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Effects of Elevated CO_2 on Creeping Bentgrass (*Agrostis stolonifera* L.) during the Ante Meridiem Photoperiod for Summer Heat Stress Tolerance

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To the Graduate Council:

I am submitting herewith a thesis written by Rodney V. Tocco Jr. entitled "Effects of Elevated CO_2 on Creeping Bentgrass (*Agrostis stolonifera* L.) during the Ante Meridiem Photoperiod for Summer Heat Stress Tolerance." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

John C. Sorochan, Major Professor

We have read this thesis and recommend its acceptance:

Robert Auge, Carl Sams

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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

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EFFECTS OF ELEVATED CO_2 ON CREEPING BENTGRASS (*Agrostis stolonifera* L.) DURING THE ANTE MERIDIEM PHOTOPERIOD FOR SUMMER HEAT STRESS TOLERANCE

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Rodney Vincent Tocco Jr.
May 2008

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I would like to thank my family first and foremost. Mom and Dad- you have always been there in times of good and bad. Your love, encouragement, and patience has made everything I've dreamed an accomplishment. Vicki, Steven, Dominic and Gino- you have been the best siblings anyone could ask for. Always there and always anchors to hold me at bay when rough seas lie ahead.

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and Wells McClure. From start to finish each of you contributed to this project and its success.

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Last but not least, I would like to give special thanks to Tim Vanini. During my undergraduate career and now distant phone conversations from so many miles away, your advice and constant pressure to succeed have kept me going when there seemed to be no light ahead. I look forward to the future now more and more each day from the wisdom you have shared with me about your many travels and experiences in life.

ABSTRACT

The demand for optimum putting conditions requires golf course superintendents to manage cool season creeping bentgrass (*Agrostis stolonifera L.*) in the transition zone and upper south. Summer heat stress combined with low mowing heights and constant traffic are challenges that superintendents must face in order to successfully manage creeping bentgrass from early May to late September. A field experiment was conducted on a Crenshaw putting green under golf course conditions during the 2006 summer and twice during the 2007 summer in Knoxville, TN. 2006 enriched air treatments of ~692 ppm CO_2 and ~891 ppm CO_2 were compared to a control of ambient air (~363 ppm CO_2). 2007 enriched air treatments of ~716 ppm CO_2 and ~1076 ppm CO_2 were compared to a control of ambient air (~451 ppm CO_2). Indirect heat stress was characterized by measuring the accumulation of total nonstructural carbohydrates (TNC) which is the sum of soluble carbohydrates and insoluble starch. The effects of CO_2 enriched air on TNC during the ante meridiem (between 12 midnight and 12 noon) photoperiod were determined using near infrared reflectance spectroscopy (NIRS). The effects of CO_2 enriched air on turfgrass quality during the ante meridiem photoperiod were determined using normalized difference vegetative index (NDVI) chlorophyll measurements. Disease and visual quality differences amongst treatments or locations were measured on an incidental basis.

No significance occurred within the 2006 and 2007 TNC or NDVI analysis for differences amongst treatments. 2006 average TNC for shoots were 24.8, 20.1, and 28.5 mg g^{-1} of tissue for the 363, 692, and 891 ppm CO_2 levels, respectively. 2006 average

NDVI for shoots were 7.2, 7.3, and 7.3 for the 363, 692, and 891 ppm CO_2 levels, respectively. 2007average TNC for shoots were 25.6, 18.9, and 23.1 mg g^{-1} of tissue for the 451, 716, and 1076 ppm CO_2 levels, respectively. 2007 average NDVI for shoots were 7.9, 8.0, and 8.0 for the 451, 716, and 1076 ppm CO_2 levels, respectively. All results were analyzed at 0.05 probability level within SAS 9.1. No incidence of disease or visual quality differences among treatments or locations occurred.

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PART I

INTRODUCTION

The climatic United States transition zone extends from northeastern New Mexico to Virginia and is the most difficult region in which to manage grasses (Christians, 2003; Fry and Huang, 2004). The transition zone covers an area 300 to 700 miles north to south in the United States whereas the northern edge is roughly defined by Interstate 70 from Maryland through eastern Kansas and the serpentine southern margin touches parts of North Carolina, Kentucky and Tennessee (Dunn and Diesburg, 2004). The transition zone is cool enough in the winter to make it difficult to maintain perennial stands of many warm-season grasses, and yet it is warm enough in the summer to make things difficult for cool-season grasses (Christians, 2003). In this zone, winter kill will affect warm season species while heat stress will harm cool season species (Fry and Huang, 2004).

In the transition zone, creeping bentgrass (*Agrostis stolonifera* L.) is the desired species for golf course putting greens. Optimum growing conditions for creeping bentgrass occur with air temperatures between 16 and 24° C (Waddington et al., 1992). Optimum growing conditions for root growth occurs when soil temperatures are between 10 and 18° C (Liu et al. 2002). However, during the summer months soil temperatures are much higher in the transition zone, thus heat stress conditions occur.

Heat stress is classified as either ‘direct’ or ‘indirect’. Direct heat stress is rapid increases in temperature causing cell death (Fry and Huang, 2004). Temperatures that approach 49°C cause direct heat stress resulting in immediate cell death (Carrow, 1996). Indirect heat stress occurs in creeping bentgrass when temperatures meet or exceed 30° C for several hours at a time (Waddington et al., 1992). Indirect heat stress is a common occurrence in the transition zone during the summer months of June, July, and August and is one of the major factors limiting use of cool-season grasses (Beard and Daniel 1965; Carrow, 1996; Beard, 1997; Huang, 2000). In addition to high daytime temperatures, high humidity acts as a buffer preventing large fluctuations from daytime to nighttime temperatures (McCarty, 2001).

During summer heat stress periods, high temperatures cause early stomatal closure resulting in increased photorespiration while net or daytime respiration increases (Huang et al, 1998). Photorespiration is viewed as a process that diminishes net photosynthesis by ~25% unfortunately due to plants having evolved when the atmosphere had much higher levels of CO_2 than it has today (Rachmiletvitch et al. 2004). Photorespiration results from the oxygenase reaction catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase in which the reaction glycollate-2-phosphate is produced and subsequently metabolized in the photorespiratory pathway forming the Calvin cycle intermediate glycerate-3-phosphate (Wingler et. al. 2000). In C_3 photosynthetic plants (cool season turfgrasses), photorespiration occurs when photosynthesis is inhibited by an over-abundant oxygen (O_2) presence that prevents carbon dioxide (CO_2) assimilation in the Calvin-Benson cycle (photosynthesis) during periods of above optimum air temperatures (Taiz and Zeiger, 1998). Oxidative stress can lead to inhibition of photosynthesis and respiration and thus plant growth (Huang 2004).

The Calvin-Benson cycle may become wasteful to plant energy with the consumption of ATP, NADPH, and other reducing equivalents for the production of CO_2 and NH_3 (Wingler et al. 2000). Rather than carbohydrates being stored, carbohydrate losses occur and often exceed net photosynthesis (carbohydrate synthesis) during the summer months. Respiratory oxygen uptake has been shown to not increase by an instantaneous elevation of CO_2 (Davey et al., 2004). Photorespiration is an energy consuming process that is detrimental when carbon is released rather than fixed. Simply put, the turf plant uses more energy than it produces during periods of indirect heat stress.

Soil temperatures above 30° C cause decreased root growth and function (Dernoeden, 2002). Fewer roots for a plant will reduce energy storage areas of carbohydrates that the plant requires for everyday processes. As a result, creeping bentgrass, in the transition zone, is especially susceptible to summer heat stress (Fry and Huang, 2004). Seaweed extract (SWE)-based cytokinin treatments showed 39% higher heat stress tolerance than that of *trans*-zeatin riboside standards (Zhang and Ervin 2008). Current recommendations for managing creeping bentgrass during the summer are increased mowing heights and reduced mowing frequency, to one or two times per week (Beard, 2002; McCarty, 2001). Common practices in the transition zone also include increasing air circulation across the turf surface using greenside fans to reduce favorable conditions for disease. Alternatively, rolling and mowing has been shown beneficial in reducing indirect heat stress (Strunk, 2005). Alternating mowing with rolling maintained higher quality putting greens versus traditional practices of mowing daily or six times per week. Increased fungicide applications and syringing are two management practices necessary to alleviate disease wilt during summer heat stress periods (Dernoeden, 2002). Syringing is a term used within the turfgrass industry for a light sprinkling of water on turf usually during the hottest part of the day to prevent wilting.

With increased awareness of global warming, governments are interested in projects to examine the effects of rising atmospheric CO_2 levels in various crops and forage production systems. Current global atmospheric CO_2 average levels are approximately 367 ppm (IPCC, 2001). These levels have increased more in the last twenty years than in the previous one-hundred on record, and levels are expected to continue increasing with the continued use of fossil fuels (IPCC, 2001). Prior research has shown CO_2 enrichment in fruit and forestry ecosystems are methods to increase plant photosynthetic processes, and thus overall plant health and growth rates through increased energy storage. Perennial ryegrass (*Lolium perenne L.*) exhibited increased root growth when enriched with CO_2 (Jongen et al., 1995). Perennial ryegrass grown for 1.8144e6 s (21 days) @ 720 ppm had 175% greater biomass than control plants grown at 450 ppm CO_2 (Hodge et al., 1998).

Respiration is a key factor to CO_2 levels within the plant that affect energy storage. High respiration rates cause negative carbon exchange rates (CER's) within the plant. Negative CER's refer to diminishing carbohydrate reserves due to the plant diverting energy towards shoot growth. When carbon is limiting, the shoots become priority for the plant and root depletion soon occurs. Root dieback is caused by the lower priority of root versus shoot growth, respectively (Carrow, 1996). Elevated atmospheric carbon (C_a) reduces stomatal conductance and transpiration, yet improves water use efficiency; while, at the same time stimulating higher rates of photosynthesis (carbohydrate production) (Drake, 1997). As temperatures increase, the rate of photosynthesis decreases (Huang, 2004). At these high atmospheric temperatures (indirect heat stress) the abundance of CO_2 available in the cells for plants in the boundary layer and stomatal cavity of the leaf decreases and O_2 increases (Taiz and Zeiger, 1998). Enriching atmospheric concentrations of CO_2 before stomatal closure, to increase carbohydrate energy production and storage during ante meridiem (AM) photoperiod hours is one way to potentially reduce the effects of summer heat stress that occurs on creeping bentgrass putting greens during the post meridiem photoperiod hours of the day in the transition zone. Thus, CO_2 enrichment has the potential to counter the negative effects of indirect heat stress.

Elevated CO_2 applications for plants in light conditions have been studied in several species. Elevated CO_2 increased yield at high N levels yet showed less N concentration within the plants in *Triticom aestivum* (Fangmeier et al., 1996). Elevated CO_2 increased biomass and leaf photosynthetic rates were always higher in elevated CO_2 of *Pinus taeda* in the summer suggesting seasonal effects of temperatures on photosynthesis (Tissue et al., 1997). Elevated CO_2 applications increased sucrose and phloridizin concentrations in apple (‘Gala’/ Malling (M9)) leaves (Kelm and Flore, 2005). A 44% increase in photosynthetic accumulation was observed in 39 tree species grown in elevated levels of CO_2 (Gunderson, 1994), and elevated CO_2 increased light-saturated net photosynthesis in sweet-gum trees (*Liquidambar styraciflua*) (Herrick and Thomas, 1999). A 64% increase in photosynthate occurred when C_3 grassland species were grown in enriched CO_2 (560 ppm) levels versus ambient CO_2 (368 ppm) levels (Jongen et al., 1999). Free Air CO_2 Enrichment (FACE) locations world-wide have been experimenting with increased CO_2 levels on crops, forestry, and other ecosystems. FACE studies showed dramatic yield increases in cotton and wheat (Culotta, 1995). Approximately 50% increases were seen in cotton yields and a more subtle but significant 10% yield increase in wheat varieties. In FACE, studies with managed grasslands, root carbon:nitrogen ratios increased in *Lolium perenne*, and root lengths increased in *Trifolium repens* (Jongen, M., et al., 1995). Atmospheric CO_2 enrichment also had significant effects on flavonoid compounds in green, well-developed flag leaves of *Triticum aestivum* (wheat) (Peñuelas, J., et al., 1999). No research has been conducted on the effects of CO_2 enrichment in managed turfgrass conditions and in particular, creeping bentgrass putting greens in the transition zone. Since the putting green is the most important facet of any golf course, the aesthetics and performance of the greens often determine the quality of a golf course. Therefore, the objective of this study is to determine if elevated CO_2 treatments provide increased photosynthetic efficiency or energy storage on creeping bentgrass putting greens during periods of indirect heat stress is warranted.

In the transition zone temperatures causing indirect heat stress do not occur until mid morning or noon (~10am-12pm) when temperatures meet or exceed 30°C. High temperatures reduced plant density, tiller density, root number, and root biomass (Xu and Huang 2001). Therefore, CO_2 enrichment during the early morning hours may reduce the effects of indirect heat stress in creeping bentgrass putting greens. C_3 grasses produce greater amounts of nonstructural carbohydrates and have greater declines in nitrogen content than do C_4 grasses under elevated CO_2 (Barbehenn et al. 2004). Hypothetically, it would be anticipated that enriching creeping bentgrass greens given CO_2 during the morning or ante meridiem hours would increase the grass's photosynthetic rate resulting in increased total nonstructural carbohydrate (TNC) production. Photosynthetic efficiency is the fraction of light energy converted into other forms of energy for use. The hypothesis tested was, "Increased CO_2 levels will counter the negative effects of indirect heat stress." This research aims to answer two questions within the hypothesis and provide valuable insight to the golf course superintendent. One, what are the effects of CO_2 enrichment on carbohydrate synthesis and metabolism in creeping bentgrass (*Agrostis stolonifera* L.*)* on putting greens in the transition zone? Two, what are the optimum CO_2 enrichment levels and timing to compensate for reduced photosynthesis (indirect heat stress) of creeping bentgrass (*Agrostis stolonifera* L.*)* grown under summer heat stress conditions in the transition zone?

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PART II

EFFECTS OF ELEVATED CO_2 ON CREEPING BENTGRASS (AGROSTIS STOLONIFERA L.) DURING THE ANTE MERIDIEM PHOTOPERIOD FOR SUMMER HEAT STRESS TOLERANCE

ABSTRACT

The demand for optimum putting conditions requires golf course superintendents to manage cool season creeping bentgrass (*Agrostis stolonifera L.*) in the transition zone and upper south. Summer heat stress combined with low mowing heights and constant traffic are challenges that superintendents must face in order to successfully manage creeping bentgrass from early May to late September. A field experiment was conducted on a Crenshaw putting green under golf course conditions during the 2006 summer and twice during the 2007 summer in Knoxville, TN. 2006 enriched air treatments of ~692 ppm CO_2 and ~891 ppm CO_2 were compared to a control of ambient air (~363 ppm CO_2). 2007 enriched air treatments of ~716 ppm CO_2 and ~1076 ppm CO_2 were compared to a control of ambient air (~451 ppm CO_2). Indirect heat stress was characterized by measuring the accumulation of total nonstructural carbohydrates (TNC) which is the sum of soluble carbohydrates and insoluble starch. The effects of CO_2 enriched air on TNC during the ante meridiem (between 12 midnight and 12 noon) photoperiod were determined using near infrared reflectance spectroscopy (NIRS). The effects of CO_2 enriched air on turfgrass quality during the ante meridiem photoperiod were determined using normalized difference vegetative index (NDVI) chlorophyll measurements. Disease and visual quality differences amongst treatments or locations were measured on an incidental basis.

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INTRODUCTION

Putting greens have become the defining characteristic that often determines the reputation and notoriety of a golf course. The golf course superintendent often spends the majority of his/her time focused on putting green speeds, playability and overall aesthetics. With the ever present player demand for optimum putting conditions, superintendents utilize a turf species not common to warm season climates. *Agrostis stolonifera* L. (creeping bentgrass) is a preferred species on putting greens for its ability to produce an ideal putting surface (Fry and Huang, 2004). Thus, superintendents within the transition zone choose to intensely manage this cool season turf species. The transition zone extends from northeastern New Mexico to Virginia, and is the most difficult region in which to manage grasses (Christians, 2003; Fry and Huang, 2004). The transition zone covers an area 300 to 700 miles north to south in the United States whereas the northern edge is roughly defined by Interstate 70 from Maryland through eastern Kansas and the serpentine southern margin touches parts of North Carolina, Kentucky and Tennessee (Dunn and Diesburg, 2004). The transition zone is cool enough in the winter to make it difficult to maintain perennial stands of many warm-season grasses, and yet it is warm enough in the summer to make growth difficult for cool-season grasses (Christians, 2003).

In the transition zone, creeping bentgrass often undergoes long periods of indirect heat stress. Indirect heat stress occurs in creeping bentgrass when temperatures meet or exceed 30° C for extended periods of time (Waddington et al., 1992). Indirect heat stress is the most common type of heat stress in the transition zone occurring in early May to late September. High daytime temperatures coupled with high humidity limit variation in evening temperatures, which causes a reduction of shoot growth, shoot density, root length, tillering, and overall turf quality (Waddington et al., 1992; Xu and Huang 2001). Maintaining creeping bentgrass putting greens during these unfavorable conditions requires creativity and intense management for golf course superintendents.

Intense management for creeping bentgrass putting greens during indirect heat stress includes syringing greens to prevent wilt and disease and frequent fungicide applications. Trinexapac-ethyl will reduce shoot elongation and growth, increase shoot density, increase rooting, and reduce the need for daily mowing (Shepard and Dipaola, 2000). Compounded with applications of trinexapac-ethyl, a superintendent often strives to reduce mowing frequency and increase mowing height during times of indirect heat stress in order to prevent injury, decreased rooting, greater water needs and reduced turf vigor (McCarty, 2001). Once again though, demands for high quality putting surfaces require the turf to be mown everyday (Beard, 2002). Mowing everyday versus less frequent mowing will reduce shoot density during periods of indirect heat stress (Beard, 2002; Huang, 2004). Alternating rolling and mowing versus daily mowing provides higher quality creeping bentgrass putting greens during periods of indirect heat stress (Strunk, 2005). Simply alternating mowing and rolling practices provides consistent putting green surfaces and reduces the frequency of wounding stress caused by daily mowing. Lastly, greenside fans are used to improve environmental conditions for creeping bentgrass putting greens during periods of indirect heat stress. Any additional management practice that will potentially reduce the negative effects of indirect heat stress on creeping bentgrass putting greens is warranted.

Elevated CO_2 applications for plants in light conditions have been studied in several species. Elevated atmospheric carbon (C_a) reduces stomatal conductance and transpiration, yet improves water use efficiency; while, at the same time stimulating higher rates of photosynthesis (carbohydrate production) (Drake, 1997). Enriching atmospheric concentrations of CO_2 before stomatal closure, to increase carbohydrate energy production and storage during ante meridiem (AM) photoperiod hours is one way to potentially reduce the effects of summer heat stress that occurs on creeping bentgrass putting greens during the post meridiem photoperiod hours of the day in the transition zone. Thus, CO_2 enrichment has the potential to counter the negative effects of indirect heat stress.

Elevated CO_2 applications increased sucrose and phloridizin concentrations in apple ('Gala'/ Malling (M9)) leaves (Kelm and Flore, 2005). A 44% increase in photosynthetic accumulation was observed in 39 tree species grown in elevated levels of CO_2 (Gunderson, 1994), and elevated CO_2 increased light-saturated net photosynthesis in sweet-gum trees (*Liquidambar styraciflua*) (Herrick and Thomas, 1999). A 64% increase in photosynthate occurred when C_3 grassland species were grown in enriched CO_2 (560 ppm) levels versus ambient CO_2 (368 ppm) levels (Jongen et al., 1995). Free Air CO_2 Enrichment (FACE) locations world-wide have been experimenting with increased CO_2 levels on crops, forestry, and other ecosystems. FACE studies showed dramatic yield increases in cotton and wheat (Culotta, 1995). Approximately 50% increases were seen in cotton yields and a more subtle but significant 10% yield increase in wheat varieties. In FACE, studies with managed grasslands, root carbon:nitrogen ratios increased in *Lolium perenne*, and root lengths increased in *Trifolium repens* (Jongen, M., et al., 1995). Atmospheric CO_2 enrichment also had significant effects on flavonoid compounds in green, well-developed flag leaves of *Triticum aestivum* (wheat) (Peñuelas, J., et al., 1999). No research has been conducted on the effects of CO_2 enrichment in managed turfgrass conditions and in particular, creeping bentgrass putting greens in the transition zone. Since the putting green is the most important facet of any golf course, the aesthetics and performance of the greens often determine the quality of a golf course.

In this experiment, elevated levels of CO_2 as treatments are examined for their potential to increase photosynthetic efficiency or energy storage to combat indirect heat stress within the transition zone on creeping bentgrass putting greens. The ultimate goal is to reduce the stress level of the turfgrass to assist the superintendent through the summer months. This research has the potential to benefit the golf industry by enabling golf course superintendents to better manage desired cool season turfgrass species in hot and humid conditions by improving the photosynthetic efficiency.

MATERIALS AND METHODS

To evaluate the effects of elevated CO_2 field experiments were conducted during the heat stress periods of June thru August in 2006 and 2007 on a creeping bentgrass (*Agrostis stolonifera* L.) putting green at the University of Tennessee Intercollegiate Golf Practice Facility in Knoxville; TN. The putting green was sodded with soil free ‘Crenshaw’ creeping bentgrass on a United States Golf Association specification sandpeat (90:10) root zone in April 2005. The putting green was mown six times per week at 4 millimeters using a Toro Flex 21 walk behind mower (The Toro Co. Minneapolis; MN). Nitrogen was applied at a rate of 4.9 kg N ha^{-1} every seven days using Harrell's 28-5-18 Bentgrass Special fertilizer (Harrell's Fertilizer, Lakeland, FL) from April thru October. Once a disease occurred and was identified, data was collected, and subsequent curative fungicide applications were applied. Trinexapac-ethyl (Syngenta Corporation, Wilmington, DE), a type II plant growth regulator, was applied to all plots at 0.4 L ha^{-1} every 21 days as part of regular management. Irrigation was applied as needed to prevent wilt (applied three times $week^{-1}$ at a 2.0 cm depth during periods of no rainfall). Light sand topdressing at $^1/_8$ to ¼ cubic yards per 93 m^2 was applied every two weeks from April thru October of each year.

Plots consisted of 0.2 m^2 chambers constructed out of Mylar® (DuPont Teijin Films; Hopewell, VA) and angle iron. Individual chambers were constructed out of Mylar® because of its ability to emit 99% infrared radiation and its low permeability to CO_2 and H_2O vapor. On 1 July 2006, CO_2 enrichment treatments were initiated five days per week from 7:00 AM to 12:00 PM for a six week period. In 2007, CO_2 enrichment treatments were repeated two times. Session one began on 6 June 2007 and concluded on 19 July 2007. Session two was initiated on 20 July 2007 and concluded on 20 August 2007. Treatments were arranged as single factors with three treatments of approximately 350, 700 and 900 ppm (+/- 25 ppm) CO_2 and 450, 725 and 1025 ppm (+/- 25 ppm) CO_2, in 2006 and 2007 respectively (Figure A-1, A-2 and A-3, respectively)[1].

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[1] All tables and figures are located in the Appendix.

In 2006, a 24 cm diameter direct drive blower furnace fan (Dayton Electric Mfg. Co.; Niles, IL) provided the air flow for all nine plots. CO_2 treatments were separated by reducing the air flow with a header to three separate 1.5 m PVC (4.55 cm diameter) pipes which in turn were reduced to 1.3 cm flexible tubing that delivered the air into the chamber. The CO_2 (Liquid CO_2, Air Gas; Knoxville, TN) was injected into the 1.5 m PVC pipes using a manifold and needle valves to regulate CO_2 flow enrichment concentrations. The delivery system was lifted on and off the green daily for treatments and management (Figure A-4). Air flow was 4 – m sec^{-1} (+/- 0.5 m sec^{-1}). CO_2 levels for each treatment were measured using a portable photosynthesis system CIRAS-1 (PP systems, Haverhill, MA). Temperature monitoring was done with an infrared thermometer (Mini IR Thermometer; Spectrum Technologies, Plainfield, IL).

In 2007, three Stanley® blower shop fans (The Stanley Works #655702; New Britain, CT) provided the air flow for each level of CO_2. Each fan connected to a 1.5 m (20 cm diameter) metal heating duct pipe which was reduced to three 4.0 cm flexible tubing that delivered the air into the chamber. The CO_2 (Liquid CO_2, Air Gas, Knoxville; TN) was injected into the 1.5 m metal heating duct using a manifold and needle valves to regulate enrichment levels. The delivery system was wheeled on and off the green daily for treatments and management (Figure A-5). Air flow was 7 – m sec^{-1} (+/- 0.5 m sec^{-1}). CO_2 levels for each treatment were measured using a portable photosynthesis system CIRAS-1 (PP systems, Haverhill, MA). Temperature monitoring was done with an infrared thermometer (Mini IR Thermometer; Spectrum Technologies, Plainfield, IL).

Turf color measurements were collected daily prior to and immediately following CO_2 enrichment treatments using a TCM NDVI turf color meter (Spectrum Technologies, Plainfield, IL). The turf color meter measures reflected light from the turfgrass in the red (660 nm) and near infrared (780 nm -NIR) spectral bands conducted with a chlorophyll meter to determine normalized difference vegetative index (NDVI) (TCM NDVI Manual). Chlorophyll absorbs the red band (660 nm) of incoming radiation and the reflectance at that wavelength is relatively low due to the strong absorption of the light by the plant pigments (TCM NDVI Manual). The high reflectance in the NIR (850nm) band is caused by the cellular structure of the plant leaves, particularly the spongy mesophyll leaf structure (TCM NDVI Manual).

One core from each plot was taken with a lever action hole cutter (Par Aide Pro; Lino Lakes, MN) to utilize in gas chromatograph and spectrophotometer TNC analysis. Cores measured 9 cm wide by 25.4 cm deep. Sand rootzone was removed by dipping the cores into a bucket of water and shaking gently. Roots were separated from the washed cores using a knife then each sample was wrapped in aluminum foil and immediately frozen in liquid nitrogen.

An additional nine cores (2 cm diameter by 25 cm deep), were taken from each plot using a Core Profile Sampler (Standard Golf Company, Cedar Falls, IA) to determine total dry mass for TNC analysis. Sand rootzone was removed by dipping the cores into a bucket of water and shaking gently. Roots were separated from the washed cores using a knife then each sample was wrapped in aluminum foil and immediately frozen in liquid nitrogen.

In 2006, total nonstructural carbohydrate and individual carbohydrate analysis was done with a spectrophotometer and gas chromatograph, respectively. TNC levels were acquired through carbohydrate extraction, derivatization, and analysis. The storage carbohydrates were extracted and analyzed by standard methods. Soluble carbohydrates were extracted 3X in 70% ethanol and analyzed using a modified phenol-sulfuric acid procedure (Dubois et al., 1962). Starch in the residual pellet was analyzed using a procedure only slightly modified from that of Keller and Loescher (1989) which was derived in part from Ebell (1969). This digestion process is with amyloglucosidase and glucose for analysis via glucose oxidase and peroxidase reactions (Sigma procedure GAGO20; or Bergmeyer and Bernt, 1974). Plant tissue analysis for the phenol-sulfuric acid method and starch analysis was done with a BioSpec-1601 spectrophotometer system (Shimadzu Scientific Instruments; Columbia, MD). An Agilent 6850 Series II gas chromatograph (Quantum Analytics, Inc.; Foster City, CA) was used for analysis.

CO_2 levels were monitored throughout the 2006 and 2007 treatment sessions (Figures A-1, A-2, and A-3, respectively)). Temperature data was collected throughout the 2006 treatment session and shown next to nearby experiment station data (Figure A-6). Temperature data was collected for session one and session two for the 2007 treatment sessions and shown next to nearby experiment station data (Figure A-7 and Figure A-8, respectively).

Statistical analysis for TNC levels and NDVI levels was completed with mixed model ANOVA and LSD means separation, on a completely random design (CRD) using

Statistical Analysis Software (SAS), version 9.1, 2003 SAS Institute Inc., Cary, NC, USA.

RESULTS

CO_2 levels for the 2006 and 2007 treatment sessions (Figures A-1, A-2, and A-3, respectively) were interesting in that overall CO_2 ambient averages were nearly 90 ppm higher for 2007. 2006 treatment levels for ambient, medium and high averaged 363, 692 and 891ppm CO_2, respectively. 2007 treatment levels for ambient, medium and high averaged 451, 716 and 1076ppm CO_2, respectively. Treatment levels for 2007 were adjusted based on the initial findings of higher ambient levels. No explanation for such variation in an identical location has been concluded to be the cause other than inter-annual variation. Daily readings were highest in the mornings and fell as the day went on but no exact trend was identified as to an hourly loss of atmospheric CO_2.

Temperature data collected throughout the 2006 treatment session (Figure A-6) was consistent in the experiment location to that of nearby experiment station data showing no significant differences that could explain our results. The same was the case with temperature data collected for session one and session two for the 2007 treatment sessions (Figures A-7 and A-8, respectively).

For 2006, total nonstructural carbohydrate levels for shoots and roots showed no significance for any level of CO_2 (Table B-1). 2006 average TNC for shoots were 24.8, 20.1, and 28.5 mg g^{-1} of tissue for the 363, 692, and 891 ppm CO_2 levels, respectively. 2006 average TNC for roots were 13.5, 13.5, and 15.6 mg g^{-1} of tissue for the 363, 692, and 891 ppm CO_2 levels, respectively. In 2006, values could not be measured for random plot samples therefore there is no statistical analysis from the gas chromatograph. The simpler starch analysis (spectrophotometer) was determined to be adequate, and therefore the GC analysis was omitted for 2007. For 2006, NDVI analysis showed no significant differences of green index measurements (Table B-2). Also for 2006 NDVI, no significance occurred between treatment measurements at 7:30 AM and 12:00PM. 2006 average NDVI for 7:30 AM were 7.2, 7.3, and 7.3 for the 363, 692, and 891 ppm CO_2 levels, respectively (Figure A-10). 2006 average NDVI for 12:00 PM were 6.9, 7.0, and 7.0 for the 363, 692, and 891 ppm CO_2 levels, respectively (Figure A-10). 2006 mean pooled ash weights for shoot and root samples utilized in analysis processes are referenced in Table B-5.

2007 analysis for session one was unable to be performed because several plots died from water deficit and high temperatures as a result of human error (Figure A-9). For 2007 session two, total nonstructural carbohydrate levels for shoots and roots showed no significance for any level of CO_2 (Table B-3). 2007 average TNC for shoots were 25.6, 18.9, and 23.1 mg g^{-1} of tissue for the 451, 716, and 1076 ppm CO_2 levels, respectively. 2007 average TNC for roots were 12.2, 15.3, and 14.1 mg g^{-1} of tissue for the 451, 716, and 1076 ppm CO_2 levels, respectively. TNC analysis was conducted using a phenol-sulfuric acid colorimetric method for two sets of sub-samples of shoots and roots. For 2007, NDVI analysis showed no significant differences of green index measurements for (Table B-4). Also for 2007 NDVI, no significance occurred between treatment measurements at 7:30 AM and 12:00PM. 2007 average NDVI for 7:30 AM were 7.9, 8.0, and 8.0 for the 451, 716, and 1076 ppm CO_2 levels, respectively (Figure A-

11). 2007 average NDVI for 12:00 PM were 7.9, 7.9, and 7.9 for the 451, 716, and 1076 ppm CO_2 levels, respectively (Figure A-11). 2007 mean pooled ash weights for shoot and root samples utilized in analysis processes are referenced in Table B-5.

All results were analyzed at 0.05 probability level within SAS 9.1. No incidence of disease or visual quality differences among treatments or locations occurred.

DISCUSSION

The lack of statistical difference for TNC during the 2006 session was determined to be a result of insufficient air flow (Flore, 2006). Temperatures within chambers consistently compared to the highest daily East Tennessee Research Station values, which subsequently may have affected photosynthesis (Figure A-6). During the 2006 session excessive condensation accumulated on the inside of the Mylar® chambers indicating insufficient air flow; thus, subsequent conditions favored photorespiration and potentially lowered levels of photosynthesis by disallowing light to the turfgrass canopy (Flore, 2006). A decrease in turf quality determined by NDVI readings was expected over the study period. NDVI measurements of 6 or greater indicate adequate turf quality. However, NDVI analysis showed no significance at any CO_2 level (Figure A-10). 2007 NDVI analysis, showed no significance at any CO_2 level (Figure A-11). Also, no significance was found between early morning measurements and subsequent daily measurements for NDVI.

Statistically, no significant difference occurred among CO_2 treatments. Throughout 2006, the lack of significance was believed to be a result of not enough air flow causing a build-up of condensation within the chambers (Figure A-12).

Condensation was prevented for 2007 sessions by increasing the air flow (Figure A-13). The first session of 2007 was unsuccessful when several plots died over a weekend from neglect due to researcher miscommunication (Figure A-14).

2007 session two results determined that the CO_2 enrichment was unable to compensate for the effects of indirect heat stress, potentially because other mechanical stresses such as frequent and low mowing added too much physiological stress. Previous FACE studies with CO_2 enrichment was shown to increase TNC in cool season grasses; however, it was un-mown perennial ryegrass grown in optimal temperatures. Possible studies could be done to investigate CO_2 enrichment on creeping bentgrass under optimal temperatures or un-mown during indirect heat stress. However, it was the goal of this study to evaluate the effects of CO_2 enrichment under actual putting green conditions. Putting greens are generally in a state of luxury consumption, in which all necessary nutrients and adequate water are provided to the plant. The only limiting factor hypothesized was levels of CO_2 in the ambient atmosphere. The levels of CO_2 treatments were determined upon ambient levels as a standard, double ambient levels as our hypothetically most advantageous level and nearly triple ambient levels to possibly curtail or negate any advantage of CO_2 fertilization. While the perennial ryegrass studies show the benefits of CO_2 enrichment, they would not represent actual creeping bentgrass putting greens for golf courses.

Additionally, CO_2 enrichment periods may have been not long enough to provide differences. As was previously mentioned, high levels of CO_2 contribute to stomatal closure (Drake et al., 1997). Therefore, coupled with high temperatures, treatments receiving elevated CO_2 may have closed their stomates sooner and photorespiration may have initiated sooner than in ambient treatments which may have offset any potential benefits being found statistically. Again, previous FACE studies on un-mown cool season grasses were under continuous CO_2 regimes. A pasture grass, Austrodanthonia caespitosa, showed inhibited growth when grown at an elevated CO_2 level of 550ppm (Williams et al., 2007). Ambient CO_2 levels in 2007 averaged 450 ppm (90 ppm higher than 2006) possibly because of urban pollution and/or seasonal climatic differences; regardless, the elevated ambient CO_2 levels may have been enough to offset the statistical significance of CO_2 treatments.

Additionally, several studies have recently shown non-successful attempts at enrichment with CO_2 in various other grass species. In a study involving Pascopyrum smithii (C_3 semi-arid grass), carboxylation/ rubisco regeneration reduced in elevated CO_2 to the point that the assimilation rate was similar in ambient vs. elevated treatments when adequate soil moisture was present (Lecain et al., 2003). Golf course greens are generally kept at adequate soil moisture levels throughout the summer months by managers with irrigation regimes which in this case may have contributed to no significant results. While elevated CO_2 increased photosynthesis and biomass production in C_3 grasses, there was observed decreases in stomatal conductance and transpiration rates (Kimball et al., 2002). This study feeds the notion that there may have been negative affects on the turf stand that off-set any potential benefit of the daily enrichment period from the treatments. Another study showed that elevated CO_2 increased net primary production (NPP) of dry matter (g m^{-2} year) but suppressed root allocation that decreased positive effects of precipitation and nitrogen (Shaw et al., 2002). Again, sufficient N and H_2O are common cultural practices carried out on golf course putting greens throughout the year. Finally, sampling error may have contributed to the lack of CO_2 treatment differences in TNC. For instance, the rinsing step and time of day (high temperature) before putting the sample in liquid nitrogen may have altered TNC levels because of varying cellular respiration rates.

CONCLUSIONS

This was an unsuccessful attempt at improving creeping bentgrass (*Agrostis stolonifera* L.) response to indirect heat stress with elevated levels of CO_2. Superintendents managing creeping bentgrass on putting greens in locations where indirect heat stress is an issue, at this time, can not consider elevated CO_2 to improve turf quality. However, this research warrants further investigations in creeping bentgrass putting green management during indirect heat stress periods to fully understand the turf quality differences in regards to high temperatures and CO_2 assimilation.

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APPENDICES

APPENDIX A

FIGURES

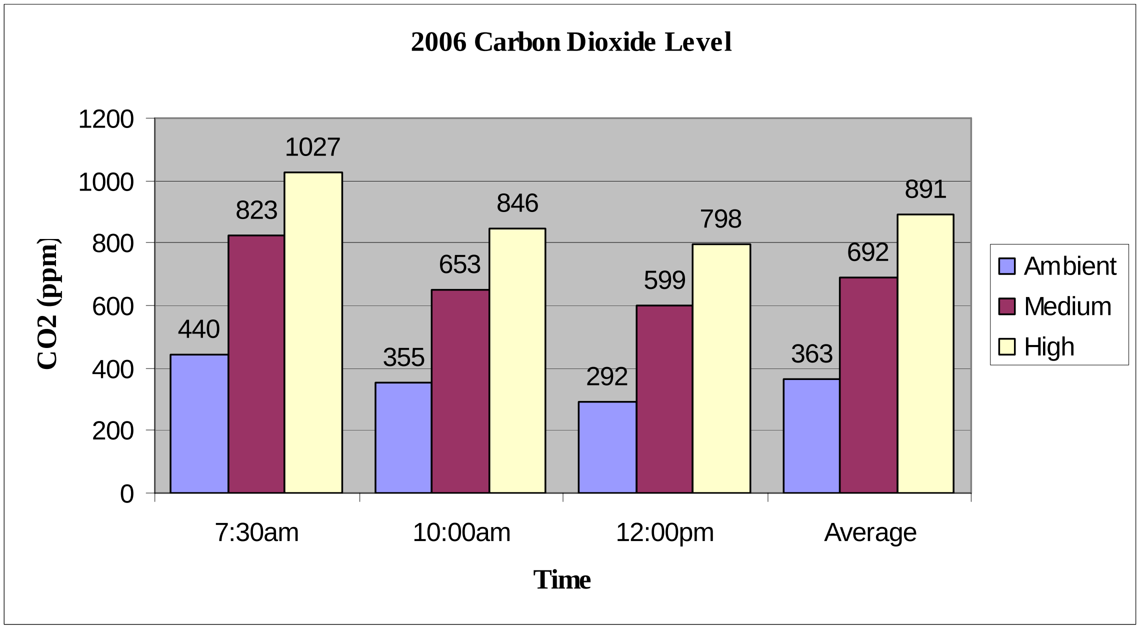

Figure A-1: Average CO_2 levels for the summer of 2006 treatment period at Knoxville, TN; 17 July- 20 August, 2006.

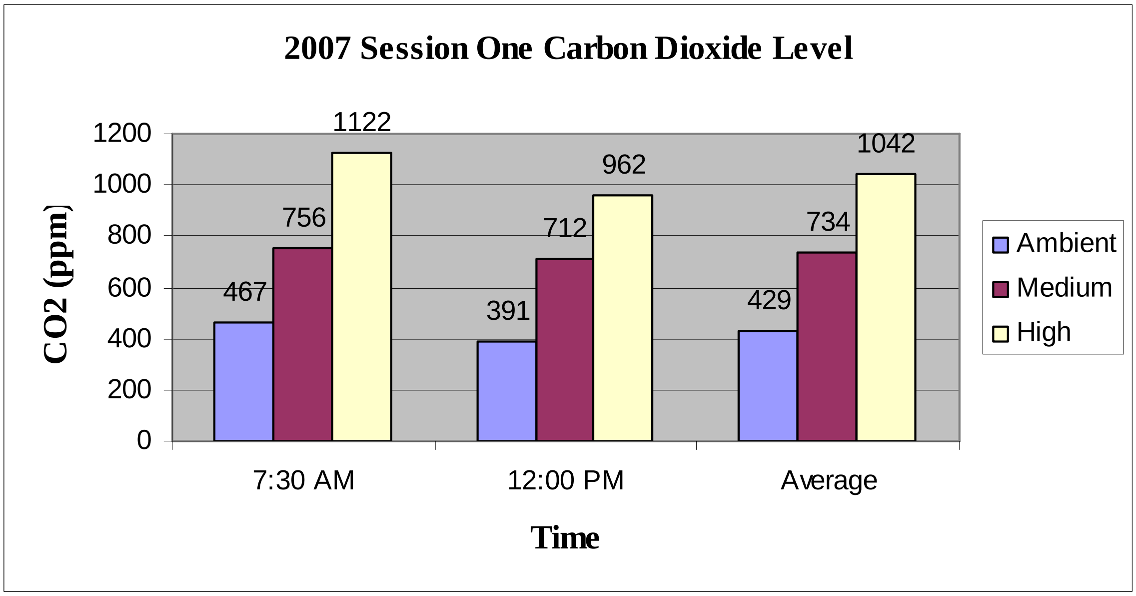

Figure A-2: Average CO_2 levels for the summer of 2007 session one treatment period at Knoxville, TN. 25 June- 19 July, 2007.

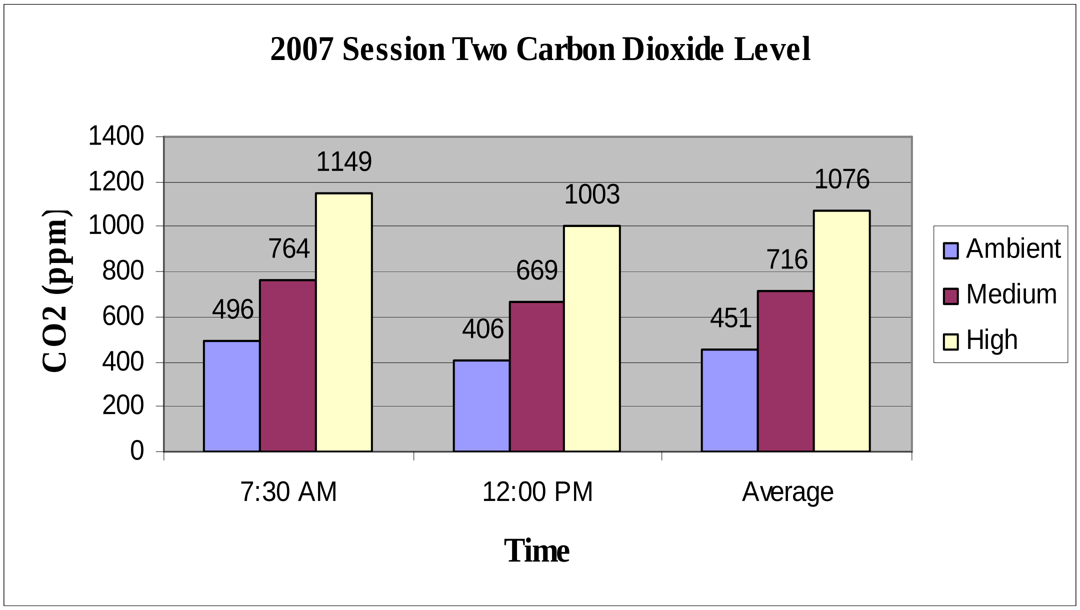

Figure A-3: Average CO_2 levels for the summer of 2007 session two treatment period at Knoxville, TN. 27 July- 20 August, 2007.

Figure A-4: 2006 delivery system at Knoxville, TN; 17 July- 20 August, 2006.

Figure A-5: 2007 delivery system at Knoxville, TN. 25 June- 20 August, 2007.

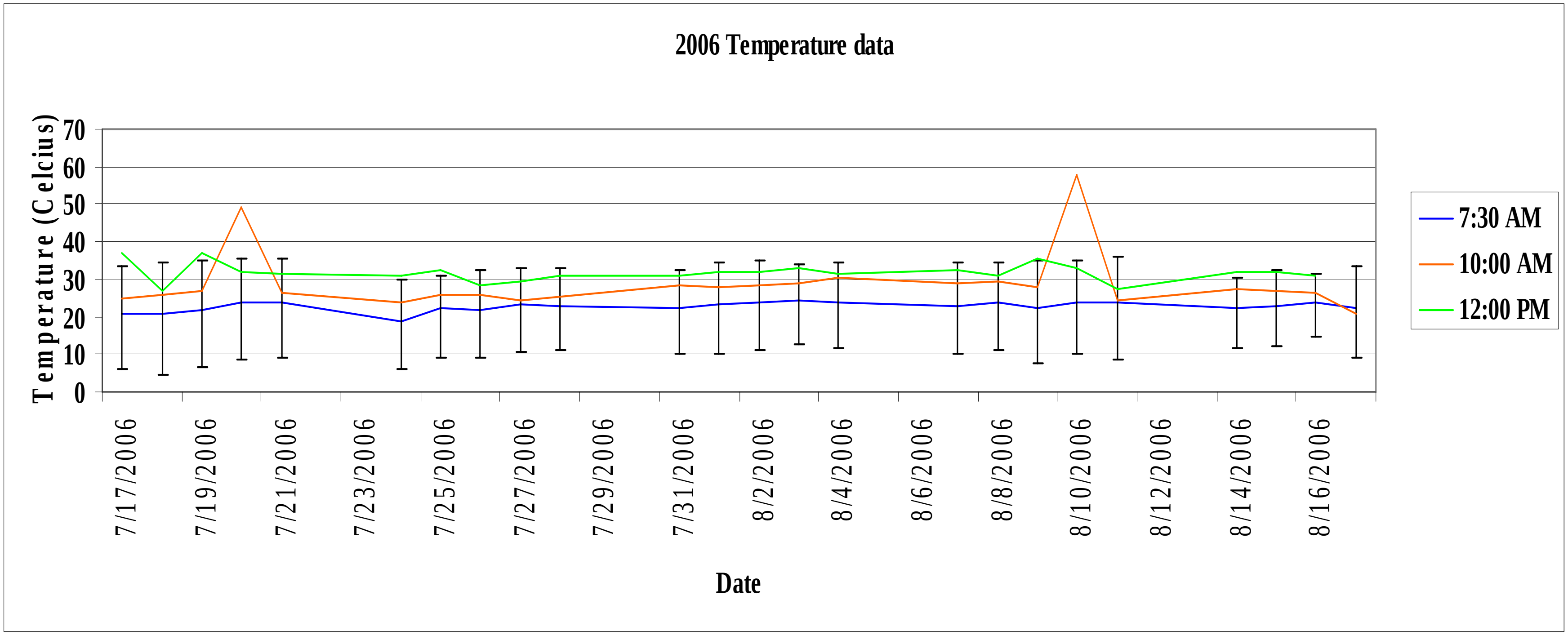

Figure A-6: Regional highs and lows for the 24-hour period (depicted by bars), and observed temperatures within chambers (depicted by three lines for varying times) at Knoxville, TN; 17 July- 20 August, 2006.

* Regional data taken from East Tennessee Research Experiment Center (ETREC)

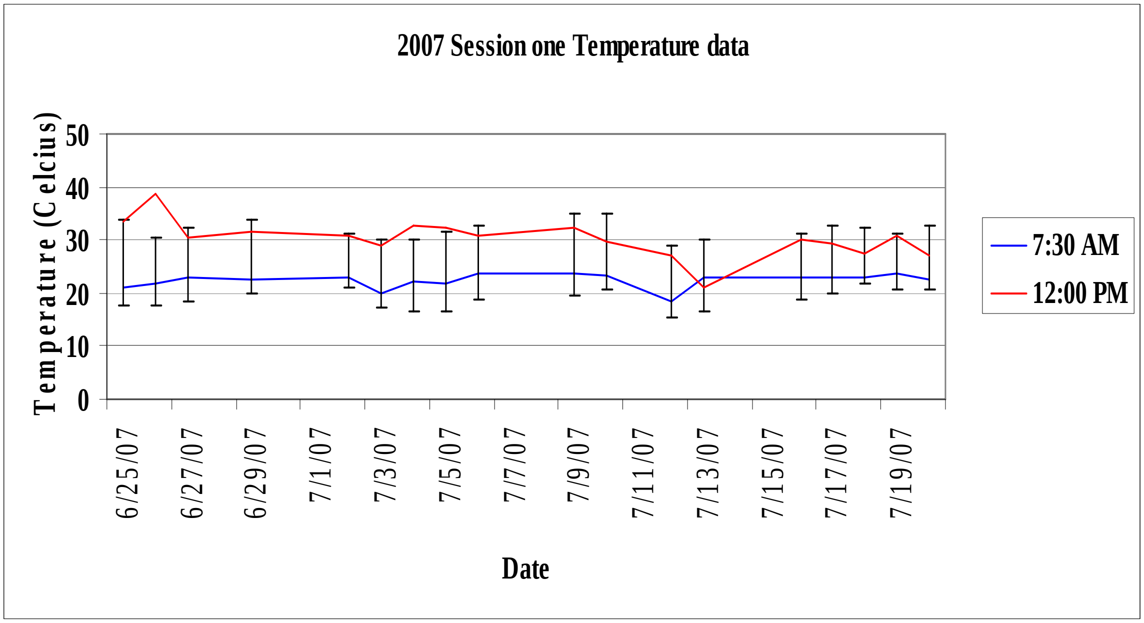

Figure A-7: Regional highs and lows for the 24-hour period (depicted by bars), and observed temperatures within chambers (depicted by three lines for varying times) at Knoxville, TN; 25 June- 19 July, 2007.

* Regional data taken from East Tennessee Research Experiment Center (ETREC)

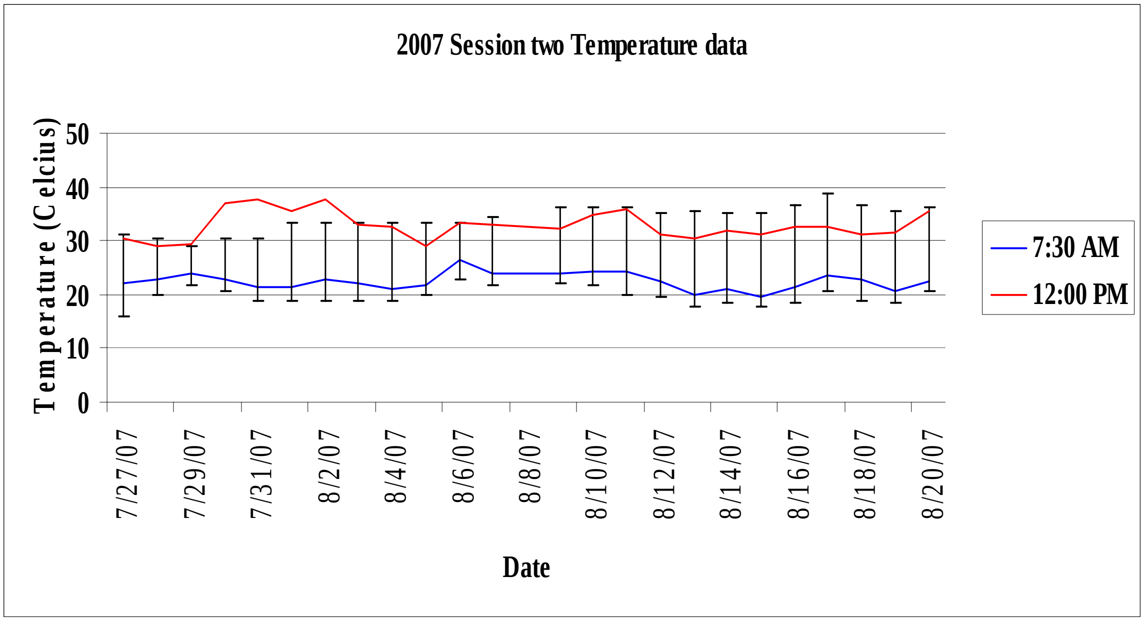

Figure A-8: Regional highs and lows for the 24-hour period (depicted by bars), and observed temperatures within chambers (depicted by three lines for varying times) at Knoxville, TN; 27 July- 20 August, 2007.

* Regional data taken from East Tennessee Research Experiment Center (ETREC)

Figure A-9: Loss of 2007 session one data due to necrosis from water deficit and a severe humidity drop at Knoxville, TN. 25 June- 19 July, 2007.

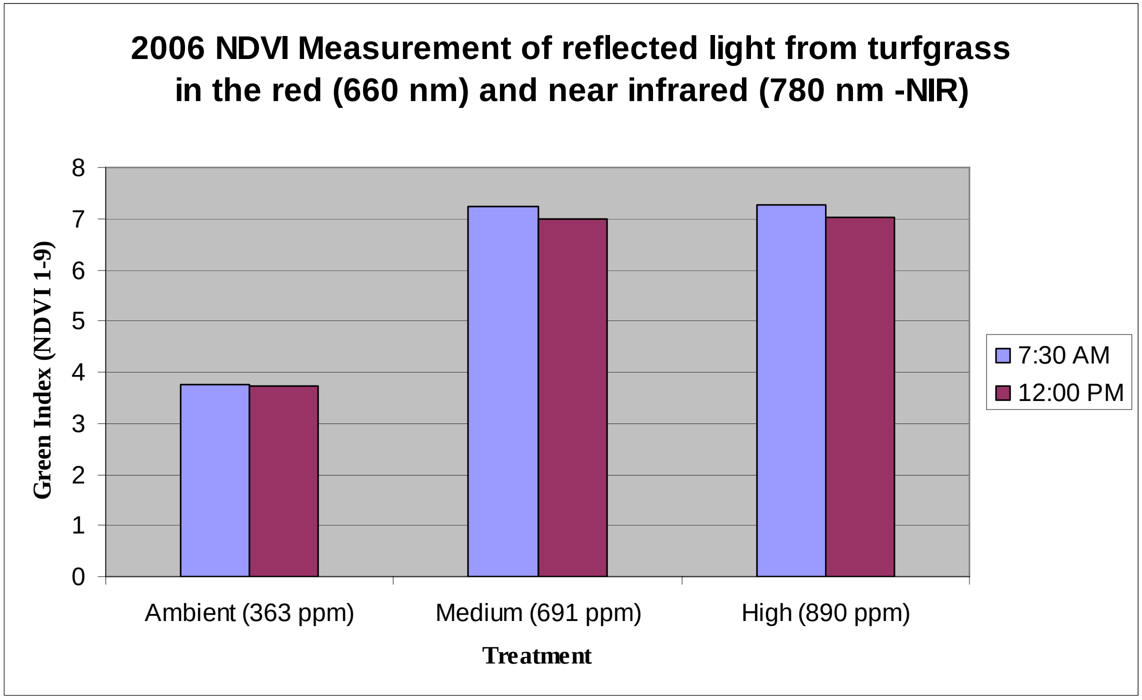

Figure A-10: Effect of elevated CO2 treatments on NDVI (green index) for creeping bentgrass (Agrostis stolonifera L.) at Knoxville, TN; 17 July- 20 August, 2006. No significant differences occurred across all averaged treatment levels. Analyzed with mixed model ANOVA. LSD means separation shown (P<0.05).

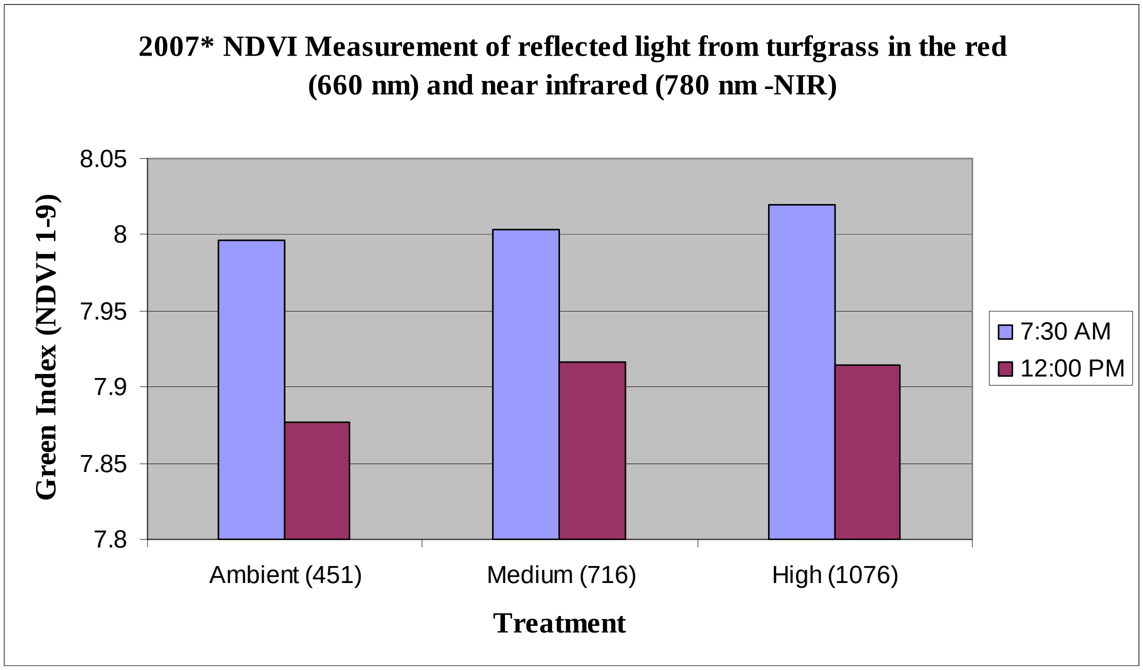

Figure A-11: Figure A-3: Effect of elevated CO2 treatments on NDVI (green index) for creeping bentgrass (Agrostis stolonifera L.) at Knoxville, TN. 27 July- 20 August, 2007. No significant differences occurred across all averaged treatment levels. Analyzed with mixed model ANOVA. LSD means separation shown (P<0.05).
*Data obtained from session two of treatments within 2007

Figure A-12: Condensation observed within chambers deemed to be from too little air flow at Knoxville, TN; 17 July- 20 August, 2006.

Figure A-13: No condensation observed within 2007 chambers due to adequate air flow at Knoxville, TN. 25 June- 20 August, 2007.

Figure A-14: Loss of several plots within session one of 2007 caused the treatments to be halted and data analysis loss at Knoxville, TN. 25 June- 19 July, 2007.

APPENDIX B

TABLES

Table B-1: Means of 2006 total nonstructural soluble carbohydrate analysis of samples with spectrophotometer (NIR at 540 nm) and gas chromatograph at Knoxville, TN. 17 July- 20 August, 2006.

Treatment†	TNC‡ Shoots (mg)	TNC Roots (mg)
Ambient	24.858	13.504
Medium	20.062	13.504
High	28.539	15.604
$LSD_{(0.05)}$	NS§	NS

† = Avg: Ambient, Medium, and High (363, 692, and 891 ppm CO_2, respectively).

‡ = Total nonstructural carbohydrates expressed as an average (mg g^{-1} of tissue).

§ = Means not significant at 0.05 probability level using mixed model analysis of variation for a completely random design ($y_{ij} = \mu + T_i + (T)_{ij}$); SAS 9.1, 2003 SAS Institute Inc., Cary, NC, USA.

Table B-2: Means§ of 2006 normalized difference vegetative index‡ at Knoxville, TN. 17 July- 20 August, 2006.

Treatment†	7:30AM	12:00PM
Ambient	7.2384 B	6.9624 B
Medium	7.251 AB	7.0254 A
High	7.277 A	7.0416 A
$LSD_{(0.05)}$		

† = Avg: Ambient, Medium, and High (363, 692, and 891 ppm CO_2, respectively).

‡ = Normalized difference vegetative index (NDVI = NIR – Red/ NIR + Red; whereas, NIR = Reflectance in the band of 850 +/- 5 nm and Red = Reflectance in the band of 660 +/- 5 nm).

§ Means followed by the same letter do not significantly differ at the 0.05 probability level using mixed model analysis of variation for a completely random design ($y_{ij} = \mu + T_i + (T)_{ij}$); SAS 9.1, 2003 SAS Institute Inc., Cary, NC, USA.

Table B-3: Means of 2007 total nonstructural soluble carbohydrate analysis of samples with spectrophotometer (NIR at 540 nm) and phenol-sulfuric assay for session two treatment period at Knoxville, TN. 27 July- 20 August, 2007.

Treatment†	TNC‡ Shoots (mg)	TNC Roots (mg)
Ambient	25.638	12.197
Medium	18.850	15.341
High	23.078	14.114
$LSD_{(0.05)}$	NS§	NS

† = Avg: Ambient, Medium, and High (451, 716, and 1076 ppm CO_2, respectively).

‡ = Total nonstructural carbohydrates expressed as an average (mg g^{-1} of tissue).

§ = Means not significant at 0.05 probability level using mixed model analysis of variation for a completely random design ($y_{ij} = \mu + T_i + (T)_{ij}$); SAS 9.1, 2003 SAS Institute Inc., Cary, NC, USA.

Table B-4: Means§ of 2007 normalized difference vegetative index‡ for session two treatment period at Knoxville, TN. 27 July- 20 August, 2007.

Treatment†	NDVI 7:30AM	NDVI 12:00PM
Ambient	7.9962	7.8773
Medium	8.0035	7.9162
High	8.0197	7.9146
LSD (0.05)	NS	NS

† = Avg: Ambient, Medium, and High (451, 716, and 1076 ppm CO_2, respectively).

‡ = Normalized difference vegetative index (NDVI = NIR – Red/ NIR + Red; whereas, NIR = Reflectance in the band of 850 +/- 5 nm and Red = Reflectance in the band of 660 +/- 5 nm).

§ Means do not significantly differ at the 0.05 probability level using mixed model analysis of variation for a completely random design ($y_{ij} = \mu + T_i + (T)_{ij}$); SAS 9.1, 2003 SAS Institute Inc., Cary, NC, USA.

Table B-5: Means for pooled ash weights of shoots and roots at Knoxville, TN. 17 July, 2006- 20 August, 2007.

Sample	2006 Ash weight (g)	2007 Ash weight (g)
Shoots	7.96	8.49
Roots	3.09	4.38

VITA

Rodney Vincent Tocco, Jr. was born in Detroit, MI, on October 18, 1982. During his childhood and college years he lived in St. Clair Shores and East Lansing, MI, respectively. He graduated from Michigan State University in May of 2005 with a Bachelor of Science degree in Crop and Soil Sciences. After graduation, Rodney started a Masters of Science program in Plant Sciences at The University of Tennessee, Knoxville, to study turfgrass physiology. He graduated in May 2008.

Rodney plans on pursuing turfgrass research and education while working in a professional setting.