 2005*b*; American Society for Testing and Materials, 2003). The NIR wavelengths are responsible for much of the heating within leaves (Forbes and Watson, 1992). Consequently, plant leaves will effectively scatter and reflect 70% of incident perpendicular infrared radiation; reducing heat-buildup in the plant and the soil (Knipling, 1970; Atwell et al., 1999; Larcher, 2003). Plants exhibiting bicoloration (abaxial surface a lighter shade of green than adaxial), a thicker cuticle, and a higher portion of mesophyll surface area exposed to intercellular air spaces exhibited predictably higher NIR reflectance values from the adaxial leaf surface. However, in plants with lower pigment content, UV-visible light absorption and NIR light reflection decrease, increasing heat buildup in soil (Knipling, 1970). Kopsell et al. (2010) reported that, among heat-tolerant cultivars of *Poa pratensis*, those highest in pigment content were the least heat tolerant.

However, also mentioned is the possibility that drought was an influencing factor in decreasing pigmentation across cultivars, which would account for a decrease in transpirational cooling and CO2 fixation. Dry plant leaves will also show diminished reflectance in the NIR, which would increase NIR absorbance by the soil (Hawley, 1971).

Light consists of both wave and particle properties. While light is propagated in wave form, its interaction with matter functions as a particle (Prasad, 1997). Planck's law describes how light exists in quanta, or bundles of energy. This theory was further developed in 1905 by Einstein, who went on to discover that radiation processes involve the emission or absorption of light quanta, or "photons" (Bohr, 1949). The energy of a photon is determined by its wavelength, the distance between repeating waves, and number of light wave repetitions per unit time. Their relationship is considered directly proportional, in that any fraction of photon wavelength equals its reciprocal in energy output, and is a function of frequency (Prasad, 1997). This is not to be confused with the intensity of light, which depends on how many photons of energy are being emitted per unit time (McDonald, 2003).

Two basic principles of light that govern the absorption properties of plant pigments are the Grotthus-Draper and Stark-Einstein laws. The Grotthus-Draper law states that photochemical processes can only occur with absorbed radiation, and the Stark-Einstein law states that each absorbed photon can only affect one molecule (Diffey, 2002). When plant pigment molecules in an unexcited ground state absorb photons of a compatible wavelength, a valance electron is quickly raised to an excited state. After returning to a ground state, the absorbed energy is released in the form of thermal dissipation, fluorescence, phosphorescence, or inductive resonance (McDonald, 2003). However, long-term exposure of plants to supraoptimal levels of light can result in the destruction of photosynthetic pigments. This chemical reaction is

considered oxygen- and light-dependent, and is defined as photooxidation (Powles, 1984). Light toxicity occurs when high flux light converts the target pigment molecules, first, into an excited state, then an initial short-lived singlet state, and finally a molecular rearrangement into a longer-lived triplet state (Larson, 1988). During this time, there is an increased chance for chemical reactions with surrounding molecules. In the formation of damaging species, triplet energy is transferred to molecular oxygen, forming singlet oxygen. This reactive oxygen species exists as a free radical, capable of oxidizing and bleaching plant pigments to an irreversible degree under extreme conditions (Larson, 1988). However, plants have evolved multiple protective mechanisms for the effective removal of this excess light energy.

Photosynthetic systems are composed of a network of principal and accessory pigments (Duysens and Amesz, 1962). While principal pigments are directly involved in the chemical conversion of energy, accessory pigments act as sinks for conducting different wavelengths, transferring the energy to the primary through inductive resonance. Together, these pigments form a network of overlapping absorption bands, improving the efficiency at which plants harvest light in the action spectrum of photosynthesis (Smith and French, 1963; McCree, 1971). However, accessory pigments also play an important role in photoprotection (Demmig-Adams and Adams III, 1996).

Functioning as accessory pigments, the six primary carotenoids in plants exist as red, orange, and yellow pigments and consist of the xanthophyll pigments zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, and lutein and the carotene pigment beta-carotene. (McElroy et al., 2006; Sandmann, 2001; Zaripheh and Erdman, 2002) Carotenoids of the xanthophyll cycle are considered essential to the dissipation of energy under conditions of excess light, through the interception and removal of excess excitation energy prior to its entrance into

the electron transport chain. Once the absorption of light exceeds a plant's capacity for CO_2 fixation, photosynthetic electron transport generates a decrease in lumen pH. This activates the conversion of xanthophylls, removing oxygen from violaxanthin to form antheraxanthin, then zeaxanthin, which dissipates the excess energy as heat (Muller, 2001). This process is reversible, where zeaxanthin will be converted back to violaxanthin in order to promote light harvesting under low-light conditions (McElroy et al., 2006). These processes allow carotenoids to function as photoprotectants by quenching free radicals such as triplet-state chlorophyll and singlet oxygen before they can cause damage to the plant. The process of releasing excess light energy as heat dissipation is known as nonphotochemical quenching (Demmig-Adams and Adams III, 1996).

Flavonoids, another group of accessory pigments, make up one of the largest known groups of phenolic compounds within plants, with over 9000 assessed from plant tissue as reported by Williams and Grayer (2004). Responsible for the many bright blue, red, and purple colors throughout nature, anthocyanins are the most widespread of the pigmented flavonoids (McDonald, 2003). The prospective roles of anthocyanin in plants are numerous, and have been contemplated by scientists for well over a century (Gould, 2010). As stated by Wheldale (1916), in one of the earliest reviews of plant anthocyanin function, "It is difficult to find a hypothesis which fits all cases of anthocyanin distribution without reduction to absurdity." However, in recent years there have been many significant advances in understanding the roles of anthocyanin pigments in plants. Related to light-attenuation properties, anthocyanin accumulation has been linked to photoprotection of chlorophyll during drought and cold stress (E. Taulavuori et al., 2010, Gould et al., 2010), improved recovery from mechanical injury (Gould et al., 2002), enhanced nutrient retrieval from senescing leaves (Hoch et al., 2003), and

delayed senescence in CO_2 rich environments (Tallis et al., 2010). Independent of light attenuation properties, anthocyanins are ascribed to many biotic-dependent roles, including: microbial defense responses (Kangatharalingam et al., 2002; Hipskind et al., 1996); herbivory avoidance (Karageorgou and Manetas, 2006); and pollination ecology (Harborne and Smith, 1978). Due to the diverse range of inducing factors associated with anthocyanin biosynthesis, correlating the transient accumulation of anthocyanin to any one function is inherently difficult. Consequently, knowledge of the localization and spectroscopic properties *in vivo* of all the pigment pools is essential for ecophysiological studies and the quantitative description of anthocyanin function (Gould et al., 2002). While its distribution in plants differs considerably across species, anthocyanin will generally localize within cell vacuoles, in or just below the adaxial epidermis, effectively providing light-protection to subjacent chloroplasts (Merzlyak et al., 2008). Abaxial accumulation in leaves has been observed, but is also considered a photoprotective adaptation of light-sensitive plants whose leaf orientation and substrate albedo may vary throughout developmental stages of the plant (Hughes and Smith, 2007). Specifically, anthocyanins have been shown to accumulate in the presence of UV light, with maximum activity occurring at 290 nm (Jenson et al., 1998; Hashimoto et al., 1991).

NEW MODELS OF PHOTINHIBITION

Photosynthesis requires the interaction between two separate, but equally complex photosystems. Photosystem I (PSI) and photosystem II (PSII) have designations of P700 and P680 respectively, named for the absorption maximum of their chlorophyll *a* molecules. Each of the photosystems contains subtle differences in protein associations, which accounts for their different absorption properties (Anderson and Andersson, 1988). Chlorophyll *a* molecules only absorb a small portion of light for use in photosynthesis. In order to better use the reaction

centers in photosynthesis, each photosystem has antenna complexes composed of several hundred pigment molecules. These light-harvesting complexes help to extend the absorption spectrum, using resonance transfer to designate the flow of excitation energy to the reaction centers (Glazer et al.,1989; Zuber, 1986).

The absorption of excess light energy has the potential to damage photosynthetic machinery, beginning with PSII. Photoinactivation of PSII is hypothesized to occur by two separate mechanisms, acceptor-side and donor-side, which both result in the inhibition of electron transfer and subsequent degradation of the D1 protein (Wei et al., 2011). Photosystem II reaction center D1 proteins, which exhibit the highest turnover rates in the thylakoid membrane, are the main target of oxidation during photodamage (Sundby et al., 1993). In the acceptor-side mechanism hypothesis, photoinhibition begins with the reduction of the plastquinone pool under intense light, causing a lack of oxidized plastoquinone to bind to the Q_B site on the D1 protein. Because Q_A^- is unable to transfer an electron to Q_B, it becomes doubly reduced to Q_A^{2-} during the second turnover of the reaction center. In order to become stable, Q_A^{2-} will become protonated, forming Q_AH_2, which is then released from the Q_A binding site on the D1 protein. The newly unoccupied Q_A site leads to the formation of the primary radical pair $P680^+Pheo^-$, and through recombination, generates triplet state P680. This reacts with oxygen to form singlet oxygen, a reactive oxygen species responsible for D1 protein degradation (Wei et al., 2011; Tyystjärvi, 2008; Anderson et al., 1998). In the donor-side mechanism hypothesis, highly reactive $P680^+$ is formed due to a lack of electron donation while under the influence of light. $P680^+$ will oxidize surrounding chlorophyll and carotenoid molecules, and lead to degradation the D1 protein (Wei et al., 2011).

Under experimental light conditions, photodamage to PSII was found to be greatest in regions of UV and yellow light exposure in *Arabidopsis thaliana* (Takahashi et al., 2010). Damage to PSII by high-energy light has recently been explained using a two-step model developed by Ohnishi et al. (2005) and further explained by Tyystjärvi (2008). This theoretical model states that photoinhibition begins with the reduction of the Mn cluster in the oxygen-evolving complex (OEC) by UV, blue, and green light, but not red (Wei et al., 2011). Following Mn inactivation, PSII becomes sensitive to light at 680 nm, experiencing inactivation from direct red and blue light exposure to its photosynthetic pigments in the donor-side mechanism (Ohnishi et al., 2005). Damage to the OEC will increase potential for PSII damage due to reductions in electron donation from the OEC to PSII undergoing oxidation (Hakala et al., 2005). Takahashi et al. (2010) attempts to explain the adverse effects of yellow light, attributing its photoinhibitory effects to the Mn light-sensitizer mechanism. While yellow light contains less excitation energy than UV and blue light, it is much more abundant in the solar spectrum (Takahashi and Badger, 2010). More of this light is able to penetrate plant tissue, due to its lack of absorption by anthocyanin (primarily blue and green light absorption), chlorophyll (primarily blue and red light absorption) and carotenoid (primarily blue and green light absorption) pigments (Takahashi et al., 2010; Solovchenko and Merzlyak, 2008). The PSII photodamage spectrum is very different from the absorption spectra of these pigments, but is closely correlated with that of Mn compounds (Wei et al., 2011). Because collimated light is scattered within leaf tissue, the efficiency of its absorption will increase with depth in the mesophyll (Vogelman et al., 1996). This allows non-photosynthetic yellow and green light to penetrate more deeply into the leaf, and trigger excitation in shade adapted chloroplasts of the lower mesophyll (Nishio, 2000). The increased presence of refracted yellow-green light in the mesophyll may have a greater influence

on Mn excitation in the absence of high energy UV light. This suggests that the mechanism of light diffusion, meant to increase the absorption of light by pigment networks, could also contribute to the indirect photodamage of PSII during high visible light irradiance (Takahasi et al., 2010). However, a recent study revealed that visible light had little impact on the production of high valent species of Mn in the OEC, while UV light did (Wei et al., 2011). Consequently, UV light inhibition of the OEC in PSII is also much faster and thus more damaging than that of visible light (Tyystjärvi, 2008). Wei et al. (2011) uses this to support the idea that photodamage from excess visible light occurs directly to PSII, without inhibiting Mn in the OEC. This supports the theory that, although donor-side photoinhibition has often been observed after chemical inactivation of the OEC by UV light, there is still potential for visible light to trigger this mechanism in the absence of UV and blue light, because the OEC will sometimes fail to reduce highly reactive $P680^{+}$ species (Anderson et al., 1998; Wei et al., 2011). The absorption peak of anthocyanin in the visible light region (450 to 550 nm), suggests that it may provide photoprotection in this mechanism as well (Solovchenko and Merzlyak, 2008). Overall, the close correlation between this photodamage spectrum and the anthocyanin absorbance spectrum supports the hypothesis that adaxial localized phenolic coumpounds are meant to act as filters for high-energy light (Takahashi and Badger, 2010).

PSII REPAIR CYCLE

Upon photodamage to PSII, the plant begins to replace damaged PSII proteins in a process known as the PSII repair cycle (Aro et al., 2004). In order to repair the damaged PSII complex, the photodamaged D1 protein is rapidly degraded, *de novo* synthesized, and incorporated back into PSII (van Wijk et al., 1997; Nishiyama et al., 2001). Environmental stresses can inhibit *de novo* synthesis of the D1 protein, and consequently limit the rate and

extent of PSII repair (Allakhverdiev and Murata, 2004). In forming a common mechanism for this inhibitory response, the role of reactive oxygen species seems likely. The fixation of CO_2 is sensitive to a wide array of environmental stresses: including light (Sun et al., 1996), temperature, drought (Cornic and Ghashghaie, 1991) and salt (Yeo et al., 1985). Limitation of CO_2 fixation decreases NADPH use efficiency, subsequently reducing $NADP^+$, a major acceptor of electrons in PSI. This accelerates the rate of electron transport to molecular oxygen, forming superoxide anions, which lead to formation of H_2O_2 by superoxide dismutase in PSI (Takahashi and Murata, 2008). The increased production of H_2O_2 can exceed the rate at which it can be scavenged in the water-water cycle (Takahashi and Murata, 2008; Asada, 1999; DeRose et al., 1994; Barber, 2008; Song et al., 2006). Unscavenged H_2O_2 inhibits the repair of PSII through blocking the synthesis of a D1 precursor (Apel and Heribert, 2004; Nishiyama et al., 2001). Though these reactions have no effect on the rate of photodamage to PSII, with repair inhibited, photoinhibition is accelerated due to on-going damage incurred from light exposure (Takahashi and Murata, 2005).

Adaxial localized screening pigments increase the reflectance of red light, the absorbance of blue light, and the attenuation of green light. In an effort to better understand the role of adaxially localized pigment compounds in preventing photoinhibition in the lower mesophyll, Hormaetxe et al. (2005) tested the filtration qualities of variously colored cultivars of *Buxus sempervirens* under photoinhibitory conditions. Adaxial sections of green, brown, orange yellow, and red colored cultivars were positioned in place of adaxial removed sections of green shade leaves. Green adaxial sections demonstrated the highest levels of absorbance, and consequently, the lowest levels of photinhibition in the lower mesophyll. These results are most likely due to the increased light absorption efficiency and photostability of high chlorophyll content leaves,

whereas the accumulation of light filtration pigments is usually associated with lower levels of chlorophyll, and thus lower light use efficiency (Close and Beadle, 2003). Assuming this higher light protection by chlorophyll in the adaxial section of the leaves of this species, researchers should begin to ask why certain plants didn't evolve specialized green pigments for the more effective filtration of excess light (Hormaetxe et al., 2005).

CHAPTER 1
ASSESSING THE ROLE OF ADAXIALLY APPLIED PIGMENTS IN CREEPING BENTGRASS (*AGROSTIS PALUSTRIS)* STRESS REPONSES TO SUPRAOPTIMAL LEVELS OF UV AND VISIBLE LIGHT.

INTRODUCTION

Pigments in Creeping Bentgrass

In recent years, there have been numerous studies investigating the impacts of supraoptimal abiotic stress factors on *Agrostis spp.* plants. As a cool-season turfgrass, the ability of creeping bentgrass to acclimate to these factors is considered essential for the maintenance of photochemical processes vital to its survival (Liu and Huang, 2008). Consequently, creeping bentgrass has evolved multiple mechanisms for pigment upregulation during acclimatization to suproptimal abiotic stress environments. McElroy et al. (2006) published a study on carotenoid production in creeping bentgrass during events of sub- and supra-optimal light exposure. The authors found that these plants will upregulate the production of xanthophyll cycle pigments during adaptation to high-irradiance. McCurdy et al. (2008) discovered a similar response in another C3 turfgrass species, perennial ryegrass (*Lolium perenne* L.). Plants treated with mesotrione, a herbicidal inhibitor of carotenoid biosynthesis in sensitive species, triggered an upregulation of the photoprotectant pigments zeaxanthin and antheraxanthin at the expense of violaxanthin, during non-target application injury to *L. perenne*. This phenomenon was thought to be associated with an effective stress-response in the plant. Significant postapplication irradiation and temperature damage following mesotrione bleaching suggests that zeaxanthin could be involved in an alternate pathway responsible for quenching excess light energy and/or reducing the size of the light-harvesting complexes in high light, during a period of time when the plant is more susceptible to attenuated light, due to an inherent reduction in chlorophyll, and thus photochemical efficiency, during bleaching (Siefermann-Harms, 1987; Baroli et al., 2003;

McCurdy et al., 2008). This idea is consistent with later findings, by Liu and Huang (2008), that showed carotenoid upregulation in creeping bentgrass during acclimation to heat stress. These findings are also in agreement with the explanation of carotenoids as having a putative role in abiotic stress avoidance, which has been implicated as a vital survival mechanism in higher plant species (Demmig-Adams and Adams III, 1996).

In cold temperatures (0 to 13 ˚C), creeping bentgrass foliar tissues will turn purple in color. A similar phenomenon will occur under drought stress; leaves produce a more bluish to purple color during periods when the turfgrass is susceptible to foot printing (Dernoeden, 2000). This response has been associated with flavonoid metabolism and increased production of anthocyanin in the leaves of the plant (Han et al., 2009). Cool-season plants exhibit a diurnal regulation of carbohydrates, producing and accumulating most of their photosynthates in the leaf tissue of the plant during the day and translocating them during the night (Geiger and Servaites, 1994). During nights when the temperature drops from warm day temperatures (18 to 24 ˚C) to cold temperatures (0 to 13 ˚C), bentgrass plants will accumulate sugars in the leaves, due to an inability to transport sugars (Dernoeden, 2000). The presence of foliar sugars has been shown to induce anthocyanin synthesis, at which point anthocyanin will bind to sugars (Chalker-Scott, 2002). Consequently, the rate at which anthocyanin dissipates from the leaves will be reduced (Dernoeden, 2000). The use of plant-growth regulators has also been implicated in the increased build-up of foliar anthocyanin, which would coincide with a mechanism where the accumulation of sugars triggers anthocyanin production and binding in the leaf tissue (Dernoeden, 2000).

Ultra-violet light can cause significant visual and photosynthetic damage to turfgrass species in only a short period of time (Ervin et al., 2004). The accumulation of foliar anthocyanin and carotenoid pigments has been associated with improved UV-B protection in the leaves of

plant species (Rao et al., 1996; Bornman et al., 1997; Pérez-Rodríguez, 1998; Kondo and Kawashima, 2000). Zhang et al. (2005) found that dark green cultivars of kentucky bluegrass (*Poa pratensis* L.) experienced reduced damage under UV light when compared to a light green cultivar. Synthetic green pigment applications to the leaves of *Poa pratensis* under UV light treatment coincided with significantly higher visual quality and photochemical efficiency when compared to controls (Ervin et al., 2004).

Chlorella

Chlorella, a genus of unicellular green algae, was first recognized through isolation by Beijernick (1890). Among known photosynthetic organisms, it is highest in chlorophyll *a* and *b* production, capable of performing photosynthesis at a rate much higher than that of many plant species. The ratio of chlorophyll *a* to chlorophyll *b* in *Chlorella* can range from approximately 3 to 6 (Reger and Krauss, 1970). Its biomass also contains high concentrations of carotenoid pigments that are capable of providing unique health benefits in humans (Cha et al., 2008). Since their introduction to the health market during the 1960s, *Chlorella* species have experienced a pronounced growth in production for use as health supplements. One of the most popular species for these applications is *Chlorella vulgaris* (Kanno, 2005). Capable of being cultivated in large-scale bioreactors, *C. vulgaris* holds a significant advantage over that of higher plant species, providing a cheap and reliable source for the mass production of beneficial nutrients (Scragg et al., 2002).

Ranging from 2 to 10 μm in diameter, *C. vulgaris* cells have a globular shape, and a strengthened cell wall that prevents its adequate digestion and beneficial uptake in humans. For this reason, *Chlorella* cells are fragmented following cultivation, allowing cell contents, particularly lutein, to have greater bioavailability in humans (Mitsuda et al., 1977; Shibata &

Hayakawa, 2009). Commercially available forms of *C. vulgaris* thus consist of fragmented cells sold as a powder (Görs et al., 2010).

The absorption spectrum of *C. vulgaris* has light attenuation properties similar to that of chlorinated copper phthalocyanine, with peaks in the NIR region at 600 to 700 nm and the UV light region at 400 to 500 nm (Ley and Mauzerall, 1982; Yun and Park, 2001). It is hypothesized that the foliar application of pulverized *C. vulgaris* cells can provide novel insight into the plant health benefits associated with phthalocyanine application in creeping bentgrass. However, data describing the effects of *Chlorella* on the adaxial attenuation, screening, and alteration of incoming light are limited.

MATERIALS AND METHODS

Chamber Trial

'Penn A-4' creeping bentgrass was seeded at 96 kg ha^{-1} into 10-cm diameter pots containing an 80:20 v/v mixture of sand (Natural Grain Silica Sand, US Silica, Frederick, MD) and sphagnum peat moss (Premier Sphagnum Peat Moss Tourbe, Québec, Canada) and maintained in a greenhouse environment. Following germination, plants were fertilized every 4 days with a complete fertilizer (Vigoro All Purpose Plant Food 10-10-10, St. Louis, MO) at 24 kg N ha^{-1} until complete groundcover was achieved, upon which plants received weekly fertilization at 4.8 kg N ha^{-1}. In order to maintain soil moisture, plants were watered twice daily with overhead irrigation as needed. The creeping bentgrass plants were manually clipped with scissors twice weekly to maintain a height of approximately 1 cm.

After two months of growth in a greenhouse, plants were placed in a controlled environmental growth chamber (Conviron Adaptis A1000, Pembina, ND) equipped with High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ) for a 3 day acclimation

phase under a relatively low UV irradiance (18.2 mol m^{-2} d^{-1} PAR; 0.25 mol m^{-2} d^{-1} UV) for the UV light stress experiment and a relatively low visible irradiance for the visible light stress experiment (6.9 mol m^{-2} d^{-1} PAR; 0.004 mol m^{-2} d^{-1} UV). For relatively high UV irradiance (21.3 mol m^{-2} d^{-1} PAR; 4.6 mol m^{-2} d^{-1} UV) treatment following acclimation, the High Output Fluorescent Lamps were alternated with Zilla Desert 21 Watt UVB 50 Fluorescent T5 Bulb-Zilla Desert Lamps (Zilla Products, Franklin, WI). For relatively high visible light irradiance (32.6 mol m^{-2} d^{-1} PAR; 0.54 mol m^{-2} d^{-1} UV) treatment following acclimation, the plants were placed in closer proximity to the High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ). Light levels were chosen based on the maximum output achievable in the chamber when controlling for each of the types of light. While the measured UV light levels increased largely as a percentage across the acclimation and treatment phases of the PAR experiment, the incident flux of this light was still low. All plants were placed under a 12h photoperiod. Temperature was measured with an EasyLog USB Data Logger (Dataq, Akrom, OH) every 30 min. Temperature data were analyzed as means (Table 2) using the PROC MEANS statement in SAS 9.2 (SAS Institute, Cary, NC). While temperature did reach daytime extremes of 29 ˚C in the treatment phase of the PAR experiment, as compared to 25 ˚C in the other experiments, this was only for a brief period of time following the activation of lights in the chamber and was not representable of the entire photoperiod.

All plant pots were placed in standing water to ensure consistent water availability. This also eliminated the need for overhead irrigation, which could influence irradiance interception by the leaf, as well as cause treatment removal. Photosynthetically active radiation (PAR) and ultraviolet radiation (UV) photon flux (μmols $s^{-1}m^{-2}$) measurements were obtained using sensors active in the wavelength ranges of 400-700 nm and 250-400 nm respectively (Apogee Quantum

and UVS Sensors, Apogee Instruments, Logan, UT). All efforts were made to maintain the visible light irradiance values across the acclimation and treatment phases in the UV light stress experiment (See Table 1.1), the UV irradiance values across the acclimation and treatment phases in the visible light stress experiment (See Table 1.2), and temperature across all experiments (See Table 2).

The pigment treatments consisted of pulverized cells of *Chlorella vulgaris* (Nuts Online, Cranford, NJ) applied at 48.8 kg ha^{-1} on a 7-day interval, Signature fungicide (Bayer Environmental Science, Research Triangle Park, NC) at the label-recommended rate of 12.2 kg ha^{-1} on a 7-day interval, and water alone as an untreated control on a 7-day interval. The treatments were applied using a handheld pressurized sprayer (Preval, Precision Valve Corporation, Yonkers, NY) calibrated to deliver 794.4 L ha^{-1} at 15.2 cm above the plant. The UV and visible light experiments were repeated twice. Plants were arranged in a completely random design with 3 replications in the first and second experimental repetitions of the visible light experiment, and in the first experimental repetition of the UV experiment. A completely random design with 2 replications was used in the second experimental repetition of the UV experiment. Replication differences between experimental repetitions were used because of insufficient plant numbers available for clipping harvest in the second repetition of the UV experiment. Plants were clipped and fertilized a final time prior to the initiation of high light and treatment phases. During this phase, plants were rerandomized 3 days after the initiation of treatments in order to account for any potential variation in the interception of light caused by differences in plant location.

Chamber Data Collection

Plants were trimmed and clippings harvested once prior to the experiment and 1 week following the initiation of high light and pigment treatments. Prior to data retrieval, *Chlorella* treated plants were flushed with deionized water to ensure the removal of pigment treatments, which could otherwise influence plant pigment analysis. Clippings were immediately placed in cold storage at -80 °C, and then later ground in liquid N. Using methods described in Kopsell et al. (2007), leaf tissue pigments were extracted and quantified using High Pressure Liquid Chromatography (HPLC). Samples measuring 0.10 g of fresh tissue were analyzed in the first experimental repetition of the UV and visible light treatments. In the second experimental run, 0.25 g samples were analyzed in each experiment. Extraction efficacy was determined through the addition of ethyl-β-8'-apo-carotenoate (Sigma Chemical Co., St. Louis) and 2.5 mL tetrahydrofuran (THF) stabilized with 25 ppm 2,6-Di-tert-butyl-4-methoxyphenol. Each sample underwent homogenization using a pestle-attached drill press (Sears, Hoffman Estates, IL) set at 540 rpm in a tissue-grinding tube (Potter-Elvehjem, Kontes, Vineland, NJ) placed in ice for heat dissipation. Following homogenization the samples were placed in a centrifuge set at 500 g_n for approximately 5 min. The obtained supernatant was removed, re-suspended, and the process was repeated 3 to 4 times until the sample pellet was colorless. Extracted supernatants were then placed under a N gas stream (N-EVAP 111, Organomation, Berlin, MA) and reduced to approximately 0.5 ml. Following the addition of approximately 4.5 mL of acetone, 2 mL of solution was filtered through a 0.2-µm polytetrafluoroethylene filter (Econofilter PTFE 25/20, Agilent Technologies, Wilmington, DE). The obtained solution was then subjected to HPLC using an Agilent 1100 HPLC unit (Agilent Technologies, Santa Clara, CA) for the separation of pigments.

Data were analyzed through analysis of variance using the MIXED procedure in SAS 9.2 (SAS Institute, Cary, NC), and least squares means compared using the Least Significant Difference method (p=0.05). The pigments that were analyzed were beta-carotene, antheraxanthin, lutein, neoxanthin, violaxanthin, zeaxanthin, chlorophyll *a*, and chlorophyll *b*. Experimental repetitions were analyzed separately due to the presence of interaction effects for the treatments across the repetitions in both the high UV and PAR light trials. Visual and digital image analysis of quality was not measured in these plants due to the influence of pigments on visual color following high light treatment. This is due to the ability of chlorinated copper phthalocyanine to dye leaves that would otherwise appear bleached. Removing the Signature pigment from the leaves proved especially difficult following weeklong application, which is perhaps attributable to the ability of chlorinated copper phthalocyanine to infiltrate the plant leaf following application.

RESULTS AND DISCUSSION

Chlorophyll

In the first experimental repetition for high UV treatment, the changes in mean total chlorophyll measurements were statistically similar, with decreases of 30 %, 41 %, and 30 % for the control, *Chlorella*, and Signature treatments respectively (Table 3). However, significant differences between these changes were detected during the second experimental run. Total chlorophyll concentrations decreased to 23 % and 40 % in the control and Signature treated plants, but increased 1 % for those treated with *Chlorella* (Table 5). These observed differences in chlorophyll concentration between *Chlorella* treatments across the two experimental repetitions can most likely be attributed to the use of a greater amount of fresh weight (FW) in

the second experimental repetition. Lower fresh weight volumes may have contributed to larger variation in output readings, creating inconsistency across the experimental repetitions.

Under supraoptimal PAR, *Chlorella* and Signature treated plants experienced degradation levels statistically similar to that of untreated controls in the first experimental repetition (Table 4). During the second experimental repetition, chlorophyll degradation was greater in Signature treated plants than *Chlorella* and the untreated control (Table 6). This consistent decrease of total chlorophyll amounts across treatments indicates that the supraoptimal visible light condition in the growth chamber was highly detrimental to leaf pigmentation, where pigment application had little impact on reducing the degradation of chlorophyll.

The absence of chlorophyll protection in Signature applications under both suproptimal UV and PAR suggests that its ability to maintain plant quality under conditions of heat stress, as demonstrated by Norton et al. (2004), may not extend to conditions of excess light. Signature's ability to improve plant quality may best be attributed to the investigated mechanism of heat avoidance. However, these results do suggest that *Chlorella* may prove effective at limiting chlorophyll degradation under suproptimal UV, but not supraoptimal PAR. These results are in agreement with the finding that Signature appears less effective than *Chlorella* at attenuating light when applied adaxially at these rates (See Chapter 2).

Across the experiments, a visible bleaching effect occurred at the tips of plants treated with Signature following one week under high light, which agrees with the greater degradation of chlorophyll in Signature treated plants (Table 6). These effects were unexpected, as this phenomenon has not been previously reported to occur under high UV light in plants treated with copper phthalocyanine (Ervin et al., 2004). It is possible that Al from the Fosetyl-Al present in Signature was responsible for toxicity effects under conditions of high light stress. Symeonidis et

al. (2004) previously demonstrated how increases in Al concentrations led to decreases in leaf chlorophyll content in *Cucumis melo*. Aluminum is believed to cause morphological damage, affecting photosynthesis by lowering chlorophyll content and reducing electron flow in the leaves of plants. It also interferes with the uptake, transport, and use of the essential elements Cu, Zn, Ca, MG, Mn, K, P, and Fe (Roy et al., 1988). Specific to turfgrass, aluminum toxicity has been shown to cause reduction in clipping yields in bentgrass plants (Kuo et al., 1992). When combined with high light irradiance, it's possible that the presence of exchangeable Al further promotes this bleaching effect in bentgrass leaves. This was further exemplified by the presence of purple leaves in the control and *Chlorella* treated plants, but not Signature treated plants, which were consistently bleached. This visible purpling is most likely a result of the build-up of anthocyanins within the bentgrass plant (Dernoeden, 2000). Visible purpling was also visibly greatest in control and *Chlorella* treated plants in the UV high light treatment. This is in agreement with the aforementioned roles of anthocyanin in plant photoprotection from UV-B, which was shown to be present in high light treatments (See Chapter 2). The absence of visible anthocyanin accumulation in the leaves of Signature treated plants, suggests that either Fosetyl-Al or chlorinated copper phthalocyanine was responsible for limiting this stress response in the plant. During HPLC analysis, particles of the synthetic pigment copper phthalocyanine were consistently visible in the fresh weight extract of Signature treated plants, suggesting that these pigment molecules did not break down in the presence of high light, and thus did not render the Cu in copper phthalocyanine to be taken up by the plant.

Carotenoids

UV and PAR light caused noticeable changes in carotenoid composition across treatments. Under conditions of supraoptimal UV light, lutein, beta-carotene, and neoxanthin

decreased in untreated controls, but significantly increased in *Chlorella* and Signature treated plants (Table 5, 5.2), suggesting that carotenoid pigment degradation had been markedly reduced by applied treatments. Lutein and beta-carotene have previously been shown to increase in the presence of high irradiance conditions in various species (Li and Walton, 1990; Hansen et al., 2002). Conversely, overall decreases in lutein and neoxanthin were observed in high irradiance, but not low irradiance (Rosevear et al., 2001). Emphasized across these experiments, is the interchangeable nature of these pigments, where one may functionally replace the absence of another during these responses (McElroy et al., 2006; Baroli et al., 2003; Niyogi et al., 1998). These results extended to changes in the total carotenoid measurements, where untreated controls decreased by 15 %, and *Chlorella* and Signature plants increased significantly by 12 % and 1 % respectively (Table 5.2). *Chlorella* treated plants also exhibited increases in the total xanthophyll cycle pigments violaxanthin, zeaxanthin, and antheraxanthin, which were found to be significantly greater than untreated controls, where slight decreases occurred (Table 5).

Across the experiments, increases in irradiance were followed by increases in zeaxanthin and antheraxanthin and decreases in violaxanthin across all treatments. This flux, or reallocation, of pigment resources in the xanthophyll cycle pool helps to better protect the plant under conditions of supraoptimal irradiance (Demmig-Adams et al., 1996). This phenomenon reflects the previous findings of McElroy et al. (2006), where bentgrass experienced similar increases in zeaxanthin and antheraxanthin and decreases in violaxanthin following 168 h of exposure to high irradiance (McElroy et al., 2006). The overall absence of significant differences between changes in these treatments indicates that the pigments were not capable of limiting the stress responses exhibited by the plant when subjected to these levels of PAR and UV light increases.

CHAPTER 2
EFFECT OF PIGMENTS ON THE TRANSMISSION SPECTRA OF UV AND VISIBLE LIGHT.

INTRODUCTION

Commercial Pigments

In recent years there has been an increased interest in the utilization of pigments in the field of turfgrass science. In 2004, a patent was filed for the commercial use of Pigment Green 7, a polychlorinated form of copper phthalocyanine, on turfgrass plants. Under supraoptimal heat stress, creeping bentgrass (*Agrostis palustris)* plants treated with copper phthalocyanine showed increases in quality, chlorophyll content, carotenoid content, and photochemical efficiency when compared to controls (Norton et al., 2004).

Copper phthalocyanine, specifically Pigment Green 7 (chlorinated copper phthalocyanine), has a long history of application across many industries. It has mainly seen use in outdoor paints due to its increased dispersibility, light fastness, heat stability, and durability (Kadish et al., 2003; Tracton, 2006). These properties make it unlikely that Cu is rendered available to the plant following application. This is supported by the visible presence of synthetic pigment in the HPLC analysis (See Chapter 1) following weeklong high light treatment, suggesting that the pigment molecules did not break down, and thus did not render Cu available for uptake in the plant.

Recently, copper phthalocyanine has begun to see use as a photosensitizer in dye-sensitized organic solar cells (Huang et al., 2009; Chu et al., 2006; Tripathi et al., 2008). This use can be attributed to the structural and spectroscopic similarities of copper phthalocyanine to plant chlorophyll compounds (Ludwig et al.,1994; Karan et al., 2007; Bohn and Walczyk, 2004). This

has resulted in many experiments evaluating their efficiency in harvesting solar energy (Farag, 2007).

Copper phthalocyanine (phthalocyanine blue) and chlorinated copper phthalocyanine (phthalocyanine green) are pigments known for their excellent light and heat stability, allowing them to maintain structure under outdoor conditions. In general, all copper phthalocyanines are considered weakly scattering pigments, with strong absorption in the red to near-infrared (NIR) portions between 500 and 700 nm, the UV region between 300 and 400 nm, and equally strong fluorescence in the UV-B between 350 and 500 nm (Levinson et al., 2005*a*; Levinson et al., 2005*b)*. Its UV-B absorption and fluorescence spectrum strongly overlap the emission band of UV light capable of high photoinhibition efficiency in the excitation of Mn in the OEC between 300 and 500 nm (Levinson et al., 2005*a*; Levinson et al., 2005*b*; Bigger and Delatycki, 1989; Saron et al., 2006; Hakala et al., 2005). Plants are only able to reflect very small amounts of UV radiation (~3%) (Larcher, 2003). Copper phthalocyanine's ability to attenuate extremely high levels of incident high energy light with minimal scattering, may allow it to effectively reduce levels of creeping bentgrass photoinhibition. By adjusting the density at which the pigment is applied via a carrier (i.e. water), the level of incident light attenuation can be adjusted to meet the high light avoidance needs of the plant (Norton et al., 2004). The largest dip in light attenuation by copper phthalocyanine occurs at 550 nm, allowing it to closely mimic the action spectrum of photosynthesis, and have a green color (McDonald, 2003; Saron et al., 2006). Phthalocyanine blue and phthalocyanine green are both insoluble, taking the appearance of powders. The weak scattering properties of the copper phthalocyanines are due to their small particle size, which is typically 120 nm in diameter (Levinson et al., 2005*b*).

Pigments more capable of reflecting NIR light are much cooler in sunlight that those that absorb NIR, such as copper phthalocyanines. This has particular importance in roofing, where the use of roofs with NIR reflecting pigments can significantly reduce building heat gain over that of roofs utilizing NIR absorbing pigments (Levinson et al., 2005*a*; Levinson et al., 2005*b*). This has led to the identification of dark colored pigments that have the ability to reflect infrared heat-building rays to the same degree that a white roof would (Miller et al., 2004). Copper phthalocyanine is considered to be an absorber of NIR light, and thus is more capable of heat build-up (Levinson et al., 2005*a*; Day and Williams, 1965).

The ability of these pigments to improve plant quality under light is possibly accomplished through reducing the quality or the quantity of the intercepted irradiance. The purpose of this study is to determine which wavelengths of light these pigments are affecting.

MATERIALS AND METHODS

Measurement of Pigment/Light Interactions

The role of exogenously applied pigments in the adaxial attenuation, filtration, and/or alteration of incoming light were studied independent of creeping bentgrass application. Sterile polystyrene petri dishes were placed under the same UV and PAR lights used in the bentgrass chamber experiments (See Chapter 1). Treatments of Signature (12.2 kg ha^{-1}), *Chlorella* (48.8 kg ha^{-1}), and a water control were applied to Fisherbrand sterile polystyrene petri dishes (Fisher Scientific, Hamptom, NH) placed randomly throughout the growth chamber and left to dry for one hour. The treatments were applied using a handheld pressurized sprayer (Preval, Precision Valve Corporation, Yonkers, NY) calibrated to deliver 794.4 L ha^{-1} at 15.2 cm above the plant. A spectroradiometer (PORTA-LIBS EPP2000, StellarNet, Tampa, FL) was positioned subjacent to the petri dishes at a random point in order to measure incident levels of UV-B (280 to 315 nm)

and PAR (400 to 700 nm) photon flux (μmols $s^{-1}m^{-2}$) measurements before and after pigment application. A completely random design with four replications was used. Data were analyzed through analysis of variance using the PROC MIXED procedure in SAS 9.2 (SAS Institute, Cary, NC), and least squares means compared using the Least Significant Difference method (p=0.05).

In order to accurately measure any spectral changes caused by the pigments, the spectroradiometer, active in the wavelength range of 200 to 1150 nm (PORTA-LIBS EPP2000, StellarNet, Tampa, FL), was also used to take images of the light transmittance spectrums using SpectraWiz (StellarNet, Tampa, FL) and Essential FTIR (Operant LLC, Sarasota, FL) software. Average spectral irradiance measurements were taken before and after pigment application to the surface of the petri dishes. The petri dishes had little effect on the spectroscopic characteristics of UV and PAR light treatments (Figures 4, 5).

RESULTS AND DISCUSSION

Spectral imaging of the UV and visible light spectrums used in the chamber experiments demonstrated differences in both the UV and PAR regions of the wavelength spectrum (Figure 1). Under high UV light, there was an increase in peak absorbance between 300 and 400 nm when compared to that of the visible light treatment, where larger characteristic peaks in the PAR region allowed for greater absorbance above 480 nm. These results coincide with the previous photon flux measurements in the chamber experiment (See Chapter 1).

Visible Light

Spectroradiometer measurements of visible light transmission through petri dishes treated with *Chlorella* and Signature pigments demonstrated decreases in photon flux when compared to that of petri dish controls. When transmitted through applications of *Chlorella*, spectral imaging

showed noticeable decreases across multiple wavelength bands of the spectrum, with the largest decreases occurring between 480 and 580 nm (Figure 2). However, when visible light was transmitted through applications of Signature, spectral imaging showed little difference in the wavelengths of transmitted light when compared to that of controls (Figure 2). This improved ability of *Chlorella* to filter visible light was shown to be significant in PAR photon flux transmittance measurements (Table 8).

UV Light

Spectral imaging of UV light transmission through petri dishes treated with *Chlorella* pigments also demonstrated localized decreases in the transmission of light, with the greatest occurring in the UV-B (280-315 nm) and UV-A (315-400 nm) regions of the spectrum (Figure 3). Conversely, Signature demonstrated very minor influences across these wavelengths (Figure 3). The photon flux decreases in the UV-B portion of the spectrum were shown to be significantly greater in measurements of *Chlorella* treated petri dishes when compared to Signature and the control (Table 8).

The results of the spectral imaging (Figures 2, 3) were in agreement with the quantitative amounts of UV and visible light photon flux changes (Table 8). In this experiment, both Signature and *Chlorella* were observed to reduce the levels of PAR and UV photon flux transmittance when compared to controls, where *Chlorella* demonstrated significantly lower light transmission than Signature under both high UV-B and PAR light. The roles of copper phthalocyanine in UV-B light absorbance are in agreement with previous findings (Levinson et al., 2005*a*; Levinson et al., 2005*b*). However, these results show significantly greater decreases in UV-B light transmittance by *Chlorella* when compared to Signature (Table 8). Considering that reduced pigment degradation was observed following *Chlorella* treatment (Chapter 1), data

in the current study suggest that retardation of UV light by *Chlorella* may be a mechanism preventing pigment degradation.

Conclusions

Under these high UV and PAR light treatments, *Chlorella*, when applied exogenously at a rate of 48.8 kg ha^{-1}, causes a reduction in the transmission of UV light between 300 and 400 nm and PAR between 480 and 580 nm, which reduces chlorophyll and carotenoid degradation under UV, but not PAR light (Figure 6). However, these effects do not extend to a measurable change in bentgrass stress response, as it relates to the buildup of xanthophyll cycle pigments. The method through which Signature reduces total carotenoid degradation under UV light, as observed in HPLC, was not readily apparent from spectral imaging, where levels of UV light between 300 and 400 nm remained largely unaffected. Signature did display a significant reduction in UV-B photon flux transmission, however the transmitted light was still significantly greater than that of *Chlorella*, suggesting that the reduced filtration of light by Signature was enough to prevent carotenoid, but not chlorophyll, degradation under UV light (Figure 7). Signature's ability to improve plant quality, as previously shown by Norton et al. (2004), may best be attributed to a mechanism of heat avoidance. Consequently, more research is needed if turfgrass reseachers are to draw definitive inferences about the relationships that exist between pigment applications incorporating copper phthalocyanine and photoprotection from high-energy light.

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APPENDIX

PRELIMINARY RESEARCH

Field Trial

A preliminary field trial was conducted during the summer of 2011 on a previously established, mixed variety, creeping bentgrass putting green constructed with a USGA (United States Golf Association) specification sand-based root-zone at the East Tennessee Research and Education Center (Knoxville, TN). This research was performed in order to determine the applicability of various pigments in the field, and determine potential mechanisms for further experimentation in a controlled setting. Plants were mown daily at a height of 0.32 cm using a reel mower. Plants were watered daily using overhead irrigation, and fertilized with urea Nitrogen (46-0-0) at 12.2 kg N ha^{-1} once a month during the growing season.

Treatments consisted of pulverized cells of *Chlorella vulgaris* (Nuts Online, Cranford, NJ) applied at 48.8 kg ha^{-1} on a 4-day interval, Signature fungicide (Bayer Environmental Science, Research Triangle Park, NC) at the label-recommended rate of 12.2 kg ha^{-1} on a 7-day interval, and Foursome Turf Pigment (Quali-Pro, Pasadena, TX) at the label-recommended rate of 1.3 L ha^{-1} on a 7-day interval. The control treatment consisted of water applied at 815 L ha^{-1} on a 7-day interval. All treatments were submersed in a water carrier and applied using a CO_2 pressurized hand-held boom sprayer with two flatfan nozzles (TeeJet 8004 XR, Spraying Systems Co., Roswell, GA) spaced 25 cm apart and positioned 25 cm above the spraying surface. The sprayer was calibrated to deliver 815 L ha^{-1} of water under a pressure of 193 kPa. Plots were arranged in a randomized complete block design with each treatment having four replications.

Field Data Collection

Visual turf quality, reflectance, and digital image analysis data were collected once prior to the experiment and 6 weeks following the initiation of treatments. Visual turf quality was assessed on a scale from 0 to 9, with 0 being the worst and 9 being the best. Canopy reflectance was measured using a Crop Circle ACS-470 spectrophotometer (Holland Scientific, Lincoln, NE) calibrated and configured for use with filters 650-40, 760/LWP, and 550-40 set for channels 1, 2, and 3 respectively. Obtained reflectance values were then used to calculate the Normalized Difference Vegetation Index (NDVI) and Ratio Vegetation Index (RVI). The obtained NDVI and RVI values were then multiplied by a factor of 10 to denote a rating scale from 0 to 10. Digital Image Analysis (DIA) was performed once prior to the experiment and 6 weeks following the initiation of treatments using still pictures taken with a Powershot G-12 Camera (Canon U.S.A., Lake Success, NY) calibrated for use in a light box equipped with approximately 120 6500 Kelvin Color Temperature Light-Emitting Diodes (LEDs). The obtained pictures were analyzed for percent green cover in Sigma Scan Pro 5 (SPSS, Chicago, IL) utilizing the methods described in Richardson et al. (2001). Analysis of variance was conducted using mixed models in ARM 7 (Gylling Data Management Inc., Brookings, SD), and least squares means were separated using the Least Significant Difference method (p=0.05).

Results

Analysis of variance in the field trial revealed significant differences in visual quality, RVI, NDVI, and DIA green cover between treatments (Table 7). All pigment treated plots had significantly higher visual quality ratings than that of the control, with Foresome and Signature treated plots measuring higher in quality than those treated with *Chlorella*. Similar relationships were detected for green cover. However, only *Chlorella* treated plots measured higher in NDVI

and RVI than the control. This could perhaps be attributed to the fact that copper phthalocyanine based pigments (i.e., Signature and Foresome) absorb NIR which consequently affects NDVI and RVI measurements (Levinson et al., 2005*a*; Day & Williams, 1965).

Response with copper phthalocyanine pigments in the current study support previous findings of Norton et al. (2004) and Ervin et al. (2004), who observed increased cool-season turfgrass quality when these materials were applied during abiotic stress. From this research, it is purported that placement of copper phthalocyanine on leaf tissue can effectively screen harmful light, preventing it from negatively impacting the leaf. Improvements in turf quality using *Chlorella* support the idea that the natural pigments found in fractured cells of the green algal protist species may function in a similar manner.

TABLES AND FIGURES

Table 1.1. Environmental light conditions during experiments measuring the response of creeping bentgrass (*Agrostis palustris*) to relatively high UV[a] irradiance in an environmental growth chamber.

	Incident[b]		Cumulative[c]	
Phase	PAR	UV	PAR	UV
	μmol m^{-2} s^{-1}		mol m^{-2} d^{-1}	
Acclimation	420.4 (±123.9)	5.80 (±1.53)	18.2	0.25
Treatment	493.9 (±108.6)	106.6 (±5.05)	21.3	4.6

[a]Abbreviations: Photosynthetically active radiation, PAR; Ultraviolet, UV.
[b]Incident light values were measured every 30 minutes during the 12 h active photoperiod.
[c]Cumulative light values were taken as cumulative average of incident light values over a 24-hour period.

Table 1.2. Environmental light conditions during experiments measuring the response of creeping bentgrass (*Agrostis palustris*) to relatively high visible irradiance in an environmental growth chamber.

	Incident[b]		Cumulative[c]	
Phase	PAR[a]	UV	PAR	UV
	μmol m^{-2} s^{-1}		mol m^{-2} d^{-1}	
Acclimation	160.8 (±14.16)	0.09 (±0.04)	6.9	0.004
Treatment	760.6 (±94.59)	12.55 (±1.31)	32.6	0.54

[a]Abbreviations: Photosynthetically active radiation, PAR; Ultraviolet, UV.
[b]Incident light values were measured every 30 minutes during the 12 h active photoperiod.
[c]Cumulative light values were taken as cumulative average of incident light values over a 24-hour period.

Table 2. Environmental temperature conditions during experiments measuring the response of creeping bentgrass (*Agrostis palustris*) to relatively high UV[a] and visible light in an environmental growth chamber.

	UV light		Visible light	
Phase	Mean	High/Low	Mean	High/Low
	C°			
Acclimation	19.02 (±3.46)	25/15	20.00 (±5.05)	25/15
Treatment	19.24 (±3.16)	24/15	22.04 (±5.75)	29/15

[a]Abbreviation: Ultraviolet, UV.
[b]Temperature and relatively humidity were recorded every 30 minutes and averaged across 24 hours during each experiment. High and low temperatures were recorded for each phase of the two experiments.

Table 3. First experimental repetition for total chlorophyll (chlorophyll a + b), total xanthophyll cycle (antheraxanthin + violaxanthin + zeaxanthin), and lutein pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}), under relatively high UV[a] light in an environmental growth chamber.

	Total Chlorophyll			Total Xanthophyll			Lutein		
	mg 100 g FW $^{-1}$[ab]								
Treatment	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	252.78	176.77	-76.02 **a**	12.36	11.06	-1.30 **a**	21.22	15.02	-6.20 **a**
Chlorella	263.88	155.25	-108.63 **a**	13.87	6.28	-7.60 **a**	21.49	11.74	-9.75 **a**
Signature	276.46	194.77	-81.68 **a**	13.44	8.08	-5.36 **a**	21.95	11.14	-10.81 **a**
LSD(0.05)[c]			234.38			11.05			22.23

[a]Abbreviations: fresh weight, FW; ultraviolet, UV.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 3.1 First experimental repetition for violaxanthin, zeaxanthin, and antheraxanthin pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}), under relatively high UV[a] light in an environmental growth chamber.

	Violaxanthin			Zeaxanthin			Antheraxanthin		
	mg 100 g FW^{-1}[ab]								
Treatment	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	3.89	6.03	2.14 **a**	1.22	1.45	0.22 **a**	7.24	3.58	-3.65 **a**
Chlorella	4.53	4.24	-0.29 **a**	1.34	0*	-1.34 **a**	8.00	2.04	-5.97 **a**
Signature	4.47	4.93	0.46 **a**	1.35	0.96	-0.40 **a**	7.61	2.19	-5.42 **a**
LSD(0.05)[c]			4.72			3.03			5.10

[a]Abbreviations: fresh weight, FW; ultraviolet, UV.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

*Statistical analysis limited by the presence of multiple values of 0 (Not Detectable in HPLC analysis)

Table 3.2 First experimental repetition for beta-carotene, neoxanthin, and total carotenoids (antheraxanthin + beta carotene + zeaxanthin + lutein + neoxanthin + violaxanthin) pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}), under relatively high UV[a] light in an environmental growth chamber.

Treatment	Beta-carotene			Neoxanthin			Total Carotenoids		
	mg 100 g FW $^{-1}$[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	4.36	2.36	-2.01 **a**	6.53	6.19	-0.34 **a**	44.47	34.63	-9.85 **a**
Chlorella	5.98	1.90	-4.08 **a**	6.33	3.78	-2.55 **a**	47.68	23.69	-23.98 **a**
Signature	5.51	1.89	-3.62 **a**	6.50	3.85	-2.65 **a**	47.40	24.96	-22.44 **a**
LSD(0.05)[c]			4.70			6.54			42.92

[a]Abbreviations: fresh weight, FW; ultraviolet, UV.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 4. First experimental repetition for total chlorophyll (chlorophyll a + b), total xanthophyll cycle (antheraxanthin + violaxanthin + zeaxanthin), and lutein pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}) while under relatively high visible light in an environmental growth chamber.

Treatment	Total Chlorophyll			Total Xanthophyll			Lutein		
	mg 100 g FW^{-1}[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	185.98	136.57	-49.41 **a**	2.79	3.84	1.05 **a**	12.30	9.14	-3.16 **a**
Chlorella	157.93	129.52	-28.41 **a**	2.34	2.79	0.45 **a**	11.98	8.20	-3.78 **a**
Signature	128.48	120.40	-8.07 **a**	2.47	3.16	0.69 **a**	9.88	8.01	-1.88 **a**
LSD(0.05)[c]	106.34			2.11			6.60		

[a]Abbreviations: fresh weight, FW.

[b]Pigment concentrations expressed as mg 100 g fresh weight^{-1} (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 4.1 First experimental repetition for violaxanthin, zeaxanthin, and antheraxanthin pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}) while under relatively high visible light in an environmental growth chamber.

Treatment	Violaxanthin			Zeaxanthin			Antheraxanthin		
	mg 100 g FW $^{-1}$[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	1.56	0.89	-0.67 **a**	0.21	0.43	0.22 **a**	1.01	2.51	1.50 **a**
Chlorella	1.52	0.91	-0.61 **a**	0.07	0.31	0.24 **a**	0.75	1.57	0.82 **a**
Signature	1.27	0.84	-0.43 **a**	0.22	0.31	0.09 **a**	0.98	2.00	1.02 **a**
LSD(0.05)[c]			1.02			0.41			1.62

[a]Abbreviations: fresh weight, FW.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 4.2 First experimental repetition for beta-carotene, neoxanthin, and total carotenoids (antheraxanthin + beta carotene + zeaxanthin + lutein + neoxanthin + violaxanthin) pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}), while under relatively high visible light in an environmental growth chamber.

Treatment	Beta-carotene			Neoxanthin			Total Carotenoids		
	mg 100 g FW $^{-1}$[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	4.19	4.13	-0.06 **a**	5.99	4.21	-1.77 **a**	25.27	21.33	-3.95 **a**
Chlorella	5.38	3.76	-1.62 **a**	5.97	4.09	-1.88 **a**	25.66	18.84	-6.83 **a**
Signature	4.15	3.79	-0.36 **a**	4.63	3.36	-1.27 **a**	21.14	18.32	-2.82 **a**
LSD(0.05)[c]			2.55			4.15			13.28

[a]Abbreviations: fresh weight, FW; ultraviolet, UV.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 5. Second experimental repetition for total chlorophyll (chlorophyll a + b), total xanthophyll cycle (antheraxanthin + violaxanthin + zeaxanthin), and lutein pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}), under relatively high UV[a] light in an environmental growth chamber.

Treatment	Total Chlorophyll			Total Xanthophyll			Lutein		
	mg 100 g FW $^{-1}$[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	814.62	626.82	-187.80 **b**	21.94	21.25	-0.69 **b**	46.65	39.05	-7.59 **b**
Chlorella	653.10	659.30	6.20 **a**	16.34	19.58	3.25 **a**	37.23	41.88	4.65 **a**
Signature	690.52	413.01	-277.51 **b**	19.17	19.50	0.32 **ab**	39.61	42.63	3.03 **a**
LSD(0.05)[c]			128.80			3.05			9.09

[a]Abbreviations: fresh weight, FW; ultraviolet, UV.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 5.1 Second experimental repetition for violaxanthin, zeaxanthin, and antheraxanthin pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}), under relatively high UV[a] light in an environmental growth chamber.

Treatment	Violaxanthin			Zeaxanthin			Antheraxanthin		
	mg 100 g FW^{-1}[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	15.21	10.47	-4.75 **a**	0.40	1.13	0.74 **a**	6.33	9.67	3.33 **a**
Chlorella	10.71	9.45	-1.26 **a**	0.29	1.18	0.88 **a**	5.33	8.95	3.62 **a**
Signature	12.30	8.70	-3.60 **a**	0.51	1.15	0.64 **a**	6.36	9.65	3.29 **a**
LSD(0.05)[c]			5.78			0.97			3.67

[a]Abbreviations: fresh weight, FW; ultraviolet, UV.

[b]Pigment concentrations expressed as mg 100 g fresh weight^{-1} (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 5.2 Second experimental repetition for beta-carotene, neoxanthin, and total carotenoids (antheraxanthin + beta carotene + zeaxanthin + lutein + neoxanthin + violaxanthin) pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}), under relatively high UV[a] light in an environmental growth chamber.

Treatment	Beta-carotene			Neoxanthin			Total Carotenoids		
	mg 100 g FW $^{-1}$[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	20.01	14.05	-5.97 **b**	17.64	15.73	-1.91 **b**	106.22	90.08	-16.14 **b**
Chlorella	16.51	15.44	-1.07 **a**	13.54	16.49	2.95 **a**	83.61	93.39	9.78 **a**
Signature	16.98	12.83	-4.15 **ab**	14.87	16.95	2.08 **a**	90.63	91.90	1.27 **a**
LSD(0.05)[c]			3.29			3.19			15.04

[a]Abbreviations: fresh weight, FW; ultraviolet, UV.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 6. Second experimental repetition for total chlorophyll (chlorophyll a + b), total xanthophyll cycle (antheraxanthin + violaxanthin + zeaxanthin), and lutein pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}) while under relatively high visible light in an environmental growth chamber.

Treatment	Total Chlorophyll			Total Xanthophyll			Lutein		
	mg 100 g FW^{-1}[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	904.45	700.53	-203.92 **a**	20.72	31.44	10.72 **a**	52.05	41.52	-10.53 **a**
Chlorella	965.68	701.82	-263.86 **a**	22.97	29.26	6.29 **a**	55.35	42.42	-12.94 **a**
Signature	983.68	458.53	-525.15 **b**	22.39	23.84	1.45 **a**	55.98	36.61	-19.37 **a**
LSD(0.05)[c]			239.60			13.33			17.39

[a]Abbreviations: fresh weight, FW.

[b]Pigment concentrations expressed as mg 100 g fresh weight^{-1} (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 6.1 Second experimental repetition for violaxanthin, zeaxanthin, and antheraxanthin pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}) while under relatively high visible light in an environmental growth chamber.

Treatment	Violaxanthin			Zeaxanthin			Antheraxanthin		
	mg 100 g FW $^{-1}$[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	15.46	7.97	-7.48 **a**	0.19	2.93	2.74 **a**	5.08	20.54	15.46 **a**
Chlorella	16.55	8.16	-8.39 **a**	0.31	2.65	2.34 **a**	6.11	18.46	12.35 **a**
Signature	16.59	6.06	-10.53 **a**	0.28	2.44	2.16 **a**	5.52	15.34	9.82 **a**
LSD(0.05)[c]			5.27			1.12			7.64

[a]Abbreviations: fresh weight, FW.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 6.2 Second experimental repetition for beta-carotene, neoxanthin, and total carotenoids (antheraxanthin + beta carotene + zeaxanthin + lutein + neoxanthin + violaxanthin) pigment concentrations in creeping bentgrass (*Agrostis palustris*) leaves emerging before (Time 0) and 1 week after (Time 1) treatment[f] with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}), while under relatively high visible light in an environmental growth chamber.

Treatment	Beta-carotene			Neoxanthin			Total Carotenoids		
	mg 100 g FW $^{-1}$[ab]								
	Time								
	0	1	Δ	0	1	Δ	0	1	Δ
Control	19.89	12.53	-7.36 **a**	14.28	13.66	-0.61 **a**	106.94	99.15	-7.79 **a**
Chlorella	20.73	13.22	-7.51 **a**	15.91	13.97	-1.95 **a**	114.97	98.87	-16.10 **a**
Signature	20.24	8.43	-11.81 **a**	15.71	12.93	-2.78 **a**	114.32	81.81	-32.51 **a**
LSD(0.05)[c]			7.98			6.46			44.37

[a]Abbreviations: fresh weight, FW; ultraviolet, UV.

[b]Pigment concentrations expressed as mg 100 g fresh $weight^{-1}$ (FW)

[c]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

[f]All treatments applied on a 7-day interval

Table 7. Plant health measurements visual quality (Quality), ratio and normalized difference vegetation indices (RVI & NDVI), and digital image analysis (DIA) of percent green cover following 6 weeks of pigment applications consisting of pulverized cells of *Chlorella vulgaris* (Nuts Online, Cranford, NJ) applied at 48.8 kg ha^{-1} on a 4-day interval, Signature fungicide (Bayer Environmental Science, Research Triangle Park, NC) at 12.2 kg ha^{-1} on a 7-day interval, Foursome Turf Pigment (Quali-Pro, Pasadena, TX) at 1.3 L ha^{-1} on a 7-day interval, and water alone as an untreated control on a 7-day interval in the summer of 2011 at the East Tennessee Research and Education Center in Knoxville, TN.

Treatment	Rate	Quality[f]	RVI[e]	NDVI[h]	DIA % Cover[gh]
Control[a]	252.78	4.00 **c**	5.48 **b**	6.89 **b**	92.29 **b**
Signature[c]	12.2 kg ha^{-1}	7.00 **ab**	5.72 **b**	6.99 **b**	98.26 **a**
Chlorella[b]	48.8 kg ha^{-1}	6.00 **b**	6.63 **a**	7.36 **a**	97.62 **a**
Foresome[c]	1.3 L ha^{-1}	7.75 **a**	5.91 **ab**	7.10 **ab**	99.03 **a**
LSD(0.05)[i]		1.47	0.73	0.29	1.94

[a]Application consisted of water applied at 814.8 L ha^{-1} every 4-days,
[b]Applied on a 4-day Interval.
[c]Applied on a 7-day Interval.
[d]Means followed by same letter do not significantly differ (P=.05, LSD).
[e]RVI and NDVI values have been scaled up by a factor of 10.
[f]Measured as visual quality on a scale from 1-9 based with 1 being the worst and 9 being the best.
[g]Measured as % green cover using methods described in Richardson et al. (2001).
[h]Abbreviations: ratio vegetation index, RVI; normalized difference vegetation index, NDVI; digital image analysis, DIA.
[i]Means separated using Fisher's least significant difference test ($p \leq 0.05$)

Table 8. Photon flux measurements (μmol m^{-2} s^{-1}) of UV-B (280-315 nm) and PAR (400-700 nm) light following transmission through sterile polystyrene petri dishes (Fisher Scientific, Hamptom, NH) treated with control[d] (794.4 L ha^{-1}), Signature (12.2 kg ha^{-1}), and *Chlorella*[e] (48.8 kg ha^{-1}) in an environmental growth chamber.

Treatment	PAR[ac]	UV-B[ab]
	μmol m^{-2} s^{-1}	
Control	490.3 **a**	3.41 **a**
Signature	467.0 **b**	3.11 **b**
Chlorella	377.5 **c**	1.82 **c**
LSD(0.05)[f]	11.67	0.11

[a]Abbreviations: photosynthetically active radiation, PAR; ultraviolet, UV.

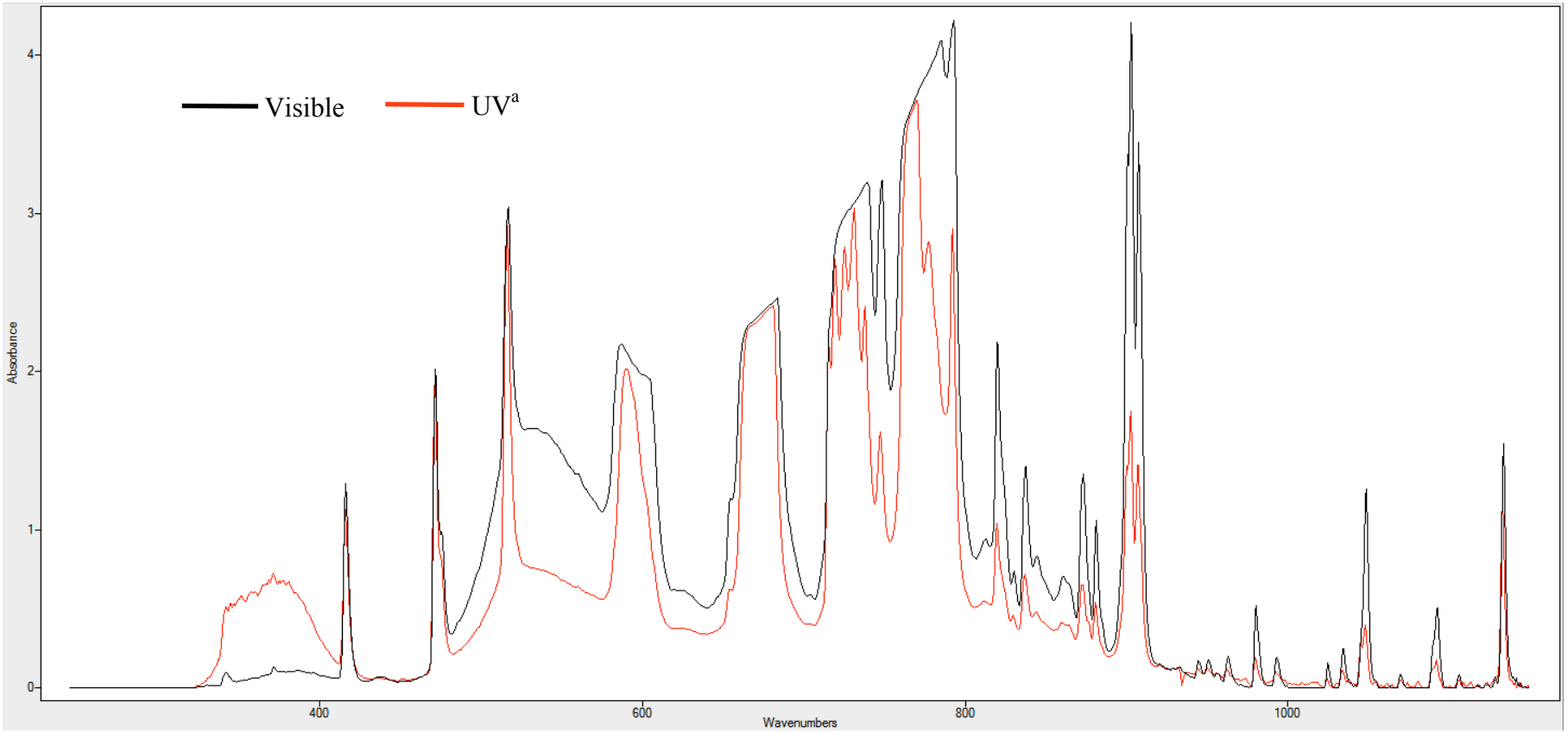
[b]Consisted of output from a mixture of High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ) and Zilla Desert 21 Watt UVB 50 Fluorescent T5 Bulb-Zilla Desert Lamps (Zilla Products, Franklin, WI).

[c]Consisted of output from High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ).

[d]Administered as water

[e]Applied as fractured cells of *Chlorella vulgaris*

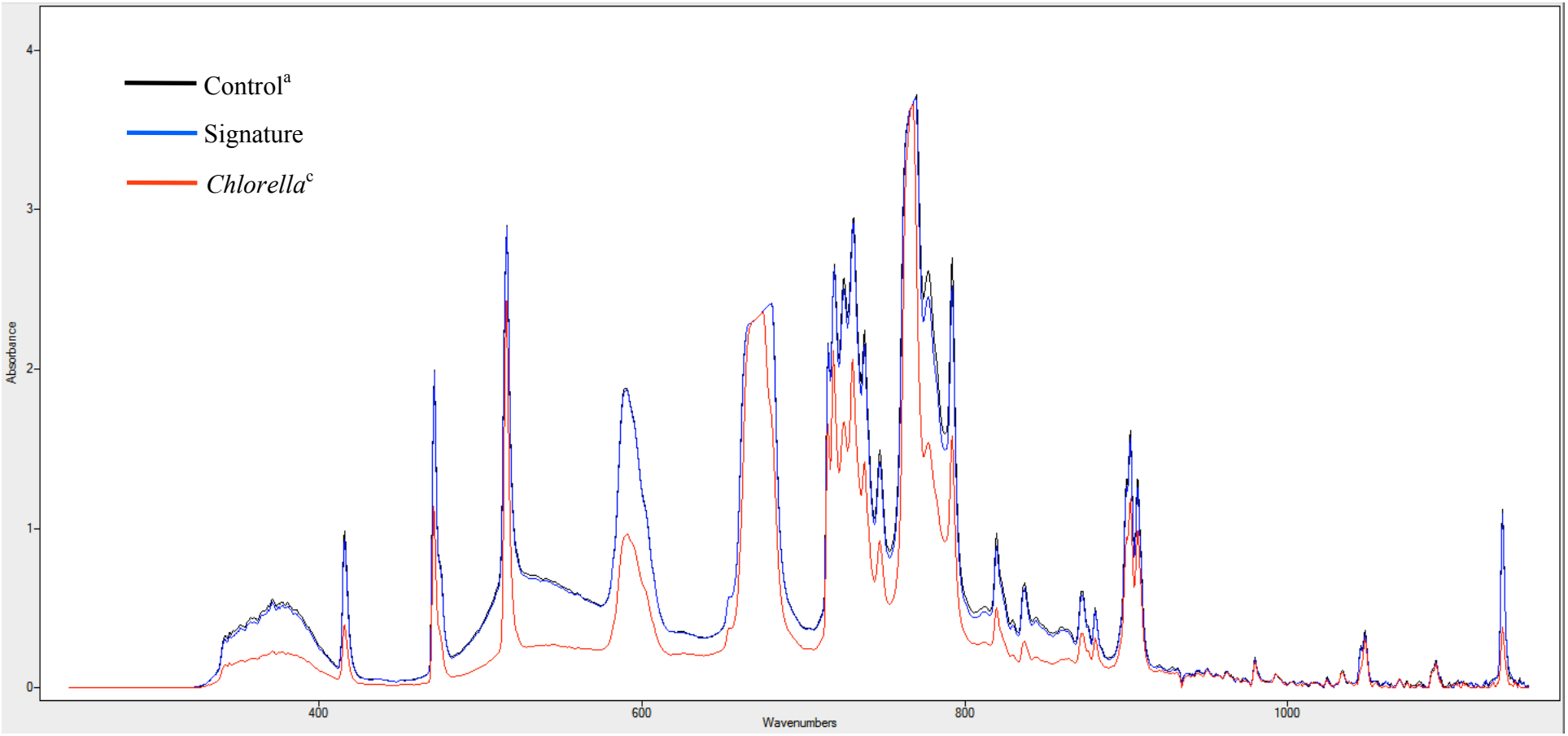
[f]Means separated using Fisher's least significant difference test, where means followed by the same letter do not significantly differ ($p \leq 0.05$)



[a]Abbreviations: ultraviolet, UV.

[b]Measurements taken between 200 and 1150 nm.

Figure 1. Irradiance spectra[b] of UV and visible light treatments used in the environmental growth chamber (Conviron Adaptis A1000, Pembina, ND). Spectral measurements were taken during mid-photo period and averaged across 10 readings. Visible light output was administered using High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ). UV light output was administered using a mixture of High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ) and Zilla Desert 21 Watt UVB 50 Fluorescent T5 Bulb-Zilla Desert Lamps (Zilla Products, Franklin, WI).

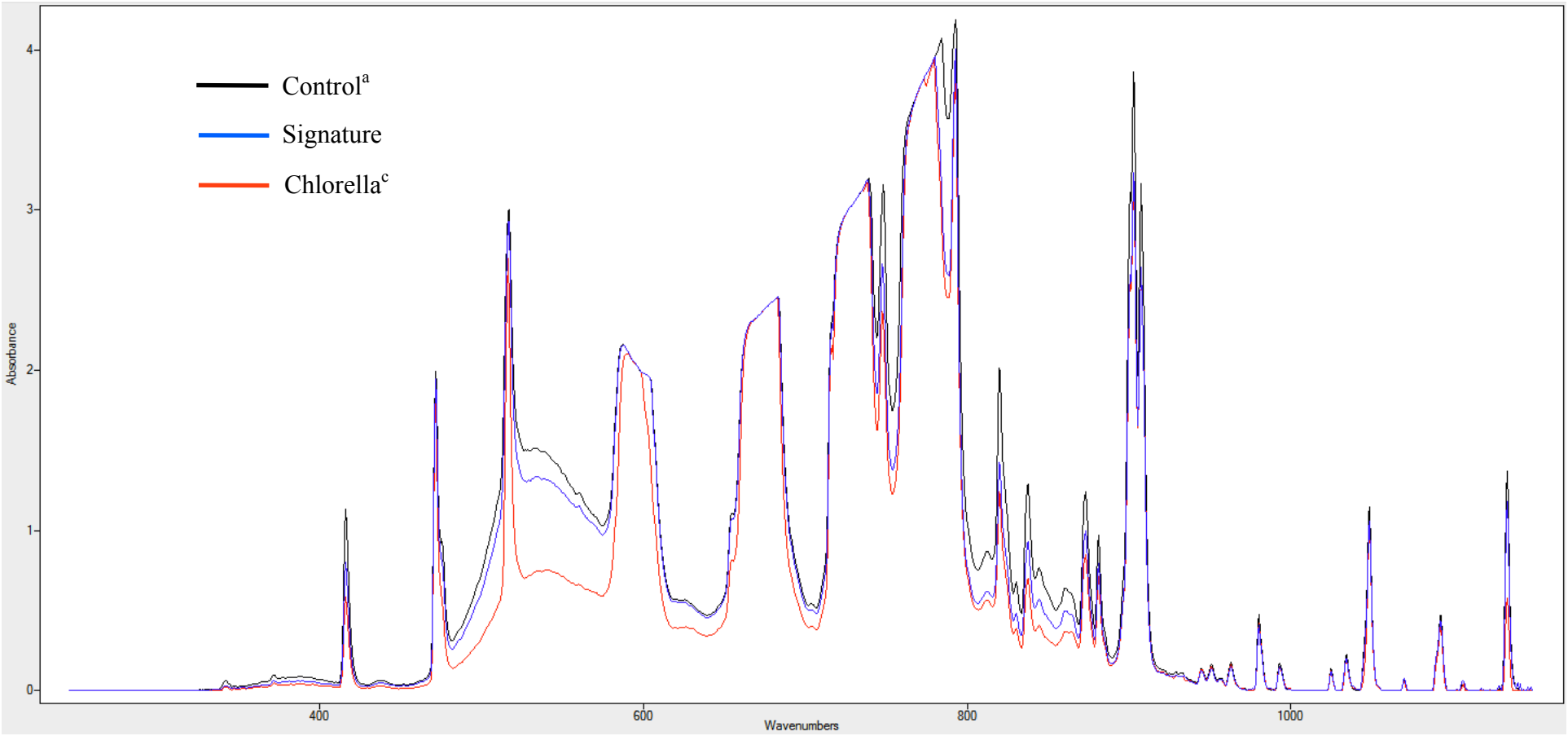


[a]Administered as water

[b]Measurements taken between 200 and 1150 nm.

[c]Applied as fractured cells of *Chlorella vulgaris*

Figure 2. Transmission spectra[b] of ultraviolet light after passing through sterile polystyrene petri dishes (Fisher Scientific, Hamptom, NH) adaxially treated with control[a] (794.4 L ha^{-1}), Signature (12.2 kg ha-1), and *Chlorella*[c] (48.8 kg ha^{-1}) in an environmental growth chamber (Conviron Adaptis A1000, Pembina, ND) equipped with a mixture of High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ) and Zilla Desert 21 Watt UVB 50 Fluorescent T5 Bulb-Zilla Desert Lamps (Zilla Products, Franklin, WI).

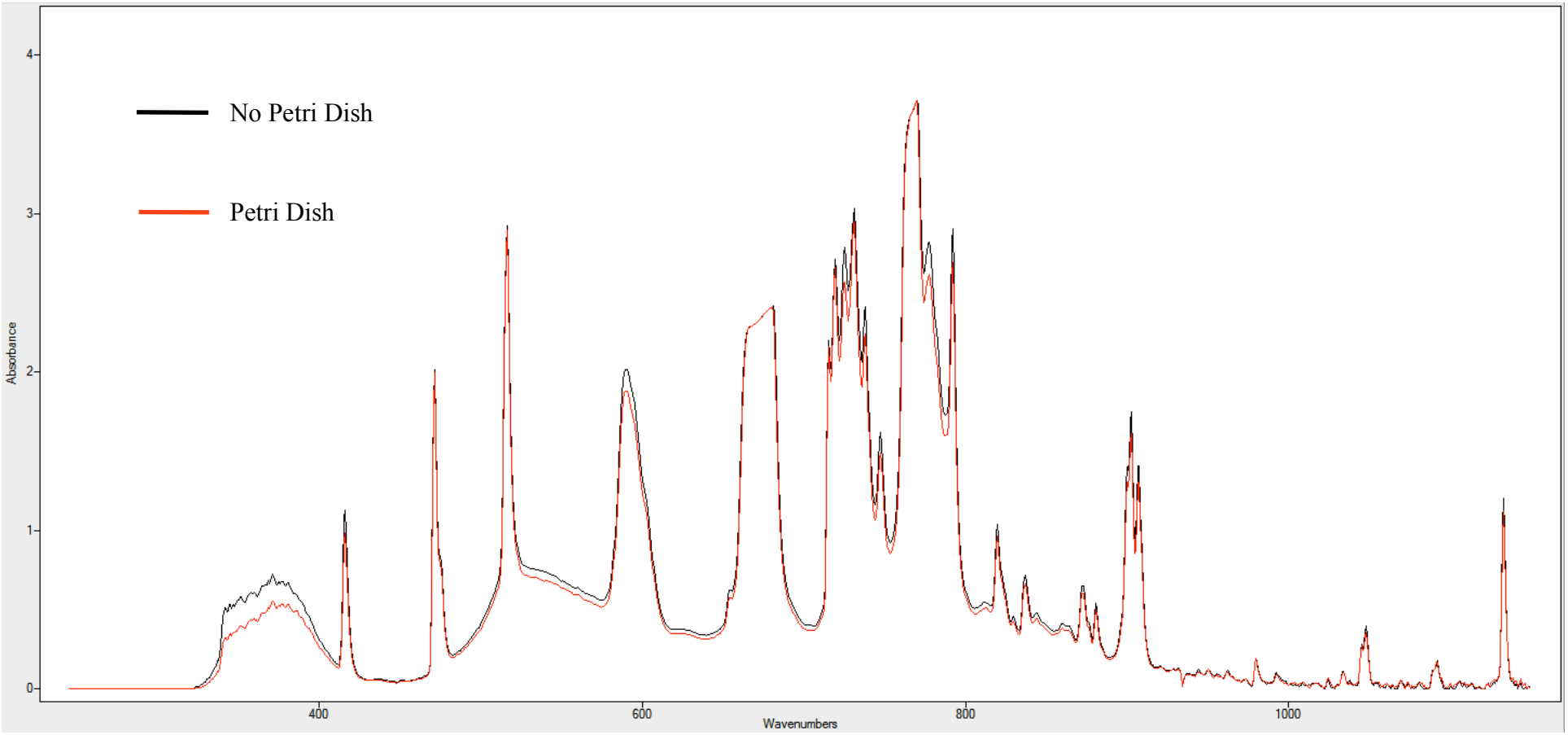


[a]Administered as water

[b]Measurements taken between 200 and 1150 nm.

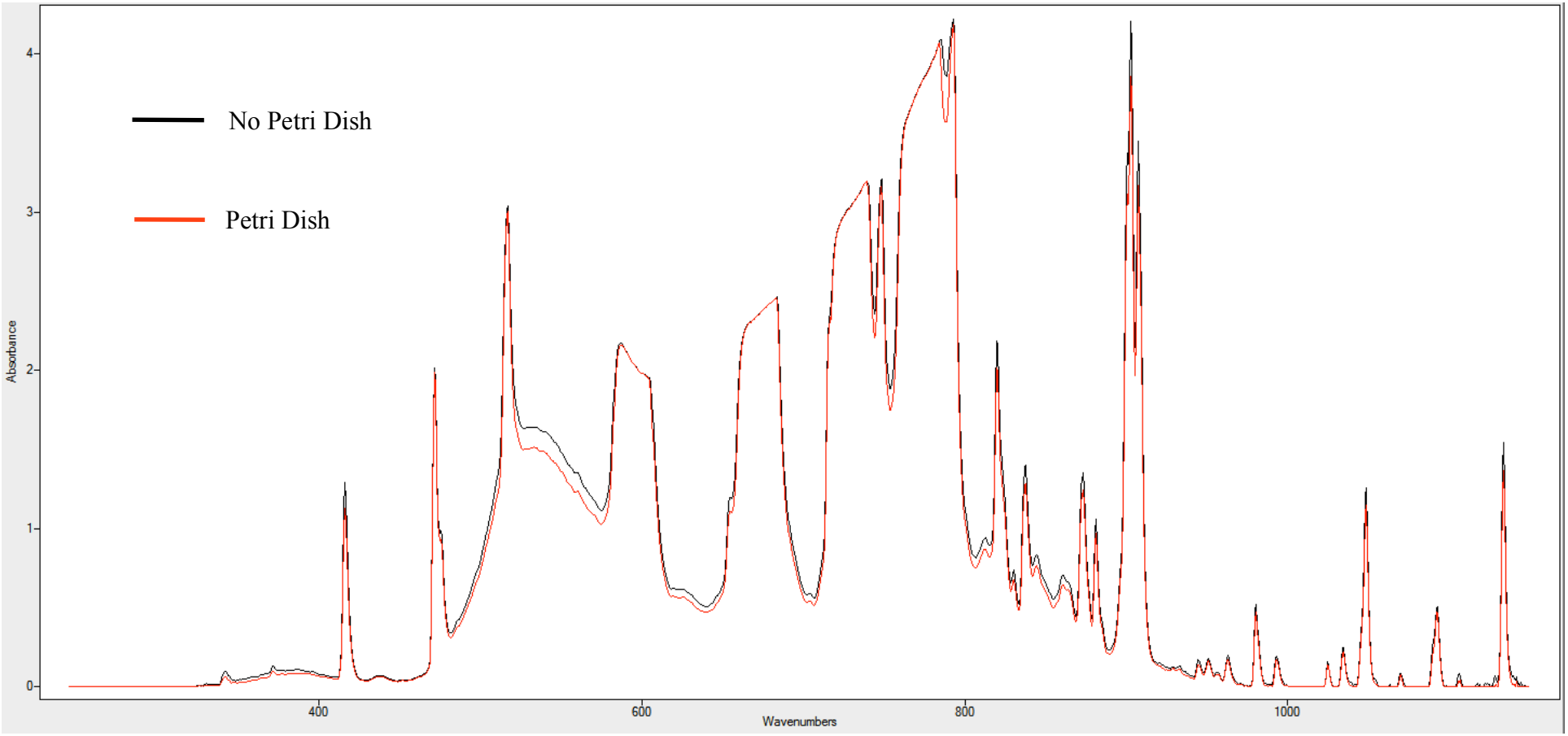
[c]Applied as fractured cells of *Chlorella vulgaris*

Figure 3. Transmission spectra[b] of visible light after passing through sterile polystyrene petri dishes (Fisher Scientific, Hamptom, NH) adaxially treated with control[a] (794.4 L ha^{-1}), Signature (12.2 kg ha-1), and *Chlorella*[c] (48.8 kg ha^{-1}) in an environmental growth chamber (Conviron Adaptis A1000, Pembina, ND) equipped with High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ).



[a]Measurements taken between 200 and 1150 nm.
[b]Abbreviations: ultraviolet, UV.

Figure 4. Irradiance and transmission spectra[a] of ultraviolet light before and after passing through sterile polystyrene petri dishes (Fisher Scientific, Hamptom, NH) in an environmental growth chamber (Conviron Adaptis A1000, Pembina, ND) equipped with a mixture of High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ) and Zilla Desert 21 Watt UVB 50 Fluorescent T5 Bulb-Zilla Desert Lamps (Zilla Products, Franklin, WI).



[a]Measurements taken between 200 and 1150 nm.

Figure 5. Irradiance and transmission spectra[a] of visible light before and after passing through sterile polystyrene petri dishes (Fisher Scientific, Hamptom, NH) in an environmental growth chamber (Conviron Adaptis A1000, Pembina, ND) equipped with High Output Fluorescent Lamps (Phillips F39T5/841 HO Alto, Somerset, NJ)

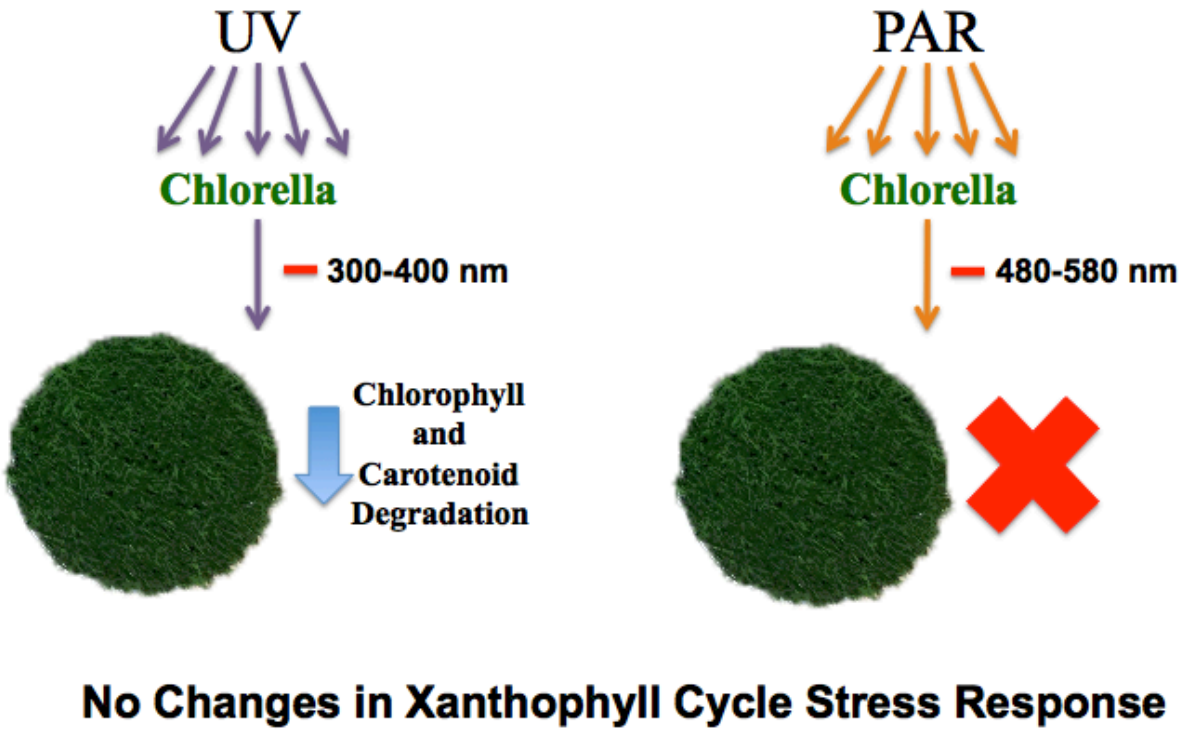


Figure 6. Diagram depicting a potential mechanism for changes observed in *Chlorella* treated plants under high UV and PAR light.

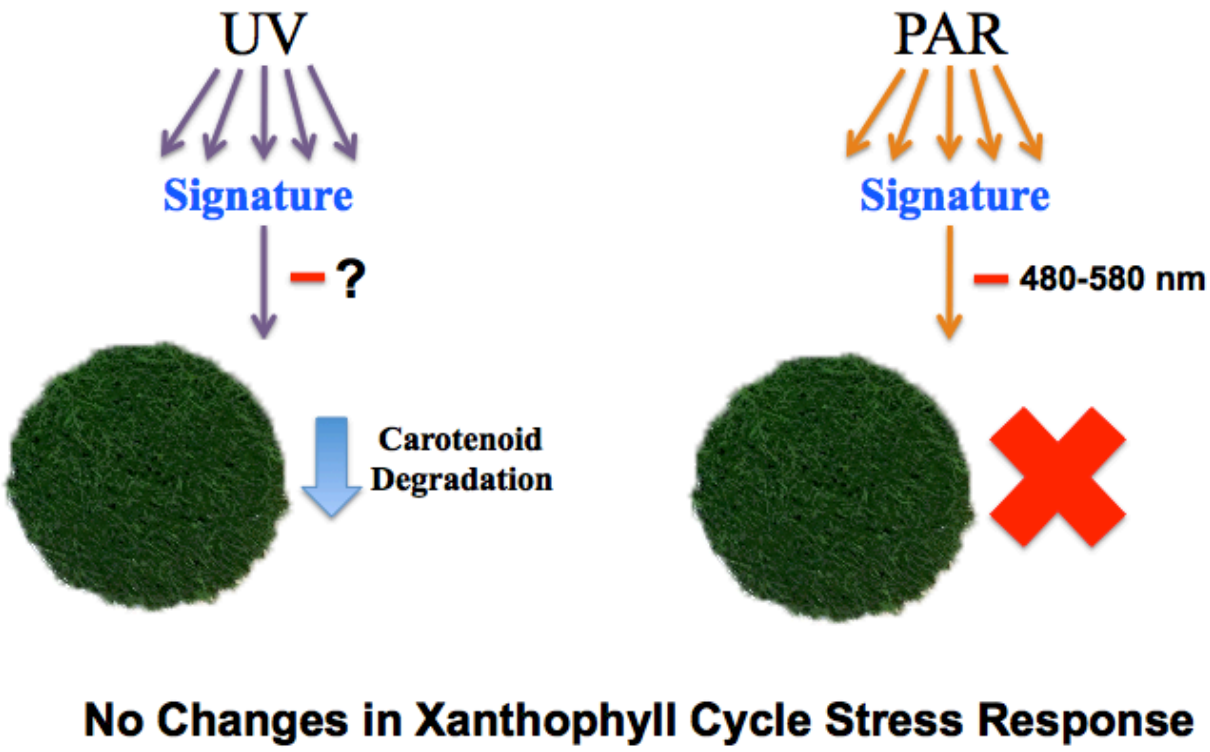


Figure 7. Diagram depicting potential mechanisms for changes observed in Signature treated plants under high UV and PAR light.

CONCLUSIONS

Chlorinated copper phthalocyanine was first investigated in a 2004 patent, where bentgrass treated plants were shown to exhibit better plant quality under high temperature stress. There was, however, no research on the roles that light played in these effects. Also done in 2004, was a study by Ervin et al., which demonstrated the detrimental effects of UV on Kentucky bluegrass. Specifically, this study found that plants treated with copper phthalocyanine had improved photochemical efficiency and forestalled superoxidedismutase decline over 1, 5, and 10 days. However, this study did not look at the comparable effects of high visible light independent of UV. Because of the inherent roles pigments play in plants, measuring chlorophyll and carotenoid content, specifically the xanthophyll cycle pigments, provided further insight into plant stress under different types of light.

Under these methods of experimentation, inconsistency in the results prevented definitive conclusions from being drawn across experimental repetitions. A possible contributor to this variation may have been due to the establishment of pots in the greenhouse, rather than the chamber. It is possible that the three-day acclimation period, following transfer to the chamber, was not sufficiently long enough to prevent potential changes in humidity, soil temperature, and soil moisture from skewing baseline HPLC readings on which later measurements would be based. Also, the lesser availability of fresh weight in the first experimental repetitions may have contributed to weak peak chromatogram readings in HPLC, leading to non-detectable values. In future research, the detrimental effects of high light on turfgrass growth should be compensated for by increasing the number of plant samples in each experimental unit. This will allow more fresh weight tissue to be drawn upon in HPLC, reducing the risk of low fresh weight volumes (< .15 g), which may contribute to large variations in output readings. In accordance with previous

HPLC research on high irradiance effects in creeping bentgrass, these experiments were performed over the course of approximately 168 hours (McElroy et al., 2006). While it is possible to detect significant changes in xanthophyll cycle stress responses over this amount of time, visual changes in plant quality were less apparent, most often due to the influence of pigments on visual color. In future experiments, it may prove beneficial for researchers to look at the use of these pigments over longer periods of time within a controlled environment. Longer time for differences based on leaf senescence may allow for more plant health analyses beyond HPLC alone. The lack of research documenting the bleaching effects of copper phthalocyanine, as observed in these experiments, suggests that the Al in Fosetyl-Al, an active ingredient in Signature, may have caused this bleaching, and thus prevented definitive conclusions from being drawn on the copper phthalocyanine pigment alone. In future experiments, efforts must be made to control for all active ingredients in pigmented products, utilizing the pigment alone whenever possible. Foliar applications of anthocyanin may also provide valuable insight into the mechanisms through which photoprotection and the adaxial screening of light may occur. Concomitantly, measuring anthocyanin buildup within the leaves may allow additional insight into the mechanisms of stress avoidance and responses to high light treatments.

The results of these experiments should prove beneficial to practitioners looking to develop methods of limiting the detrimental effects of abiotic stress responses in the field. However, more research is needed if practitioners are to draw definitive inferences about the relationships that exist between exogenous pigment applications and photoprotection. Up until this point, little research has been conducted on these potential methods of light protection. This can most likely be attributed to a lack of research on the implications of high light stress in bentgrass maintenance, specifically the roles that UV light plays in bentgrass decline in both the

field and environmentally controlled settings. As these roles are uncovered, this research will hopefully provide further insight into the importance of photoprotective mechanisms in the plant, and what turfgrass managers can do to improve them. As the popularity of pigment applications continues to grow in the marketplace, the interest in the empirical research documenting their effects should also gain priority. Overall, this research further emphasizes the roles that light plays in bentgrass quality, as well as a new technique for reducing UV light damage through the application of fractured cells of *Chlorella* biomass.

VITA

After graduating high school, Keith pursued a Bachelors of Science degree from the University of Tennessee in Pre-Med, before switching to Plant Sciences in his second year. In the spring of 2010, he accepted a graduate research position at the University of Tennessee in Plant Sciences under the direction of Dr. Brandon Horvath. Upon graduating, Keith will be pursuing work as a research assistant in autism research.