LABORATORY AND FIELD INVESTIGATIONS ON CREEPING BENTGRASS (*Agrostis palustris* Huds.) GOLF GREENS

By

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GOLF GREENS

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INTRODUCTION

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CHAPTER I

LITERATURE REVIEW

Literature Review

ANNUAL BLUEGRASS: HISTORICALLY CONSIDERED A WEED

Managing annual bluegrass (*Poa annua* L.) in creeping bentgrass (*Agrostis palustris* Huds.) golf greens is a concern for golf course superintendents in most areas of the United States. Annual bluegrass is sometimes used as turf, but is usually considered a weed for aesthetic, functional, and economic reasons in creeping bentgrass greens. A most undesirable trait is the species' propensity for flowering (Johnson and White 1998). Unlike most grasses, annual bluegrass can produce inflorescence even at mowing heights of 3 to 6 mm (Sprague and Burton 1937). Flowering and its typical yellow-green color, disrupt the uniformity and consistency of putting surfaces. Annual bluegrass effectively competes for resources with desirable species (Ashton and Monaco 1991) and poses several other management problems. Poor heat tolerance, overall poor cold tolerance, poor drought tolerance, and susceptibility to death from ice encasement, combine to make annual bluegrass undesirable and difficult to manage effectively. It is susceptible to fungal attack under low nutrient levels, to other fungal diseases under high nutrient levels, and to annual bluegrass weevil and other insect pests. Therefore, a mixed sward of annual bluegrass and creeping bentgrass requires more precise management practices and more inputs (nutrients, water, and pesticides) than a pure stand of creeping bentgrass.

ANNUAL BLUEGRASS LIFE CYCLE AND PREVALENCE

The life cycle of annual bluegrass is highly variable (Johnson and White 1998; Mitich 1998). In cooler climates, it often survives as a perennial, while in warmer regions it usually persists as an annual. Historically, the perennial forms of the species

have proven the most difficult to control (Johnson and Murphy 1996; Bell et al. 1999). Annual bluegrass is ubiquitous (Mitich 1998; Bell, et al. 1999), suggesting that control measures have worldwide importance. High seed production and high seed viability contribute to the success of the species as a colonizer (Mitich 1998). Its presence has been recorded on all continents of the world, even Antarctica (Scott, 1987). Annual bluegrass seed can be spread by birds and transported on shoes, tools, mowers, and other equipment, creating opportunities for invasion of existing turf (Mitich 1998).

HISTORICAL METHODS FOR ANNUAL BLUEGRASS CONTROL

Despite over 50 years of scientific research, annual bluegrass remains a tenacious weed in cool-season turf. Possible control methods have included the following: pre-emergence herbicides (Callahan and Shepard 1991; Callahan and McDonald 1992), plant growth regulators (Breuninger and Watschke 1982; Shoop, et al. 1986; Gaussoin and Branham 1989; Johnson and Murphy 1996), selective herbicides (Gaul and Christians 1988; Branham 1990), varying irrigation regimes (Sprague and Burton 1937; Gaussoin and Branham 1989), various nutritional inputs (Waddington, et al. 1978; Eggens and Wright 1985; Dest and Guillard 1987; Goss 1974), and phytopathogenic bacteria (Savage, et al. 1993; Zhou and Neal 1995; Imaizumi, et al. 1997).

ECOLOGICAL MANIPULATION FOR ANNUAL BLUEGRASS CONTROL

Variations in photosynthetic efficiency and pigment levels between annual bluegrass and creeping bentgrass have been suggested as a possible means of exploitation (Bell, et al. 1999). In that study, researchers were able to significantly reduce young (1-2

years of growth) annual bluegrass populations by 63.7% in a creeping bentgrass fairway study using foliar iron/magnesium applications; while traditional methods of control, [plant growth regulator (trinexapac-ethyl), selective herbicide (ethofumesate), and pre-emergence herbicide (bensulide)] had no significant effects.

PIGMENT EXTRACTION AND ANALYSIS BACKGROUND

Pigment analysis of the chlorophylls and carotenoids, including the xanthophylls is important in the plant sciences. The examination of these pigments can help scientists determine plant health status since each pigment exhibits special functions that are integral to the photosynthetic process (Cogdell 1988). The extraction and isolation of plant pigments is a critical part of pigment analysis. Various solvents have been used for pigment extraction and subsequent analysis.

HISTORICAL METHODS OF PIGMENT ANALYSIS AND EXTRACTION PROCEDURES

Acetone has long been used in the laboratory as an extraction solvent for higher plant study (MacKinney 1941; Arnon 1949). The most widely accepted extraction procedure uses 80% aqueous acetone or 100% acetone and some sort of homogenizer to macerate the tissue and isolate plant pigments for further analysis (Holden 1976; Porra, et al. 1989; De las Rivas, et al. 1991; Gilmore and Yamamoto 1991; Svec 1991; Almela 1992;Val and Monge 1994; Bell and Danneberger 1999).

N, N dimethylformamide (DMF) is another organic solvent that has been used successfully for pigment extraction in previous studies (Moran 1982; Moran and Porath

1980; Inskeep and Bloom 1985; Bergweiler and Lutz 1986; Yadava 1986; Marquard and Tipton 1987; Danehower and Kelley 1990) suggesting it may be an acceptable substitute for acetone. Most pigment extraction studies with DMF used spectrophotometry for chlorophyll measurement (Bergweiler and Lutz 1986; Yadava 1986; Marquard and Tipton 1987). Only one known study combines the use of DMF extraction with high performance liquid chromatography (HPLC) for the rapid assessment of all major plant pigments (Danehower and Kelley 1990).

Few investigations have taken place within turfgrass research in the area of DMF extraction or HPLC pigment analysis. In a study testing chlorophyll contents of turfgrasses after application of plant growth regulators, Gaussoin, et al. (1997), used DMF for pigment extraction along with spectrophotometric analysis. Bell and Danneberger (1999) used acetone extraction and HPLC to simultaneously assess the chlorophylls and carotenoids, including the xanthophylls of creeping bentgrass under shade and full sun conditions.

OPTICAL SENSING BACKGROUND

The use of optical sensors to assess plant status may play an integral part in the future of turfgrass management. If sensors can replace wet chemical plant analysis procedures (soil nutrient analysis or pigment analysis) and give managers the same information in real-time, sensor measurements may help field-practitioners achieve healthier plants with less inputs (fertilizer, chemicals, water, labor). Optical sensing of spectral reflectance has been used to measure plant disease levels (Nutter 1989; Nutter, et al. 1993), plant nutrient deficiencies (Bausch and Duke, 1996; Masoni, et al. 1996),

phytomass (Daughtry, et al. 1992), herbicide injury (Adcock, et al. 1990), leaf water content (Thomas, et al. 1971), leaf nitrogen content (Thomas and Oerther, 1972), leaf chlorophyll (Gitelson and Merzlyak, 1994), pigment content (Thomas and Gausman, 1977; Chappelle et al. 1992; Datt, 1998;), and photosynthetic performance (Gamon, et al., 1990).

OPTICAL SENSING: A CASE STUDY

Stone et al. (1996) successfully scanned fields of winter wheat using a tractor-mounted optical sensor. It was possible to vary nitrogen applications based on this mapping technique to correct nitrogen deficiencies in specific local areas. It was estimated that the use of sensor technology resulted in savings between 32 and 57 kg N ha^{-1} when compared to normal broadcast applications with no loss of yield. Instruments, such as the one employed by Stone et al. (1996), can be calibrated to examine turfgrass quality. Turfgrass managers and researchers could implement this technology in many ways. Early detection of pest-related problems, the accurate assessment of plant nutrient status, the recognition of certain weed species, especially in dormant bermudagrass, and the development of a water deficit management program are potential areas of implementation.

SPECTRAL REFLECTANCE, PIGMENT ABSORPTION, AND NDVI

Solar radiation striking the surface of a plant is reflected in wavelengths that have characteristic frequencies and energies (Raun, et al. 1998). Only a portion of the incident energy is reflected from the leaf. The remainder is either absorbed or transmitted. In the

visible spectral region radiation is absorbed by leaf pigments, primarily chlorophylls. Carotenoids, including xanthophylls, and anthocyanins also affect absorption (Gates, et al. 1965; Rabideau et al., 1946). Variations in pigment levels within the plant cause different amounts of radiant energy absorption, reflection, and transmission. The differences in reflection are determined by optical sensing to provide indirect, real-time measurements of plant status. HPLC analysis offers the opportunity to assess carotenoid levels, including the xanthophylls and chlorophylls simultaneously (Bell and Danneberger 1999).

The most widely used vegetation index applied to optical sensor data has been the normalized difference vegetation index (NDVI) (Rouse, et al. 1973). This index relies on the spectral contrast between the near-infrared and the red regions of the spectrum to measure plant status. NDVI may be defined as the difference between the reflectances in the near infrared and red regions of the spectrum normalized to the sum of these reflectances (Tucker 1979). Turf leaves absorb red and reflect near-infrared radiation. NDVI has been shown to correlate closely with nitrogen content (Howell 1998), nitrogen rate (Bell, et al. 2001), biomass, leaf area index, and chlorophyll absorption (Datt, 1998).

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CHAPTER II

FOLIAR APPLICATIONS OF IRON AND MAGNESIUM FOR SUPPRESSION OF ANNUAL BLUEGRASS IN CREEPING BENTGRASS PUTTING GREENS

Foliar Applications of Iron and Magnesium for Suppression of Annual Bluegrass in Creeping Bentgrass Putting Greens

J.C. Stiegler, G.E. Bell*, and D.L. Martin

Foliar iron and magnesium applications have indicated potential as a safe, low-cost control method for annual bluegrass (*Poa annua* L.) in creeping bentgrass (*Agrostis palustris* Huds. var. 'Penncross') turf. A two-year field study was conducted in Stillwater, OK to evaluate the efficacy of iron/magnesium treatments alone, and in combination with conventional control methods or activity enhancers for suppressing indigenous annual bluegrass in an established creeping bentgrass golf green. The research site received full deciduous shade until early afternoon. Treatments included: Fe/Mg, Fe/Mg + surfactant, trinexapac-ethyl (TE) [4-(cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester], Fe/Mg + TE, ammonium nitrate (AN), Fe/Mg + AN, ammonium sulfate (AS), Fe/Mg + AS, dithiopyr (Di) [S,S-dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)-3,5-pyridinedicarbothioate], Fe/Mg + Di, and control. None of the experimental treatments were effective in reducing the population of annual

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bluegrass at the site. Fe/Mg + dithiopyr, however, slowed annual bluegrass encroachment compared to the control. Annual bluegrass proportion increased significantly in plots treated with Fe/Mg. With the exception of Di treatments, plots treated with Fe/Mg experienced larger increases in annual bluegrass than plots treated with corresponding herbicides or nutrients alone. Contrasts between treatments receiving Fe/Mg and treatments not receiving Fe/Mg indicated a significant increase in annual bluegrass proportion in the Fe/Mg treatments. These results suggest that Fe/Mg applied to shaded sites favors annual bluegrass growth compared to creeping bentgrass.

INTRODUCTION

Managing annual bluegrass (*Poa annua* L.) in creeping bentgrass (*Agrostis palustris* Huds.) golf greens is a concern for golf course superintendents in most areas of the United States. Possible control methods include: pre-emergence herbicides (Callahan and Shepard 1991; Callahan and McDonald 1992), plant growth regulators (Breuninger and Watschke 1982; Shoop, et al. 1986; Gaussoin and Branham 1989; Johnson and Murphy 1996), selective herbicides (Gaul and Christians 1988; Branham 1990), varying irrigation regimes (Sprague and Burton 1937; Gaussoin and Branham 1989), various nutritional inputs (Waddington, et al. 1978; Eggens and Wright 1985; Dest and Guillard 1987; Goss 1974), and phytopathogenic bacteria (Savage, et al. 1993; Zhou and Neal

1995; Imaizumi, et al. 1997). Despite over 50 years of scientific research, annual bluegrass remains a tenacious weed in cool-season turf.

The life cycle of annual bluegrass is highly variable (Johnson and White 1998; Mitich 1998). In cooler climates, it often survives as a perennial, while in warmer regions it usually persists as an annual. Historically, the perennial forms of the species have proven the most difficult to control (Johnson and Murphy 1996; Bell et al. 1999).

Annual bluegrass is sometimes used as turf, but is usually considered a weed for aesthetic, functional, and economic reasons. A most undesirable trait is the species' propensity for flowering (Johnson and White 1998). Unlike most grasses, annual bluegrass can produce inflorescence even at mowing heights of 3 to 6 mm (Sprague and Burton 1937). Flowering and its typical yellow-green color, disrupt the uniformity and consistency of putting surfaces. Annual bluegrass effectively competes for resources with desirable species (Ashton and Monaco 1991) and poses several other management problems. Poor heat tolerance, overall poor cold tolerance, poor drought tolerance, and susceptibility to death from ice encasement, combine to make annual bluegrass undesirable and difficult to manage effectively. It is susceptible to fungal attack under low or high nutrient levels and to insect attack by annual bluegrass weevil and other pests. Therefore, a mixed sward of annual bluegrass and creeping bentgrass requires more precise management practices and more inputs (nutrients, water, and pesticides) than a pure stand of creeping bentgrass.

Annual bluegrass is ubiquitous (Mitich 1998; Bell, et al. 1999), suggesting that control measures have worldwide importance. High seed production and high seed viability contribute to the success of the species as a colonizer (Mitich 1998). Its

presence has been recorded on all continents of the world, even Antarctica (Scott, 1987). Birds can easily spread annual bluegrass seed and it is also transported on shoes, tools, mowers, and other equipment, creating opportunities for invasion of existing turf (Mitich 1998).

Variations in photosynthetic efficiency and pigment levels between annual bluegrass and creeping bentgrass have been suggested as a possible means of exploitation (Bell, et al. 1999). In that study, researchers were able to significantly reduce young (1-2 years of growth) annual bluegrass populations in a creeping bentgrass fairway study by 63.7% using foliar iron/magnesium applications; while traditional methods of control, [plant growth regulator (trinexapac-ethyl), selective herbicide (ethofumesate), and pre-emergence herbicide (bensulide)] had no significant effects. Annual bluegrass is believed to be more efficient, photosynthetically, than creeping bentgrass (Gaussoin 1988; Vargas 1996), and its yellow-green color suggests that it contains high levels of accessory pigments or low levels of chlorophyll compared with creeping bentgrass. Enhancement of chlorophyll synthesis can be obtained from inputs of atomic iron in combination with atomic magnesium (Parekh and Puranik 1992; Walker et al. 1997). The enhancement of chlorophyll synthesis and resulting increase in chlorophyll concentrations, may cause increases in plant excitation energy. Photoinhibition (Barenyi and Krause 1985; Critchley 1988; Long et al. 1994), membrane degradation (Fuerst and Norman 1991), and increased photorespiration (Ogren 1984; Watschke, et al. 1972) are negative physiological processes that may result. Although increases in chlorophyll content may occur in both species within a sward treated with iron/magnesium, the treatment should be increasingly detrimental to the more photosynthetically efficient annual bluegrass in

comparison to creeping bentgrass (Bell, et al. 1999). Bell, et al. (1999) suggested that applications of iron/magnesium created a competitive advantage for creeping bentgrass, which over time resulted in reductions of annual bluegrass populations in a newly-seeded turf. Xu and Mancino (1998) found that applications of iron and magnesium encouraged growth of creeping bentgrass, but did not affect annual bluegrass. For these reasons, iron/magnesium applications have promise for effective, inexpensive, and environmentally sound reduction of annual bluegrass in creeping bentgrass turf. Continued research with foliar iron/magnesium applications, under varying parameters, is necessary to establish practical management techniques for use by field practitioners.

The objectives of this study were to evaluate the efficacy of iron/magnesium treatments alone, and in combination with other conventional methods (pre-emergence, plant growth regulator, and ammonium sulfate) or activity enhancers (surfactant and ammonium nitrate) for suppressing indigenous annual bluegrass in a creeping bentgrass golf green.

MATERIALS AND METHODS

A research site was selected at the Oklahoma State University Turfgrass Research Center in Stillwater, OK. The site consisted of 'Penncross' creeping bentgrass and indigenous annual bluegrass. The site was seeded to Penncross in 1992 on a sand base and maintained as a creeping bentgrass putting green. Mowing was performed six days per week. Before the start of the project, the mowing height was set at 4.1 mm and was gradually reduced to 3.1 mm in fall 1998. Pesticides were applied to the area as needed to maintain turf health and nitrogen was applied at 159 kg ha^{-1} yr^{-1}. Phosphorus,

potassium and other nutrients were applied based on annual soil tests. The site was shaded throughout the morning and historically supported annual bluegrass invasion from fall until mid-summer. When the first treatments were applied on September 1, 1998, the site was free of annual bluegrass.

The research site was divided into 0.9 m x 1.5 m plots and arranged in a completely randomized design. Each plot received one of 11 treatments: 1) foliar iron + foliar magnesium (Fe/Mg); 2) Fe/Mg + surfactant (Fe/Mg/S); 3) trinexapac-ethyl (TE), a plant growth regulator; 4) Fe/Mg + TE (Fe/Mg/TE); 5) ammonium nitrate fertilizer (NH_4NO_3); 6) Fe/Mg + NH_4NO_3 (Fe/Mg/NH_4NO_3); 7) ammonium sulfate fertilizer [$(NH_4)_2SO_4$)]; 8) Fe/Mg + $(NH_4)_2SO_4$ [Fe/Mg/$(NH_4)_2SO_4$]; 9) dithiopyr (Di), a pre-emergence herbicide; 10) Fe/Mg + Di (Fe/Mg/Di); 11) no treatment (NT), a control. All treatments except Di were applied on or around the first day of each month from September 1998 through November 1998; March 1999 through November 1999; and March 2000 through October 2000. Di was applied twice each fall (September and October) and once each spring (March) over the course of the study. Fe ($FeSO_4 \cdot 7H_2O$) and Mg ($MgSO_4 \cdot 7H_2O$) was applied at 1.68 kg ai ha^{-1}, TE (Primo formulation) at 0.4 L ha^{-1}, NH_4NO_3, and $(NH_4)_2SO_4$ at 6.1 kg N ha^{-1}, and Di (Dimension formulation) at 3.5 L ha^{-1} . All treatments were applied using a wheelchair CO_2 sprayer (374 L ha^{-1}) configured to apply uniformly across the full 0.9 m width of the plot. Visual ratings for color on a 1 through 9 scale (1=brown; 5=yellow-green; 9=blue-green) and density by percentage of potential (Bell, et al. 1999) were determined visually on or around the 15th day of each month that treatments were applied.

Species composition was rated in November 1998, March 1999, November 1999, March 2000, and again at the end of the study in November 2000. The actual rating area of the experimental plots was 0.7 m by 1.3 m separated by 0.3 m buffer strips.

Evaluation of species proportions were performed using a template that divided each plot into eight equal 0.3048 m by 0.3048 m sections. Species proportion was determined for each section and a mean calculated for each plot.

Data were analyzed using analysis of variance for a completely randomized design and mean separations were determined using LSD (least significant difference) developed with SAS Version 7 (Cary, NC). To test whether Fe/Mg had any effect on annual bluegrass populations, a contrast was formed between those treatments containing Fe/Mg applications and those without. All results were tested at $\alpha = 0.05$ (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Two seasons research was concluded at the site in October 2000. The overall color ratings for both seasons show that inputs of Fe/Mg usually increased visual quality of the creeping bentgrass/annual bluegrass mixture compared with plots not treated with those nutrients (Table 1). The duration of this color enhancement was at least two weeks, which is consistent with a previous study where Fe was applied to creeping bentgrass (Snyder and Schmidt 1974). During the first year of the trial, plots treated with Fe/Mg or any combination treatment including Fe/Mg, excluding dithiopyr, along with the ammonium nitrate treatment alone, averaged significantly higher visual color ratings than the control plots (Table 1). In the second year, Fe/Mg in combination with both nitrogen sources and trinexapac-ethyl averaged significantly higher color ratings. Significant color enhancement was not consistent from month to month and was usually more prevalent during spring and early summer. Seasonal variation in color intensity has also been reported by Glinski, et al. (1992), when evaluating various Fe

sources on creeping bentgrass golf greens. Due to phytotoxicity associated with dithiopyr applications during high temperatures in 1998, visual color ratings of plots treated with this herbicide were overall significantly lower than the control plots.

The overall density ratings at the site were lower than the normal density potential of a creeping bentgrass green in full sun. Shade causes leaf blades to elongate and grow more upright in an effort to reach more sunlight (Qian and Engelke 1999). The creeping bentgrass cultivar, 'Penncross' also has lower density than most new cultivars used on creeping bentgrass golf greens. Results of a 1998 NTEP Creeping Bentgrass Trial located at Oklahoma State University support this contention, where 'Penncross' received significantly lower density ratings than most cultivars in the trial (Dr. Dennis Martin, personal communication). From September 1998 to September 1999, the overall mean for turf density was significantly higher than the control for the ammonium sulfate, trinexapac-ethyl, Fe/Mg + ammonium nitrate, Fe/Mg + ammonium sulfate, and Fe/Mg + trinexapac-ethyl treatments (Table 2). Dithiopyr alone and in combination with Fe/Mg, produced significantly lower density during the same time. Trinexapac-ethyl treatments improved density in both years. Bell, et al. (1999) also found TE to mathematically increase density when compared with control.

From October 1999 to October 2000 (Table 2), the overall mean turf density ratings at the site remained fairly consistent from treatment to treatment. The only plots that were significantly different from the control, were those that received dithiopyr alone. These plots were significantly lower in their ratings due to phytotoxicity and the added influence of a reduction in annual bluegrass in the second year with this treatment (Table 2).

Table 3 displays the results of our two-year annual bluegrass suppression study. Overall, most of the significant findings throughout the study resulted from

major increases instead of decreases in annual bluegrass populations. These significant increases corresponded with either Fe/Mg alone, or some combination of the treatment (Table 3). In the first year of the trial, the proportion of annual bluegrass to creeping bentgrass at the site increased from an average of 9% in November 1998 to 14% in March 1999 to 17% in November 1999 (data not shown). Annual bluegrass population increased 134% in the control plots between November 1998 and November 1999 (Table 3). This increase was due to a combination of low mowing height, a shaded site, and mild winter weather. Annual bluegrass is a shallow rooted species (Murphy, et al. 1994) and is more adapted to low mowing heights than 'Penncross' creeping bentgrass. The 3.0 mm-mowing height favors annual bluegrass development. A mild, wet 1998/1999 winter increased annual bluegrass survival and germination. In addition, the site chosen for this research was shaded throughout the morning and into the afternoon, making annual bluegrass more competitive with creeping bentgrass. None of the treatments, during the first year, were effective for reducing the population of annual bluegrass at the site. Fe/Mg + dithiopyr, however, slowed annual bluegrass encroachment significantly compared to control. Annual bluegrass proportion increased significantly in plots treated with Fe/Mg. In addition, with the exception of dithiopyr treatments, plots treated with Fe/Mg experienced larger mathematical increases in annual bluegrass than plots treated with corresponding herbicides and nutrients alone.

During the second year, annual bluegrass populations at the site continued to increase, however the increase was noticeably less dramatic (Table 3). Annual bluegrass populations remained steady in the control (+134 % to +133 %) and dithiopyr (+59 % to +60 %) plots, while the addition of Fe/Mg continued to enhance annual bluegrass populations (Table 3). As in the first year, a significant contrast was found between treatments including Fe/Mg and those without Fe/Mg. In each case, except with

dithiopyr treatments, plots treated with Fe/Mg experienced larger mathematical increases in annual bluegrass than plots treated with corresponding herbicides and nutrients alone. Fe/Mg + trinexapac-ethyl significantly increased annual bluegrass populations during the second year of the trial from +91 % to +259 %. Trinexapac-ethyl trials have demonstrated successful annual bluegrass suppression in a creeping bentgrass sward, over several months, with no adverse affects on the creeping bentgrass (Neylon, et al. 1998). Bell, et al. (1999) found no significant difference compared to control when evaluating trinexapac-ethyl for annual bluegrass suppression in a creeping bentgrass fairway. Based on these earlier findings, the significant increase found in the second year of our trial may have been attributed more to the inclusion of Fe/Mg in the treatment, rather than a result of the trinexapac-ethyl applications.

In September 1998, dithiopyr applications caused a severe phytotoxic reaction in creeping bentgrass and possibly slowed stoloniferous growth in fall 1998 and spring 1999. As a result, plots treated with dithiopyr did not maintain acceptable density (Table 2) or color (Table 1), but were usually low in annual bluegrass content. Again, in fall 1999, dithiopyr caused phytotoxicity, this time only slight, which resulted in data and results similar to the previous year (Tables 1 & 2).

The results obtained from this two-year field trial, suggest that Fe/Mg applied to shaded sites favors annual bluegrass compared to creeping bentgrass. Combinations of these nutrients are known to encourage chlorophyll synthesis (Parekh and Puranik 1992, Walker, et al. 1997). High concentrations of chlorophyll in a photosynthetically efficient species like annual bluegrass may help it out-compete a less efficient species like creeping bentgrass. In full sun, however, the opposite may be true, as proposed in earlier work by Bell, et al. (1999). Increasing chlorophyll concentrations in annual bluegrass in full sun could lead to energy-enhanced decline such as photoinhibition and lipid

peroxidation. When in shaded environments the light compensation point of the annual bluegrass plants may not be reached with the Fe/Mg inputs, negating degradation caused by excess excitation energy.

Beyond the shade influence, several other factors may have contributed to conflicting results when compared with the novel work by Bell, et al. (1999). It is important to note that in that study, successful reductions in annual bluegrass populations were attained on young (1-2 yr old) turfgrass. The area studied here was established in 1992 and consisted of annual bluegrass plants that were indigenous and well adapted to the area. Older plants have a less absorptive cuticle (Ashton and Monaco 1991), which may diminish nutrient uptake and decrease the treatment efficacy. In addition, individual leaf photosynthetic rate decreases with age (Morgan and Brown 1983; Woledge and Leafe 1976), which may make reaching energy enhanced decline with Fe/Mg inputs more difficult in more mature polystands. Another possible reason for the differences in results from this experiment and the positive results in earlier literature may be the differences in local annual bluegrass biotypes. Annual bluegrass biotypes in Ohio are different from those in Oklahoma. In the northeastern US, the predominant subspecies inhabiting golf course turf is *P. annua* ssp. *reptans*, a perennial with a prostrate growth habit (Zhou and Neal 1995). In the transition zone, both annual and perennial biotypes may occur together on the same site making consistent control strategies more complicated. Inconsistent results from site to site with post-emergence annual bluegrass control with chlorsulfuron, led Gaul and Christians (1988) to conclude that the great diversity in the annual bluegrass species was the probable cause. An annual bluegrass control measure that may work in one climate or environment may not be effective, or could in fact be counter-effective, in another.

SUMMARY

While these results were not consistent with earlier work by Bell, et al. (1999), they are valuable in learning more about the potential of this novel weed-control approach. Future efforts with Fe/Mg to reduce annual bluegrass populations in creeping bentgrass greens may be limited to newly established areas and/or sites in full sun. Mature turfgrass plants could have low photosynthetic rate and shaded sites may not receive the necessary amount of photosynthetically active radiation for the ecological manipulation of the turfgrass stand with Fe/Mg inputs. Similar studies at different turfgrass sites, under varying environmental parameters are needed for a more accurate assessment of this weed control strategy.

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Table 1. Mean turf visual color ratings. Means were calculated from three replications of each treatment each month.

September 1998 to September 1999

Treatment	9/17/98	10/21/98	11/19/98	3/18/99	4/20/99	5/20/99	6/24/99	7/20/99	8/19/99	9/23/99	Overall Mean
	1 to 9 = best										
Control	7	7	6	8	8	7	8	8	7	7	7
NH_4NO_3	8	7	7	8	8	8	8	7	8	8	8
$(NH_4)_2SO_4$	7	7	7	7	8	8	8	8	8	8	7
Dithiopyr	7	5	5	5	6	6	7	7	7	6	6
Trinexapac-ethyl	8	8	6	7	8	8	9	8	7	8	7
Fe/Mg	8	8	7	8	8	8	9	8	8	8	8
Fe/Mg + NH_4NO_3	8	8	8	9	8	8	9	9	8	8	8
Fe/Mg + $(NH_4)_2SO_4$	8	8	9	9	8	8	8	9	8	8	8
Fe/Mg + dithiopyr	7	6	6	6	7	7	7	7	7	6	7
Fe/Mg + trinexapac-ethyl	7	7	7	7	8	8	9	8	8	8	8
Fe/Mg + surfactant	8	7	7	7	8	8	9	9	8	7	8
LSD(p=0.05)†	1.2	0.8	0.6	1.3	0.8	0.8	0.9	0.8	0.7	1.1	0.3

October 1999 to October 2000

Treatment	10/19/99	11/16/99	3/20/00	4/13/00	5/12/00	6/26/00	7/21/00	8/18/00	9/21/00	10/20/00	Overall Mean
	1 to 9 = best										
Control	7	6	8	8	8	7	7	6	6	6	7
NH_4NO_3	7	7	8	7	8	8	8	7	7	7	7
$(NH_4)_2SO_4$	7	6	7	7	8	8	7	7	7	7	7
Dithiopyr	6	5	8	8	8	6	6	6	6	5	6
Trinexapac-ethyl	7	6	7	7	9	8	8	7	7	6	7
Fe/Mg	7	6	7	7	9	8	8	7	7	6	7
Fe/Mg + NH_4NO_3	8	8	8	8	8	8	7	8	8	7	8
Fe/Mg + $(NH_4)_2SO_4$	8	7	7	7	8	8	8	8	7	7	8
Fe/Mg + dithiopyr	6	5	8	8	8	7	6	6	6	6	7
Fe/Mg + trinexapac-ethyl	8	7	8	8	8	9	8	8	7	7	8
Fe/Mg + surfactant	8	7	8	7	8	8	8	8	7	6	7
LSD(p=0.05)†	0.7	0.9	0.8	0.8	0.9	1.3	1.1	1.1	1.6	0.9	0.5

† If the difference between any two means in a column is equal to or greater than the LSD value, the means are statistically different.

Table 2. Means of turf density ratings by percentage of potential. Means were calculated from three replications of each treatment each month.

September 1998 to September 1999

Treatment	9/17/98	10/21/98	11/19/98	3/18/99	4/20/99	5/20/99	6/24/99	7/20/99	8/19/99	9/23/99	Overall Mean
	% of potential										
Control	77	77	75	77	81	82	83	82	78	75	79
NH_4NO_3	78	78	80	75	78	88	88	83	82	78	81
$(NH_4)_2SO_4$	80	81	78	81	80	93	90	84	82	80	83
Dithiopyr	72	65	62	62	62	60	72	73	68	63	66
Trinexapac-ethyl	88	86	80	87	85	87	90	88	86	78	86
Fe/Mg	82	78	75	77	80	90	90	80	77	80	81
Fe/Mg + NH_4NO_3	81	82	80	82	81	87	89	85	80	80	83
Fe/Mg + $(NH_4)_2SO_4$	76	82	80	80	87	93	88	83	80	80	83
Fe/Mg + dithiopyr	74	66	63	63	68	67	85	83	80	70	72
Fe/Mg + trinexapac-ethyl	85	87	75	87	87	82	83	85	88	77	84
Fe/Mg + surfactant	80	78	77	75	78	82	85	83	83	75	80
LSD(p=0.05)†	5.1	7.2	9.0	5.3	4.7	9.6	7.2	5.7	5.9	12.3	3.7

October 1999 to October 2000

Treatment	10/19/99	11/16/99	3/20/00	4/13/00	5/12/00	6/26/00	7/21/00	8/18/00	9/21/00	10/20/00	Overall Mean
	% of potential										
Control	80	70	87	87	83	90	78	77	80	75	81
NH_4NO_3	77	72	90	90	90	90	85	85	85	82	85
$(NH_4)_2SO_4$	75	73	90	90	87	90	85	83	80	73	83
Dithiopyr	74	57	75	80	77	77	72	65	65	65	71
Trinexapac-ethyl	86	72	87	83	90	90	82	85	85	80	84
Fe/Mg	82	77	90	90	88	88	85	83	80	77	84
Fe/Mg + NH_4NO_3	78	78	90	87	87	92	87	83	85	82	85
Fe/Mg + $(NH_4)_2SO_4$	78	78	90	90	92	90	85	83	78	78	85
Fe/Mg + dithiopyr	72	62	80	80	85	82	77	78	78	70	76
Fe/Mg + trinexapac-ethyl	80	77	88	87	88	92	87	88	80	75	84
Fe/Mg + surfactant	77	72	88	90	88	90	85	82	80	78	83
LSD(p=0.05)†	8.3	10.6	4.7	5.9	5.5	6.4	9.2	15.5	ns	15.7	6.1

† If the difference between any two means in a column is equal to or greater than the LSD value, the means are statistically different.

Table 3. Visual estimation of annual bluegrass proportion. Initial evaluation (Nov. 98) is estimated annual bluegrass proportion. Remaining observations represent the percent increase/decrease of annual bluegrass populations compared to the initial rating date.

Treatment	Nov 98		Mar 99		Nov 99		Mar 00		Nov 00	
	—%—		% change							
Control	6		+41		+134		+170		+133	
NH_4NO_3	15	*	+40		+80		+78		+86	
$(NH_4)_2SO_4$	12		+67		+82		+139		+103	
Dithiopyr	15	*	-3		+59		+104		+60	
Trinexapac-ethyl	11		+53		+53		+102		+93	
Fe/Mg	6		+197	*	+244	*	+302		+321	
Fe/Mg + NH_4NO_3	5		+118		+168		+278		+329	*
Fe/Mg + $(NH_4)_2SO_4$	6		+139		+232		+256		+333	*
Fe/Mg + dithiopyr	11		-8		+16	*	+71		+49	
Fe/Mg + trinexapac-ethyl	6		+22		+91		+199		+259	
Fe/Mg + surfactant	6		+219	*	+183		+267		+274	
Mean	9		+81		+122		+179		+186	

* Indicates significant difference compared to control ($p<0.05$)

CHAPTER III

COMPARISON OF ACETONE AND N, N DIMETHYLFORMAMIDE (DMF) FOR PIGMENT EXTRACTION IN TURFGRASS

Comparison of Acetone and N, N Dimethylformamide (DMF) for Pigment Extraction in Turfgrass

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The extraction and isolation of plant pigments is a critical part of pigment analysis procedures used by plant scientists. Various organic solvents have been used to liberate the chlorophylls and carotenoids, including the xanthophylls from plant tissue. Acetone extraction is the most common method, but other solvents, such as N, N dimethylformamide (DMF), have also been used successfully. The objectives of this study were to compare the repeatability of acetone extraction with DMF extraction for turfgrass pigment research and investigate which extraction method was more appropriate, under the existing parameters, for use in preparation for HPLC analysis, and to develop an improved protocol for HPLC analysis for use in future turfgrass research. Results indicate that while both acetone and DMF procedures exhibited acceptable variation, DMF extraction was more consistent at each procedural level examined and yielded more pigment. Overall, pigment extraction with DMF produced quicker, more quantitative, and more reliable results than the presented acetone extraction procedure.

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INTRODUCTION

In 1903, M.S. Tswett began a novel investigation into plant pigment analysis (Danehower and Kelley 1990). Almost a century later, the separation and analysis of the chlorophylls and carotenoids, including the xanthophylls is still an important analytical procedure in plant science. The examination of these pigments can help scientists determine plant health status since each pigment exhibits special functions that are integral to the photosynthetic process (Cogdell 1988). The extraction and isolation of plant pigments is a critical part of pigment analysis procedures used by plant scientists. The goal of this procedure is to liberate the compound or compounds of interest in a reproducible manner, in a stable form which can be analyzed to reveal the original form in the host plant, plant part, or product, and in a sufficiently concentrated form to accommodate analysis.

Acetone has long been used in the laboratory as an extraction solvent for higher plant study (MacKinney 1941; Arnon 1949). The most widely accepted extraction procedure uses 80% aqueous acetone or 100% acetone and some sort of homogenizer to macerate the tissue and isolate plant pigments for further analysis (Holden 1976; Porra, et al. 1989; De las Rivas, et al. 1991; Gilmore and Yamamoto 1991; Svec 1991; Almela 1992;Val and Monge 1994; Bell and Danneberger 1999). While this method has certainly been effective, there are some areas with opportunity for improvement. The acetone extraction process is time consuming, especially when dealing with high sample numbers. The low vapor pressure of acetone causes it to evaporate easily during and after tissue extraction. This usually means high volumes of solvent must be used that

dilute pigment concentrations in the final solution (Moran and Porath 1980). This phenomenon may eventually cause inaccuracies in pigment concentration data and affect the reproducibility of the procedure.

N, N dimethylformamide (DMF) is another organic solvent that affords laboratory technicians with an alternative to acetone when choosing an extraction chemical. DMF has been used successfully for pigment extraction in previous studies (Moran 1982; Moran and Porath 1980; Inskeep and Bloom 1985; Bergweiler and Lutz 1986; Yadava 1986; Marquard and Tipton 1987; Danehower and Kelley 1990) suggesting it may be an acceptable substitute for acetone. Several advantages of DMF compared with acetone extraction have been discovered through prior investigations. DMF quickly and efficiently extracted pigments, allowed for more storage time without significant degradation (Bergweiler and Lutz 1986), and could be implemented without complete tissue maceration, multiple extractions, or the centrifugation necessary with acetone (Danehower and Kelly 1990). It is also less volatile than acetone and less error involving evaporative losses may be possible when using DMF as opposed to acetone as solvent.

Most pigment extraction work with DMF has been completed in plant studies using spectrophotometry for chlorophyll measurement (Bergweiler and Lutz 1986; Yadava 1986; Marquard and Tipton 1987). These previous papers used extinction coefficients and formulas obtained from work by Moran (1982), or more recently by Inskeep and Bloom (1985) for pigment quantification. Similar to the study presented here, only one known study combines the use of DMF extraction with high performance liquid chromatography (HPLC) for the rapid assessment of all major plant pigments (Danehower and Kelley 1990).

Few investigations have taken place within turfgrass research in the area of DMF extraction or HPLC pigment analysis. In a study testing chlorophyll contents of turfgrasses after application of plant growth regulators, Gaussoin, et al. (1997) used DMF for pigment extraction along with spectrophotometric analysis. Bell and Danneberger (1999) used acetone extraction and HPLC to simultaneously assess chlorophylls and carotenoids, including the xanthophylls of creeping bentgrass under shade and full sun conditions.

The objectives of our study were to compare the repeatability of analytical results obtained with acetone extraction and DMF extraction for turfgrass pigment research in order to evaluate which extraction method was more appropriate for use in preparation for HPLC analysis. In addition, it was our objective to incorporate DMF extraction into an improved protocol for HPLC analysis for use in future turfgrass research.

MATERIALS AND METHODS

Tissue samples for extraction were obtained from a research putting green at the Oklahoma State University Turfgrass Research Center in Stillwater, OK. The site consisted of 'Penncross' creeping bentgrass (*Agrostis palustris* Huds.) and indigenous annual bluegrass (*Poa annua* L.). The site was seeded to Penncross in 1992 on a sand base and was maintained as a creeping bentgrass putting green. Mowing was performed six days per week. Before the start of the project, the mowing height was set at 4.06 mm and was gradually reduced to 3.05 mm in fall 1998. Pesticides were applied to the area as needed to maintain turf health. Core aerification was performed on the area once in 1998, and twice in both 1999 and 2000. Nitrogen was applied to all of the plots as a

general maintenance task at 159 kg ha^{-1} yr^{-1}. General maintenance applications of nitrogen were carefully timed so that they would not mask treatment effects or when readily available as a source, isobutylenediurea (IBDU), a slow-release fertilizer was used to minimize this interference. Phosphorus, potassium and other nutrients were applied based on annual soil tests.

The site was divided into 0.9 m x 1.5 m plots and arranged in a completely randomized design. On June 8, 1999 each plot received one of 3 treatments: 1) foliar iron + foliar magnesium (Fe/Mg); 2) Fe/Mg + trinexapac ethyl (TE) (Fe/Mg/TE); 3) no treatment (NT), a control. Fe ($FeSO_4 \cdot 7H_2O$) and Mg ($MgSO_4 \cdot 7H_2O$) was applied at 1.68 kg ai ha^{-1}, TE (Primo formulation) at 0.4 L product ha^{-1}. Treatments were chosen to induce some change in pigment concentrations. Inputs of atomic iron in combination with atomic magnesium were used to promote chlorophyll synthesis (Parekh and Puranik 1992; Walker et al. 1997). All treatments were applied using a wheelchair CO_2 sprayer (374 L ha^{-1}) configured to apply uniformly across the full 0.9 m width of the plot.

Sample Collection and Preparation

Freezing is the most widely used collection and preservation method for plants that contain high amounts of chlorophyll. Canjura and Schwartz (1991) found that mild heat treatments induced the formation of chlorophyll C-10 epimers (prime derivatives) and that prolonged heating, degraded chlorophylls to olive-colored pheophytins and pyropheophytins. Carotenoids, including the xanthophylls, are also subject to oxidation and isomerization losses during storage and heating (Livingston et al. 1968; Davies 1976). Bailey and Chen (1988) reported that field-drying resulted in carotene and

xanthophyll concentrations of only 15 and 52 mg/kg, whereas freeze-drying yielded 499 and 886 mg/kg, respectively. To avoid pigment degradation, tissue freezing and lyophilization (freeze-drying) were used during collection and preservation of turfgrass samples.

On June 24, 1999, turf clippings were collected for analyses. Tissue collection occurred 15 days after treatments were applied. A standard greens mower and collection basket were used to harvest clippings. Collections were performed at or near solar noon. The selected interval of 15d between treatment application and tissue harvesting was based on prior knowledge obtained by Bell et al. (1999), using similar foliar iron/magnesium applications. In order to limit pigment degradation, samples were collected into a square section of cloth, tied off with string and immediately dipped into a duer containing liquid nitrogen (N_2). Samples were left in the duer with lid closed to block light that could degrade pigments, while the remaining monthly samples were collected. Samples remained in liquid nitrogen at least until the chemical stopped boiling. At that point the reaction had reached equilibrium and the sample was completely frozen. The intent of rapid freezing of the sample was to limit cellular damage by forming many small ice crystals, rather than the few, large ice crystals associated with slow freezing. In general, the freezing of samples lowered the water activity and reduced microbial attraction that could ultimately affect sample integrity. Samples were transported from the field still in duer (N_2), then transferred into a box with a lid to block light, and placed into a -20°C freezer to await lyophilization.

Samples were lyophilized in a Consol 12 shelf freeze dryer (Virtis, Gardiner, NY). The sample chamber was covered with aluminum foil to avoid exposure of samples

to light. Samples were held in the freeze dryer with shelf temperature at 15°C for 48 hours. Samples were then placed into a darkened dessicator overnight to await homogenization.

Samples were removed individually from the dessicator and ground in a cyclone sample mill (U/D, Boulder, CO) under a fume hood. The fins on the cyclone mill were wiped clean with cloth and all areas of the mill were air blown to prevent contamination between samples. Sample output from the mill was contained in a brown bottle and stored in a freezer at -20°C, until extraction.

Sample Extraction

Three bottles of plant tissue, corresponding with three experimental plots were randomly selected for analysis. One bottle of tissue corresponded to a plot treated with Fe/Mg, another corresponded to an Fe/Mg + trinexapac-ethyl treatment, and the third corresponded with a non-treated (control) plot. Tissue (30 mg) was accurately weighed into 10 disposable test tubes. Half of these test tubes received an acetone extraction procedure, the remaining five received a DMF extraction procedure. These same turf samples were subsampled for repetitions of the extraction procedure on later dates to determine consistency in extraction methods.

The acetone and DMF extractions were prepared one set (10 test tubes) at a time. After tissue was weighed (30 mg) and recorded, 2mls of a mixture of acetone + 1% butylated hydroxytoluene (BHT-synthetic antioxidant) was added to the first five test tubes and 2 mls DMF was added to the remaining five. All ten test tubes then had micro-

stir bars placed inside and were situated into a 250 ml beaker and stirred in darkness for 30 minutes. Following agitation, the DMF extracts were decanted with a Pasteur pipet through Whatman 41 filter paper into a clean 10 ml volumetric flask. The acetone extracts were centrifuged in a Speed Vac centrifuge (Savant, Holbrook, NY) for 10 minutes, then decanted similarly, taking care not to loosen the pellet or micro-stirring bar. This process was repeated two more times for the acetone extracted samples, for a total of three extractions. Though current literature suggests that DMF negates the need for further extraction (Danehower and Kelly 1990), we discovered that the turfgrass tissue was not always completely bleached after the first extraction. In order to accommodate more complete extraction and sample transfer, the DMF assigned test tubes were again filled with 2 mls DMF (taking care to distribute chemical on all areas inside the test tube), stirred for 2 minutes, and decanted into their respective volumetrics. This process was repeated using only 1 ml DMF and again using 2 mls DMF, for a total of three mini-extractions. All extractions were brought to volume (10 ml) by adding their respective chemicals. The extracts were agitated by hand and transferred into labeled brown vials. These vials were sealed with Teflon®-lined caps and stored overnight in a freezer at -20°C to protect against evaporation while awaiting HPLC analysis.

HPLC Preparation

The brown vials of extracts were removed from the freezer and allowed to equilibrate to room temperature before transfer to brown-colored auto-sampler vials for HPLC analysis. Preliminary work with our HPLC system suggested that 800 µl minimum volume would be the appropriate volume per vial and that drying samples in

the Speed-Vac (Savant) and re-solubilizing resulted in sharper peaks than running samples "wet" (data not shown). Replicated HPLC runs from the same extract were used to test HPLC machine variation.

Preliminary investigations revealed that dry down time should be kept to a minimum to limit pigment degradation. Degradation may occur when light enters the vial and/or heat develops inside the Speed-Vac (Savant) chamber during long centrifugal drying times. Because of the differences in boiling point between extraction materials, it was deemed important to determine the exact time necessary to dry down acetone and DMF for minimal pigment degradation. Preliminary tests indicated that 15 minutes was required for a full rack (24 auto-sampler vials with 800 µl) of acetone extracts and 65 minutes for a full rack of DMF extracts. After drying both acetone and DMF extracts, they were immediately filled with 800 µl of a 1:1 ratio mixture of acetonitrile and acetone + 1% BHT, capped with a septum seal and placed into the auto sampler. Because DMF extracts required much longer to dry down, there was a problem with pigments adhering to the bottom of the vial. When the solvents were added the mass of pigment was not completely dissolved into solution with agitation alone. To avoid this problem, it was necessary to add an additional step to the protocol. All DMF extracts were sonicated for 10 minutes prior to being placed into the autosampler to await injection into the HPLC unit.

High performance liquid chromatography techniques are considered to be among the most reproducible methods for analyzing complex mixtures of pigments (Bushway, 1986). HPLC determinations were measured using procedures consistent with Gilmore and Yamamoto (1991) and Bell and Danneberger (1997). A loosely packed, non-

endcapped C18, spherisorb ODS-1 column (Alltech, Deerfield, IL) was preceded by an ODS-1 guard column (Alltech) for pigment separation. Sample extract (25 µl) was automatically injected using a Dionex (Sunnyvale, CA) AS3500 autosampler into a Series 4500I HPLC unit (Dionex) with flow rate adjusted to 2.0 ml min^{-1}. Pigment content was measured using a Dionex variable wavelength detector set at 440 nm. The initial solvent used for separation was a mixture of 86.8% acetonitrile, 9.6% methanol, and 3.6% 0.1 M Tris-HCl buffer (pH 8.00) for 4.0 min followed by a gradient to 20.0% hexane and 80.0% methanol. After 2.5 min. of the hexane/methanol mixture, separation was completed using the initial solvent mixture. The total run time was 18.0 min. All solvents were pressurized under helium gas to minimize pump pressure problems.

Results were compared using area units developed by the PeakNet version 4.3 Chromatography Workstation (Dionex). Area units for each pigment eluted (neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll b, chlorophyll a, and β-carotene) were recorded for each run. Peaks were identified by comparison with previous HPLC literature and by coelution with authentic standards for chlorophyll b, chlorophyll a, lutein, and β-carotene (Thayer and Bjorkman 1990; Gilmore and Yamamoto 1991).

Data analysis was consistent with Raun, et al. (1998), who suggested using coefficients of variation (CVs) to explain the repeatability of different parameters (soil pH, organic carbon, P, and K) obtained from chemical soil test extraction procedures. There is a strong parallel between the results examined in that particular research and the study presented here. Lopez-Hernandez, et al. (1993) also used CVs to determine measurement and method precision for HPLC determination of major pigments in beans (*Phaseolus vulgaris* L.). Data were analyzed using SAS software version 7 (Cary, NC).

Area units of the chlorophylls were considerably higher than those of the carotenoids, including the xanthophylls, so a square root transformation was used to encourage homogeneity of variance before analysis of area unit data (Steel and Torrie 1997). Simple statistics for mean, standard deviation, standard error, and variance were also calculated to examine relationships with pigment amounts obtained by each procedure. Data were reported as actual values, not as transformed variables.

Three levels of variation were examined: 1) tissue variation; 2) extraction variation; and 3) machine variation. Further discussion will be limited to these identified terms. Coefficients of variation were calculated and compared for each pigment, by sample batch ("tissue" variation), by procedure ("extraction" variation), and by subsample ("machine" variation). The best procedure was selected by establishing which procedure, acetone or DMF extraction, exhibited the overall least variation for combined pigments. Taylor et al., (1999) noted that large CVs (>30%) are often associated with increased experimental variability. This threshold was used to establish acceptable or non-acceptable variation among data.

RESULTS AND DISCUSSION

The DMF extraction exhibited lower relative variation than the acetone extraction at each procedural level (Table 4). The mean of CVs for tissue variation using acetone or DMF extraction were 8.1 and 5.5, respectively. Both of these statistical measurements are well under the acceptable CV level described by Taylor, et al. (1999). The mean CV for machine variation with acetone was 8.6 and for DMF was 5.8.

Upon examination of extraction variation, DMF proved to be the more repeatable extractor (Table 4). Mean acetone or DMF CVs for extraction variation were 10.1 and 8.8, respectively. After the initial 30 minutes of stirring, the extracts treated with DMF were noticeably darker green and had less green tissue remaining than extracts with acetone. This suggested that DMF extracted pigments faster and more thoroughly than acetone. This visual observation was supported by the HPLC area unit results. DMF extractions yielded substantially more neoxanthin, violaxanthin and lutein, and over twice as much chlorophyll compared with acetone (Table 5). Extraction of β-carotene was also mathematically greater using DMF compared to acetone, but to a lesser degree than the other pigments (Table 5). β-carotene is a non-polar, lipid soluble pigment (De Las Rivas 1991; Zhang and Schmidt 1999). This may lend to a limited ability for DMF to increase the quantitative extraction of this particular pigment. The DMF procedure was able to extract measurable quantities of antheraxanthin, but acetone extraction did not consistently produce this pigment (Table 5). Antheraxanthin is an unstable pigment produced during the reversible conversion of violaxanthin to zeaxanthin (Thayer and Bjorkman 1990). The pigment is normally found in plants in very small quantities but is important for elucidation of the xanthophyll pool [violaxanthin (V) + antheraxanthin (A) + zeaxanthin (Z)]; Bell and Danneberger 1999). Zeaxanthin was not found in plant tissue regardless of extraction method used because the light environment was not conducive to its formation during tissue collection. Demmig, et al. (1987) reported that zeaxanthin responds to excessive light treatments (100 $\mu mol\ m^{-2}\ s^{-1}$) with an instantaneous increase in content, peaking at ~60 min, forming a plateau, then declining at ~180 minutes. This suggests that elucidation of substantial quantities of zeaxanthin is a time-sensitive

phenomenon, that may not have occurred during our tissue collections. The research site received full, deciduous shade until early afternoon. Turfgrass plants at the site may not have received enough solar radiation energy to cause the formation of zeaxanthin. Demmig-Adams (1990) reported that under excessive light conditions, photosynthetically active radiation exceeds the capacity of the photosynthetic reactions and violaxanthin is deepoxidized to zeaxanthin via antheraxanthin. However, this process is readily reversed under limiting light, suggesting that the shade conditions observed at our site would influence most of the xanthophyll pool to remain in the epoxidized state.

Both acetone and DMF procedures consistently extracted measurable quantities of β-carotene. Bell and Danneberger (1999) were unable to elucidate this pigment in creeping bentgrass. This, along with acceptable levels of variation for both extraction procedures presented in this paper (Table 4), suggest that the methods presented here are indeed an improved HPLC protocol for turfgrass pigment extraction.

SUMMARY

These results indicate that while the acetone procedure produced acceptable variation, the DMF procedure was more consistent and yielded more pigment. The material presented here provides a description and comparison of acceptable procedures for use in future plant pigment analysis. The use of the presented DMF procedure in subsequent research efforts should produce quicker, more quantitative, and more reliable results for turfgrass scientists than the more common acetone extraction methods.

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Table 4. Mean coefficient of variation (CV) for each procedural step in pigment analysis. Mean CVs were calculated from HPLC measurements of eight plant pigments in three tissue samples, 15 extractions, and 45 machine procedures replicated three times on different dates.

Procedural Level	Acetone	DMF
	%	
Tissue	8.1	5.5
Extraction	10.1	8.8
Machine	8.6	5.8

Table 5. Pigment area unit (AU) means determined by HPLC and compared by extraction procedure. Means were calculated from the results of five extractions and 15 machine measurements replicated three times on different dates.

Pigment	Acetone	DMF
	AU	
Neoxanthin	2084	3827
Violaxanthin	2076	3177
Antheraxanthin	NA	2042
Lutein	8934	13151
Chlorophyll b	6627	13495
Chlorophyll a	13842	33970
ß-carotene	5943	6802

CHAPTER IV

SPECTRAL DETECTION OF PIGMENT CONCENTRATIONS IN CREEPING BENTGRASS GOLF GREENS

Spectral Detection of Pigment Concentrations in Creeping Bentgrass Golf Greens

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The use of optical sensors to assess plant status may play an integral part in the future of turfgrass management. Varying pigment concentrations affect the amount of reflectance obtained from the plant canopy and, consequently, may affect the reliability of these sensor-based indirect measurements. The objectives of this study were to determine the influence of the chlorophylls and carotenoids, including xanthophylls on spectral reflectance of turfgrass; and to examine the impact of iron/magnesium applications on spectral acquisition data (NDVI-normalized difference vegetation index) and pigment concentrations. Data from optical sensors was statistically compared to individual pigment concentrations, obtained from HPLC, to determine significant correlation. Pigments were highly correlated to one another. Significant relationships were found between NDVI and pigments, however pigment concentrations never accounted for more than 38% of the variation associated with NDVI. This suggests that there may be many variables and factors that contribute to the overall influence on NDVI, besides pigments.

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INTRODUCTION

The use of vehicle-mounted optical sensors to assess plant status has potential for practical management of turfgrass areas. Optical sensing of spectral reflectance has been used to measure plant disease (Nutter, et al. 1993), plant nutrient deficiencies (Bausch and Duke, 1996; Masoni, et al. 1996), phytomass (Daughtry, et al. 1992), herbicide injury (Adcock, et al. 1990), leaf nitrogen content (Thomas and Oerther, 1972), leaf chlorophyll estimation (Gitelson and Merzlyak, 1994), pigment content (Chappelle et al. 1992; Datt, 1998; Thomas and Gausman, 1977), and photosynthetic performance (Gamon, et al., 1990). Stone et al. (1996) successfully scanned fields of winter wheat using a tractor-mounted optical sensor. It was possible to vary nitrogen applications based on this mapping technique to correct nitrogen deficiencies in specific local areas. It was estimated that the use of sensor technology resulted in savings between 32 and 57 kg N ha^{-1} when compared to normal broadcast applications with no loss of yield. Instruments, such as the one employed by Stone et al. (1996), can be calibrated to examine turfgrass quality. Turfgrass managers and researchers could implement this technology in many ways. Early detection of pest-related problems, the accurate assessment of plant nutrient status, the recognition of certain weed species, especially in dormant bermudagrass, and the development of a water deficit management program are potential areas of implementation.

There are many factors that may affect the information obtained from optical sensors. To understand spectral reflectance, some knowledge of how solar radiation interacts with vegetation is necessary. Solar radiation striking the surface of a plant is reflected in wavelengths that have a characteristic frequency and energy (Raun, et al.

1998). Only a portion of the incident energy is reflected from the leaf. The remainder is either absorbed or transmitted. In the visible spectral region radiation is absorbed by leaf pigments, primarily chlorophylls. Carotenoids, including xanthophylls, and anthocyanins also affect absorption (Gates, et al. 1965; Rabideau et al., 1946). Variations in pigment levels within the plant cause different amounts of radiant energy absorption and reflection. It is these differences in reflectivity that are determined by optical sensing to provide indirect, real-time measurements of plant status.

The most widely used vegetation index applied to optical sensor data has been the normalized difference vegetation index (NDVI) (Rouse, et al. 1973). This index relies heavily on the spectral contrast between the near-infrared and the red regions of the spectrum to measure plant status. NDVI is defined as the difference between the reflectances in the near infrared and red regions of the spectrum normalized to the sum of these reflectances (Tucker 1979). Turf leaves absorb red and reflect or transmit near-infrared radiation. Leaf reflectance in the visible region of the spectrum is affected by leaf thickness and water content (Wooley, 1971). By computing the difference between reflectances at a visible wavelength and at an infrared wavelength, NDVI should allow for the removal of these confounding physical effects (thickness and water content) and allow it to be correlated with leaf pigment concentration (Maas and Dunlap 1989). NDVI has shown high correlations with plant biomass (Sellers 1987), leaf area index (Gallo, et al. 1985), canopy photosynthetic capacity (Sellers 1987), and chlorophyll absorption (Asrar, et al. 1984; Gallo, et al. 1985; Datt, 1998). NDVI has also been shown to strongly correlate with nitrogen content (Howell 1998) and applied nitrogen fertilization rate (Bell, et al. 2001) in turfgrass.

Several studies have attempted to optically sense chlorophyll levels to determine plant stress (Carter and Miller 1994; Gitelson and Merzylak 1994; Lichtenthaler, et al. 1996). During stressful periods, plants are known to exhibit a measurable decrease in chlorophyll, known as chlorosis (Lichtenthaler, et al. 1996). It is evident, however, that carotenoids, including xanthophylls, also have very important functions in the process of photosynthesis (Thayer and Bjorkman 1990, Gilmore and Yamamoto 1991; Bell and Danneberger 1999). The ability to sense all pigments simultaneously may eventually give researchers a better indicator of plant health than chlorophylls alone.

The objectives of this study were to determine the influence of chlorophylls and accessory pigments on spectral reflectance of turfgrass, and to provide information that may improve future sensor design.

MATERIALS AND METHODS

Research was conducted at the Oklahoma State University Turfgrass Research Center in Stillwater, OK. The research site consisted of 'Penncross' creeping bentgrass (*Agrostis palustris* Huds.) and indigenous annual bluegrass (*Poa annua* L.). The site was seeded to Penncross in 1992 on a sand (pH 6.9) base and maintained as a creeping bentgrass putting green. Mowing was performed six days per week. Before the start of the project, the mowing height was set at 4.1 mm and was gradually reduced to 3.1 mm in fall 1998. Pesticides were applied to the area as needed to maintain turf health and nitrogen was applied at 159 kg ha^{-1} yr^{-1}. Phosphorus, potassium and other nutrients were applied based on annual soil tests. The site was shaded for the entire morning and has historically supported annual bluegrass invasion from fall until mid-summer.

The site was divided into 0.9 m x 1.5 m plots and arranged in a completely randomized design. Each plot received one of 11 treatments: 1) foliar iron + foliar magnesium (Fe/Mg); 2) Fe/Mg + surfactant (Fe/Mg/S); 3) trinexapac-ethyl (TE), a plant growth regulator; 4)Fe/Mg + TE (Fe/Mg/TE); 5) ammonium nitrate fertilizer (NH_4NO_3); 6) Fe/Mg + NH_4NO_3 (Fe/Mg/NH_4NO_3); 7) ammonium sulfate fertilizer ($(NH_4)_2SO_4$); 8) Fe/Mg + $(NH_4)_2SO_4$ (Fe/Mg/$(NH_4)_2SO_4$); 9) dithiopyr (Di), a pre-emergence herbicide; 10) Fe/Mg + Di (Fe/Mg/Di); 11) no treatment (NT), a control. All treatments except Di were applied on or around the first day of each month from September 1998 through November 1998; March 1999 through November 1999; and March 2000 through October 2000. Di was applied twice each fall (September and October) and once each spring (March) over the course of the study. Fe ($FeSO_4 \cdot 7H_2O$) and Mg ($MgSO_4 \cdot 7H_2O$) was applied at 1.68 kg ai ha^{-1}, TE (Primo formulation) at 0.4 L product ha^{-1}, NH_4NO_3, and $(NH_4)_2SO_4$ at 6.1 kg N ha^{-1}, and Di (Dimension formulation) at 3.5 L product ha^{-1}. Treatments were chosen to induce some change in pigment concentrations. Nitrogen was applied to increase chlorophyll levels and inputs of Fe in combination with Mg were applied to promote chlorophyll synthesis (Parekh and Puranik 1992; Walker, et al. 1997). All treatments were applied using a wheelchair CO2 sprayer (374 L ha^{-1}) configured to apply uniformly across the full 0.9 m width of the plot. Visual ratings for color on a 1 through 9 scale (1=brown; 5=yellow-green; 9=blue-green) and density by percentage of potential (estimated visually; Bell, et al. 1999) were made on or around the 15^{th} day of each month that treatments were applied.

Data from optical sensors were collected on the same day as visual ratings. Plots were scanned with Patchen (Patchen, Los Gatos, CA) Weed Seeker PhD 600 Sensors

mounted on a John Deere 4100 Tractor. The sensors project red (680 nm) and near infrared (780 nm) pulsed light from integrated light emitting diodes (LED's). The optical sensor filtered sunlight and collected energy only from these integrated LED's. The sensor employed a phase shift technique and high pass filters were used to exclude natural sunlight. The optical sensor collected radiant energy from a 1 by 30 cm field of view. This energy was converted to voltage measurements and processed through an analog to digital converter. Data were collected by an on-board portable computer and were converted to NDVI using derived calculations (Bell, et al. 2001). These calculations compensated the readings of each individual sensor for temperature as well as converting the voltage readings to NDVI. Optical measurements and clipping collections were performed at or near solar noon.

Sample Collection and Preservation

Turf clippings were collected for this study in September 1998, October 1998, and November 1998, then, again, in 1999 during the months of March, April, May, June, July, and August (approx. 15 days after treatments were applied). Samples were collected by mowing each plot with a standard greensmower with collection basket, immediately following spectral data collection. The selected interval of 15 days between treatment application and tissue harvesting was based on knowledge of plant response obtained by Bell et al. (1999), who used similar foliar iron/magnesium applications.

Canjura and Schwartz (1991) found that mild heat treatments induced the formation of chlorophyll C-10 epimers (prime derivatives) and that prolonged heating,

degraded chlorophylls to olive-colored pheophytins and pyropheophytins. To limit pigment degradation, samples were collected into a square section of cloth, tied off with string and immediately dipped into a duer of liquid N_2. Samples were left in the duer with lid closed to block light that could degrade pigments, while the remaining samples were collected. Samples remained in liquid nitrogen at least until the chemical stopped boiling. At that point the reaction had reached equilibrium and the samples were completely frozen. Rapid freezing of each sample was done to limit cellular damage by forming many small ice crystals, rather than the few, large ice crystals associated with slow freezing. In general, the freezing of samples lowered the water activity and reduced microbial attraction that could ultimately affect sample integrity. Samples were then transported from the field in liquid N_2, then enclosed in a cardboard box, and placed into a -20°C freezer to await lyophilization (freeze-drying).

Samples were lyophilized in a Consol 12 shelf freeze dryer (Virtis, Gardiner, NY) that could accommodate a high sample number. The sample chamber was covered with aluminum foil to exclude light and samples were held in the freeze dryer with shelf temperature at 15°C for 48 hours. Samples were then placed into a darkened dessicator overnight to await homogenization. Samples were removed individually from the dessicator and ground in a cyclone sample mill (U/D, Boulder, CO) under fume hood. The fins on the cyclone mill were wiped clean with cloth and all areas of the mill were air blown to prevent contamination between samples. Sample output from the cyclone mill was contained in a brown bottle and stored in a freezer at -20°C until extraction.

Sample Extraction

Extracting an entire month at once proved too cumbersome, so monthly samples were divided into three equal replicates each corresponding to the 11 treatments. Tissue (30 mg) from each replicate was placed into disposable test tubes and processed using the DMF extraction procedure described in Chapter III.

HPLC Procedure

In order to quantitate actual pigment concentrations (mg/g), a standard was used that was chemically similar to the compounds of interest. Bell and Danneberger (1999) used α-carotene, a pigment not found in creeping bentgrass, as a standard in their analysis. α-carotene is no longer commercially available. Instead, to accommodate quantitative analysis of the major pigments, an internal standard in the form of a β-carotene "spike" was used in our procedure. Each sample corresponding to one experimental unit was followed by a sample containing the same extract plus a known amount of β-carotene, during HPLC processing. Authentic standards for lutein, chlorophyll a, chlorophyll b, and β-carotene were obtained from Sigma (Sigma Chemical Co., St. Louis, MO). Standards were prepared in a 1:1 ratio of acetone plus 1% BHT and acetonitrile, so that each would equal a concentration of 0.25 nmoles/25 µl injection. To have a measure of machine performance during multiple HPLC runs, samples containing equal amounts (200 µl) of each standard were prepared and run after every eight samples. Standard runs were comparable to sample runs in concentration and volume. To assure

this, the same amount (200 µl) of β-carotene standard was added to each of the spiked samples.

The brown vials of pigment extracts (DMF) were removed from the freezer and allowed to equilibrate to room temperature before transfer into auto-sampler vials for HPLC analysis. Preliminary work with our HPLC system suggested that 800 µl would be the appropriate volume per vial in order to achieve optimum performance from the 25 µl automatic injector (data not shown). Samples were prepared by pipetting 800 µl of one sample extract into each of two auto-sampler vials. One was labeled with the corresponding sample number and the other with sample number plus spike. Samples were dried in a Speed-Vac centrifuge for 65 minutes and resolubilized by adding 800 µl of a 1:1 ratio of acetone plus 1% BHT and acetonitrile. Samples containing β-carotene spikes were re-solubilized with 600 µl of 1:1 ratio of acetone plus 1% BHT and acetonitrile with an additional 200 µl β-carotene for a final volume of 800 µl. Samples and spiked samples were capped and sonicated for 10 minutes and placed in an auto-sampler to await HPLC injection.

High performance liquid chromatography techniques are considered to be among the most reproducible methods for analyzing complex mixtures of pigments (Bushway, 1986). HPLC determinations were measured using procedures consistent with Gilmore and Yamamoto (1991) and Bell and Danneberger (1999). A loosely packed, non-endcapped C18, spherisorb ODS-1 column (Alltech, Deerfield, IL) was preceded by an ODS-1 guard column (Alltech) for pigment separation. Sample extract was automatically injected at 25 µl per sample using a Dionex AS3500 autosampler (Dionex, Sunnyvale, CA) onto a Dionex Series 4500I HPLC unit with flow rate adjusted to 2.0 ml min^{-1}.

Pigment content was measured using a Dionex variable wavelength detector set at 440 nm. The initial solvent used for separation was a mixture of 86.8% acetonitrile, 9.6% methanol, and 3.6% 0.1 M Tris-HCl buffer (pH 8.00) for 4.0 min followed by a gradient to 20.0% hexane and 80.0% methanol. After 2.5 min. of the hexane/methanol mixture, separation was completed using the initial solvent mixture. The total run time was 18.0 min. All solvents were HPLC-grade chemicals and pressurized under helium gas to minimize pump pressure problems.

Results were compared using area units (AUs) developed by the PeakNet version 4.3 Chromatography Workstation (Dionex). AUs for each pigment eluted (neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll b, chlorophyll a, and β carotene) were recorded for each sample. Peaks were identified using previous HPLC literature (Thayer and Bjorkman 1990; Gilmore and Yamamoto 1991) and by coelution with authentic standards.

Quantitative Procedure

Quantification of pigment concentrations for lutein, chlorophyll a and b, and β-carotene was possible because of the availability of authentic standards. The formula for quantification of lutein, chlorophyll a, and chlorophyll b was the following:

$$\text{Pigment Content} = \frac{\text{Pigment of interest in unknown / (β-carotene spiked sample) – (β-carotene non-spiked sample)}}{\text{Pigment of interest in standard / β-carotene in standard}}$$

The formula for the quantification of β-carotene was the following:

$$\beta\text{-carotene content} = \frac{\beta\text{-carotene in non-spiked sample}}{\beta\text{-carotene in standard}}$$

The result of the above formulae were then multiplied by the concentration of each standard (~0.25 nmoles/25 µl injection) corresponding to the compound of interest. The resulting calculation reveals a number expressed in nmoles pigment/injection, which was converted to mg pigment/ g tissue for presentation, using the molecular weights of β-carotene (536.9), lutein (568.9), chlorophyll a (893.5) and chlorophyll b (907.5). Because standards were not available, neoxanthin, violaxanthin, antheraxanthin, and zeaxanthin were compared using AUs.

Interactions between chlorophyll b, chlorophyll a, lutein, and β-carotene were examined and the Pearson's correlation (r) between the vehicle-mounted optical sensor data (NDVI) and pigment concentrations (mg/g) were developed using SAS Version 7 (Cary, NC). Coefficient of determination (r^2) and scatter plots were also developed between data variables to find the best fit simple equation using TableCurve 2D 5.0 Software (AISN Software, SPSS Science, Chicago, IL). Data were pooled over months and orthogonal contrasts of all variables involved were developed using SAS Version 7 (Cary, NC). The use of multiple regression analysis was negated by preliminary findings. High correlations between pigments indicated that concentrations did not vary independently.

RESULTS AND DISCUSSION

The effects of the nutrients and chemicals in our treatments and the influence of each on NDVI and the individual pigments are shown in Tables 6 & 7. Dithiopyr had a significant influence on each variable, except ß-carotene (Table 6). This can be explained by the phytotoxicity associated with the application of this chemical during hot weather. NDVI was more sensitive to the increases in pigment concentrations associated with Fe/Mg applications than from nitrogen sources (Tables 6 & 7). However, nitrogen applications caused significant increases in every pigment concentration, while main effect interactions found that Fe/Mg treatments only increased chlorophyll b and total chlorophyll significantly (Table 6).

Regression analyses were performed to examine the relationships among NDVI and each pigment evaluated (neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, chlorophyll b, chlorophyll a, and ß-carotene). NDVI was not strongly correlated with any individual pigment concentration, however, there was a significant relationship between NDVI and each pigment, except when NDVI was compared with antheraxanthin and zeaxanthin (pigments found in relatively small quantities in creeping bentgrass). Individual pigment concentrations never accounted for more than 38% of the variation associated with NDVI among quantifiable pigments (Figures 1, 2, & 3). Of the pigments compared only by AUs, violaxanthin and neoxanthin showed significant relationships with NDVI, but explained only 30% and 24% of the variation associated with the vegetation index, respectively. NDVI has been shown to correlate well with biomass and pigment composition when expressed as area or volume (Sellers 1987; Penuelas, et al.

1993; Datt 1998), rather than concentration. Based on our results, either sensor technology may not be sensitive enough at this time to predict plant pigment concentrations (mg/g), or NDVI is a vegetation index that is not sensitive enough to predict plant pigment concentrations (mg/g). Another possible reason for the low correlation (r^2) values between NDVI and pigment concentrations may be that the expression of pigment values on a concentration basis is not representative of the amount of matter interacting with the light emitted by the sensors per unit surface area. Datt (1998) reported that pigment content (mg/cm^2) may be more suitable than pigment concentration (mg/g) when comparing with remote sensing applications.

Upon further evaluation of the scatter plots in Figures 1, 2, & 3, many outlying data points are apparent. It is interesting to note that averaging each variable (NDVI, total chlorophyll, lutein, and ß-carotene) by treatment over the nine months of data (n=11) resulted in r^2 values that would make NDVI very highly correlated with total chlorophyll (r^2=0.85), lutein (r^2=0.76), and ß-carotene (r^2=0.59). The significance of this finding may be diluted, however, since this averaging procedure would obviously increase the correlation (r^2) of any given regression data set. Using the F-value and subsequent F-test of these averaged scatter plots still resulted in significance at $p<0.05$ and would be scientifically acceptable on those terms, however.

Highly significant correlations were seen from pigment to pigment. (Table 8). Regression analyses of total chlorophyll and lutein; total chlorophyll and ß-carotene; lutein and ß-carotene produced coefficients of determination (r^2) values of 0.82, 0.71, and 0.87, respectively. This finding agrees with prior plant pigment research, as Maas and Dunlap (1989) also reported high correlation between carotenoid and chlorophyll

concentrations in a study using corn leaves. Pigment concentrations of creeping bentgrass did not vary independently, suggesting that increases in chlorophyll concentrations of creeping bentgrass were also followed by an increase in accessory pigments. This may be of significance in future research in this area, since accurate sensor (reflectance)-based assessment of carotenoid concentration in the absence of a significant correlation between chlorophyll and carotenoid levels has been questioned (Maas and Dunlap 1989).

There may be many variables and factors that contribute to the overall influence on NDVI. Blackshaw, et al. (1998) used a similar type of sensor (red and infrared) while attempting to detect weeds for the eventual integration into a spot-spraying system. They also realized and attempted to determine the influence of a wide array of environmental and physical factors that they believed could affect the reliability of information obtained through optical sensing. Based on their findings, and our results it is most likely that several biological and physical factors, not just pigment concentration information alone, need to be incorporated into a model in order for researchers to reliably predict plant status or health via optical sensing.

SUMMARY

NDVI was more influenced by Fe/Mg applications than nitrogen or plant growth regulator applications. Phytotoxicity from dithiopyr applications was detected by the subsequent lowering of NDVI, chlorophyll, and xanthophyll, but not ß-carotene. NDVI was not well correlated with any individual pigment. Regression analysis indicated a

significant relationship between NDVI and density, chlorophyll b, chlorophyll a, total chlorophyll, lutein, and ß-carotene, however, only 30-40% of the variation in NDVI could be explained with any single variable (pigment concentration or density). This suggests that there may be many variables and factors, both physical and biological, that contribute to the overall influence on NDVI, besides pigment levels. More studies involving optical sensing and HPLC analysis are needed in order to learn more about the influence of varying pigment concentrations on NDVI or other viable vegetation indices.

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Table 6. The influence of treatment main effects (nitrogen, forms of nitrogen, iron and magnesium, surfactant, dithiopyr, and trinexapac-ethyl) on normalized difference vegetation index (NDVI) and pigment concentrations. Means were pooled over months because of no significant (ns) month by treatment interaction.

Contrasts	NDVI	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Lutein	ß-carotene
Nitrogen						
vs. no nitrogen†	ns	*	*	*	**	**
Ammonium nitrate						
vs. ammonium sulfate	ns	ns	ns	ns	ns	ns
Fe/Mg vs. no Fe/Mg	**	ns	**	*	ns	ns
Fe/Mg						
vs. Fe/Mg + surfactant	ns	ns	*	ns	ns	ns
Dithiopyr						
vs. no dithiopyr	**	**	**	**	*	ns
Trinexapac-ethyl						
vs. no trinexapac-ethyl	ns	ns	ns	ns	ns	ns

ns, *, **Not significant or significant at P<0.05 or 0.01, respectively.

† Four treaments including ammonium nitrate or ammonium sulfate (nitrogen) contrasted against control and Fe/Mg treatments (no nitrogen).

Table 7. Influence of treatments on normalized difference vegetation index (NDVI) and pigment concentrations. Means were pooled over months because of no significant (ns) month by treatment interaction.

Treatment	NDVI	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Lutein	ß-Carotene
		mg/g				
Control	0.67	4.88	1.54	6.42	0.57	0.25
NH_4NO_3	0.68	5.08	1.65 *	6.73	0.62 *	0.27 *
$(NH_4)_2SO_4$	0.67	4.92	1.63 *	6.55	0.61 *	0.26
Dithiopyr	0.64 *	4.42 *	1.47	5.89 *	0.54 *	0.23
Trinexapac-ethyl	0.66	4.89	1.63 *	6.52	0.58	0.24
Fe/Mg	0.68	4.87	1.66 *	6.53	0.58	0.24
Fe/Mg + NH_4NO_3	0.69 *	5.10	1.66 *	6.76	0.61 *	0.25
Fe/Mg + $(NH_4)_2SO_4$	0.69 *	5.31 *	1.73 *	7.04 *	0.62 *	0.26
Fe/Mg + dithiopyr	0.65 *	4.62	1.52	6.14	0.57	0.25
Fe/Mg + trinexapac-ethyl	0.68	4.96	1.60	6.56	0.59	0.26
Fe/Mg + surfactant	0.68	4.81	1.56	6.37	0.58	0.25
Mean	0.67	4.90	1.60	6.50	0.59	0.25

* Indicates significant difference compared to control ($p<0.05$).

Table 8. Pearson correlation coefficients (r) matrices showing relationship among pigments (n=297).

Pigment	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Lutein	ß-Carotene
Chlorophyll a	-				
Chlorophyll b	0.84	-			
Total Chlorophyll	0.99	0.91	-		
Xanthophyll	0.91	0.72	0.89	-	
ß-Carotene	0.86	0.58	0.81	0.93	-

Figure 1

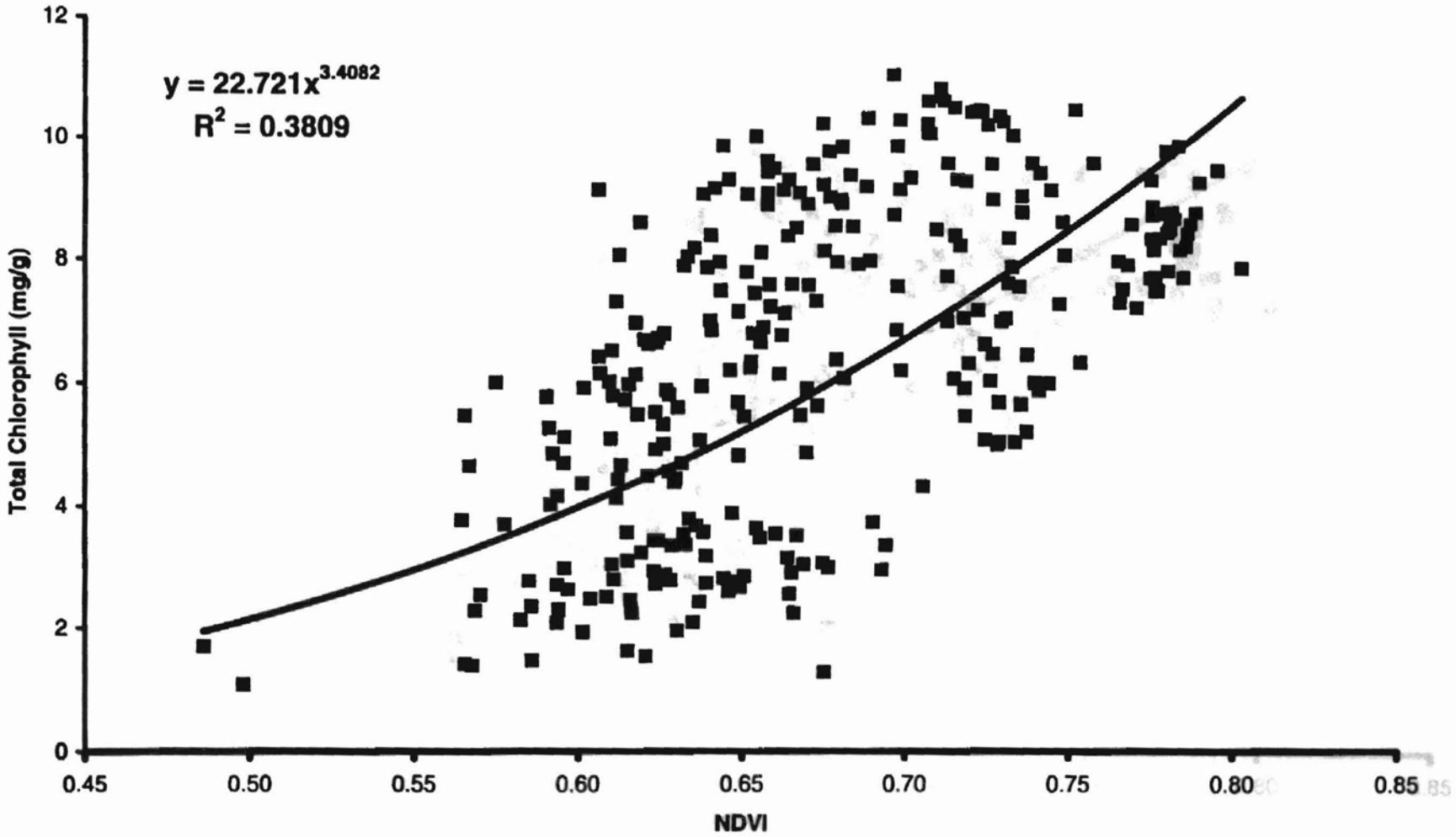

y = 22.721x^3.4082
R^2 = 0.3809
Total Chlorophyll (mg/g)
NDVI
0
2
4
6
8
10
12
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85

Figure 2

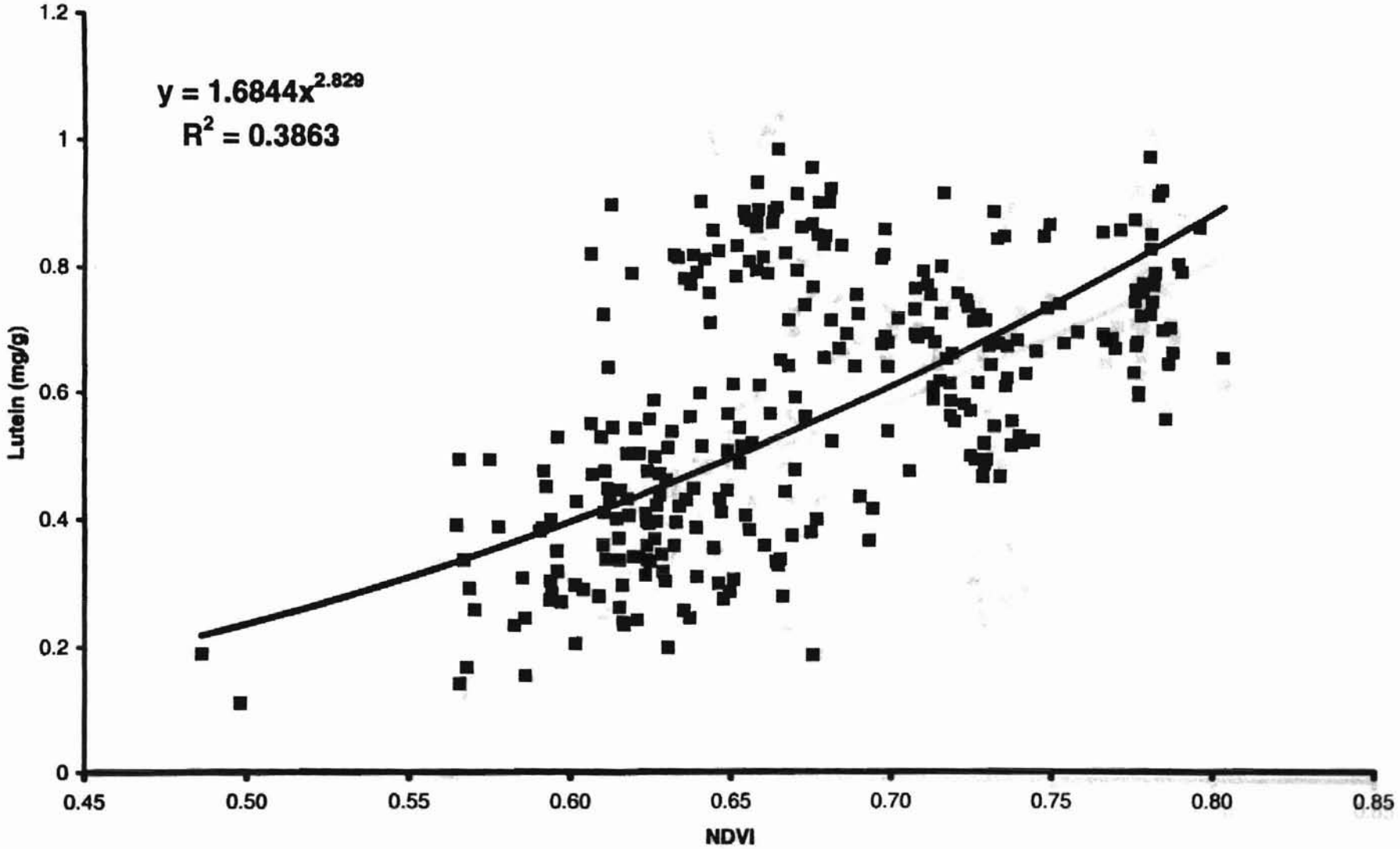
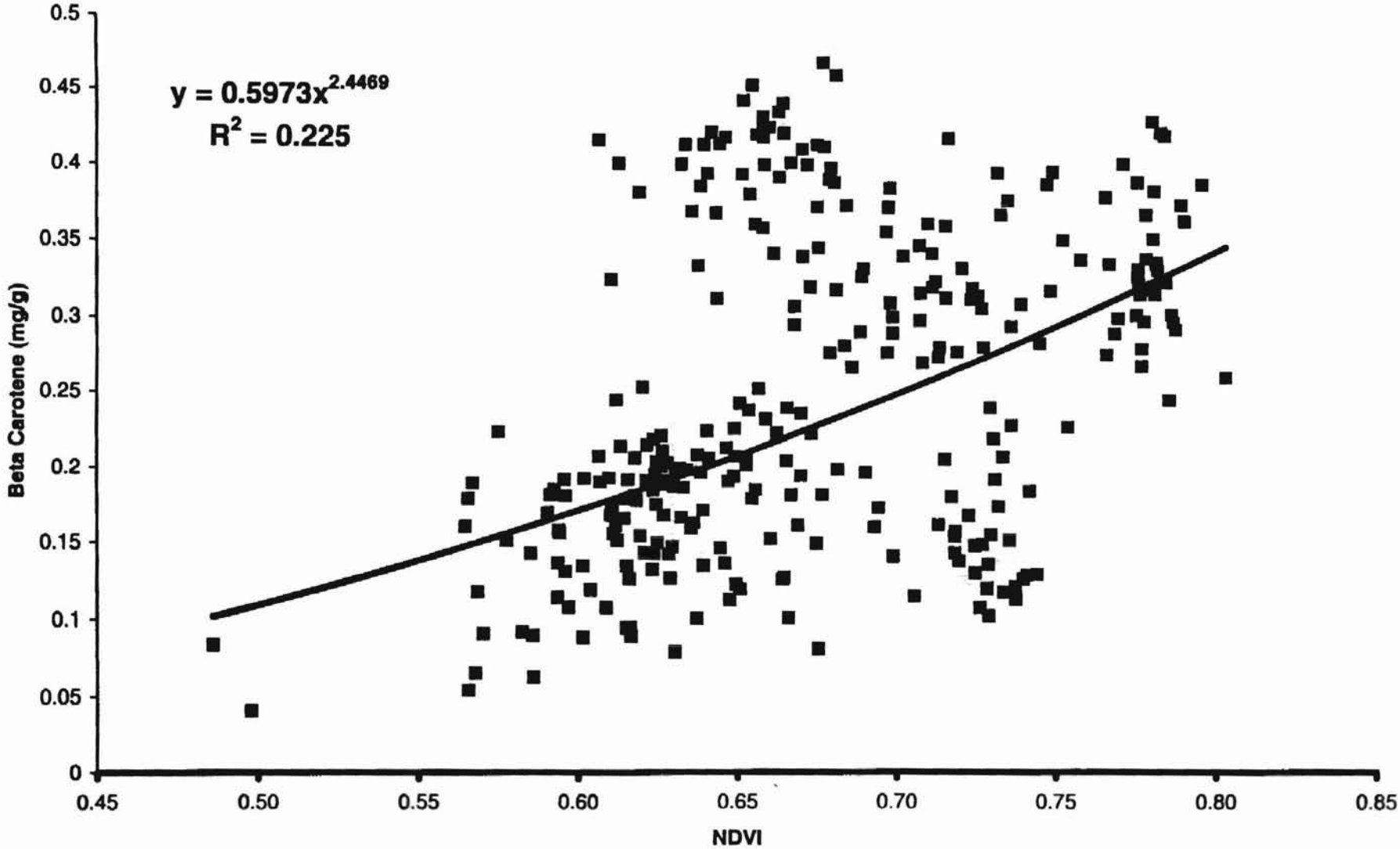

y = 1.6844x^2.829
R^2 = 0.3863
Lutein (mg/g)
NDVI
1.2
1
0.8
0.6
0.4
0.2
0
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85

Figure 3


y = 0.5973x^2.4469
R^2 = 0.225
Beta Carotene (mg/g)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.45
0.50
0.55
0.60
0.65
0.70
0.75
0.80
0.85
NDVI

VITA

James Christopher Stiegler

Candidate for the Degree of

Master of Science

Thesis: LABORATORY AND FIELD INVESTIGATIONS ON CREEPING BENTGRASS (*Agrostis palustris* Huds.) GOLF GREENS

Major Field: Horticulture

Biographical:

Personal Data: Born in Stillwater, Oklahoma, on January 28, 1975, the son of Jim and Connie Stiegler.

Education: Graduated from Stillwater High School, Stillwater, Oklahoma in May 1993; received Bachelor of Science degree in Agricultural Sciences and Natural Resources, majoring in Horticulture and Landscape Architecture from Oklahoma State University, Stillwater, Oklahoma in May 1998. Completed the requirements for the Master of Science degree with a major in Horticulture at Oklahoma State University in May, 2001.

Experience: Employed by Oklahoma State University, Stillwater, OK as an undergraduate working at OSU Turfgrass Research Center in 1996 and Soil, Water, and Forage Analytical Laboratory during summer 1993. Completed two turfgrass management internships, one at Bear Creek Golf Club in Denver, CO during summer 1997 and one with the United States Golf Association-Green Section during summer 2000. Completing a stint as Graduate Research Assistant, Oklahoma State University, Department of Horticulture & LA, August 1998-present.

Professional Memberships: Golf Course Superintendent's Association of America, American Society of Agronomy, Crop Science of America