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Potential Health Benefits of Pigment-Containing Products on Creedping Bentgrass [Agrostis stolonifera L. var palustris (Huds.)] and Hybrid Bermudagrass [Cynodon dactylon (L.) Pers. × C. transvaalensis Burtt-Davy]

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POTENTIAL HEALTH BENEFITS OF PIGMENT-CONTAINING PRODUCTS ON CREEPING BENTGRASS [*Agrostis stolonifera* L. var *palustris* (Huds.)] AND HYBRID BERMUDAGRASS [*Cynodon dactylon* (L.) *Pers.* × *C. transvaalensis* Burtt-Davy]

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ABSTRACT

With the ever changing market of the golf course industry, turfgrass managers are constantly exploring options of promoting healthier turf while also maintaining an appropriate budget. One constant problem is how to manage and relieve summer stress on bentgrass [*Agrostis stolonifera* L. var *palustris* (Huds.)] putting greens. Application of pigmented products is an increasingly popular management practice attempting to relieve some of this stress associated with high temperatures and light intensity. Several of these products are also marketed for use on warm-season grasses such as hybrid bermudagrass as a mean of providing winter green color and improving or hastening breaking of winter dormancy. Research on use of these products on creeping bentgrass has increased in recent years but is still limited, while research on warm-season grasses is sparse. The objective of this study was therefore to investigate the impacts of pigment-containing products on turfgrass physiology of warm- and cool-season grasses during periods of respective stress.

Three pigment-containing products and three pigment-free products were selected for testing alone and in combinations for two field studies in 2013 and 2014 on creeping bentgrass and hybrid bermudagrass: Turf Screen (zinc oxide and titanium dioxide), PAR (copper-based pigment), Chipco Signature (fosetyl-aluminum and a copper-based pigment), Title Phyte (potassium phosphite), Turf Screen + Title Phyte, PAR + Title Phyte, and Fosetyl-Al (fosetyl-aluminum). Products were applied bi-weekly for twelve weeks. Civitas (mineral oil) + Harmonizer (copper based pigment) and Harmonizer alone were added for 2014 field study on hybrid bermudagrass. All products were used in two

separate growth chamber studies investigating health of bentgrass in high temperatures and bermudagrass in freezing temperatures. All products were also used in a bermudagrass dormancy breaking study.

In field studies, application of products caused a general increase in canopy temperatures (~0.5 to 3°C) compared to untreated controls of both grass species. Bentgrass treated with pigmented products exhibited greater (~6 to 20 μmol CO_2 cm^{-2} s^{-1}) carbon dioxide exchange rates (CER) than that of the untreated control indicating a reduction in photosynthesis. Applications of Chipco Signature to hybrid bermudagrass in year two resulted in a more negative CER (-28.295 μmol CO_2 cm^{-2} s^{-1}) indicating greater photosynthetic activity. Decreased (24-50 relative chlorophyll) chlorophyll concentration in creeping bentgrass was observed in both study years by all treatments compared to the untreated while no effect was observed in bermudagrass. No effect on root mass following product use was observed in either grass species. Tissue and soil analysis of creeping bentgrass indicated that Turf Screen and Turf Screen + Title Phyte applications increased zinc concentration in both the plant and soil by an average of 820 ppm and 4.75 kg ha^{-1}, respectively. Applications of PAR, PAR + Title Phyte, and Turf Screen + Title Phyte caused an increase of ~27 ppm of copper in plant tissue of bentgrass. Applications to bermudagrass had similar results with zinc in soil and tissue analysis.

Growth chamber studies on creeping bentgrass further confirmed field studies. Application of treatments in study one resulted in statistically greater CER than unstressed control by an average of ~15.4 μmol CO_2 cm^{-2} s^{-1}. Fluorescence ratings in study two yielded greater (~13) F_v/F_m values in the unstressed control than any other treatment indicating reduced photosynthetic efficiency.

Growth chamber studies on hybrid bermudagrass focusing on freezing stress indicated a net increase (~8 to 21 μmol CO_2 cm^{-2} s^{-1}) in CER of Title Phyte, stressed control, and Turf Screen in both studies over the unstressed control. However, overall fluorescence of all treatments was reduced compared to the unstressed control by a F_v/F_m value of ~12.

Spring green-up study revealed no differences among treatments on earlier breaking of dormancy of hybrid bermudagrass.

The increased bentgrass CER of treated turf indicates a reduction in net photosynthesis while increased canopy temperatures promote a more stressful environment. Results suggest that several products investigated may promote greater heat stress on creeping bentgrass during times of hot, humid weather. Applications to bermudagrass during the same time period did not show negative effects, however the concentration of heavy metals could create future toxicity problems.

DEDICATION

I dedicate this work to my father, mother, sister, and brother. They have all set examples throughout my life on how to live as happily as possible. Their forgiving nature is one that I can never forget and will carry throughout my life, and their support for whatever I do is more than I could ever ask for.

ACKNOWLEDGEMENTS

I would like to thank Dr. Bert McCarty, my major advisor. Though we have not always agreed on the methods with which he taught, nor the way that I worked, his perseverance and knowledge are things that I will always carry with me.

I would also like to thank Dr. Bruce Martin and Dr. Christina Wells, the other members of my graduate committee. Dr. Martin, for helping me get started in this journey with ideas which helped me understand what I was looking for when I myself was unsure, and for additional ideas on how to further my research. Dr. Wells, for her willingness to work with me, even when I would not always know what I was searching for and her teaching of equipment usage. Their advice, knowledge, and equipment got me further along in this research than I would have been able to alone.

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CHAPTER I

INTRODUCTION

Creeping Bentgrass

Creeping bentgrass [*Agrostis stolonifera* L. *var palustris* (Huds.)] is a cool season turfgrass native to central Europe often grown in the cool and humid environments of northern United States (McCarty, 2011). Due to its ability to tolerate low mowing heights (3 mm), soft texture, superb density, and narrow leaf blade (0.62 to 0.92 mm) (Beard and Sifers, 1997), creeping bentgrass is the most commonly used cool season turfgrass species on golf greens (Beard, 1982). Due to its popularity, bentgrass greens have been constructed in warmer regions of the United States. However, as a result of these higher temperatures and greater humidity, quality of creeping bentgrass often declines in summer months; a condition referred to as “summer bentgrass decline” (Lucas, 1995; Carrow, 1996; Beard, 1997).

Creeping bentgrass is a C_3 plant which is more suited for temperatures of 15 to 24°C (Beard, 1997). Transitional zones, where bentgrass has been used, often experience temperatures greater than 30°C in summer months. These temperatures play a major factor in suppressing growth and quality of cool season grasses (Carrow, 1996; Beard, 1997). This suppression is observed through root loss, increased disease incidence, reduced vegetative growth, and reduced shoot density (Krans and Johnson, 1974; DiPaola and Beard, 1992; Carrow, 1996; Huang et al., 1998a, b). In addition to high temperatures, summer decline is also intensified by improper soil water levels, poor soil aeration, and soil-borne disease organism (Lucas, 1995; Carrow, 1996).

Hybrid Bermudagrass

Hybrid bermudagrass [*Cynodon dactylon* (L.) *Pers.* × *C. transvaalensis* Burtt-Davy], a warm-season turfgrass, is one of the four main turf-type bermudagrasses used as a fine-turf (McCarty, 2011). Native to the hot, dry summers in Africa around the Indian Ocean, the common type of bermudagrass [*Cynodon dactylon* (L.) *Pers.*] was often used on all playing surfaces of golf courses prior to the mid-1940's (McCarty, 2011). Hybrid bermudagrass is favored over its common type form in warm subtropical and tropical climates due to its ability to tolerate very low mowing heights (3.2 to 4 mm) while sustaining a dense stand of turf and exhibiting good recovery potential (Turgeon, 2008; Stier et al., 2013).

Though advantageous in climates experiencing hotter temperatures of 27 to 38°C, bermudagrass is used less in cooler climates due to a dormancy (brown) state that occurs below 10°C with potential for chilling and freezing damage (McCarty, 2011). Bermudagrass is also susceptible to high thatch accumulation due to its rapid growth, exhibits poor shade tolerance, and is susceptible to insect and disease problems (Brede, 2000; McCarty, 2011).

Fungicidal Effects on Plant Health

Fungicides are commonly used on creeping bentgrass during the summer, both as a way to prevent the occurrence of several diseases as well as a way to combat summer bentgrass decline (McCarty, 2011). Dernoeden and Fu (2008) concluded that applications of aluminum tris + chlorothalonil and potassium salts of phosphite fungicides + mancozeb improved the overall quality of creeping bentgrass during the summer in addition to reducing scalping injury. Though not proven, it was suggested the fungicides modified the plant morphology, growth habit, and/or rate.

Lucas (1995) observed that applications of aluminum tris + mancozeb or aluminum tris + chlorothalonil at 14 day intervals could reduce summer bentgrass decline. Building upon that, Lucas and Mudge (1997) enhanced bentgrass quality using a monoester salt of phosphorous acid and an ethylene bisdithiocarbamate fungicide. The eventual conclusion was that the mixture of aluminum tris + mancozeb (as Fore 80WP, Rohm and Hass company), which contains Pigment Blue 15, provided greater quality and color than any other fungicide combinations. Pigment Blue 15 reportedly enhanced the activity of mancozeb and aluminum tris (Lucas and Mudge, 1997).

In addition, sequential applications of KH_2PO_3 (potassium phosphite), either applied alone or in combination with iprodione, were found to reduce the occurrence of *Michrodochium nivale* (Fr.) Samuels and Hallett and other disease such dollar spot (*Sclerotinia homeocarpa* F.T. Benn.) and possibly Pythium (*Pythium spp.*), as well as improve overall quality of turf canopy (Dempsey et al. 2012).

Air and Soil Temperature Effects

Bentgrass:

Premier temperatures for cool-season grasses range from 15 to 24°C for shoot growth and 10 to 18°C for root growth (Beard, 1973). A study on root growth of Kentucky bluegrass (*Poa pratensis* L.) indicated an increase of soil temperatures up to 25°C had a negative impact on root growth (Aldous and Kaufman, 1979). Little root initiation occurs at high soil temperatures except following a 2 to 3 day period of cooler soil temperatures (Beard and Daniel, 1966). The greater sensitivity of roots to soil temperature may promote direct injury of roots as an initial factor in a plant's response to high temperatures, thus lower soil temperatures may reduce the occurrence of summer bentgrass decline. This reduction of soil temperatures allows for better plant health in areas of greater root growth, leaf photosynthesis, and shoot growth (Skene and Kerridge, 1967; Aldous and Kaufman, 1979; Kuroyanagi and Paulsen, 1988).

Hybrid Bermudagrass:

Optimum soil and air temperatures for bermudagrass growth are 24 to 35°C and 29 to 37°C, respectively (Lovvorn, 1945). Bermudagrass is prone to low temperature injury, particularly in late winter and early spring, and most commonly occurs during periods of alternating freezing and thawing and is aggravated by increased crown hydration (Beard, 1973), shade, traffic, and crown dessication (McCarty, 2011). To prevent potential damage caused by freezing temperatures, plants can obtain chilling and freezing tolerance when exposed to low temperatures that are still greater than freezing temperature (Hughes and Dunn, 1996). Chilling and freezing stress result in the reduction of photosynthetic assimilation of CO_2 via the reduction of stomatal conductance, modification of thylakoid lipids, and restriction of electron transport, as well as loss of chlorophyll and cessation of growth (Miller, 1960; DiPaola et al., 1981; Karnok and Beard, 1983; Allen and Ort, 2001; Adams et al., 2002). A 45% decrease in photosynthetic rates was reported by Miller (1960) when temperatures were decreased from 35°C to 15°C. Chilling injury has been shown to increase the leakage of ions and amino acids (McKersie and Leshem, 1994). Additionally, production of reactive oxygen species (ROS) such as superoxide (O_2^-), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2) is also induced by chilling and freezing stress which can cause severe cellular injury (Inze and Van Montagu, 1995).

Pigments

The application of pigments and dyes has become a common practice on golf courses. Most of these products have a green-based color, thus, help create more aesthetically pleasing turf. However, repeated applications of pigment-containing products have been reported to reduce overall stand quality. This may be due, in part, to the altering of light intensity and spectral quality (Reynolds et al., 2012). Pigments consist of dry powders with varying chemical compositions based on desired color. Common pigments in white, black, and red paint are TiO_2 (titanium dioxide), C (carbon), and Fe_2O_3 (iron oxide), respectively (Reynolds et al., 2012).

Many pigments have a molecular structure similar to chlorophyll, however, pigment centered molecules are copper ions instead of magnesium as with chlorophyll. This similar molecular structure of pigment molecules to chlorophyll is one possible means of increasing a plant's photosynthetic efficiency. Visible light and photosynthetically active radiation (PAR) share the same range of wavelengths, 400 to 700 nm. Within PAR are two specific wavelengths, grouped by color, that are most effectively absorbed for photosynthesis - blue light and red light with wavelengths of 400 to 500 nm and 600 to 700 nm, respectively (Taiz and Zeiger, 2006). Reynolds et al. (2013) determined that the long-term application of pigments, specifically those of darker colors such as green, black, and dark blue, reflected 87 to 95% of PAR. Summer applications of pigmented products to creeping bentgrass decreased carbon dioxide exchange rate as well as normalized difference vegetation index (McCarty et al., 2014).

The application of pigments onto playing areas to provide desirable winter color is an increasing trend in lieu of overseeding. Application of a colorant to buffalograss (*Bouteloua dactyloides* (Nutt.) Columbus) was found to provide similar color and quality as adjacent cool season turfgrasses (Shearman et al., 2005). Applications of pigmented products can enhance spring green-up when compared to traditional overseeding practices and dormant turf, due in part to the increased soil and air temperatures (Liu et al., 2007). Previous work by Kreuser and Rossi (2014) found that summer applications of a mineral oil containing product, Civitas, caused chlorosis and decreased visual quality on creeping bentgrass. They also noted that the use of an accompanying pigment-containing product, Harmonizer, did not alleviate any symptoms and appeared only to mask stress issues by providing green color.

Fluorescence

Chlorophyll fluorescence is a means of explaining photosynthetic efficiency and the effect of abiotic stresses on that efficiency (Adams et al., 2004; Maxwell and Johnson, 2000). Stress that causes the formation of various active oxygen radicals decreases quantum efficiency of photosystem II (F_v/F_m). The reactive nature of F_v/F_m makes for an indicator of plant stress which can be quantitatively compared to stress responses of other plant species (Cessna et al, 2010).

The fluctuation of solar radiation throughout the day has a significant impact on photosynthetic light reactions in the thylakoid membrane system inside chloroplasts (Kirchoff, 2014). When solar radiation is absorbed at levels that exceed rates of photosynthesis, an event typically seen mid-day under full sun exposure, or when plants are experiencing sub-par environmental conditions, excess energy is more likely transferred to reactive oxygen species (ROS) (Demmig-Adams and Adam, 2006). Plants have developed various forms of photoprotection as a way to prevent damaging effects of the various ROS.

In response to high light situations, plants will avoid absorbing excessive photons through heliotropic movements as well as reducing their chlorophyll content (Heber, 2002). Redirection of electrons from the electron transport chain to reactions other than the Calvin cycle such as the water-water cycle also allow for the dissipation of light energy (Heber, 2002).

Heavy Metal Toxicity

The presence of heavy metals in a soil environment may be beneficial or toxic to plants, depending on their concentrations (Rout and Das, 2003). Zinc is required in the synthesis of growth hormones and proteins (Marschner, 1995) requiring rapid incorporation of the element allowing for high potential of phototoxicity (Rout and Das, 2003). Zinc toxicity may result in reduced root growth, yellowing of leaves, and eventual plant death (Havlin et al., 2005). A sufficient zinc level is suggested between 20 to 200 mg kg^{-1} in various turf tissues with variation occurring between grass species (Boehle and Lindsay, 1969; Jones, 1980; McCarty, 2011). Xu and Mancino (2001) observed that maintaining a concentration below 109 mg kg^{-1} prevented phytotoxicity in creeping bentgrass, while annual bluegrass (*Poa annua* L.) was able to tolerate a level of 200 mg kg^{-1}.

Copper is an essential element involved in various redox reactions (Taiz and Zeiger, 2006). Soil concentrations of copper may increase over time due to the repeated application of Cu-containing fungicides, organic fertilizers, and effluent irrigation water (Marschner, 1995; Brun et al., 2001). Faust and Christians (2000) reported soil copper levels increasing from 0 to 600 mg kg^{-1} caused a 16% decrease in bentgrass dry clipping weight as well as 52% lower dry root mass than the untreated.

Photosynthesis

Photosynthesis is an essential plant process, thus making it an effective indicator of plant health when subjected to stress (Salisbury and Ross, 1978). The net uptake or efflux of CO_2 from a given area of turf canopy provides a measure of net photosynthesis (Kosugi et al, 2010). Positive measurements of CER indicate that respiration rates exceed photosynthesis rates, while negative measurements indicate that photosynthesis predominates. Measurements of photosynthesis are often conducted with hand-held chambers placed over the turf canopy and soil and measure CO_2 exchange (Bremer and Ham, 2005).

The CIRAS-2 Portable Photosynthesis System (PP System, Haverhill, MA 01912) has been used previously to measure CO_2 exchange rates in turfgrass scenarios. The system was used to determine canopy photosynthetic rates in an assessment of lowlight tolerance of seashore paspalum (*Paspalum vaginatum* Swartz) and hybrid bermudagrass (Jiang et al, 2004). Ambient light and CO_2 were used to measure net canopy photosynthetic rate in micromoles of CO_2 per meter squared per second (μmol cm^{-2} s^{-1}). To obtain measurements, a 150 millimeter diameter clear polyethylene chamber was firmly pressed to the turfgrass surface to seal the chamber, allowing the CIRAS-2 two to three minutes per sample. A similar system and method was used in this study to test the hypothesis that the application of pigment-containing products causes reduced photosynthesis and inferior turf quality.

CHAPTER II

MATERIALS AND METHODS

Field Studies

Field research was conducted in Clemson, SC at the Clemson University Turfgrass Research Facility on two 14 year old putting greens, L-93 bentgrass and TifEagle bermudagrass, both constructed to USGA specifications (USGA Green Section Staff 1993). This research was conducted from 24 June to 16 September, 2013 and 7 July to 29 September, 2014. Treatments consisted of an untreated control, zinc oxide + titanium dioxide + pigment (Turf Screen) (TurfMax LLC., Erdenheim. PA) at 2.5 oz/1,000 ft^2 (7.97 L ha^{-1}), copper phtalocyanine pigment (PAR) (Harrell's LLC., Lakeland, FL) at 0.37 oz/1,000 ft^2 (1.17 L ha^{-1}), potassium phosphite (Title Phyte) (Harrell's LLC., Lakeland, FL) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}), Turf Screen and Title Phyte at 2.5 oz/1,000 ft^2 (7.97 L ha^{-1}), and 4 oz/1,000 ft^2 (12.57 L ha^{-1}) respectively, PAR and Title Phyte at 0.37 oz/1,000 ft^2 (1.17 L ha^{-1}) and 4 oz/1,000 ft^2 (12.57 L ha^{-1}) respectively, fosetyl-aluminum (Chipco Signature + Stressgard) (Bayer CropScience AG, Monheim Am Rhein, Germany) at 6 oz/1,000 ft^2 (19.13 L ha^{-1}), and Fosetyl-al (fosetyl-aluminum) (Quali-Pro, Pasadena, TX) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}) (Table 2-1). Mineral oil (Civitas)(Petro-Canada, Mississauga,Ontario) at 0.367 oz/1000 ft^2 (1.17 L ha^{-1}) plus a proprietary pigment (Civitas Harmonizer) (Petro-Canada, Mississauga,Ontario) at 0.023 oz/1000 ft^2 (0.073 L ha^{-1}) and Civitas Harmonizer alone at 0.023 oz/1000 ft^2 (0.073 L ha^{-1}) were added to 2014 bermudagrass field trials (Table 2-2). Due to the potassium content of Title Phyte, soluble potash derived from potassium phosphite (Stress Relefe, 0-0-25) (Harrell's LLC., Lakeland, FL) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}) was added to non-potassium treated plots. Treatments were applied every 14 days for twelve weeks at the labeled rates using a CO_2 back pack sprayer delivering 20 gal/acre (187.3 L ha^{-1}). Plots were 2.5 x 1.5 meters and replicated 4 times in each experiment. Both greens were maintained at mowing heights of 3.175 mm. Plots were arranged using a randomized complete block design and results analyzed using Analysis of Variance and Fisher's LSD (alpha=0.05).

Turf Quality

To quantify treatment effects on turfgrass quality, two measurements were recorded. The Normalized Difference Vegetation Index (NDVI) was measured twice weekly using a Field Scout Turf Color Meter (Spectrum Technologies, Plainfield, IL). The device estimates turf quality by measuring the red and near-infrared light reflected off of the plant's surface. A "greener" surface is indicated by a higher NDVI ratio ([near infrared light(NIR)-Red light)]/[NIR + Red]) (Bremer et al, 2011) measured on a 0-1 scale. Turfgrass quality was also measured visually (1-9, 9 = best) twice weekly.

Canopy Temperatures and Chlorophyll Content

Due to the presence of pigments in products tested (Figures 2-1 and 2-2), quality based on plant color or appearance could be misleading. Therefore, physiological measurements not dependent on plant color were also performed. Daily canopy temperatures (°C) were taken at approximately 2 pm EST (1 hour past solar noon) using a handheld infrared thermometer (Raytek Corporation, Santa Cruz, CA). Three readings per plot were averaged. Chlorophyll content was measured daily based on relatve concentration of chlorophyll using a Field Scout CM 1000 Chlorophyll Meter (Spectrum Technologies, Plainfield, IL) at the same time as canopy temperatures. Three readings per plot were averaged. In addition, CO_2 exchange rates were measured twice weekly using a CIRAS-2 Portable Photosynthesis System (PP Systems, Haverhill, MA USA) which included the differential CO_2/H_2O gas analyzer attachment. Two readings per plot were averaged.

Volumetric Soil Moisture Content

Volumetric soil moisture content in the top 12 cm was recorded from each plot twice weekly (% volumetric water cm soil^{-2}) using a FieldScout TDR 100 (Spectrum Technologies, Plainfield, IL) to indicate if turf quality was associated with soil moisture stress.

Roots

Root weights were collected at the beginning and conclusion of each field trial. Three cores (2.5 cm diameter x 15 cm deep) were removed from each plot. Green shoots and the next 1.5 cm were removed. Remaining vegetation was used for root weight. To determine actual weights, both the root layer was dried at 80°C for 7 days, weighed, then incinerated at 500°C for 3 hours. Weight of ash was then subtracted from the dried weight to determine root mass.

Nutrient Analysis

Soil nutrient analysis was performed by extracting 5 cores (2 cm diameter x 15 cm deeper) per plot, blending each plot's respective cores, and then placing the mixed soil into individual paper bags. Tissue analysis was performed by obtaining clippings from each plot using a standard walk-behind greens mower with bucket attachment. Three passes were made on each plot before removing the clippings and placing them in paper bags. Due to the limited growth of the bermudagrass plots, tissue analysis was averaged per treatment, as opposed to one analysis per plot on the bentgrass green, thus statistical analysis was not possible since samples were combined over all replicates, however data is presented for comparison. Both tissue and soil samples were sent to the Clemson University Agriculutural Service Laboratory for heavy metal analysis.

Figure 2-1 Creeping bentgrass (*Agrostis stolonifera* L. *var palustris* (Huds.)) appearance following foliar application of various pigmented products.

Figure 2-2 TifEagle bermudagrass (*Cynodon dactylon* (L.) *Pers.* X *C. transvaalensis* Burtt-Davy) appearance following foliar application of various pigmented products.

Bentgrass Growth Chamber Experiment

Two separate 10 day studies were conducted in growth chambers (Conviron, Pembina, ND) located in the Clemson University greenhouse facility in Clemson, SC to determine potential impact of pigment containing products on bentgrass health when exposed to a highly stressful environment. ‘L-93’ bentgrass plugs (10 cm diameter x 10 cm deep) were removed from the Clemson University Turfgrass Research Facilities bentgrass putting green and placed into greenhouse pots containing 85:15 sand/peat rootzone mix. Turf was established for 3 weeks in the greenhouse facility at optimum temperature of 25°C (77°F). Turf plugs were allowed to grow to a 15 cm diameter and 13 mm height. Established turf pots were then moved into the growth chamber and maintained at 35°C

(95°F) and 24°C (75.2°F) for 12 hours each. To reduce the potential for localized effects of pot placement in the chamber, pot positions were rotated daily. All treatments received 100 ml of water every three days to maintain field capacity.

Treatments included a stressed and unstressed untreated control, zinc oxide + titanium dioxide + pigment (Turf Screen) (TurfMax LLC., Erdenheim. PA) at 2.5 oz/1,000 ft^2 (7.97 L ha^{-1}), copper phytalocyanine pigment (PAR) (Harrell's LLC., Lakeland, FL) at 0.37 oz/1,000 ft^2 (1.17 L ha^{-1}), potassium phosphite (Title Phyte) (Harrell's LLC., Lakeland, FL) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}), Turf Screen plus Title Phyte at 2.5 oz/1,000 ft^2 (7.97 L ha^{-1}) and 4 oz/1,000 ft^2 (12.57 L ha^{-1}) respectively, PAR plus Title Phyte at 0.37 oz/1,000 ft^2 (1.17 L ha^{-1}) and 4 oz/1,000 ft^2 (12.57 L ha^{-1}) respectively, Chipco Signature (Bayer CropScience AG, Monheim Am Rhein, Germany) at 6 oz/1,000 ft^2 (19.13 L ha^{-1}), Fosetyl-al (Quali-Pro, Pasadena, TX) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}), mineral oil (Civitas) (Petro-Canada, Mississauga, Ontario) at 0.367 oz/1000 ft^2 (1.17 L ha^{-1}) with a proprietary pigment (Civitas Harmonizer) (Petro-Canada, Mississauga, Ontario) at 0.023 oz/1000 ft^2 (0.073 L ha^{-1}), and Civitas Harmonizer at 0.023 oz/1000 ft^2 (0.073 L ha^{-1}). Due to the potassium content of Title Phyte, a K-containing fertilizer was added to the other treated plots using potassium acetate (Stress Relefe, 0-0-25) (Harrell's LLC., Lakeland FL) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}) (Table 2-3). All treatments were replicated 3 times. The unstressed untreated control pots remained in the greenhouse facility at an optimal temperature of 28°C (82.4°F).

Carbon-dioxide exchange rates (CER) were measured using a CIRAS-2 Portable Photosynthesis System (PP Systems, Haverhill, MA USA) which included the differential

CO_2/H_2O gas analyzer attachment. This system included a clear polyethylene 150 mm diameter chamber placed around the plug for 75 seconds for each reading. These ratings were taken prior to placement in the growth chamber, on day 5, and at the end of the study.

Variable chlorophyll fluorescence (F_v/F_m) of turf plugs was documented using a FluorPen FP100 (Photon Systems Instruments, Drasov, Czech Republic). Ratings on a 0-1 were taken at the end of the study.

Bermudagrass Growth Chamber Study

Two separate studies were conducted in the Clemson University greenhouse facility in Clemson, SC to determine potential impact of the pigment-containing products on bermudagrass health when exposed to freezing temperatures. TifEagle bermudagrass plugs (10 cm diameter x 10 cm deeper) were removed from the Clemson University Turfgrass Research Facilities bermudagrass putting green and placed into greenhouse pots containing 85:15 sand/peat rootzone mix. Turf plugs were established over 3 weeks in the greenhouse facility at optimal temperatures, ~35°C (95°F) during the day, allowing foliage to grow to a 15 cm diameter and 13 mm height. Two applications were then made to foliage separated by two weeks. One week after the second treatment, plugs were placed into growth chambers (Conviron, Pembina, ND) at -5°C (23°F) for 3 hours simulating a rapid hard freeze on green tissue. To reduce potential for localized effects, pots were rotated daily.

Treatments included a stressed and unstressed untreated control, zinc oxide + titanium dioxide + pigment (Turf Screen) (TurfMax LLC., Erdenheim. PA) at 2.5 oz/1,000 ft^2 (7.97 L ha^{-1}), copper phytalocyanine pigment (PAR) (Harrell's LLC., Lakeland, FL) at 0.37 oz/1,000 ft^2 (1.17 L ha^{-1}), potassium phosphite (Title Phyte) (Harrell's LLC., Lakeland, FL) at 4 oz/1,000 ft^2 (12.572 L ha^{-1}), Turf Screen plus Title Phyte at 2.5 oz/1,000 ft^2 (7.97 L ha^{-1}) and 4 oz/1,000 ft^2 (12.572 L ha^{-1}) respectively, PAR plus Title Phyte at 0.37 oz/1,000 ft^2 (1.17 L ha^{-1}) and 4 oz/1,000 ft^2 (12.57 L ha^{-1}) respectively, Chipco Signature (Bayer CropScience AG, Monheim Am Rhein, Germany) at 6 oz/1,000 ft^2 (19.128 L ha^{-1}), Fosetyl-al (Quali-Pro, Pasadena, TX) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}), mineral oil (Civtas) (Petro-Canada, Mississauga, Ontario) at 0.367 oz/1000 ft^2 (1.17 L ha^{-1}) with a proprietary pigment (Civitas Harmonizer) (Petro-Canada, Mississauga, Ontario) at 0.023 oz/1000 ft^2 (0.073 L ha^{-1}), and Civitas Harmonizer at 0.023 oz/1000 ft^2 (0.073 L ha^{-1}) (Table 2-3). Due to the potassium content of Title Phyte, a K-containing fertilizer was added to non-potassium containing treatments using potassium acetate (Stress Relefe, 0-0-25) (Harrell's LLC., Lakeland FL) at 4 oz/1,000 ft^2(12.57 L ha^{-1}) (Table 2-3). All treatments were replicated 4 times.

Carbon-dioxide exchange rates (CER) were measured using a CIRAS-2 Portable Photosynthesis System (PP Systems, Haverhill, MA USA). This system included a polythylene 150 mm diameter chamber placed around the plug for 75 seconds for each reading. Ratings were taken prior to placement in chamber and at the conclusion of the 3 hours.

Variable chlorophyll fluorescence of plugs was documented using a FluorPen FP100 (Photon Systems Instruments, Drasov, Czech Republic). Ratings on a 0-1 scale were taken at the end of the study.

Spring Greenup Study

Two spring field trials were performed to evaluate the possible effects of pigmented products on timing of bermudagrass recovery from winter dormancy. Treatments were an untreated control, zinc oxide + titanium dioxide + pigment (Turf Screen) (TurfMax LLC., Erdenheim. PA) at 2.5 oz/1,000 ft^2 (7.97 L ha^{-1}), copper phytalocyanine pigment (PAR) (Harrell's LLC., Lakeland, FL) at 0.37 oz/1,000 ft^2 (1.17 L ha^{-1}), potassium phosphite (Title Phyte) (Harrell's LLC., Lakeland, FL) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}), Turf Screen plus Title Phyte at 2.5 oz/1,000 ft^2 (7.97 L ha^{-1}) and 4 oz/1,000 ft^2 (12.57 L ha^{-1}) respectively, PAR plus Title Phyte at 0.37 oz/1,000 ft^2 (1.17 L ha^{-1}) and 4 oz/1,000 ft^2 (12.57 L ha^{-1}) respectively, Fosetyl-al plus StressGard (Chipco Signature)(Bayer CropScience AG, Monheim Am Rhein, Germany) at 6 oz/1,000 ft^2 (19.13 L ha^{-1}), Fosetyl-al (Quali-Pro, Pasadena, TX) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}), mineral oil (Civtas) (Petro-Canada, Mississauga, Ontario) at 0.367 oz/1,000 ft^2 (1.17 L ha^{-1}) with a proprietary pigment (Civitas Harmonizer) (Petro-Canada, Mississauga, Ontario) at 0.023 oz/1,000 ft^2 (0.073 L ha^{-1}), and Civitas Harmonizer at 0.023 oz/1,000 ft^2 (0.073 L ha^{-1}) (Table 2-4). Due to the potassium content of Title Phyte, a K-containg fertilizer was added to other treated plots using potassium acetate (Stress Relefe, 0-0-25) (Harrell's

LLC., Lakeland FL) at 4 oz/1,000 ft^2 (12.57 L ha^{-1}). All treatments were replicated 4 times.

Applications were made every 14 days beginning 7 October to 18 November, 2013 with late winter applications beginning 17 February to 15 April, 2014. Plots were 2.5 by 1.5 meters on a 14 year old TifEagle bermudagrass green maintained to USGA specifications.

Greenup was as a measurement of NDVI compared to the untreated. The NDVI was measured using a Field Scout Turf Color Meter (Spectrum Technologies, Plainfield, IL). Measurements were taken weekly. Three ratings were taken per plot and averaged.

Table 2-1. Treatments and rates applied to L-93 creeping bentgrass green field studies bi-weekly in 2013 and 2014 at Clemson University, Clemson, SC.

Treatment	Rate
zinc oxide + titanium dioxide + pigment (Turf Screen)	2.5 oz/1,000 ft^2 (7.97 L ha^{-1})
copper phytalocyanine pigment (PAR)	0.37 oz/1,000 ft^2 (1.17 L ha^{-1})
potassium phosphite (Title Phyte)	4 oz/1,000 ft^2 (12.57 L ha^{-1})
Turf Screen + Title Phyte	2.5 oz/1,000 ft^2 (7.97 L ha^{-1}) + 4 oz/1,000 ft^2 (12.57 L ha^{-1})
PAR + Title Phyte	0.37 oz/1,000 ft^2 (1.17 L ha^{-1}) + 4 oz/1,000 ft^2 (12.57 L ha^{-1})
Fosetyl-Al + StressGard (Chipco Signature)	6 oz/1,000 ft^2 (19.13 L ha^{-1})
Fosetyl-Al	4 oz/1,000 ft^2 (12.57 L ha^{-1})

All treatments not including Title Phyte received a potassium supplement using potassium acetate (Stress Relefe, 0-0-25) (Harrell's LLC., Lakeland FL) at 4 oz/1,000ft^2 (12.572 L ha^{-1}).

Table 2-2. Treatments and rates applied bi-weekly to Tifeagle bermudagrass green field studies in 2013 and 2014 at Clemson University, Clemson, SC.

Treatment	Rate
zinc oxide + titanium dioxide + pigment (Turf Screen)	2.5 oz/1,000 ft^2 (7.97 L ha^{-1})
copper phytalocyanine pigment (PAR)	0.37 oz/1,000 ft^2 (1.17 L ha^{-1})
potassium phosphite (Title Phyte)	4 oz/1,000 ft^2 (12.57 L ha^{-1})
Turf Screen + Title Phyte	2.5 oz/1,000 ft^2 (7.97 L ha^{-1}) + 4 oz/1,000 ft^2 (12.57 L ha^{-1})
PAR + Title Phyte	0.37 oz/1,000 ft^2 (1.17 L ha^{-1}) + 4 oz/1,000 ft^2 (12.57 L ha^{-1})
Fosetyl-Al + StressGard (Chipco Signature)	6 oz/1,000 ft^2 (19.13 L ha^{-1})
Fosetyl-Al	4 oz/1,000 ft^2 (12.57 L ha^{-1})
mineral oil (Civitas) + proprietary pigment (Civitas Harmonizer)*	0.367 oz/1000 ft^2 (1.17 L ha^{-1}) + 0.023 oz/1000 ft^2 (0.073 L ha^{-1})
proprietary pigment (Civitas Harmonizer)*	0.023 oz/1000 ft^2 (0.073 L ha^{-1})

All treatments not including Title Phyte received a potassium supplement using potassium acetate (Stress Relefe, 0-0-25) (Harrell's LLC., Lakeland FL) at 4 oz/1,000ft^2 (12.572 L ha^{-1}).
*Treatments added after the end of 2013 field trial

Table 2-3. Treatments and rates applied bi-weekly to L-93 creeping bentgrass and Tifeagle bermudagrass for growth chamber studies in 2013 and 2014 at Clemson University, Clemson, SC.

Treatment	Rate
zinc oxide + titanium dioxide + pigment (Turf Screen)	2.5 oz/1,000 ft^2 (7.97 L ha^{-1})
copper phytalocyanine pigment (PAR)	0.37 oz/1,000 ft^2 (1.17 L ha^{-1})
potassium phosphite (Title Phyte)	4 oz/1,000 ft^2 (12.57 L ha^{-1})
Turf Screen + Title Phyte	2.5 oz/1,000 ft^2 (7.97 L ha^{-1}) + 4 oz/1,000 ft^2 (12.57 L ha^{-1})
PAR + Title Phyte	0.37 oz/1,000 ft^2 (1.17 L ha^{-1}) + 4 oz/1,000 ft^2 (12.57 L ha^{-1})
Fosetyl-Al + StressGard (Chipco Signature)	6 oz/1,000 ft^2 (19.13 L ha^{-1})
Fosetyl-Al	4 oz/1,000 ft^2 (12.57 L ha^{-1})
mineral oil (Civitas) + proprietary pigment (Civitas Harmonizer)	0.367 oz/1000 ft^2 (1.17 L ha^{-1}) + 0.023 oz/1000 ft^2 (0.073 L ha^{-1})
mineral oil (Civitas)	0.367 oz/1000 ft^2 (1.17 L ha^{-1})
proprietary pigment (Civitas Harmonizer)	0.023 oz/1000 ft^2 (0.073 L ha^{-1})

All treatments not including Title Phyte received a potassium supplement using potassium acetate (Stress Relefe, 0-0-25) (Harrell's LLC., Lakeland FL) at 4 oz/1,000ft^2 (12.572 L ha^{-1}).

Table 2-4. Treatments and rates applied bi-weekly to Tifeagle bermudagrass green green-up studies in 2013 and 2014 at Clemson University, Clemson, SC.

Treatment	Rate
zinc oxide + titanium dioxide + pigment (Turf Screen)	2.5 oz/1,000 ft^2 (7.97 L ha^{-1})
copper phytalocyanine pigment (PAR)	0.37 oz/1,000 ft^2 (1.17 L ha^{-1})
potassium phosphite (Title Phyte)	4 oz/1,000 ft^2 (12.57 L ha^{-1})
Turf Screen + Title Phyte	2.5 oz/1,000 ft^2 (7.97 L ha^{-1}) + 4 oz/1,000 ft^2 (12.57 L ha^{-1})
PAR + Title Phyte	0.37 oz/1,000 ft^2 (1.17 L ha^{-1}) + 4 oz/1,000 ft^2 (12.57 L ha^{-1})
Fosetyl-Al + StressGard (Chipco Signature)	6 oz/1,000 ft^2 (19.13 L ha^{-1})
Fosetyl-Al	4 oz/1,000 ft^2 (12.57 L ha^{-1})
mineral oil (Civitas) + proprietary pigment (Civitas Harmonizer)	0.367 oz/1000 ft^2 (1.17 L ha^{-1}) + 0.023 oz/1000 ft^2 (0.073 L ha^{-1})
proprietary pigment (Civitas Harmonizer)	0.023 oz/1000 ft^2 (0.073 L ha^{-1})

All treatments not including Title Phyte received a potassium supplement using potassium acetate (Stress Relefe, 0-0-25) (Harrell's LLC., Lakeland FL) at 4 oz/1,000ft^2 (12.572 L ha^{-1}).

CHAPTER III

RESULTS AND DISCUSSION

Field Studies

Due to the addition of Civitas and Civitas Harmonizer in the second study, bermudagrass field studies were analyzed spearately. In bentgrass field studies, results varied between studies, and data were therefore also analyzed separately.

Bentgrass Field Studies

Canopy Temperatures

The reduction of summer canopy temperatures has been shown to positively affect overall bentgrass health and is a major claim for several health promoting products, including several in this study. None of the studied products, however, produced lower canopy temperatures. In fact, treated turf exhibited higher temperatures than the untreated controls in study two. All treatments in study one showed similar average summer temperatures to untreated control with canopy temperatures between 98.6 and 100°F (37 and 37.8°C) (Figure 3-1). In study two, the untreated control (37.6°C) exhibited significantly lower temperatures than Turf Screen, PAR, and PAR + Title Phyte with an average difference of 2.8°F (~1.39°C) suggesting these treatments may actually cause more stressful environments.

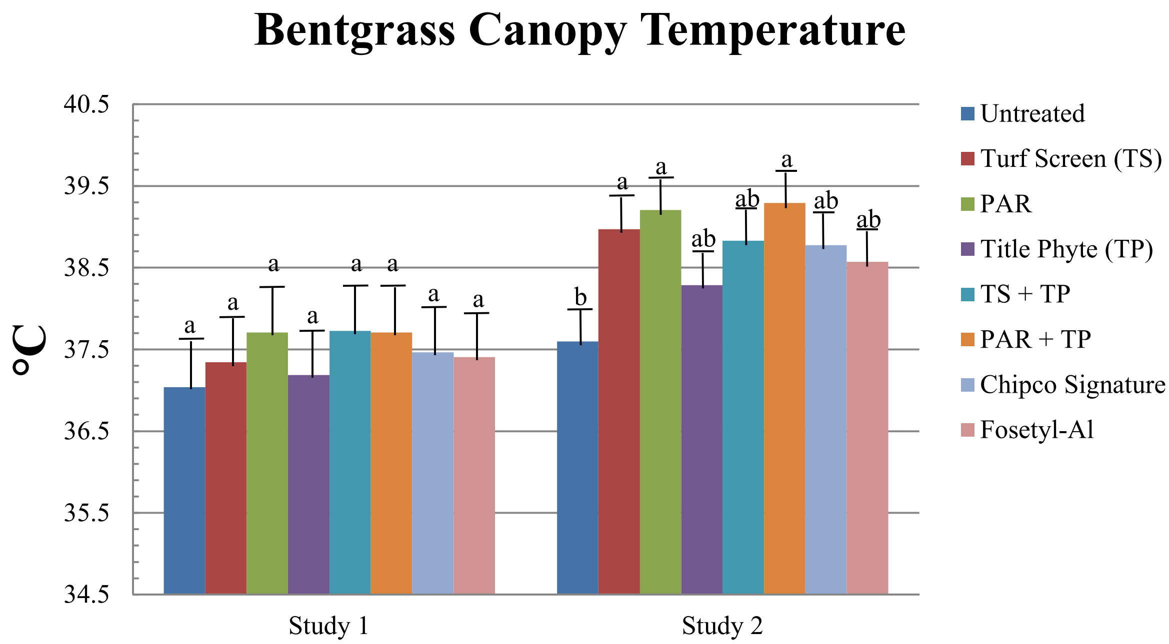

Figure 3-1. Canopy temperature averages for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Chlorophyll Content

Reduced chlorophyll content is indicative of possible stress having occurred. In both studies the untreated control exhibited significantly higher levels of chlorophyll (~30 relative concentration) than all treatments in study one and 24 in study two (Figure 3-2). In study one, Chipco Signature exhibited higher chlorophyll content averaging 297 compared to 282 of PAR but less than the untreated (327 relative concentration). No differences were observed between Fosetyl-Al, PAR + Title Phyte, Title Phyte, Turf Screen, and Turf Screen + Title Phyte in study one. In study two, differences were observed between Title Phyte (290), Signature (283), Fosetyl-Al (276), Turf Screen

(273), Turf Screen + Title Phyte (268), PAR (267), and PAR + Title Phyte (262). Decreased chlorophyll concentration is symptomatic of plant stress.

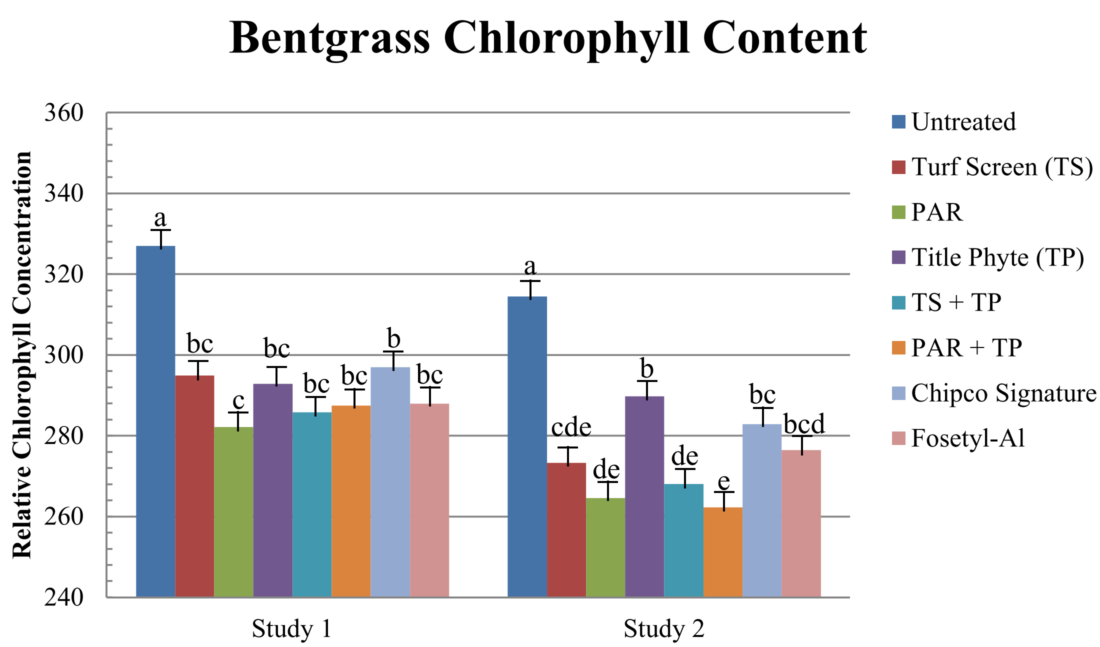

Figure 3-2. Chlorophyll content averages for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Visual Turf Quality

Visual quality for areas treated with Title Phyte and Fosetyl-Al averaged 5.79 and 5.46 in study one, significantly less than all other treatments which averaged ~6.8 (Figure 3-3). In study 2, turf quality for Fosetyl-Al was significantly less than Signature, PAR, untreated control, and PAR + Title Phyte averaging 5.4 compared to ~6.3 for treatments tested. However, Fosetyl-Al showed similar visual quality to that of Turf Screen, Turf Screen + Title Phyte, and Title Phyte averaging ~5.9.

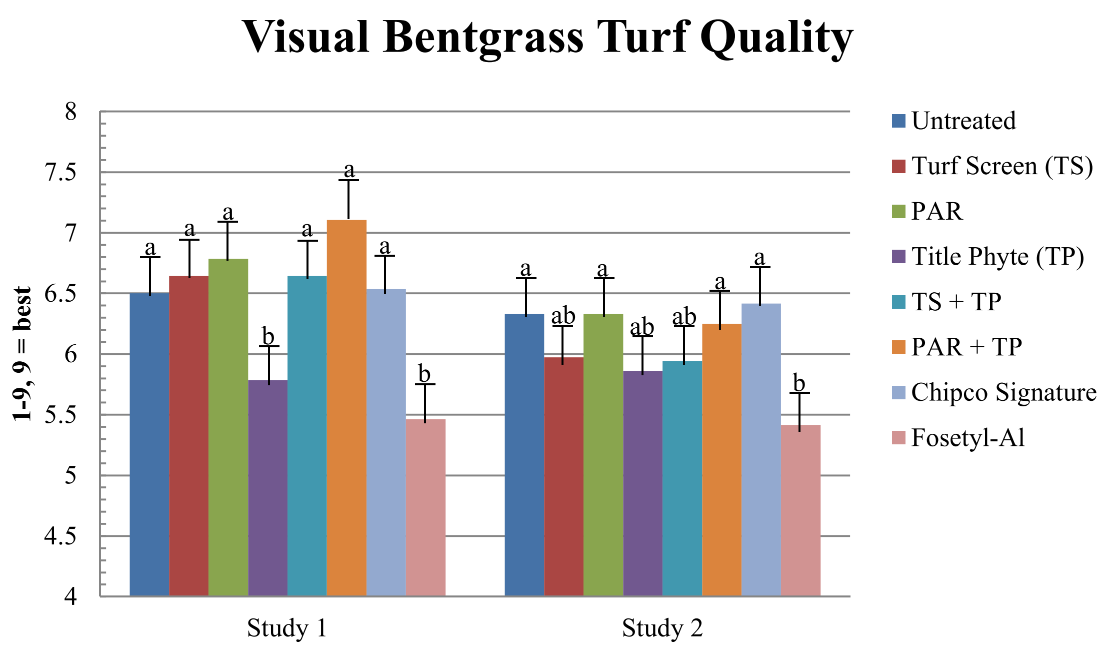

Figure 3-3. Average visual turfgrass quality for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Normalized Difference Vegetation Index

In both studies, the NDVI [(near infrared light (NIR) - red light)/(NIR + red light)] average of the untreated control was similar to Fosetyl-Al (~0.74 in study 1, ~0.71 in study 2) (Figure 3-4). Differences were not observed between Turf Screen, Turf Screen + Title Phyte, PAR + Title Phyte, and PAR in either study (~0.72 in study 1, ~0.68 in study 2). Similar NDVI averages were observed between Title Phyte and Signature in both studies (~0.73 in study 1, ~0.7 in study 2).

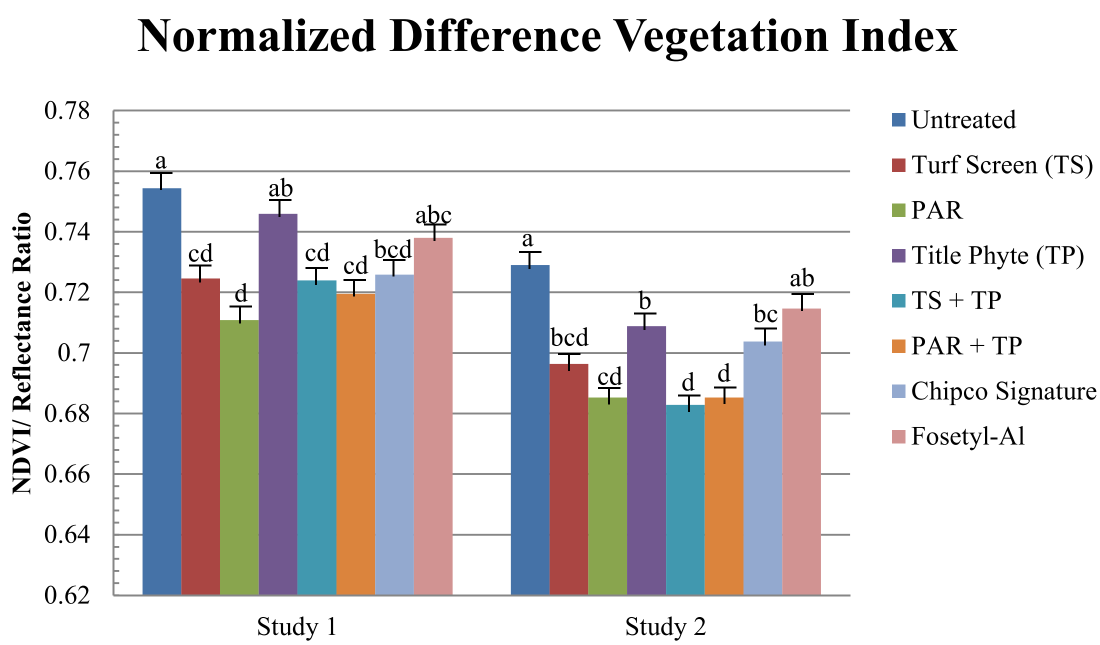

Figure 3-4. Normalized difference vegetation index (NDVI) averages for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Soil Moisture

Similar levels of volumetric soil water content were observed for all treatments in both studies. Average volumetric soil water content for all treatments ranged between 9 and 13 % in study one and between 9 and 11% in study two (Table 3-1).

Table 3-1. Volumetric soil water content data for two studies following bi-weekly applications of various nonpigmented and pigment-containing products to creeping bentgrass.

	Soil Moisture	
Treatment	**Study 1**	**Study 2**
	--------%--------	
Untreated	10.975	10.473

Turf Screen (TS)	9.375	9.578
PAR	9.479	9.478
Title Phyte (TP)	11.204	10.23
TS + TP	9.789	9.865
PAR + TP	9.929	9.595
Chipco Signature	12.687	9.894
Fosetyl-Al	10.075	9.732
	NS	NS

‡Means within columns analyzed according to Fisher's protected LSD (p = 0.05). NS = nonsignificant

Carbon Dioxide Exchange Rate

Carbon dioxide exchange rate (CER) measures the net CO_2 exchange from the surface of the turfgrass. A positive measurement is indicative of respiration exceeding photosynthesis and negative if photosynthesis exceeds respiration. Differences were not observed between treatments in study one, with CER levels ranging between 2.4 and 21.5 μmol CO_2 cm^{-2} s^{-1} (Figure 3-5). In study two, significantly lower CER (10.67 μmol CO_2 cm^{-2} s^{-1}) was measured in the untreated control compared to PAR + Title Phyte (30.35 μmol CO_2 cm^{-2} s^{-1}), Turf Screen + Title Phyte (29.42 μmol CO_2 cm^{-2} s^{-1}), Fosetyl-Al (27.52 μmol CO_2 cm^{-2} s^{-1}), PAR (27.02 μmol CO_2 cm^{-2} s^{-1}), and Turf Screen (22.27 μmol CO_2 cm^{-2} s^{-1}). Similar levels of CER were observed between Title Phyte (18.78 μmol CO_2 cm^{-2} s^{-1}), Signature (20.99 μmol CO_2 cm^{-2} s^{-1}), Turf Screen + Title Phyte, Fosetyl-Al, PAR, and Turf Screen in study two. All levels indicate failure to reduce net CO_2 exchange rate suggesting that products do not alleviate summer photosynthetic stress on creeping bentgrass.

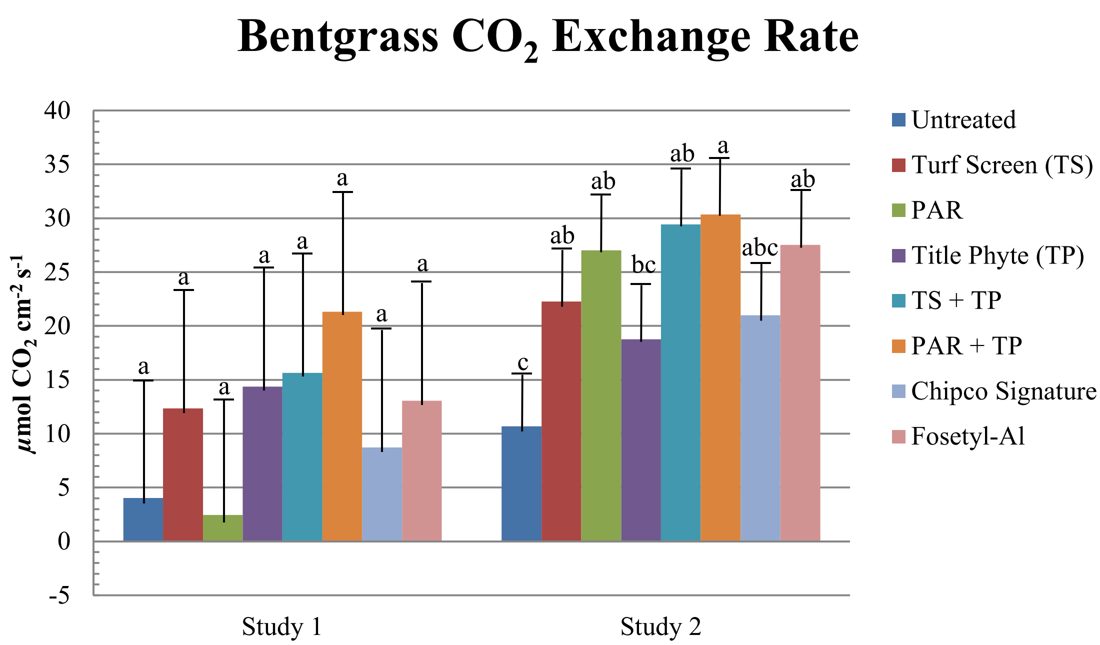

Figure 3-5. Carbon dioxide exchange rates for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Root Weight

No differences between treatments were observed in net root weight in either study. In study one, all treatments averaged between 0.02 and 0.05 g 200 cm^{-3}, and between 0.02 and 0.08 g 200 cm^{-3} in study two (Table 3-2).

Table 3-2. Net root dry weight of creeping bentgrass following bi-weekly applications of various nonpigment and pigment-containing products.

	Dry Root Weight change	
Treatments	**Study 1**	**Study 2**
	-------g 200 cm^{-3}------	
Untreated	0.036	0.063
Turf Screen (TS)	0.047	0.073

PAR	0.039	0.043
Title Phyte (TP)	0.024	0.038
TS + TP	0.039	0.036
PAR + TP	0.024	0.026
Chipco Signature	0.027	0.041
Fosetyl-Al	0.025	0.043
	NS	NS

‡Means within columns analyzed according to Fisher's protected LSD (p = 0.05). NS = nonsignificant

Heavy Metals

Due to the presence of metal molecules in the pigmented products, heavy metal concentrations of plant tissue and soil was analyzed. Turf Screen and Turf Screen + Title Phyte exhibited significantly higher net change of zinc concentrations in bentgrass plant tissue in both studies (~825 mg/kg in study 1, ~920 mg/kg in study 2) (Figure 3.6). Additionally, plots treated with both products were significantly higher than all other products in net soil concentration of zinc (~4.5 kg ha^{-1} greater in both studies) (Figure 3.8). This was assumed to be from applying Turf Screen which contained zinc.

The concentration of copper in bentgrass plant tissue and soil was also of interest due to the presence of copper-based pigments in several of the other products. In study one, PAR + Title Phyte (50 mg/kg), PAR (45 mg/kg), Turf Screen + Title Phyte (41 mg/kg), and Signature (37 mg/kg) exhibited significantly higher increases in copper concentration in plant tissue than the untreated control (1.25 mg/kg) (Figure 3.6). Turf Screen (34 mg/kg), Fosetyl-Al (30 mg/kg), and Title Phyte (8 mg/kg) also had increases compared to the control. In study two, PAR + Title Phyte (56 mg/kg), Turf Screen + Title Phyte (43 mg/kg), PAR (4 mg/kg), and Turf Screen (34 mg/kg) exhibited significantly greater net increases in soil copper concentration than the control (11 mg/kg) and Title Phyte (11 mg/kg). No observed differences occurred in net soil copper concentration in either year with study one net ranging between 0.03 and 0.4 kg Cu ha^{-1} in both studies (Table 3.3).

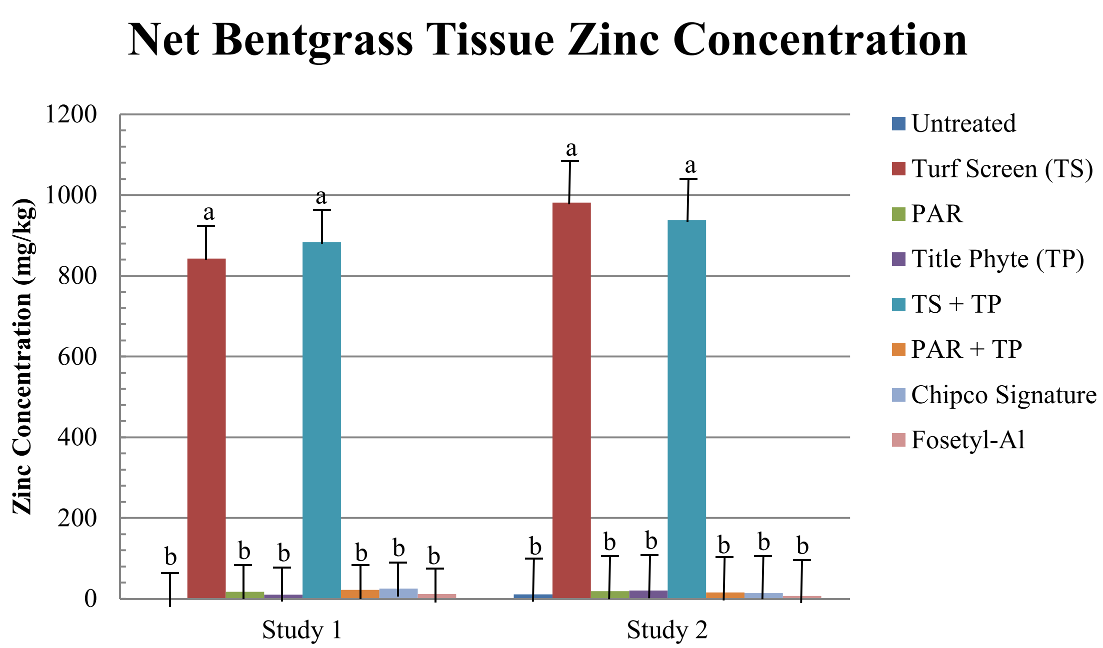

Figure 3-6. Net plant tissue zinc concentration for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

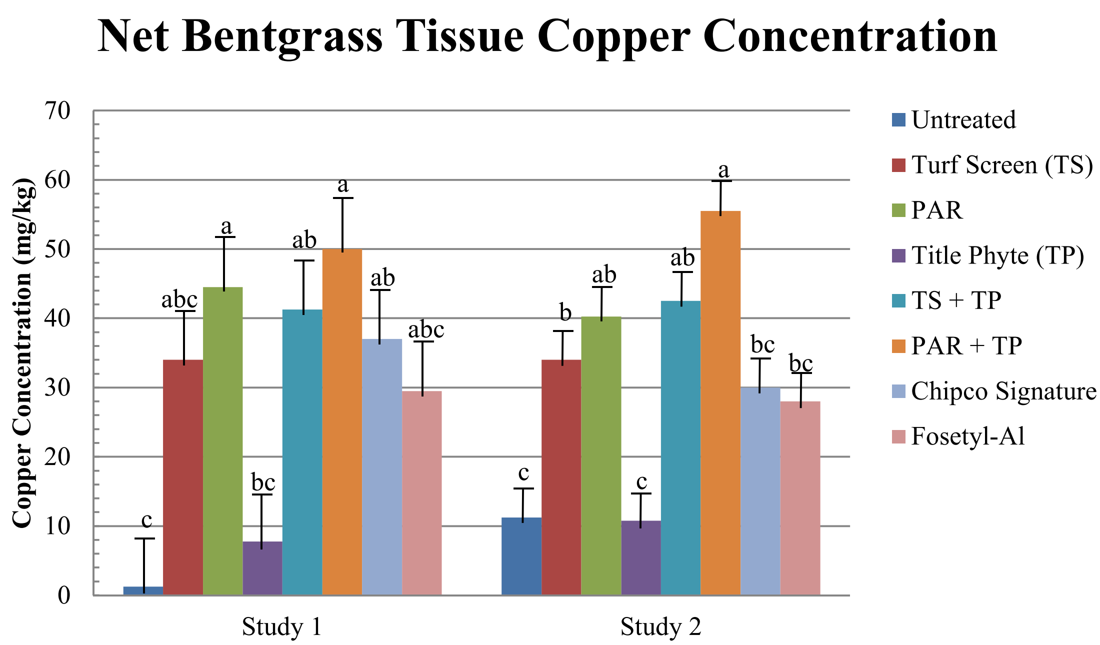

Figure 3-7. Net plant tissue copper concentration for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

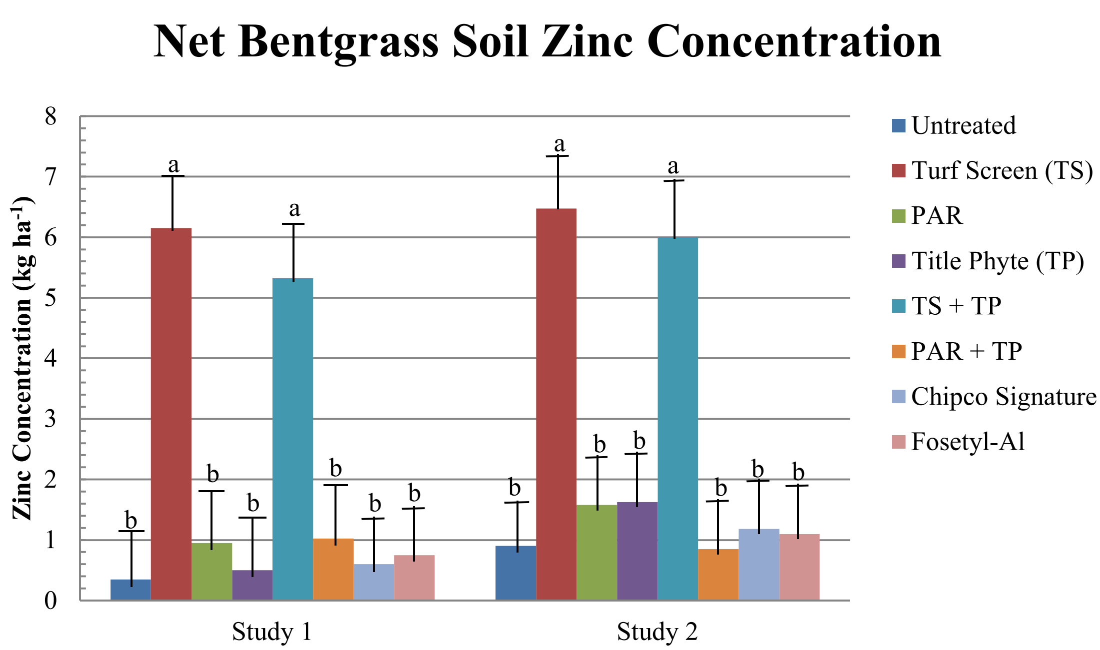

Figure 3-8. Net soil zinc concentration for two summer studies following bi-weekly applications to creeping bentgrass of various nonpigmented and pigment-containing

products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Table 3-3. Net soil copper concentration data for two bentgrass studies following bi-weekly applications of various nonpigmented and pigment-containing products.

	Net Soil Copper Concentration	
Treatment	**Study 1**	**Study 2**
	-------- kg ha^{-1}--------	
Untreated	0.07	0.18
Turf Screen (TS)	0.32	0.18
PAR	0.09	0.15
Title Phyte (TP)	0.05	0.28
TS + TP	0.22	0.1
PAR + TP	0.2	0.28
Chipco Signature	0.03	0.18
Fosetyl-Al	0.11	0.33
	NS	NS

‡Means within columns analyzed according to Fisher's protected LSD ($p = 0.05$). NS = nonsignificant

Bermudagrass Field Studies

Canopy Temperature

High temperature stress is not a major concern on hybrid bermudagrass due to its C_4 physiology. All treatments in study one showed similar canopy temperatures to that of the untreated with temperatures between 98.9 to 100.4°F (37 to 39°C) (Figure 3-9). Temperatures in study 2, however, exhibited greater differences (~2 to 3.5°C) to that of the untreated (36.6°C) with only Title Phyte (37.9°C) showing similar temperatures.

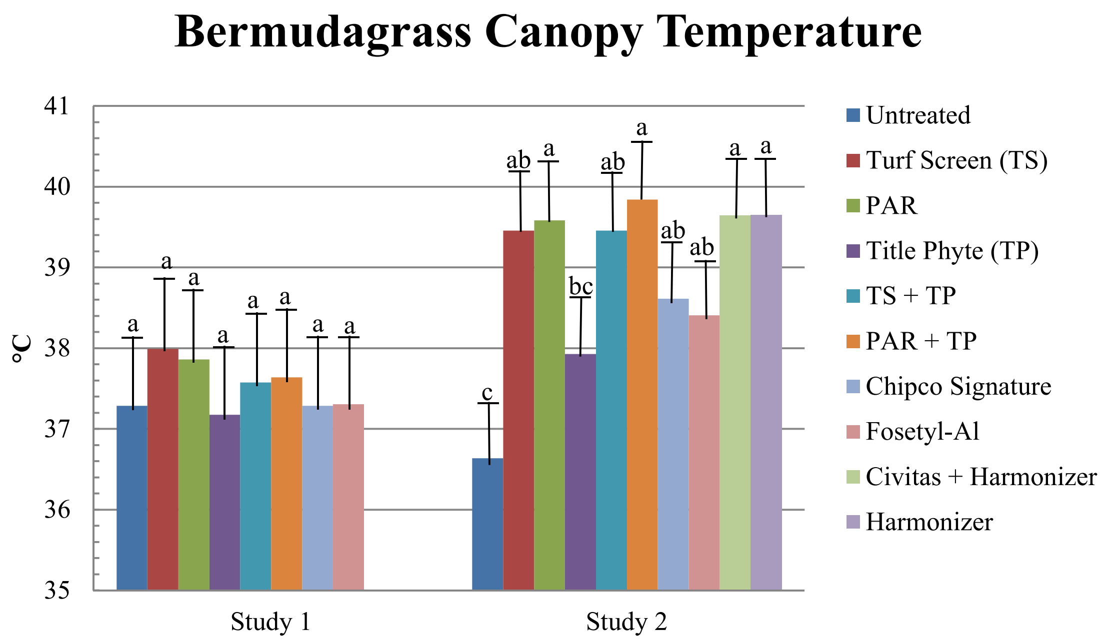

Figure 3-9. Canopy temperature averages for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

<u>Chlorophyll Content</u>

No significant differences were observed in either year of study in chlorophyll content. Study 1 observed concentration averages of 261to 298 (Figure 3-10). In study 2, content concentrations ranged between 310 to 339.

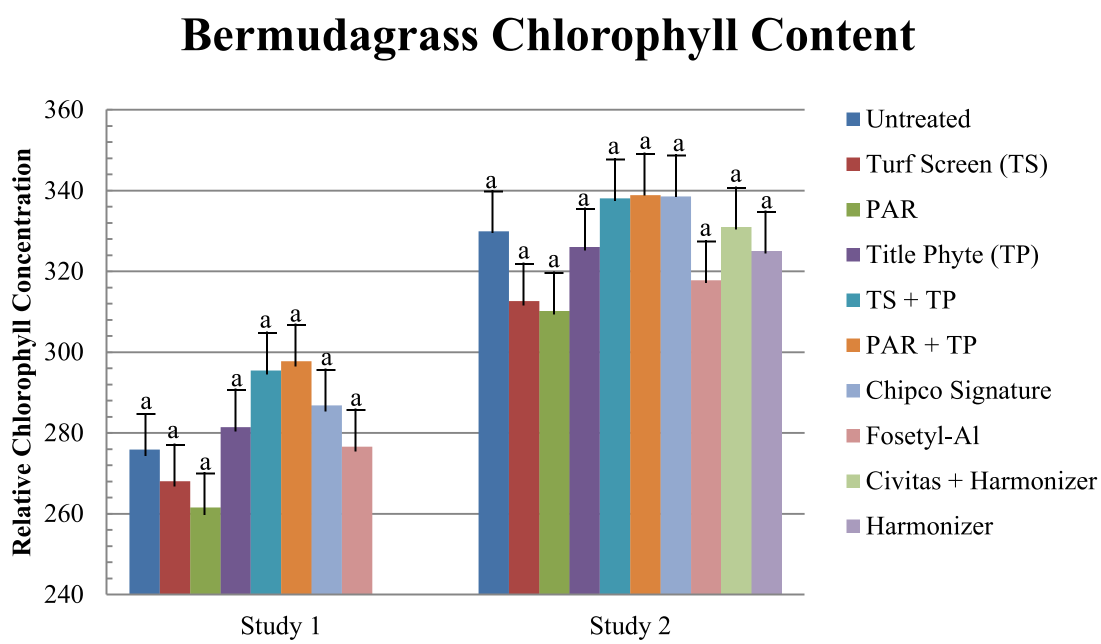

Figure 3-10. Chlorophyll content averages for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

<u>Turf Quality</u>

In study 1, visual turf quality of PAR, Chipco Signature, Title Phyte, and Fosetyl-Al were similar to the untreated control with average ratings between 5.7 and 6.4 (Figure 3-11). PAR + Title Phyte, TurfScreen + Title Phyte, and Turf Screen yielded greater visual ratings of 6.9, 6.8, and 6.7, respectively. None of these ratings, however, meet an average acceptable turf quality level of 7. No differences in visual quality were observed in study 2, with all treatments ranging between 6.6 and 7.1.

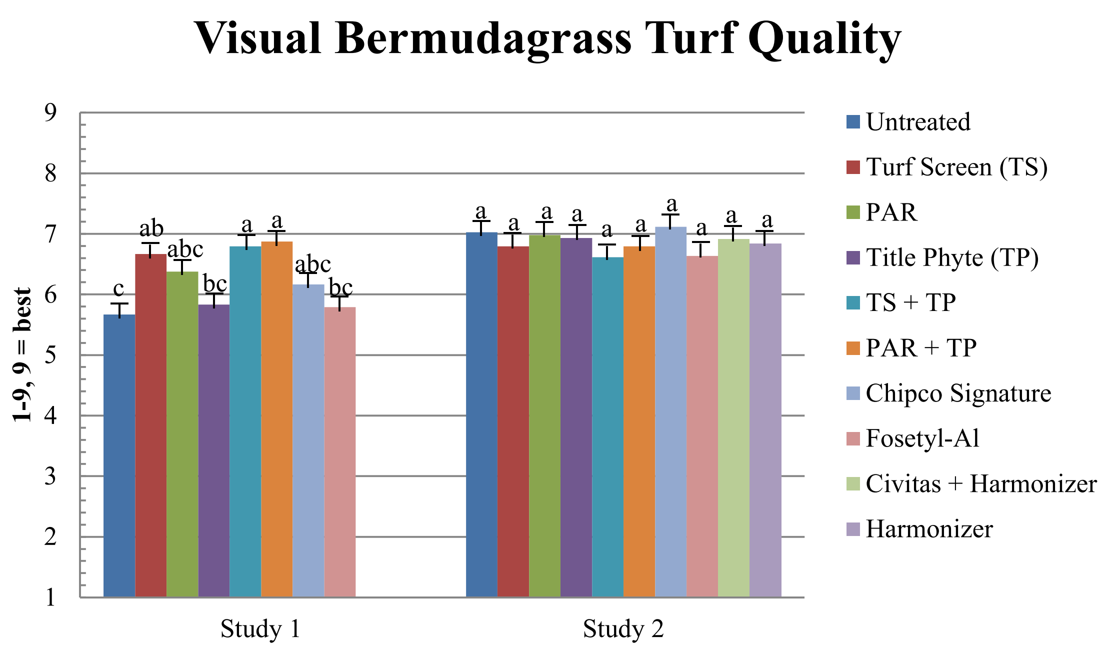

Figure 3-11. Average visual turfgrass quality for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Normalized Difference Vegetation Index

Differences in NDVI ratings were not observed in either study between untreated control and treated plots. Study 1 yielded reflectance averages between 0.65 – 0.69 (Figure 3-12). Similar observations were made in study 2 with averages between 0.64 – 0.68. This indicates that though products may include a pigment or dye, the leaf of a plant still maintains a similar level of green.

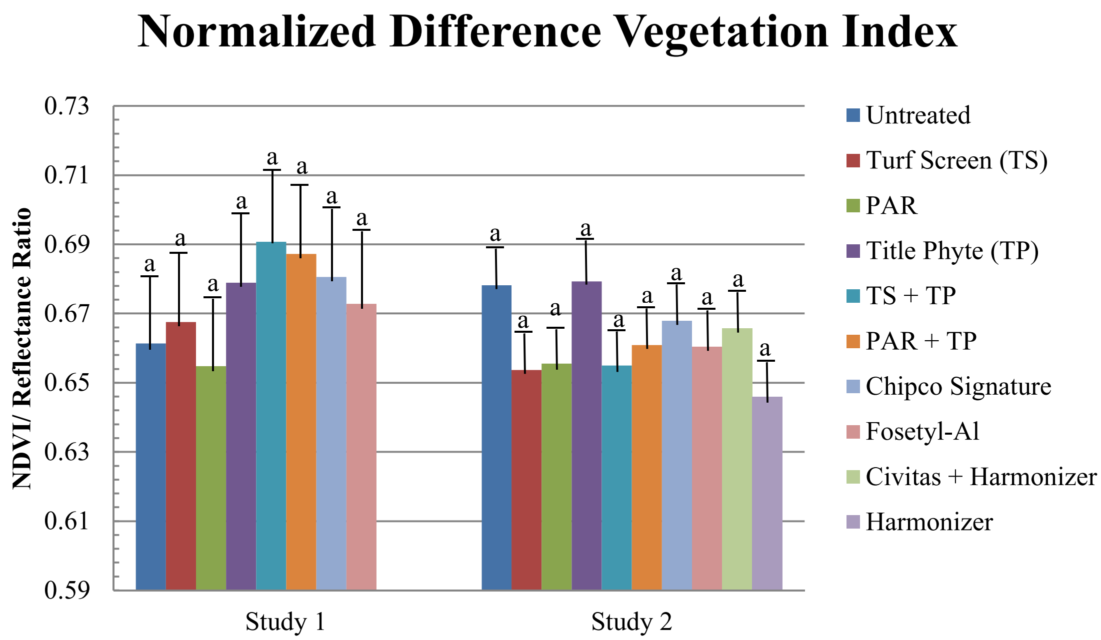

Figure 3-12. Normalized difference vegetation index (NDVI) averages for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Soil Moisture

Similar levels of volumetric soil moisture were observed throughout both studies, with no impact on turf quality. Study 1 moisture content ranged between 6.3 and 8.0%, while study 2 averages were between 6.8 and 8.3% (Table 3-4).

Table 3-4. Volumetric soil water content data for two studies following bi-weekly applications of various nonpigmented and pigment-containing products to hybrid bermudagrass.

	Soil Moisture	
Treatment	**Study 1**	**Study 2**
	--------%--------	
Untreated	7.693	7.875
Turf Screen (TS)	8.029	7.507
PAR	6.268	6.929
Title Phyte (TP)	7.471	7.564
TS + TP	7.15	7.332
PAR + TP	7.182	7.893
Chipco Signature	7.355	7.879
Fosetyl-Al	6.254	6.807
Civitas + Harmonizer	-	8.275
Harmonizer	-	7.964
	NS	NS

‡Means within columns analyzed according to Fisher's protected LSD (p = 0.05). NS = nonsignificant

Carbon Dioxide Exchange Rate

Study one yielded no differences between treated areas and untreated with rates between 14.3 and 24.4 ppm CO_2 (Figure 3-13). In field study 2, Signature (-28.295 ppm) was the only treatment that statistically different from the untreated control of -8.972 ppm. A more negative number indicates a greater photosynthetic efficiency.

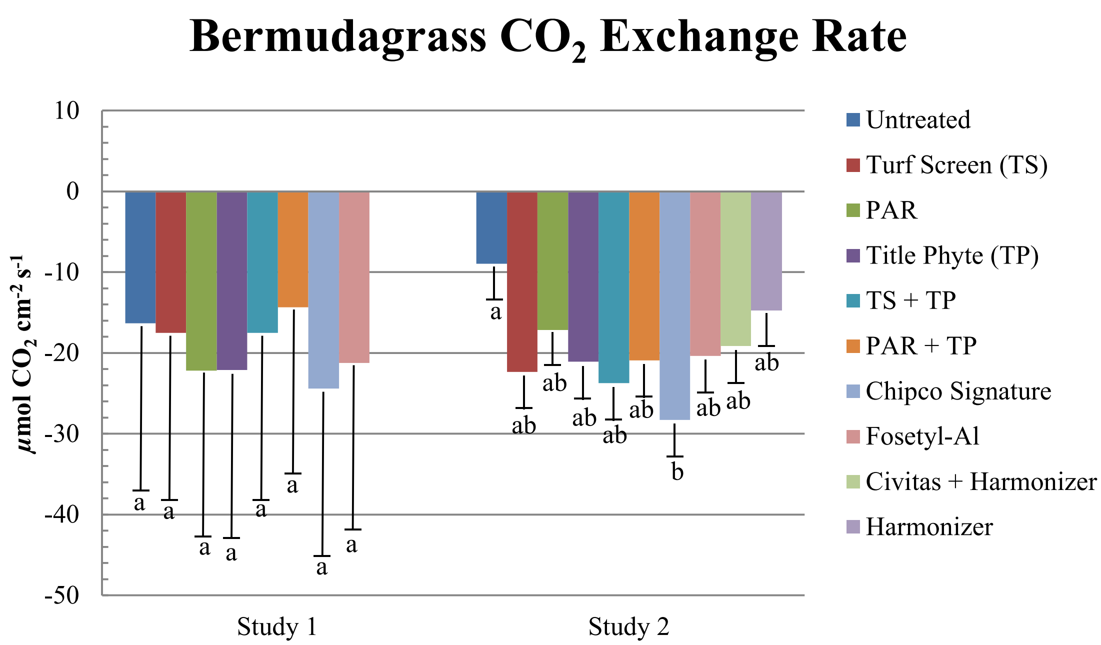

Figure 3-13. Carbon dioxide exchange rates for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products. A more negative number indicates a greater photosynthetic efficiency. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Root Weight

Differences between treatments were not observed in net root weight in either study. In study one, all treatments averaged between 0.09 and 0.11 g 200 cm^{-3}, while in study two, root weights were between 0.08 and 0.1 g 200 cm^{-3} (Table 3-2).

Table 3-5. Net root dry weight of hybrid bermudagrass following bi-weekly applications of various nonpigment and pigment-containing products.

	Dry Root Weight change	
Treatments	**Study 1**	**Study 2**
	----g 200 cm^{-3}----	
Untreated	0.09	0.08

Turf Screen (TS)	0.11	0.08
PAR	0.1	0.09
Title Phyte (TP)	0.11	0.09
TS + TP	0.09	0.1
PAR + TP	0.11	0.09
Chipco Signature	0.11	0.1
Fosetyl-Al	0.09	0.08
Civitas + Harmonizer	-	0.09
Harmonizer	-	0.09
	NS	NS

‡Means within columns followed by the same letter are not significantly different according to Fisher's protected LSD ($p = 0.05$). NS = nonsignificant

Heavy Metals

Due to lack of comparable samples as a result of limited bermudagrass vegetative growth, statistical analysis of tissue analysis could not be performed. However, areas treated with Turf Screen and Turf Screen + Title Phyte appeared to have higher bermudagrass tissue concentrations of zinc than other treatments in both studies (Figure 3-14). Additionally, both treatments were significantly higher than all others in zinc concentration in soil (Figure 3-16).

In study one, Turf Screen + Title Phyte (51 mg/kg), PAR (45 mg/kg), and Turf Screen (39 mg/kg), exhibited higher copper concentrations in tissue than the untreated control (0 mg/kg) (Figure 3-15). Signature (34 mg/kg), PAR + Title Phyte (32 mg/kg), Fosetyl-Al (28 mg/kg), and Title Phyte (11 mg/kg) all showed increases as well. In study two, Turf Screen (50 mg/kg), Civitas (42 mg/kg), PAR (41 mg/kg), and Turf Screen + Title Phyte (38 mg/kg) exhibited net increases in soil copper concentration while the control had an increase of 9 mg/kg. No observed differences occurred in net soil copper concentration in either year with either study ranging between 0.05 and 0.45 kg Cu ha^{-1} (Table 3-6).

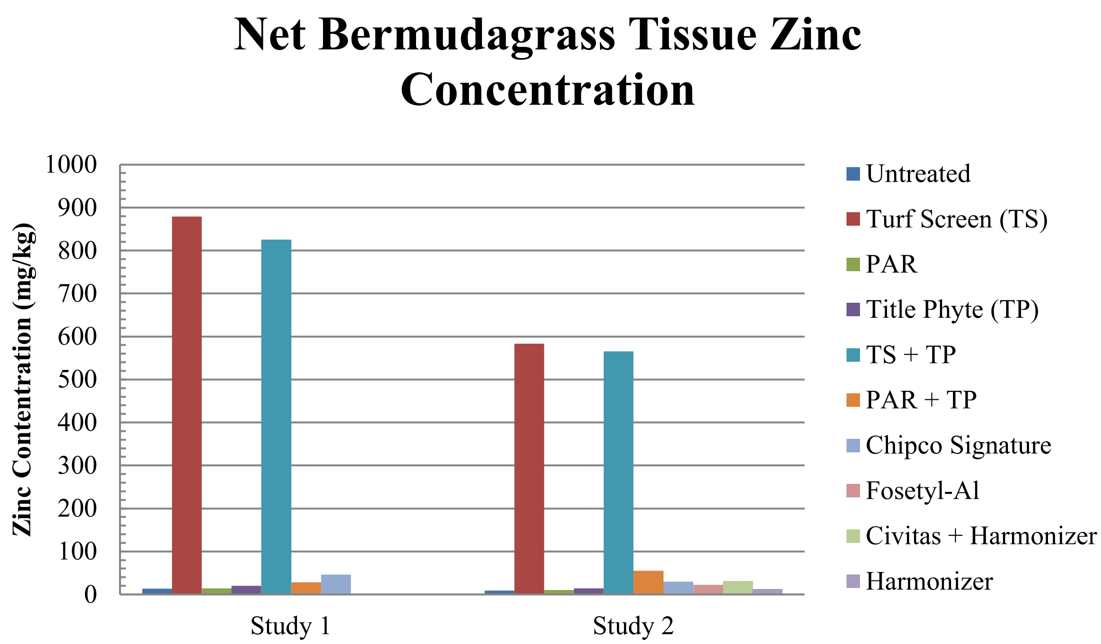

Figure 3-14. Net plant tissue zinc concentration for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products.

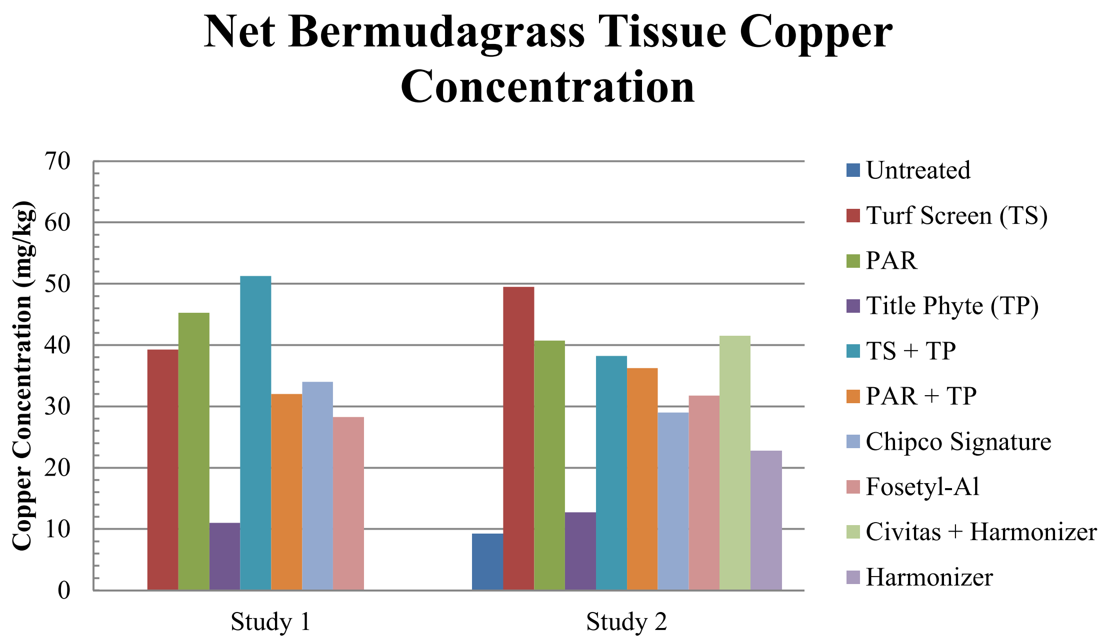

Figure 3-15. Net plant tissue copper concentration for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing products.

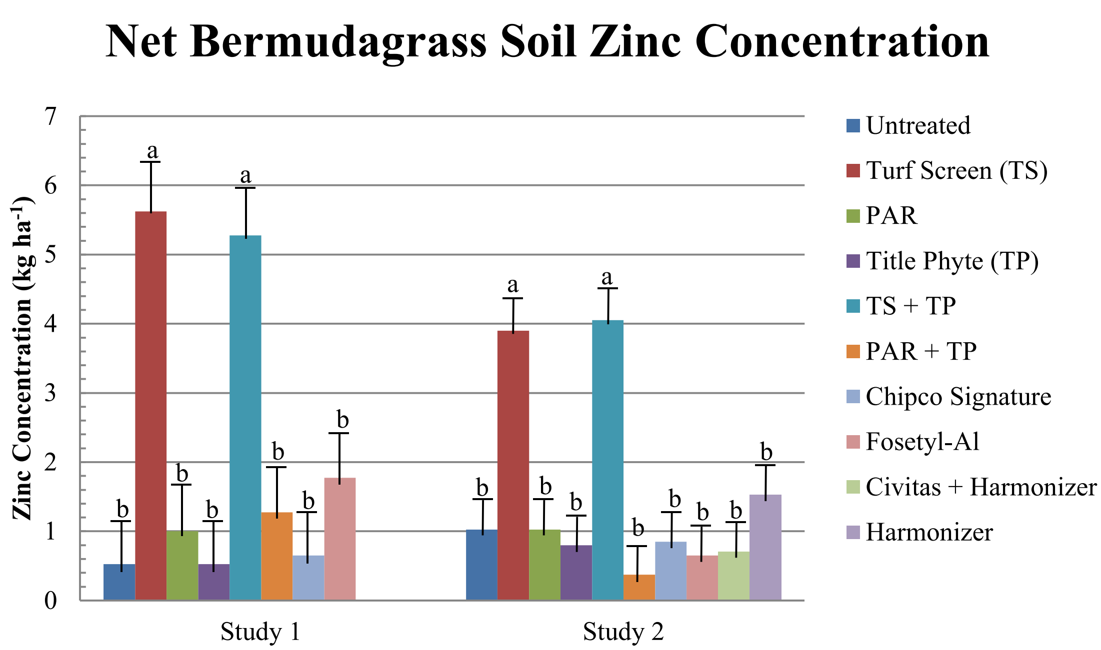

Figure 3-16. Net soil zinc concentration for two summer studies following bi-weekly applications to hybrid bermudagrass of various nonpigmented and pigment-containing

products. Vertical bars represent standard errors. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$).

Table 3-6. Net soil copper concentration data for two studies following bi-weekly applications of various nonpigmented and pigment-containing products to hybrid bermudagrass.

	Net Soil Copper Concentration	
Treatment	**Study 1**	**Study 2**
	-------- kg ha^{-1}--------	
Untreated	0.05	0.14
Turf Screen (TS)	0.41	0.14
PAR	0.3	0.32
Title Phyte (TP)	0.23	0.16
TS + TP	0.25	0.45
PAR + TP	0.23	0.32
Chipco Signature	0.12	0.21
Fosetyl-Al	0.27	0.24
Civitas + Harmonizer	-	0.2
Harmonizer	-	0.25
	NS	NS

‡Means within columns analyzed according to Fisher's protected LSD ($p = 0.05$). NS = nonsignificant

Growth Chamber Studies

Bentgrass Heat Stress

Carbon Dioxide Exchange and Fluorescence

In study one, the unstressed creeping bentgrass control (-13.548 μmol CO_2 cm^{-2} s^{-1}) had significantly more negative CO_2 exchange rates than all stressed treatments, none of which differed from each other. Exchange rates for stressed plants ranged from 2.13 to 8.28 μmol CO_2 cm^{-2} s^{-1} (Figure 3-17). A higher CER value is indicative of a decrease in net photosynthesis. There were no significant differences among treatments in study two.

Variable chlorophyll fluorescence (F_v/F_m) measurements in study two yielded statistical differences between F_v/F_m values of all stressed creeping bentgrass treatments compared to that of the unstressed control (0.785) (Figure 3-18). The decrease in average F_v/F_m values suggest decreased photosynthetic efficiency in addition to potential damage of the photosynthetic pathways.

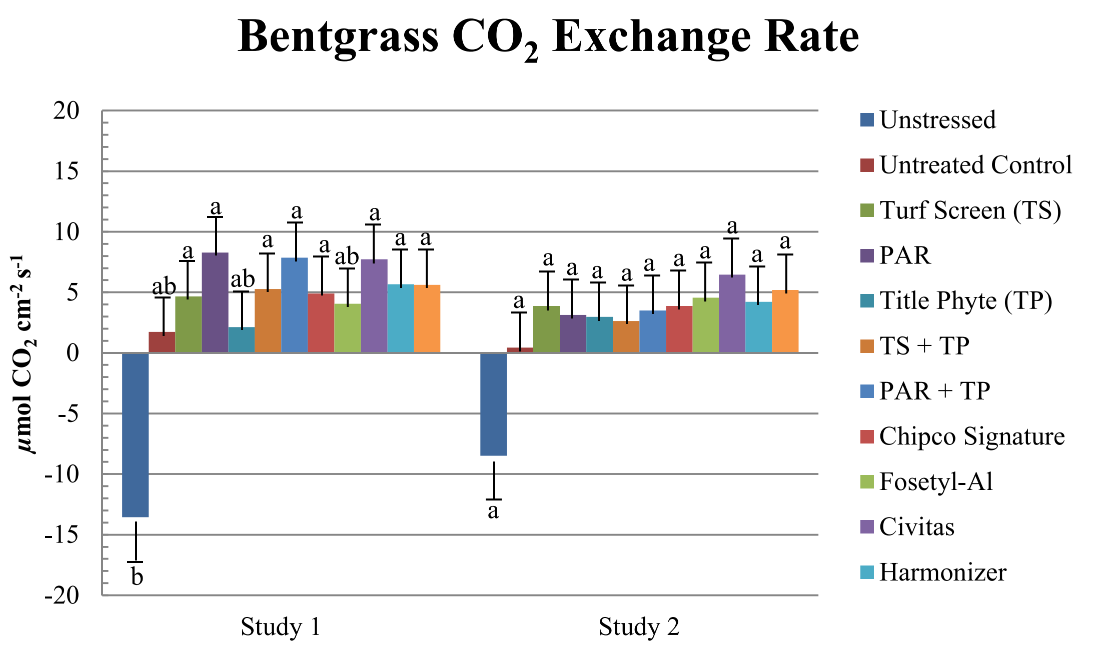

Figure 3-17. Carbon dioxide exchange rates for two growth chamber studies at Clemson University Greenhouse Facility following treatments with pigment-containing products on creeping bentgrass. Vertical bars represent standard errors. Lower values indicate a plant experiencing less stress. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$). Stressed control = 35°C. Unstressed control = 27°C.

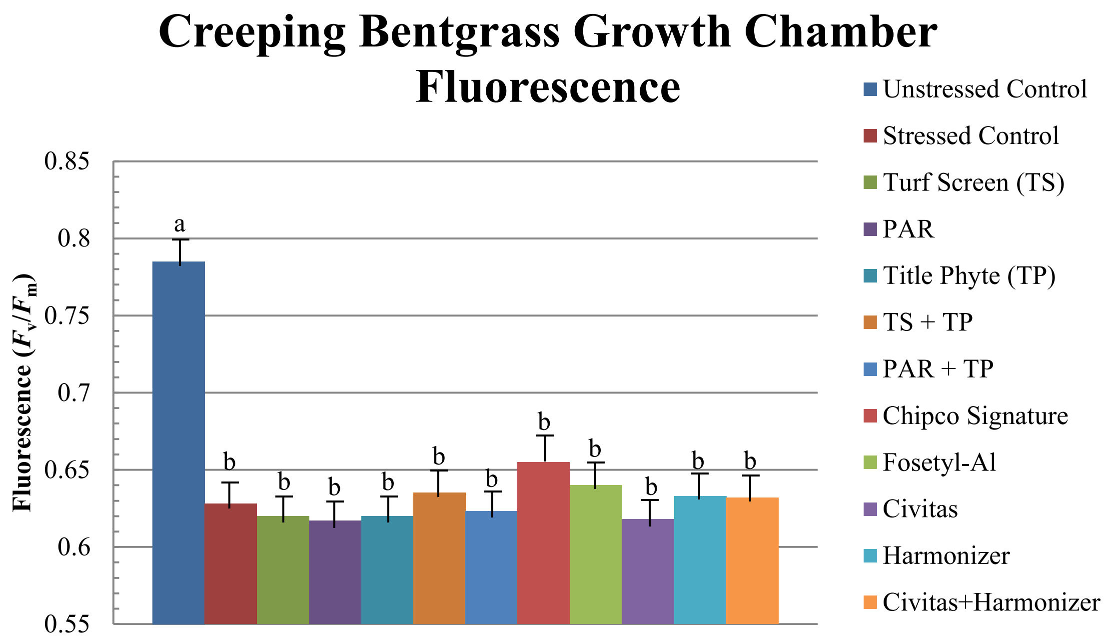

Figure 3-18. Average FluorPen readings for growth chamber study following treatments with pigment-free and pigment-containing products on creeping bentgrass. Vertical bars represent standard errors. Higher values are indicative of plant experiencing less stress. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$). Stressed control = 35°C. Unstressed control = 27°C.

<u>Bermudagrass Cold Stress</u>

Greenhouse study one had differences in the net CO_2 exchange rate between the unstressed bermudagrass control (3.1 μmol CO_2 cm^{-2} s^{-1}) and stressed control (40.2), Title Phyte (37.5), Turf Screen (33.4), and PAR + Title Phyte (32.4 μmol CO_2 cm^{-2} s^{-1}) (Table 3 -7). Differences were also observed in study two with Turf Screen + Title Phyte (38.5), stressed control (34.5), Fosetyl-Al (34.4), Title Phyte (34.1), PAR (32.1), and Turf Screen (29.2 μmol CO_2 cm^{-2} s^{-1}) all being statistically greater than the unstressed control (-2.1 μmol CO_2 cm^{-2} s^{-1}).

Fluorescence ratings in the study showed a statistical difference between the unstressed control (0.76) and all other treatments (Table 3-7). These findings suggest that the photosynthetic activity is heavily reduced by the freezing temperatures which may be caused by ice formation or reduced chlorophyll molecular integrity and/or capability (Allen and Ort, 2001).

Table 3-7. Net carbon dioxide exchange rate of hybrid bermudagrass subjected to simulated freeze following bi-weekly applications of various nonpigment and pigment-containing products. Lower values indicate plant experiencing less stress.

	Net CER rate	
Treatments	**Study 1**	**Study 2**
	μmol CO_2 cm^{-2} s^{-1}	
Unstressed Control	3.093b	-2.07b
Stressed Control	40.183a	34.497a
Turf Screen (TS)	33.38a	29.16a
PAR	23.3ab	32.077a
Title Phyte (TP)	37.537a	34.093a
TS + TP	29.98ab	38.517a
PAR + TP	32.377a	22.747ab
Chipco Signature	28.58ab	24.113ab
Fosetyl-Al	31.653ab	34.43a
Civitas	22.187ab	24.447ab
Harmonizer	31.883ab	26.73a
Civitas + Harmonizer	29.27ab	25.703ab
p-value	0.0181	0.0038

‡Means within columns followed by the same letter are not significantly different according to Fisher's protected LSD (p = 0.05).

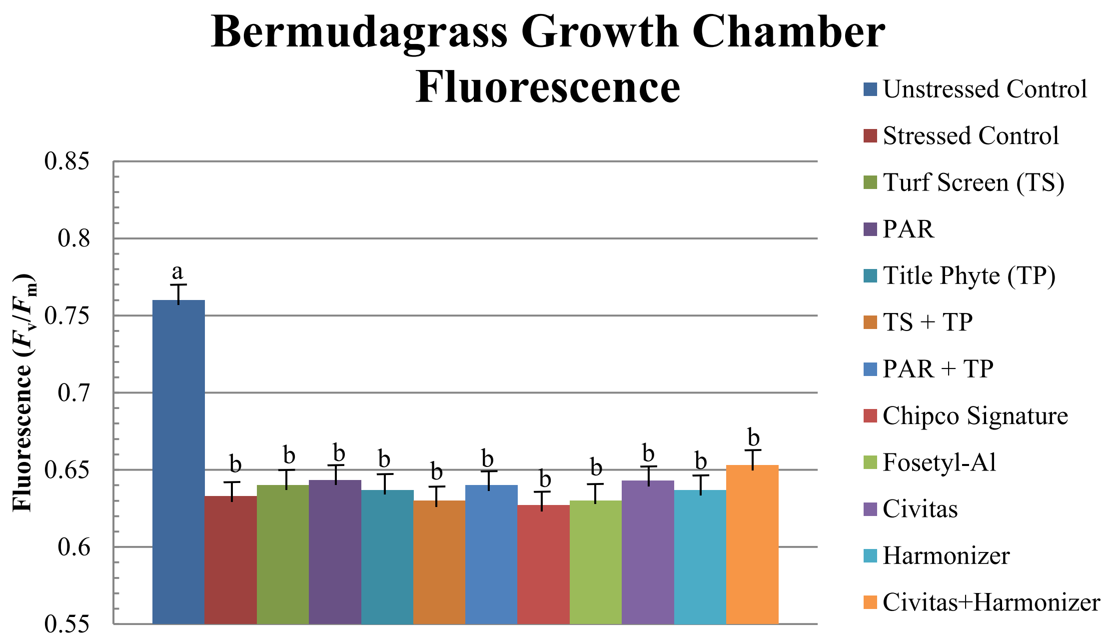

Figure 3-19. Average FluorPen readings for growth chamber study following treatments with pigment-free and pigment-containing products on hybrid bermudagrass. Vertical bars represent standard errors. Greater values indicate plant experiencing less stress. Different letters indicate significant differences between treatments within each study by LSD test ($p \leq 0.05$). Stressed control = °C. Unstressed control = 27°C.

Green-up Study

No differences were observed on any date comparing spring green-up of bermudagrass to that of the untreated (0.083, 0.129, 0.134, and 0.178) (Figure 3-20).

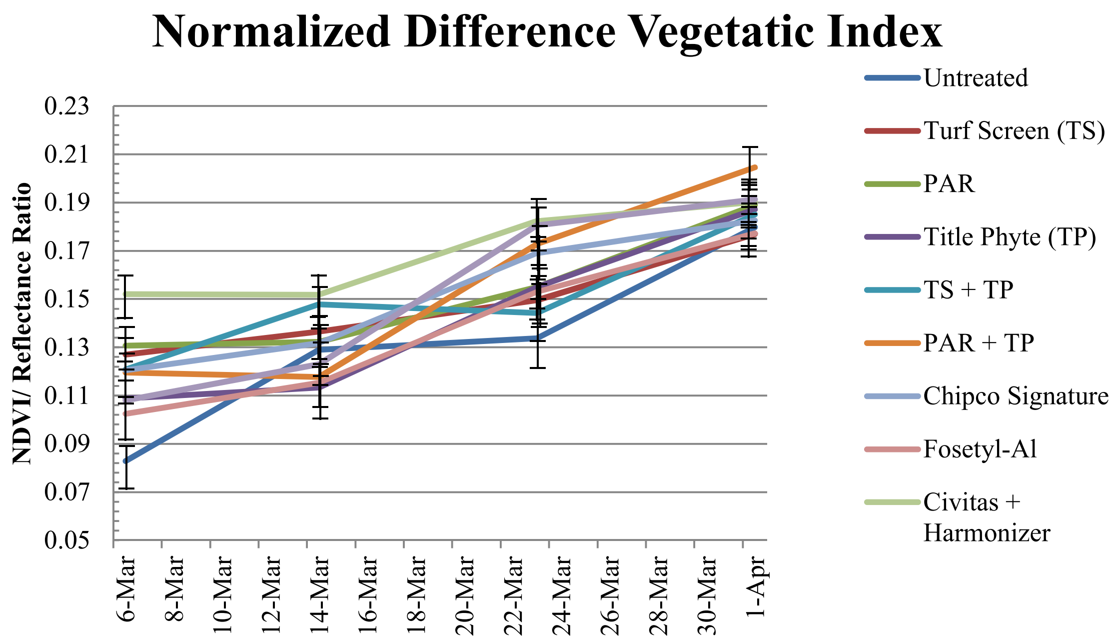

Figure 3-20. Normalize difference vegetative index for spring green up on hybrid bermudagrass treated with nonpigmented and pigment-containing products. Vertical bars represent standard errors. Means analyzed according to Fisher's protected LSD ($p \leq 0.05$).

CHAPTER IV

CONCLUSION

Bentgrass

Pigment and sunscreen-containing products are marketed as capable of relieving summer stress by way of reducing canopy temperatures while increasing a plant's basic photosynthetic efficiency. However results of this study largely do not support these claims. Repeated product applications on creeping bentgrass, a turfgrass that historically suffers from heat and high light stress, caused an increase in canopy temperatures in addition to a reduction in overall efficiency as indicated by greater CO_2 exchange rates when compared to the untreated in field studies. The reduction in F_v/F_m in field study two also indicated the inability of products to reduce stress typically seen during extended times of supraoptimal temperatures. In 2014, McCarty et al. linked the increase in temperatures to the covering and/or entering ("clogging") of stomata by the products thus reducing the transpiration of the plant.

Greenhouse studies supported results from the field. Both greenhouse studies reported all treatments in growth chamber (35°C) experienced greater CO_2 exchange rates when compared to an unstressed control. Greenhouse study two also confirmed the inability of tested products to reduce stress when the stressed control averaged a F_v/F_m value of 0.63 which was similar to all other stressed treatments.

Bermudagrass

While high temperatures are not of as great concern to bermudagrass compared to bentgrass, the effects of these products is. Similar to the bentgrass study, all products caused an increase in canopy temperature, however, this may be beneficial to bermudagrass because a more negative CO_2 rate was observed with the application of Signature. The cause of this is a potential study as no major difference in chlorophyll was observed in either study between any plots.

Greenhouse studies focused on cold stress as continued applications of these products later into the year may cause a decrease in cold acclimation of the plants due to higher soil temperatures causing a greater susceptibility to cold and freezing stress. However, no major differences were observed in carbon exchange rates between any stressed plants. All stressed plant measurements showed greater CO_2 exchange rates than that of the unstressed control. F_v/F_m rates in study two were reduced compared to unstressed, however showed no differences between stressed plants.

SUMMARY

These products exhibited mixed results depending on which species of grass to which they were applied. When applied to creeping bentgrass, products decreased overall turf health via a reduction in basic photosynthetic properties. Conversely, applications to bermudagrass during times of high temperatures may improve various aspects of the physiological functions. However, both studies indicated that long term use pigmented products can cause a significant increase in heavy metal concentrations which may lead to later toxicity though no such symptoms were observed in any of these studies. The impact of these concentrations may be a point of further research. The potential fungicidal effect of products was not investigated. Results for these products may vary depending on environmental conditions as these studies were conducted under hot, humid conditions. Research should continue to investigate potential stress relieving strategies as well as possible methods to improving and increasing spring green-up timing.

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