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#### RESPONSE OF WARM SEASON TURFGRASSES TO REDUCED LIGHT ENVIRONMENTS

A Thesis Presented to The Graduate School of Clemson University

In Partial Fulfillment of the Requirements for the Degree Masters of Science Plant and Environmental Science

> by Jeffrey Lansing Atkinson December 2010

Accepted by: Dr. L. B. McCarty, Committee Chair Dr. Haibo Liu Dr. Jim Faust Dr. Joe Toler

#### <u>ABSTRACT</u>

Shade or low light tolerance is an increasingly important issue to turf managers as they are often expected to grow turf in less than ideal agronomic conditions. As permanent structures such as residential buildings add to already problematic shade caused by trees, and other barriers, new solutions are needed to help turf managers provide acceptable turf conditions. The plant growth regulator trinexapac-ethyl (TE) can lessen negative responses of turfgrass to shade.

Two experiments were conducted during the summers of 2008 and 2009 to evaluate various grasses under a reduced light environment (RLE). In the first study, performance of 'Diamond' zoysiagrass in a RLE was evaluated when maintained under putting green conditions. In a second study, performance of various cultivars of zoysiagrass [*Zoysia japonica* Steud.] [*Zoysia matrella* (L.) Merr.] and bermudagrass [*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy] were evaluated under a RLE.

Both studies included three levels of shade: (0, 60, and 90%) and two levels of trinexapac-ethyl (0 and 0.57 kg ai ha<sup>-1</sup> wk<sup>-1</sup> Primo MAXX 1 EC). TE treatments were applied with a CO<sub>2</sub> backpack operating at 189.5 L ha<sup>-1</sup> (20 GPA) with an 8003 flat flan nozzle. Application of shade was initiated on 23 May 2008 and removed 30 October 2008. In 2009 application of shade was initiated 24 May and removed 31 October. Plot size in the first study was 2m by 1.5m. Shade structures were maintained at a height of 45 cm above ground level to maintain proper airflow. Shade tents were removed 2 hours weekly to take measurements and perform maintenance.

ii

In the first study, application of Trinexapac-ethyl to 90% shade increased turf quality by  $\sim 4$  units from 1 to  $\sim 5$  at the end of each study year. While still not commercially acceptable (TQ $\geq$ 7), some turf cover was still preserved. Other plant responses measured included percent lateral regrowth (LR), total non-structural carbohydrates (TNC), clipping yield, ball roll distance, and total shoot chlorophyll. Lateral regrowth increased initially with shade application until plant health declined, leading to decreased LR. Application of TE decreased LR by limiting plant growth. Total non-structural carbohydrates decreased with increasing shade application, and increased with TE application. Clipping yield initially increase in both 60% and 90% RLE, then declined as plant health declined. Application of TE slowed clipping yield production at the beginning of the study, then increased clipping yield at the end due to increase in plant health from TE application compared to treatments not receiving TE. Ball roll distance was decreased by 60% and 90% RLE initially. As plant health in 90% RLE without TE and 90% RLE + TE declined, ball roll distance increased due to declining turf cover. Chlorophyll concentration was increased by both 60% RLE with and without TE. Ninety percent RLE with and without TE reduced chlorophyll concentration.

In the second study, TQ decreased with increasing RLE level in all cultivars. At the end of both years, Diamond and Meyer zoysiagrass demonstrated the highest TQ in a 60% RLE out of all cultivars. In a 90% RLE, Meyer zoysiagrass demonstrated the highest TQ at the end of each year. Application of TE increased TQ of cultivars grown in

iii

60% and 90% RLE. At the end of 2009, Meyer zoysiagrass + TE application was the only cultivar to maintain turf cover in a 90% RLE.

Clipping yield was initially increased in all cultivars by increased levels of RLE. Application of TE decreased clipping yield. As plant health declined, clipping yield also decreased. At the end of the study, TE application increased clipping yield as a result of increased plant health.

Initially, an increase was seen in chlorophyll concentration with increased levels of RLE. Application of TE to RLE treatments further increased chlorophyll concentration. At the end of each study, chlorophyll concentration decreased in 60% and 90% RLE treatments as plant health declined. Once again, as TE application increased plant health, chlorophyll concentration was increased.

## **DEDICATION**

I dedicate this work to my mother, father and sister who have encouraged me to take advantage of every opportunity.

## TABLE OF CONTENTS

TITLE PAGE	i
ABSTRACT	ii
DEDICATION	V
LIST OF FIGURES	viii
LIST OF TABLES	ix
CHAPTER	
1. INTRODUCTION	1
'Diamond' Zoysiagrass	1
Plant Response to Reduced Light Environments	2
Gibberellic Acid	4
Gibberellin Biosynthesis	5
Trinexapac-ethyl	8
2. 'DIAMOND' ZOYSIAGRASS RESPONSE TO REDUCED LIGHT ENVIRONMENTS Introduction	9 9
Materials and Methods	11
Results and Discussion	17
Summary	33
3. RESPONSE OF ZOYSIAGRASS [Zoysia spp.], BERMUDAGRASS [Cynodon spp.], AND SEASHORE PASPALUM [Paspalum vaginatum Sw.] RESPONSE TO REDUCED LIGHT ENVIRONMENTS AND TRINEXAPAC-	

ETHYL	,	35
	Introduction	35
	Materials and Methods	37
	Results and Discussion	42
	Summary	75
APPENDICES		

A. Procedure for Nelson's Assay and Glucose Standard Curves for TNC Analysis	77
B. Chlorophyll Extraction with DMSO	81
REFERENCES	82

## LIST OF FIGURES

Figure		Page
	1. Simplified gibberellins synthesis pathway	7
	A-1. Glucose standard curve for TNC calculation for zoysiagrass	80

## LIST OF TABLES

Table		Page
	1. Analysis of variance (ANOVA) table of a split plot with whole plots arranged in a randomized complete split block design for 'Diamond' zoysiagrass shade application study	13
	2. Average, maximum, and minimum daily total of Photosynthetic Active Radiation (DTP mol $m^{-2} d^{-1}$ ) of three levels of shade (0, 60, 90%) in Clemson, SC during June - October 2008 and 2009	17
	3. Turf quality response means of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009	19
	4. Percentage lateral re-growth response of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009	21
	5. Total non-structural carbohydrate response of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009	23
	6. Clipping yield response means of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009	26
	7. Ball roll distance response means of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009	29
	8. Chlorophyll concentration response means of various reduced light environment (RLE) and trinexapac-ethyl (TE) treatments on 'Diamond' Zoysiagrass from May to October 2008 and 2009	32
	9. Analysis of variance (ANOVA) table of a combined analysis for six randomized complete block design studies.	40
	10. Average, maximum, and minimum daily total of Photosynthetic Active Radiation (DTP mol $m^{-2} d^{-1}$ ) (PAR) of three levels of shade (0, 60, 90%) during June - October 2008 and 2009 in Clemson, SC	42

11. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments and trinexapac-ethyl treatments from June through October 2008.	4
12. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl treatments within reduced light environment (RLE) treatments from June through October 2008	5
13. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments and trinexapac-ethyl treatments from June through October 2009	5
14. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl treatments within reduced light environment (RLE) treatments from June through October 2009.	5
15. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008	6
16. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to trinexapac-ethyl (TE) within reduced light environment (RLE) treatments from June through October 2008	6
17. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2009	6
18. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and	

'SeaIsle Supreme' seashore paspalum to trinexapac-ethyl (TE) treatment within reduced light environment (RLE) treatments from June through	
October 2009	67
19. Chlorophyll concentration response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl (TE) treatments within reduced light environment (RLE) treatments from June through October 2008 and 2009	71
20. Chlorophyll concentration response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl (TE) treatments within reduced light environment (RLE) treatments from June through October 2008 and 2009	73

## CHAPTER 1 INTRODUCTION

#### 'Diamond' Zoysiagrass

Zoysiagrass is a warm-season grass native to China, Japan and other parts of Southeast Asia (Duble 1989). Due to its excellent wear tolerance, unique blue green color, salt tolerance, better shade tolerance and lower fertility requirements than bermudagrass, zoysiagrass is being more widely used on golf courses. Compared to bermudagrass, zoysiagrass possesses a slower rate of regrowth from injury due to slower lateral growth (McCarty 2011). A highly stoloniferous grass, zoysiagrasses are susceptible to thatch and organic matter accumulation that can potentially provide environments ideal for disease development. An excessive thatch layer and excessive N application can cause a decrease in low temperature tolerance in zoysiagrass and reduces watering efficiency (Fry and Huang 2004).

*Zoysia* is a genus with two species commonly used for turf. Meyer Zoysiagrass (*Zoysia japonica* Steud.) is a popular turfgrass species utilized by many turf managers in the transition zone. Although possessing improved shade tolerance when compared with bermudagrass [*Cynodon dactylon* (L.) Pers.], Meyer zoysiagrass still exhibits reduced functionality in heavily shaded areas (Ervin 2002). Compared to *Zoysia japonica, Zoysia matrella* L. Merr. is generally regarded as being more shade tolerant species. "Diamond," a variety of *Zoysia matrella* released in 1996 by the Texas Agricultural

Experimental Station, has shown acceptable turf quality under 73 percent shade (Qian and Engelke 1999). With its improved turf characteristics, such as decreased shoot width and increased shoot density, "Diamond" zoysiagrass may be a possible alternative for use by turf managers on putting green surfaces (Qian and Engelke 1999).

In the same study, "Diamond" zoysiagrass showed more vertical shoot growth when shaded at 87 percent compared to the "Diamond" grown in full sun. With frequent trinexapac-ethyl applications, excessive vertical growth was significantly inhibited. Turf quality also improved with trinexapac-ethyl applications along with enhanced color, tiller number and turf density (Qian and Engelke 1999).

#### Plant Response to Reduced Light Environments

As a  $C_4$  subtropical species, zoysiagrass depends on higher light intensities to supply enough energy to perform photosynthesis without a depletion of carbohydrate reserves. Compared to  $C_3$  turfgrasses,  $C_4$  turfgrasses are more efficient consumers of carbon dioxide ( $CO_2$ ) and water ( $H_2O$ ), allowing for photosynthesis to continue in hot environments that cause  $C_3$  plant stomata to close, reducing the concentration of  $CO_2$ inside the plant available for fixation (Sinha 2004). To perform the  $C_4$  photosynthetic pathway, two additional ATP molecules are needed compared to the  $C_3$  pathway (Sage and Monson 1999). The higher demand for ATP requires higher levels of light intensity to reach maximum photosynthetic capability in a  $C_4$  species (Cooper 1970). If  $C_4$  plants are grown in a reduced light environment (RLE) the light intensity may not be adequate

to perform the required amount of photosynthesis to keep up with the depletion of carbohydrate reserves. A common plant avoidance mechanism to a RLE is an increase in stem elongation (Gawronska et. al 1995). In 1986, Ingram found a strong correlation between gibberellin  $A_1$  (GA<sub>1</sub>) concentration and internode length. Tan and Qian (2003) found 'KenblueTimes', 'Livingston', and 'NuGlade' Kentucky bluegrass (Poa pratensis L.) in a 73% RLE demonstrated a 44% to 47% increase of GA<sub>1</sub> concentration. Enhanced shoot elongation and stem internode length is a shade-avoidance mechanism that causes the depletion of plant carbohydrate concentration at a pace quicker than the plant can replenish (Burton 1959). Depletion of plant carbohydrate concentration results from the removal of increased amounts of plant tissue from mowing due to increased vertical growth. Accelerated depletion of plant energy reserves leads to decreased rooting and tillering with an overall decrease in turf quality (Qian et. al., 1998). Another morphological plant response is an increase of leaf surface area in order to intercept more sunlight. In turf, this translates into wider leaf blades that are detrimental to putting surface quality.

Physiological responses are also seen by plants in a RLE. As light travels through the upper tree canopy, much of the photosynthetic active radiation (PAR) spectrum (400-700 nm) is filtered out while more of the far-red portion of the spectrum (700-750 nm) is able to pass through, increasing the abundance of far-red light at ground level in a RLE. Since far red light is primarily absorbed by photosystem I, some plants have adapted to a RLE by increasing the ratio of photosystem II to photosystem I from 2:1 to 3:1 in order to maintain a better balance of energy flow in an environment of abundant far red light

(Anderson 1986). Other shade species have adapted by increasing the number of antennae chlorophyll in photosystem II to also maintain a balance of energy flow through photosystem I and II (Melis 1996).

#### Gibberellic Acid

Gibberellins (GA) are a group of tetracylic diterpenoids best known for their promotion of stem elongation (Taiz and Zeiger 2006). Brian and Hemming showed in 1955 that the application of GA to pea seedlings increased the growth rate of shoots relative to the amount of GA applied. GAs are also involved in seed germination, transition to flowering, anther development, pollen tube growth, floral development and seed development (Taiz and Zeiger 2006). During plant development, changes in GA concentration of different tissues and organs occur. High GA levels are usually correlated with phases of active growth (Mohr et. al. 1995). In plants, GA appears in many chemically similar forms. The basic structure of the more than 80 identified GAs is the tetracyclic ring system of the *ent-gibberellan* with two rings of six C atoms and two rings of five C atoms, often supplemented by an additional lactone ring (Mohr et. al. 1995). All GAs that have been chemically characterized are assigned an "A" number. This number does not represent a chemical relationship; instead it represents the order of discovery (Hopkins 1995). Most plants contain many forms of GA while the function of these are mostly unknown. It is hypothesized that many forms are intermediates in GA synthesis. Many forms of GA can be found in Poaceae species. GA<sub>53</sub>, GA<sub>44</sub>, GA<sub>19</sub>, GA<sub>17</sub> and GA<sub>1</sub> have been identified from leaf tissues of *Poa pratensis* L. cv. Holt; however,

early studies of activity and metabolism of GAs in Poaceae species have found  $GA_1$  as the biologically active GA for vegetative growth (Ingram 1986).

#### **Gibberellin Biosynthesis**

Gibberellins (GA) are diterpenoid acids chemically related to other terpenoids. The biosynthesis of GA can be divided into three parts, each occurring in a different cellular compartment (Taiz and Zeiger 2006) (Fig. 1). Because of the terpenoid relation, the first stage of GA synthesis, occurring in the plastids, is the mevalonic acid pathway that leads the formation of other terpenoids. In this step, a 5-carbon isoprenoid unit isopentenylpyrophosphate (IPP) is synthesized from acetyl coenzyme A (acetyl-CoA). Isoprene units are then added to the IPP successively to a  $C_{20}$  geranylgeranylpyrophosphate (GGPP). At this point the biosynthesis process is not yet GA specific. GCPP is a precursor to many terpenoids compounds found in plants. The conversion of GCPP to *ent*-kaurene is the first step that is GA biosynthesis specific. The second stage occurs in the endoplasmic reticulum. In this stage an oxidation reaction occurs that converts *ent*-kaurene to a carboxylic acid. A contraction of *ent*-kaurene's 6 carbon ring to a 5 carbon ring converts *ent*-kaurene to GA<sub>12</sub>-aldehyde. GA<sub>12</sub>-aldehyde is oxidized to form GA<sub>12</sub> the first gibberellin in the pathway and the precursor to all other forms of GA.

The third step of GA biosynthesis is the conversion of  $GA_{12}$  to all other forms of GAs. This is a multiple step process occurring in the cytosol by a group of soluble dioxgenases. Also included in this step is the up or down regulation of active forms of

GA biosynthesis. (Hopkins 1995 ; Taiz and Ziegler 2006). The first two steps of the GA biosynthesis pathway are the same in all plants. The third step can vary between genera and even vary between different tissues of the same plant (Hopkins 1995). Regulation of the GA biosynthetic pathway is important so that excessive stem elongation does not occur.

Regulation is achieved through the up or down regulation of genes that encode enzymes responsible for the conversion of  $GA_{20}$  to the biologically active form of  $GA_1$ and the conversion of  $GA_1$  to non-biologically active forms of GA, mainly  $GA_8$ . The three main enzymes in the regulation of GA biosynthesis are GA 20-oxidase (GA20ox), GA 3-oxidase (GA3ox), and GA 2-oxidase (GA2ox). To maintain hormone homeostasis, the presence of bioactive GA triggers a down regulation of the gene encoding GA20ox and GA3ox enzymes which are responsible for the final conversion of the inactive form of  $GA_{20}$  to the biologically active form of  $GA_1$ . When more GA deactivation is needed, the gene that encodes the GA2ox enzyme is up-regulated. GA2ox is responsible for deactivation of  $GA_1$  to inactive forms of GA (Taiz and Zeiger 2006).

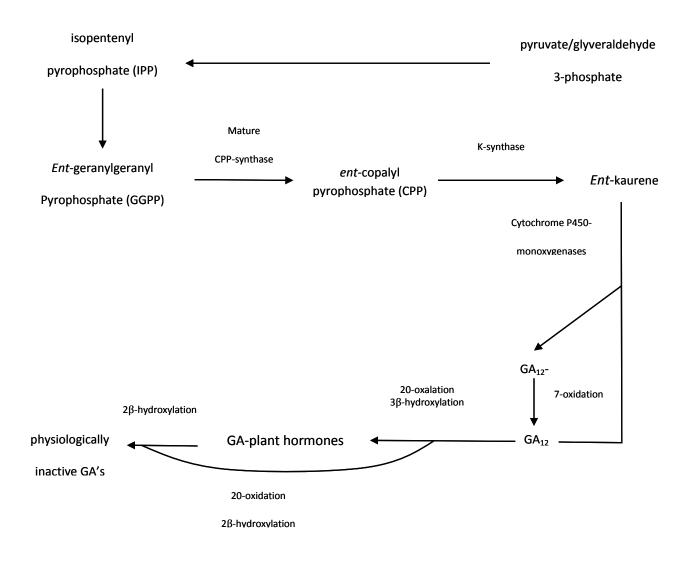


Fig. 1. Simplified gibberellin synthesis pathway (Hopkins 1995).

#### Trinexapac-Ethyl

Trinexapac-ethyl (TE) is a commercial anti-gibberellin plant growth regulator (PGR) that inhibits formation of 3ß-hydroxylase (Adams et al., 1992). Inhibition of 3ßhydroxylase formation blocks conversion of GA<sub>20</sub> to GA<sub>1</sub>, the active form of gibberellin (Adams et al., 1992). In Kentucky bluegrass, application of TE has shown a reduction of GA<sub>1</sub> content by 49% and an increase in GA<sub>20</sub> content by two fold (Tan and Qian 2003). Fagerness and Penner (1998) noted the largest amount of TE absorption occurred at leaf sheaths at the base of the plant with a maximum absorption level of 94%. The reduction of vertical shoot growth under shade conditions preserves canopy density and leaf color, increasing photosynthesis and carbohydrates (Engelke and Qian 1999). With increased photosynthesis and carbohydrate production, plants are better able to cope with reduced light.

#### **CHAPTER 2**

## 'DIAMOND' ZOYSIAGRASS [Zoysia matrella (L.) Merr.] RESPONSE TO REDUCED LIGHT ENVIRONMENTS

#### Introduction

Shade or low light tolerance is an increasingly important issue to turf managers as they are often expected to grow turf in less than ideal agronomic conditions. As permanent structures such as residential buildings add to already problematic shade produced by trees, and other barriers, new solutions are needed to help turf managers provide acceptable turf conditions.

Plants require light to perform photosynthesis. In a reduced light environment (RLE), plants respond with elongated stems, increased upright growth, lengthened leaf sheaths, increased root-to-shoot ratios, decreased leaf thickness and reduced tillering (Taiz and Zeiger 2006). With increases in upright growth and elongated stems, carbohydrate reserves are stressed through tissue removal from increased mowing frequency. With depleted carbohydrate reserves and reduced tillering, turf plants are less able to recover, increasing susceptibility to environmental stresses and other damaging forces such as traffic. Engelke and Qian (1999) noted an increase in gibberellic acid (GA) levels contributed to excessive shoot growth in pea (*Pisum sativum* L.) plants. In turfgrasses, GA inhibitors reduce vertical growth and control internode elongation (Ervin et. al. 2002).

Although improvements have been made in low light tolerance for certain bermudagrass cultivars, progress has been inadequate to solve shade issues, especially on closely mowed putting surfaces. Zoysiagrass species have shown better low light tolerance compared to bermudagrass (Bunnell, et. al. 2005). As zoysiagrass varieties improve, their tolerable mowing heights are shortened; allowing for the possibility of use on putting surfaces. Past research illustrates zoysiagrass's superiority over bermudagrass regarding shade tolerance when maintained at higher mowing heights; however, little research exists evaluating the species when maintained in a reduced light environment under putting green conditions.

#### Materials and Methods

An experiment was conducted during the summers of 2008 and 2009 to evaluate the performance of 'Diamond' zoysiagrass [*Zoysia matrella* (L.) Merr.] in a Reduced Light Environment (RLE) when maintained under golf course putting green conditions.

Location was the Clemson University turf plots in Clemson, South Carolina. All research was conducted on a 'Diamond' zoysiagrass research putting green established during the summer of 2007. The study included three levels of RLE: none (0%), low (60%), and high (90%). The three levels of shade were applied continuously to all plots using a neutral density, poly-fiber black shade cloth (model no. SC-ST60 and SC-ST90; International Greenhouse, Sidel, IL). The study was conducted during a 22-week period beginning 4 weeks prior to the summer solstice and ending 18 weeks past it. Timing coincided with maximum seasonal growth of zoysiagrass. In year one, applications began on May 23, and ended on October 30. In year two, applications began on May 24, and ended on October 31. Being a longitudinal study, RLE treatments were imposed on the same pots for the two year study to determine long term effects of shade on 'Diamond' zoysiagrass.

Two treatments were within each level of shade. These two included the application of trinexapac-ethyl (TE) with a CO<sub>2</sub> backpack sprayer operating at 189.5 L ha<sup>-1</sup> (20 GPA) at a rate of 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup> (1.5 oz/1000ft<sup>2</sup> wk<sup>-1</sup> of a 1EC formulation) and an untreated control. TE was first applied on the initial date of imposing shade with repeat applications every two weeks.

Low and high RLE applications were administered with a neutral density, polyfiber black shade cloth removing 60% and 90% full sunlight respectively. Percent shade was determined by comparing PPF under the shade cloths to full sun at turf canopy level using a LI-28663 quantum light sensor (LiCor, Inc., Lincon, NE) [(PAR<sub>full sun</sub> – PAR<sub>under</sub> shade cloth)/PAR<sub>full sun</sub>] × 100. Measurements were recorded twice yearly on a clear, cloudless day at solar-noon.

Individual shade cloth tent frames were 2 m by 1.5 m, constructed with 2.5 cm diameter polyvinyl chloride (PVC) pipes. Shade tents were fixed 45 cm above ground level by 60 cm legs placed 15 cm into the ground to prevent movement due to environmental conditions. Shade cloths were attached to PVC frames with zip-ties so as to keep shade cloths at a consistent height above ground level.

Cultural practices requiring the removal of shade cloth tents were performed with all tents removed for the same amount of time and did not exceed two hours of additional full sunlight wk<sup>-1</sup>. Fertilizer was applied monthly at a rate of 24.21 kg N ha<sup>-1</sup> with a 20N-8.8P-16.6K (Harrell's, Lakeland, FL) fertilizer throughout the growing season. Mowing height was maintained at 3.18 mm by a walk-behind commercial mower (John Deere, model 180C, Moline, Illinois). Plots were mowed 6 times wk<sup>-1</sup> and clippings removed. Plots were irrigated three times weekly, and hand watered on an as needed basis to prevent stress. Aerification was preformed once yearly using 1.59 cm inside diameter tines at a spacing of 5.08 cm by 5.08 cm. No thatch removal was preformed through the duration of the study. Monitoring for pests was conducted bi-weekly. No treatments were necessary for pest control.

#### Statistical Design

Statistical design was a split plot with whole plots arranged in a randomized complete block design. Whole plots were three levels of shade, (0, 60 and 90%), and split by trinexapac-ethyl treatment. Studies were conducted during the summers of 2008 and 2009. Data was analyzed separately for each year due to effects on turf from the 2008 study year. All data were analyzed using analysis of variance (ANOVA) at a significance level of 0.05 (Table 1). The General Linear Model procedure (GLM) of SAS (SAS institute, 2005) was used for all separations.

Table 1. Analysis of variance (ANOVA) table of a split plot with whole plots arranged in arandomized complete split block design for 'Diamond' zoysiagrass shade application study.

Source		Degrees of Freedom
Block		2
Shade		2
Error A		4
Trinexapac-ethyl		1
Trinexapac-ethyl*Shade		2
Error B		6
	Total	17

#### **Measurements**

Daily light integral (DLI) was recorded using a model 305 datalogger fitted with a quantum light sensor (Spectrum Technologies, Inc, Plainfield, II). Readings were recorded every minute and reported as mol  $m^2 d^{-1}$ . Total DLI was averaged over both growing seasons. DLI for RLE treatments was calculated by subtracting 60% and 90% of full sun DLI for the respective RLE treatments.

Turf quality was visually measured on a 1-9 scale, with 1 representing dead turf and 9 representing flawless turf. A TQ rating <7 was deemed unacceptable.

Total non-structural carbohydrates (TNC) of below ground tissue, including roots and rhizomes were measured on the last day of the study for both years. Two samples 2.5 cm in diameter to a depth of 6.5 cm were extracted per plot and kept separate for evaluation. All samples were taken before sunrise to prevent diurnal fluctuations in carbohydrate levels. Harvested roots and rhizomes were washed free of sand and organic matter. Washed roots were wrapped with aluminum foil and frozen at -75 ° C to cease biological activity and stored at this temperature for 14 days. After 14 days, a 50 mg dry tissue sample was obtained from each stored package and placed into a 13 x 100 mm test tube and re-hydrated with 100  $\mu$ L of an 80% ethanol solution. Two mL of 0.1 M sodium acetate buffer was added to maintain a consistent pH. Test tubes were placed in boiling water for one hour, allowed to cool, and then placed in boiling water a second time to stop any extraneous enzyme activity. After test tubes cooled, 1.0 mL enzyme solution, invertase and amyloglucosidase was added and allowed to incubate for 3 days at 40-45° C. Samples were vortexed 3 times daily. Following post incubation period, samples were allowed to settle until clear. Twenty-five  $\mu$ L of aliquot was removed for TNC analysis.

Nelson's Assay was used to measure TNC, quantifying the reducing sugars, glucose and fructose, in plants. A copper reagent and an arsenomolybdate color reagent were added to each sample solution to quantify reducing sugars. The arsenomolybdate color reagent reacts with the reduced copper to produce a stable change in photo-transmission of the solution. This allows for the quantification of reducing sugars with the use of a spectrophotometer at 520 nm wavelength when compared to known reducing sugar values. A complete description of the procedure can be found in Appendix A.

Percentage lateral re-growth (RG) was evaluated by removing a 10.8 cm diameter plug from each plot at the initiation of the study. Holes were backfilled with a sand:peat media similar to the original media. A wire grid was constructed to match the removed 'Diamond' zoysiagrass plug. The wire grid was placed over the plugged area in each plot, and a shoot observed in a grid square denoted a hit. Percent lateral re-growth was calculated as number of hits divided by total number of squares in grid (total hits/total squares) multiplied by 100.

Clipping yield was collected once every two weeks from  $1.1 \text{ m}^2$  of each plot. Collected clippings were dried at  $70^{\circ}$  C for 24 hours, weighed, and converted into g m<sup>2-1</sup>.

Chlorophyll content was measured 10 and 20 weeks after implementation of shade treatments. Fresh clippings were collected from individual plots, and chlorophyll was extracted using dimethyl sulfoxide (DMSO). The DMSO method can be performed without grinding or maceration of the plant tissue (Hiscox and Israelstam 1979). A

spectrophotometer was used at 645 and 663 nm to determine chlorophyll content as suggested by the Arnon Equation (Arnon 1949). Details of the procedure can be found in Appendix B.

Putting greens speed (distance) was measured using a stimpmeter. A flat surface approximately 0.93 m<sup>2</sup> was selected for each treatment. The tapered end of the stimpmeter was placed at the edge of the area. The notched end was then raised until the ball began to roll down the stimpmeter. Once the ball began to roll, the stimpmeter was held steady at the position of rolling initiation. The stopping point of the ball was marked. This process was repeated three times at 90° angles on each individual replicate with the distances of each ball roll averaged (USGA 2008).

#### **Results and Discussion**

#### Daily Light Integral

Daily light integral (DLI) maximum, minimum, and average were determined

each year for each shade level. DLI measures light quantity and ranged from 44.95 to

 $4.495 \text{ mol m}^{-2} \text{ d}^{-1} \text{ in } 2008 \text{ and } 43.18 \text{ to } 4.32 \text{ mol m}^{-2} \text{ d}^{-1} \text{ in } 2009.$ 

Table 2. Average, maximum, and minimum daily total of Photosynthetic Active Radiation (DTP mol  $m^{-2} d^{-1}$ ) of three levels of shade (0, 60, 90%) in Clemson, SC during June - October 2008 and 2009.

		Daily PAR Total					
		% Shade					
Year	Quantity	0	60	90			
		-		$- mol m^{-2} d^{-}$			
			1				
2008	Mean	44.95	17.98	4.495			
	Maximum	67.5	27	6.75			
	Minimum	4.5	1.8	0.45			
2009	Mean	43.18	17.27	4.32			
	Maximum	67.7	27.08	6.17			
	Minimum	3.6	1.44	0.36			

<sup>†</sup> DTP of 2008 Study measured from May 20 – October 31; 2009 study from May 21 – October 31.

<sup>‡</sup>Maximum and minimum DTP for 2008 occurred on July 4 and October 24, respectively. In 2009, maximum and minimum DTP occurred on June 23 and October 27, respectively.

#### Turf Quality

Turf quality (TQ) was impacted by shade level, trinexapac-ethyl (TE) treatment, and year. In 2008, a 90% reduced light environment (RLE) caused a reduction in TQ from 7.4 in the untreated to 5.9 during July. The application of TE increased TQ in 90% RLE from 5.9 to 6.6. In August, 90% RLE without TE application reduced TQ from 8.3 in 0% RLE without TE to 3.8. Application of TE increased TQ in a 90% RLE from 3.8 to 5.6. Full sun and 60% RLE treatments both with and without TE maintained commercial acceptable TQ throughout 2008. These trends continued through the duration of the study year. At the final rating, 90% RLE treatments not receiving TE were absent of turf cover. Application of TE to 90% RLE increased TQ by ~4 points (Table 3).

In 2009, TE application continued to improve TQ. Throughout the 2009 study, 90% RLE treatments without TE were without turf cover. TE application to 90% RLE increased TQ by ~5 points throughout the 2009 study year. All 0% and 60% RLE treatments continued to have statistically similar TQ until September 2009 when 60% RLE without TE reduced TQ from 8.5 to 7.5 when compared to the untreated. TE application to 60% RLE improved TQ from 7.5 to 8.3. In the final month of the 2009 study year, 60% RLE TQ continued to deteriorate while the application of TE improved TQ from 5.6 to 7.9. Sixty percent RLE with the application of TE and 0% RLE with and without TE showed similar TQ throughout both study years (Table 1).

		—Ju	ine	J	uly——	—Au	gust—	—Septe	ember—	—Oct	tober—
Year	RLE Level	-TE††	+TE‡‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						1-9					
2008	0%	7.3§	7.5	7.4	7.6	8.3	8.4	7.6	7.5	8.0	7.8
	60%	7.9	7.9	8.2	8.2	8.5	8.4	8.1	7.8*	8.3	7.9
	90%	7.3	7.6	5.9†	6.6*†	3.8†	5.6*†	1.8†	5.3*†	1.0†	4.8*†
2009	0%	8.2§	8.2	8.3	8.3	8.3	8.3	8.5	8.4	8.3	8.3
	60%	7.9	8.3	8.2	8.3	7.7	8.3	7.5†	8.3*	5.6†	7.9*
	90%	1.0†	6.2*†	1.0†	6.3*†	1.0†	5.8*†	1.0†	6.2*†	1.0†	6.0*†

Table 3. Turf quality response means of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

\* Denotes a significant difference between TE treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

† Denotes a significant difference compared to 0% RLE within TE treatments according to Fisher's LSD (α=0.05) test.

 $\dagger$ † -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup> wk<sup>-1</sup>.$ 

§ Turf quality rated from 1 - 9 where 9 = best turf and values <7.0 are unacceptable.

#### Lateral Regrowth

Analysis of LR revealed interaction between shade level, TE treatments, and years (P< 0.05); therefore, results are presented and discussed separately for each year. In 2008 TE application reduced LR 22% in 60% reduced light environments (RLE) and 12% in 90% RLE in July. In August, 90% RLE reduced LR by 18% compared to 0% RLE. TE application decreased LR 21% in 60% RLE and 17% receiving 90% RLE. LR receiving 90% RLE declined steadily through the rest of the 2008 study year as turf cover declined (Table 4).

In 2009, all treatments had similar LR with exception of 90% RLE without TE. The lack of LR in 90% RLE without TE resulted from dead turf caused by shade application in 2008. LR was reduced 10% in October by TE application in 60% RLE (Table 4).

	• • • •	——Ju	ne	Jı	ıly——	—Au	gust—	—Septe	mber—	—Oct	ober—
Year	RLE Level	-TE††	+TE <b>‡‡</b>	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						%					
2008	0%	39	33	71	58*	92	83	97	93	98	98
	60%	42	36	76	59*	97	76*	98	84	96	88*
	90%	44	41	66	59	83	68*	67†	76†	0†	76*†
2009	0%	69	61	75	76	95	84	98	89	98	91
	60%	65	63	76	78	92	83	100	85*	100	90*
	90%	0†	52*	0†	70*	0†	78*	0†	83*	0†	86*

Table 4. Percentage lateral re-growth response of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

\* Denotes a significant difference between TE treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  Denotes a significant difference compared to 0% RLE within TE treatments according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$ <sup>†</sup> -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup> wk<sup>-1</sup>.$ 

#### Total Non-Structural Carbohydrates

Analysis of TNC concentrations revealed interaction between shade level, TE treatments, and years (P< 0.05); therefore, results are presented and discussed separately for each year. In 2008, all treatments receiving TE had similar TNC concentrations. Zero and 60% RLE without TE also had similar TNC concentrations, however, 90% RLE without TE did not have any measureable carbohydrates after the 2008 study year. The application to TE to 90% RLE increased TNC concentration from 0 to 35 mg g<sup>-1</sup>.

In 2009, 0% and 60% RLE had similar TNC concentration while 90% RLE did not have any measurable carbohydrates. Treatments receiving 0% and 60% RLE + TE had similar TNC concentrations while 90% RLE + TE had a 45% reduction compared to 0% RLE + TE. Trinexapac-ethyl + 0% RLE in 2009 increased TNC concentration by 27% compared with 0% RLE without TE and increased TNC concentration in 90% RLE from 0 to 19.44 mg g<sup>-1</sup> (Table 5).

	20	08	2	009
RLE Level	- TE††	+ TE‡‡	- TE	+ TE
		mg g	-1	
0%	36.0§	38.0	27.9	35.5*
60%	35.2	37.0	34.0	34.8
90%	$0.0^{+}$	34.9*	0.0†	19.4* <b>†</b>

Table 5. Total non-structural carbohydrate response of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

\* Denotes a significant difference between TE treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

<sup>†</sup> Denotes a significant difference compared to 0% RLE within TE treatments according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger \dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup> wk<sup>-1</sup>.$ 

§ Total non-structural carbohydrates were measured at the end of each study year.

#### **Clipping Yield**

Analysis of clipping yields revealed interaction between shade level, TE treatments, and years (P< 0.05); therefore, results are presented and discussed separately for each year. In July 2008, shade increased clipping yield in 60% reduced light environment (RLE) treatments with and without TE application compared to 0% RLE with and without TE, respectively. Application of TE in 60% RLE treatments reduced clipping yield from 2.0 to 1.4 g m<sup>-2</sup>. Compared to 0% RLE, clipping yield was increased in 90% RLE treatments without TE from 0.9 to 2.92 g m<sup>-2</sup> and increased from 0.8 to 1.5 g m<sup>-2</sup> in 90% RLE treatments with TE. Application of TE in 90% RLE treatments reduced clipping yield by from 2.9 to 1.5 g m<sup>-2</sup> (Table 6).

In August 2008, 60% and 90% RLE treatments continued to increase clipping yields by from 1.0 g m<sup>-2</sup> in the untreated to 3.1 and 4.4 g m<sup>-2</sup>, respectively. TE application reduced clipping yield in 60% RLE from 3.1 to 1.6 g m<sup>-2</sup> and from 4.38 to 2.5 g m<sup>-2</sup> in 90% RLE. These trends continued through the end of the study year. The last measurement showed a reduction in clipping yield by 90% RLE without TE due to a decline in turf cover. Sixty percent RLE without TE continued to have higher clipping yields compared to all other treatments at the end of the 2008 study year potentially due to increased gibberellin (GA) production causing excessive cell elongation as a response to the RLE. Application of TE to 60% RLE may have reduced GA production, thereby reducing clipping yield from 2.0 to 1.0 g m<sup>-2</sup> (Table 6).

In 2009, 60% RLE treatments not receiving TE had significantly higher clipping yields compared to the untreated throughout the duration of the study year. Ninety

percent RLE treatments not receiving TE had no turf cover during the 2009 study year, therefore lacked measureable clipping yield. Zero percent RLE treatments with and without TE application and 60% RLE treatments with TE application had similar clipping yields throughout the 2009 study (Table 6).

		—Ju	ne	J	uly——	—Au	ıgust—	—Septe	ember—	—Oct	ober—
Year	RLE Level	-TE††	+TE‡‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						g	m <sup>-2</sup>				
2008	0%	2.1	1.6	0.9	0.8	1.0	1.5	1.1	0.5	0.6	0.4
	60%	2.9	2.8	2.0†	1.4*†	3.1†	1.6*	4.5†	1.6*†	2.0†	1.0*
	90%	3.2	3.3†	2.9†	1.5*†	4.4 <b>†</b>	2.5*	3.4†	1.8*†	0.0†	1.0*
2009	0%	0.8	0.1	0.5	0.2	0.9	0.4*	1.5	1.1	2.3	2.6
	60%	2.1†	0.5*	1.2†	0.8†	1.6†	0.7*	2.6†	1.4*	3.9†	2.3*
	90%	0	0.4	0	0.4*	0	0.5*†	0	1.4* <b>†</b>	0	1.8*†

Table 6. Clipping yield response means of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

\* Denotes a significant difference between TE treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

† Denotes a significant difference compared to 0% RLE within TE treatments according to Fisher's LSD (α=0.05) test.

 $\dagger$  + -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup> wk<sup>-1</sup>.$ 

## Ball Roll Distance

Shade level, trinexapac-ethyl treatment (TE), and year impacted golf ball roll distance. In 2008, 60% reduced light environments (RLE) without TE decreased ball roll distance by 7% compared to the untreated in July. In August, shade continued to effect ball roll distance in 60% RLE treatments while the application of TE increased ball roll distance 10%. In September, 90% RLE treatments without TE decreased ball roll distance 16% compared to the untreated due to increased vertical growth. Sixty percent RLE treatments with and without TE continued to decrease ball roll distance in September 18% and 37% compared to 0% RLE with and without TE, respectively. TE application to 0% RLE treatments increased ball roll distance 19% and 9% under 60% RLE treatments. In the final month of the 2008 study, 90% RLE with TE increased ball roll distance 22%. This was due to declining turf cover under 90% RLE treatments at the end of the study year (Table 7).

In 2009, shade and TE treatment continued to effect ball roll distance. In June, 90% RLE + TE increased ball roll distance 9% compared to the 0% RLE + TE. Once again this is from declining turf cover in 90% RLE. In July, 60% RLE + TE decreased ball roll distance 12% compared to 0% shade + TE. These trends continued through the end of 2009 with the exception of TE application increasing ball roll distance in 90% RLE by 34% compared to 0% RLE + TE. At the end of 2009, 90% RLE treatments without TE lacked turf cover from the previous year's study, exposing the soil profile. While originally increasing ball roll distance as the plant material decomposed in the soil, a softer surface was produced. This softer surface decreased ball roll distance over time.

The increase in ball roll distance in 90% RLE treatments with TE was due to declining turf cover over time. As the turf density thinned, the plants ability to produce leaf tissue diminished. The lack of turf cover and lack of upright growth resulted in reduced friction that lessens ball roll, resulting in increased ball roll distance (Table 7).

		——Ju	ne——	——Jı	ıly——	—Au	ıgust—	—Septe	ember—	October	
Year	RLE Level	-TE††	+TE <b>‡</b> ‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
							cm———				
2008	0%	124	133	119	127	112	124*	113	140*	137	141
	60%	122	132	111†	116	97†	107*†	89†	115*†	134	138
	90%	122	135	118	122	110	114	115	117†	176†	155*†
2009	0%	128	135	119	116	108	109	102	102	89	84
	60%	117	124	102	112	89	102	83	91	87	75
	90%	148	131*	135	132†	130	123	131	105	129	82*

Table 7. Ball roll distance response means of 'Diamond' zoysiagrass to various reduced light environments (RLE) and trinexapacethyl (TE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

\* Denotes a significant difference between TE treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  Denotes a significant difference compared to 0% RLE within TE treatments according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$ <sup>†</sup> -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup> wk<sup>-1</sup>.$ 

## Chlorophyll Concentration

Analysis of chlorophyll content revealed interaction between shade level, TE treatments, and years (P < 0.05); therefore, results are presented and discussed separately for each year. In 2008, 10 weeks after initial shade application, the chlorophyll concentrations of all treatments were similar. Twenty weeks after application of shade, 60% reduced light environment (RLE) treatments without TE increased chlorophyll concentration to 1.54 g kg<sup>-1</sup> from 0.98 g kg<sup>-1</sup> in the untreated. Ninety percent RLE treatments without TE did not have leaf tissue for chlorophyll concentration analysis. Sixty percent RLE treatments with TE reduced chlorophyll concentration to 0.81 g kg<sup>-1</sup> from 1.08 g kg<sup>-1</sup> in 0% RLE with TE. As the Ninety percent RLE with TE reduced chlorophyll content to 0.38 g kg<sup>-1</sup>, a 68% reduction compared to 0% shade with TE. TE application reduced chlorophyll content in 60% RLE 20 weeks after shade application by 47% compared to 60% RLE without TE. The reduction seen in 60% RLE may be a plant response to declining plant health. A signaling mechanism for reduced light stress may increase chlorophyll production, thereby increasing concentration as plant health reaches a threshold in its decline. TE application increases plant health, potentially decreasing the need for increased levels of chlorophyll compared to more stressed plants (Table 8).

In 2009, shade and TE treatment affected chlorophyll concentration 10 weeks after shade application. In treatments not receiving TE, 60% RLE treatments increased chlorophyll concentration by 34% from 1.86 g kg<sup>-1</sup> in the untreated to 2.81 g kg<sup>-1</sup>. Ninety percent RLE treatments without TE did not have any leaf tissue chlorophyll concentration

analysis by studies end. In treatments receiving TE, chlorophyll concentration in 0% RLE was increased by 65% from 1.01 g kg<sup>-1</sup> to 2.85 g kg<sup>-1</sup> in 60% RLE treatments and by 67% to 3.04 g kg<sup>-1</sup> in 90% RLE environments. TE application decreased chlorophyll concentration in 0% RLE treatments by 46% from 1.88 g kg<sup>-1</sup> to 1.01 g kg<sup>-1</sup>.

Twenty weeks after shade application, shade and TE application continued to impact chlorophyll concentration. Sixty percent RLE treatments without TE increased chlorophyll concentration by 62% compared to the untreated from 0.57 g kg<sup>-1</sup> to 1.52 g kg<sup>-1</sup>. In treatments receiving TE, chlorophyll increased to 1.52 g kg<sup>-1</sup> in 60% RLE from 0.23 g kg<sup>-1</sup> in 0% RLE. Ninety percent RLE treatments increased chlorophyll by 1.00 g kg<sup>-1</sup>(Table 8).

		20	008		2009					
	10 V	WAS	20 V	WAS	10 V	WAS	20 WAS			
RLE Level	-TE†	+TE‡	-TE	-TE +TE		+TE	-TE	+TE		
				g kg	-1					
None	1.11	1.71	0.98	1.08	1.01	1.88*	0.57	0.23		
Low (60%)	1.65	1.78	1.54†	0.81*†	2.81†	2.85†	1.52†	1.55†		
High (90%)	1.44	1.45	0.00†	0.38* <b>†</b>	$0.00^{+}$	3.04*†	0.00	1.23*†		

Table 8. Chlorophyll concentration response means of various reduced light environment (RLE) and trinexapac-ethyl (TE) treatments on 'Diamond' Zoysiagrass from May to October 2008 and 2009 in Clemson, SC.

\* Denotes a significant difference between TE treatments receiving the same shade treatment. ( $\alpha$ =0.05)

<sup>†</sup> Denotes a significant difference compared to 0% RLE within TE treatments. ( $\alpha$ =0.05)

 $\dagger$   $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup> wk<sup>-1</sup>.$ 

§ Chlorophyll concentration was measured 10 and 20 weeks after the application of shade during each study year.

## Summary

Reduction of light quantity is a growth limiting factor in all turfgrasses including 'Diamond' zoysiagrass. Growth of 'Diamond' zoysiagrass is affected morphologically and physiologically in a reduced light environment (RLE). A study during the summers of 2008 and 2009 in Clemson, SC demonstrated the response of 'Diamond' zoysiagrass to three levels of RLE, 0%, 60% and 90% and two trinexapac-ethyl (TE) treatments, 0.57 kg ai ha<sup>-1</sup>, and no TE application.

Plant responses such as turf quality (TQ), lateral regrowth (LR), total nonstructural carbohydrates (TNC), clipping yield, ball roll distance, and chlorophyll concentration were measured to determine morphological and physiological responses of 'Diamond' zoysiagrass to various levels of RLE. Zero percent and 60% RLE maintained commercially acceptable TQ ( $\geq$ 7) through 2008. In 2009, both treatments remained commercially acceptable until 60% RLE without TE declined to unacceptable TQ levels between September and October. Ninety percent RLE treatments both with and without TE application remained below commercially unacceptable TQ levels throughout both years, however TE application increased TQ by ~5 points throughout 2009.

Lateral regrowth was most affected by 90% RLE treatments not receiving TE. After August 2008, LR of 90% RLE without TE dropped significantly due to declining turf cover. This trend continued through 2009 as lack of turf cover prevented any LR. Application of TE to 90% RLE increased TNC concentration in 2008 and 2009. Ninety percent RLE without TE application lacked measurable root tissue for the determination

of carbohydrate levels. Clipping yield increased as light quantity decreased due to a plant response of increased gibberellin production increasing cell elongation. The application of TE, a gibberellin synthesis inhibitor, reduced the production of gibberellin, reducing overall clipping yields for 60% and 90% RLE. Ball roll distance was reduced by RLE application. As a result of increased leaf tissue in RLE, the increased friction decreased ball roll distance. Application of TE to RLE increased ball roll by decreasing leaf tissue production. Chlorophyll concentration was increased in both 60% and 90% RLE in 2008 and 2009. Ninety percent RLE treatments not receiving TE did not have measurable leaf tissue for chlorophyll concentration analysis after the 10 weeks after shade application (WAS) measurement in 2008.

The study conducted by Qian and Engelke in 1999 compares favorably with the study conducted at Clemson University. The reduction of TQ due to RLE along with the increase of TQ after TE application is supported by both studies. While the lower mowing heights used in the Clemson study may cause a faster depletion of carbohydrate reserves due to less leaf tissue to perform photosynthesis, the general carbohydrate depletion pattern in the studies is comparable.

Future research is needed to quantify a daily light integral (DLI) for 'Diamond' zoysiagrass to maintain commercially acceptable TQ. Research is also needed to determine how varying levels of nutrient application will interact with TE application. Lastly, research determining the ability of other GA synthesis inhibiting growth regulators to improve morphological and physiological responses of 'Diamond' zoysiagrass to RLE is needed.

## CHAPTER 3

# RESPONSE OF ZOYSIAGRASS [Zoysia spp.], BERMUDAGRASS [Cynodon spp.], AND SEASHORE PASPALUM [Paspalum vaginatum Sw.] CULTIVARS TO REDUCED LIGHT ENVIRONMENTS AND TRINEXAPAC-ETHYL

### Introduction

Zoysiagrass [*Zoysia matrella* (L.) Merr.], [*Zoysia japonica* Steud.], bermudagrass [*Cynodon dactylon* (L.) Pers.], [*Cynodon dactylon* (L.) Pers. x *C. traansvaalensis* Burtt-Davy], and seashore paspalum [*Paspalum vaginatum* Sw.] are popular grasses for use on golf courses and athletic fields in the southern United States.

Due to their C<sub>4</sub> physiology, high levels of light are needed to perform photosynthesis at optimum levels. In a reduced light environment (RLE), plants respond with elongated stems, increased upright growth, lengthened leaf sheaths, increased rootto-shoot ratios, decreased leaf thickness and reduced tillering (Taiz and Zeiger 2006). With increases in upright growth and elongated stems, carbohydrate reserves are stressed through tissue removal from increased mowing frequency due to the increased vertical growth. With depleted carbohydrate reserves and reduced tillering, turf plants are less able to recover, from environmental stresses and other damaging forces such as traffic. Engelke and Qian (1999) noted an increase in gibberellic acid (GA) levels contributed to excessive shoot growth in pea plants (*Pisum sativum* L). In turfgrasses, GA inhibitors reduce vertical growth and control internode elongation (Ervin et. al. 2002).

Shade or low light tolerance is becoming an increasingly important issue to turf managers as they are often expected to grow turf in less than ideal agronomic conditions. As permanent structures such as residential buildings add to already problematic shade caused by trees, and other barriers, new research is needed to determine the tolerance of several commonly used turf species to a reduced light environment (RLE).

## Materials and Methods

An experiment was conducted during the summers of 2008 and 2009 to evaluate the performance of 'Diamond' zoysiagrass (*Zoysia matrella* (L.) Merr.), 'Meyer' zoysiagrass (*Zoysia japonica* Steud.), 'Champion,' 'MiniVerde,' 'Tifway,' 'TifGrand' bermudagrasses (*Cynodon dactylon* (L.) Pers. x *C. traansvaalensis* Burtt-Davy), 'Celebration' bermudagrass (*Cynodon dactylon* (L.) Pers.) and 'SeaIsle Supreme' seashore paspalum (*Paspalum vaginatum* Sw.) to a reduced light environment (RLE).

Location was the Clemson University turf plots in Clemson, South Carolina. Samples were grown in 15 cm diameter by 30 cm deep pots. Growing media was an 85:15 (v:v) sand:peat mixture, meeting USGA greens specifications (USGA, 2008). Samples were established using washed, certified sod. All samples were watered to field capacity daily to aid establishment. Ten-centimeter diameter plugs were taken from each variety and allowed to establish for three weeks prior to shade application.

The study included three levels of shade: none (0%), low (60%), and high (90%). The three levels of shade were applied continuously to all plots using a neutral density, poly-fiber black shade cloth (model no SC-ST60 and SC-ST90; International Greenhouse, Sidel, IL). The study was conducted during a 22-week period beginning 4 weeks prior to the summer solstice and ending 18 weeks past it. Timing coincided with maximum seasonal growth of turf species in the study. In year one, applications began on May 23 and ended on October 30. In year two, applications began on May 24 and ended on October 31.

Two TE treatments were included within each level of shade: 0.57 kg ai ha<sup>-1</sup> of TE applied with a  $CO_2$  backpack sprayer operating at 189.5 L ha<sup>-1</sup> (20 GPA) and an untreated control. TE was first applied at initiation of shading treatments and repeated weekly.

Low and high RLE applications were administered with a neutral density, polyfiber black shade cloth removing 60% and 90% of full sunlight respectively. Percent shade was determined by comparing photosynthetic photon flux density (PPFD), the amount of photosynthetically active radiation expressed on a quantum basis (mol m<sup>-2</sup> s<sup>-2</sup>), under the shade cloths to full sun at turf canopy level using a LI-28663 quantum light sensor (LiCor, Inc., Lincon, NE). Measurements were recorded twice yearly on a clear, cloudless day at solar-noon.

Individual shade cloth tent frames were 2 m by 1.5 m, constructed with 2.5 cm diameter PVC pipes. Shade tents were fixed 45 cm above ground level by 60 cm legs placed 15 cm into the ground to prevent movement due to environmental conditions. Shade cloths were attached to PVC frames with zip-ties so as to keep shade cloths at a consistent height above ground level.

Cultural practices requiring the removal of shade cloth tents were performed with all tents removed for the same amount of time and did not exceed two hours of additional full sunlight wk<sup>-1</sup>. Fertilizer was applied monthly at a rate of 24.21 kg N ha<sup>-1</sup> with a 20N-8.8P-16.6K (Harrell's, Lakeland, FL) fertilizer throughout the growing season. Mowing height simulated those on highly maintained turf and included 3.18 mm for 'Diamond' zoysiagrass, 'Champion' bermudagrass, 'Mini-verde' bermudagrass and 'Sea Isle

Supreme' seashore paspalum and 12.7 mm for 'Meyer' zoysiagrass, 'Tifway' bermudagrass, 'TifGrand' bermudgrass and 'Celebration' bermudagrass using hand clippers (Wahl, model 8886, Sterling, Illinois). Pots were mowed 3 times wk<sup>-1</sup> and clippings removed. Pots were irrigated three times weekly to field capacity and supplemental hand watering applied on an as needed basis to prevent stress. Monitoring for pests was conducted bi-weekly. No treatments were necessary for pest control.

# Statistical Design

Statistical design was a combined analysis of six separate experiments in a randomized complete block design (Table 15). The six experiments consisted of three shade treatments (0, 60, and 90%) each with two separate TE treatments. Data was analyzed separately for each year due to separate turf samples used for each year. All means separations were conducted using Fishers LSD at a significance level of 0.05. The General Linear Model procedure (GLM) of SAS (SAS institute, 2005) was used for all separations.

Source		Degrees of Freedom
Experiments*		5
Block		12
Cultivar		7
Cultivar*Experiment		35
Error		84
	Total	143

Table 15. Analysis of variance (ANOVA) table of a combined analysis for six randomized complete block design studies.

\*Experiments include 3 shade levels (0, 60 and 90% shade), and two trinexapac-ethyl treatments (+TE and -TE)

#### Measurements

Daily light integral (DLI) was recorded using a model 305 datalogger fitted with a quantum light sensor (Spectrum Technologies, Inc, Plainfield, II). Readings were recorded every minute and reported as mol  $m^2 d^{-1}$ . Total DLI was averaged over both growing seasons. DLI for RLE treatments was calculated by subtracting 60% and 90% of full sun DLI for the respective RLE treatments.

Turf-quality (TQ) ratings were taken on a weekly basis. TQ was measured on a 1-9 scale, with 1 representing dead turf and 9 representing ideal turf. A TQ rating <7 was deemed unacceptable.

Clipping yield was collected once every two weeks; samples were weighed and converted to  $g m^{-1}$ .

Chlorophyll content was measured 10 and 20 weeks after implementation of shade treatments. Fresh clippings were collected from individual replicates and chlorophyll was extracted using dimethyl sulfoxide (DMSO). The DMSO method is beneficial since it involves no grinding or maceration of the plant tissue (Hiscox and Israelstam 1979). A spectrophotometer was used at 645 and 663 nm to determine chlorophyll content as suggested by the Arnon Equation (Arnon). A detailed procedure can be found for this in Appendix B.

## **Results and Discussion**

# Daily Light Integral

Daily light integral (DLI) maximum, minimum, and average were determined each year for each shade level. DLI measures light quantity and ranged from 44.95 to  $4.495 \text{ mol m}^{-2} \text{ d}^{-1}$  in 2008 and 43.18 to 4.32 mol m<sup>-2</sup> d<sup>-1</sup> in 2009.

Table 10. Average, maximum, and minimum daily total of Photosynthetic Active Radiation (DTP mol  $m^{-2} d^{-1}$ ) (PAR) of three levels of shade (0, 60, 90%) during June - October 2008 and 2009 in Clemson, SC.

			Daily PAR T	otal
			% Shade	
Year	Quantity	0	60	90
			mol m <sup>-7</sup>	<sup>2</sup> d <sup>-1</sup>
2008	Mean	44.95	17.98	4.495
	Maximum	67.5	27	6.75
	Minimum	4.5	1.8	0.45
2009	Mean	43.18	17.27	4.32
	Maximum	67.7	27.08	6.17
	Minimum	3.6	1.44	0.36

<sup>†</sup> DTP of 2008 Study measured from May 20 – October 31; 2009 study from May 21 – October 31.

<sup>‡</sup>Maximum and minimum DTP for 2008 occurred on July 4 and October 24, respectively. In 2009, maximum and minimum DTP occurred on June 23 and October 27, respectively.

## Turf Quality

Analysis of TQ ratings revealed interaction between shade level, TE treatments, cultivars, and years (P< 0.05); therefore, results are presented and discussed separately for each year. In 2008, no cultivars demonstrated a statistical TQ superiority in 0% RLE without TE. In 0% RLE + TE treatments, all cultivars demonstrated higher TQ then TifGrand between June and September with the exception of Champion and SeaIsle Supreme in July, and Celebration and SeaIsle Supreme in September. By the end of 2008 no cultivars demonstrated a statistically superior TQ in 0% RLE + TE (Table 11). The application of TE to 0% RLE did not statistically improve any individual cultivar's TQ during 2008 (Table 12).

In 60% RLE without TE, Champion, MiniVerde, Diamond, and Meyer demonstrated superior TQ during June. In July, Diamond and Meyer zoysiagrass had statistically higher TQ compared to all other cultivars. Meyer zoysiagrass continued to have statistically superior TQ to all other cultivars in 2008 between August and September, with the exception of Diamond having statistically similar TQ to Meyer during October (Table 11).

Sixty percent RLE + TE treatments showed more variability in TQ compared to non TE treatments. In June, Champion MiniVerde, Diamond, and Meyer were statistically superior to all other cultivars, similar to non TE treatments. This trend began to differentiate in July when the same treatments with the addition of TifGrand and Tifway had higher TQ compared to Celebration and SeaIsle Supreme. This trend can also be seen in September. At the end of 2008, only Meyer zoysiagrass demonstrated a

statistical superiority to other cultivars in 60% RLE treatments receiving TE. Meyer was also the only cultivar to remain at commercially acceptable TQ levels in 60% RLE + TE treatments during 2008 (Table 11).

Application of TE to 60% RLE increased TQ in Champion during August from 5.8 to 6.8, from 5.5 to 7.2 in September, and from 5.2 to 6.7 in October. MiniVerde TQ was improved by TE application in September from 5.7 to 7.0 and October from 6.0 to 6.5. Diamond TQ improved during September from 6.2 to 7.2. TifGrand TQ improved from TE application during September from 5.5 to 7.2 and October from 5.3 to 6.8. Tifway TQ was improved by TE application during September from 5.5 to 7.2 and October from 5.3 to 6.8 and October from 5.2 to 6.5. Meyer, Celebration, and SeaIsle Supreme TQ did not improve with TE application in 60% RLE during 2008 (Table 12).

In 90% RLE without TE, Diamond, Meyer, TifGrand, and Celebration initially showed statistically superior TQ compared to other cultivars. As the study progressed, Diamond and Meyer zoysiagrass demonstrated higher TQ to other cultivars between July and September. At the end of 2008, only Meyer had a statistically higher TQ then all other cultivars in 90% RLE without TE (Table 11).

In treatments receiving TE Champion, MiniVerde, Diamond and Meyer demonstrated superior TQ during June. In July Diamond and Meyer zoysiagrass showed higher TQ compared to all other cultivars. Meyer zoysiagrass continued to demonstrate statistically higher TQ then all other cultivars during 2008 in 90% RLE + TE treatments (Table 11).

The application of TE to 90% RLE improved TQ in MiniVerde during June by from 5.7 to 6.8 and Meyer in October from 2.3 to 3.2 (Table 12).

In 2009, Champion, Diamond, Meyer, TifGrand, and Celebration demonstrated better TQ during June in 0% RLE without TE then all other cultivars. In July through October, all cultivars in 0% RLE without TE demonstrated similar TQ with the exception of Champion bermudagrass having statistically lower TQ then all other cultivars during August and Celebration and SeaIsle Supreme having statistically lower TQ during September. All 0% RLE treatments without TE kept TQ and commercially acceptable levels throught 2009 (Table 13).

In treatments receiving TE, Diamond, Meyer, TifGrand, Tifway, and Celebration demonstrated stastically superior TQ compared to SeaIsle Supreme, MiniVerde and Champion during June. Similar trends continued through August with the exception of Champion TQ increasing to statiscally higher levels in July and MiniVerde TQ increasing in August. In September, all 0% RLE + TE cultivars had similar TQ levels. At the end of 2009, Diamond, Meyer, TifGrand, Tifway, and Celebration had higher TQ compared to Champion, MiniVerde, and SeaIsle Supreme (Table 13).

In 60% RLE treatments without TE, Champion, Diamond, Meyer, Celebration, and SeaIsle Supreme demonstrated higer initial TQ then all other cultivars. As the study progressed, Diamond and Meyer zoysiagrass demonstrated statistically superior TQ in 60% RLE without TE throughout 2009 (Table 13).

In 60% RLE treatments receiving TE, all cultivars with the exception of Champion demonstrated similar TQ initially. In July, Champion, MiniVerde, Diamond,

Meyer, and TifGrand demonstrated superior TQ compared to Tifway, Celebration, and SeaIsle Supreme. This trend continued in August with the exception of TifGrand TQ declining. At the end of 2009 Diamond and Meyer zoysiagrass had statistically higher TQ compared to all other cultivars in 60% RLE + TE (Table 13).

In 90% RLE treatments without TE, Diamond, Meyer, and Celebration demonstrated statistically superior TQ duing June and July. In August and September only Diamond and Meyer continued to demonstrate higher TQ then all other cultivars. At the end of 2009, no cultivars receiving 90% RLE continued to maintain turf cover (Table 13).

In 90% RLE + TE treatments, Diamond, Meyer, and Celebration initially demonstrated higher TQ then all other cultivars. This trend continued in July and August with the addition of MiniVerde, TifGrand, and Tifway to stastically superior TQ levels. As the study progressed into September, only Diamond, Meyer, Tifway and Celebration continued to demonstrate statistically higher levels to TQ compared to other cultivars. At the end of 2009, only Meyer zoysiagrass continued to maintain some turf cover (Table 13).

Application of TE improved TQ in MiniVerde during July from 6.7 to 7.7 in a 60% RLE. In 90% RLE treatments, TQ improved in Champion, MiniVerde, Diamond, Tifway, TifGrand, Celebration, and SeaIsle Supreme after TE application. In August application of TE to 60% RLE treatments increased turf quality in Meyer, Tifway, TifGrand, Celebration, and SeaIsle supreme. TQ was improved during August in 90% RLE by TE application in Meyer zoysiagrass by from 5.3 to 6.7. In September, TE

application increased turf quality in Champion bermudagrass by from 2.0 to 3.5. At the end of 2009, in a 90% RLE, only Meyer + TE maintained turf cover, with TE application increasing TQ  $\sim$ 2 points from 1 to 3 (Table 14).

		——Ju	ne	—July——August—			—Septe	ember—	—October—		
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						TQ	1-9 —				
Champion	0%	7.3§*	7.3*	7.7	7.5	8.0	7.8*	8.0	8.0*	8.3	8.3
MiniVerde	0%	7.7*	7.7*	7.8	7.7*	7.8	7.8*	8.0	8.0*	8.3	8.3
Diamond	0%	7.3*	7.3*	7.7	7.8*	7.8	8.0*	8.0	8.0*	8.3	8.3
Meyer	0%	7.3*	7.5*	7.5	8.2*	7.8	8.7*	8.0	8.7*	8.3	8.5
TifGrand	0%	7.2*	7.0	7.5	7.2	7.8	7.5	7.8	7.5	8.0	8.2
Tifway	0%	7.7*	7.2*	7.8	8.0*	7.8	8.7*	7.8	8.3*	8.3	8.2
Celebration	0%	7.3*	7.2*	7.7	7.8*	7.9	7.8*	7.8	7.7	8.2	8.2
SeaIsle Supreme	0%	7.0	7.2*	7.8	7.5	7.8	7.7*	7.8	7.7	8.2	8.0

Table 11. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments and trinexapac-ethyl treatments from June through October 2008 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1-9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

		Ju	ne	——Ju	ıly——	—Aug	gust—	—Sept	ember—	October		
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE	
				TQ 1-9								
Champion	60%	7.3*	7.3*	6.7	7.0*	5.8	6.8	5.5	7.2*	5.2	6.7	
MiniVerde	60%	7.3*	7.3*	6.5	6.8*	6.0	6.5	5.7	7.0*	5.0	6.5	
Diamond	60%	7.3*	7.3*	7.2*	7.3*	6.5	7.3*	6.2	7.2*	6.7*	6.8	
Meyer	60%	7.8*	7.7*	7.5*	7.2*	7.5*	7.7*	7.3*	7.3*	7.3*	7.8*	
TifGrand	60%	6.8	6.8	6.5	7.2*	6.8	6.8	5.5	7.2*	5.3	6.8	
Tifway	60%	6.7	7.2	6.3	6.8*	6.3	6.7	5.3	6.8*	5.2	6.5	
Celebration	60%	6.8	6.8	6.2	5.3	5.8	6.3	4.7	5.3	3.3	4.2	
SeaIsle Supreme	60%	6.8	7.0	6.3	6.3	6.2	6.3	5.8	6.2	4.7	5.0	

Table 11 contd. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments and trinexapac-ethyl treatments from June through October 2008 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1-9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

		Ju Ju	ne	——Ju	ıly——	—Aug	gust—	—Septe	ember—	—October—	
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						TQ	1-9 —				
Champion	90%	6.2	6.5*	5.2	4.2	3.5	3.3	2.3	2.3	2.0	2.2
MiniVerde	90%	5.7	6.8*	5.2	5.8	3.7	4.3	2.5	2.8	2.0	2.0
Diamond	90%	7.0*	7.3*	7.0*	7.2*	6.2*	5.3	5.2*	4.3	2.0	2.0
Meyer	90%	7.0*	6.8*	6.8*	6.7*	6.3*	6.2*	5.3*	5.2*	2.3*	3.2*
TifGrand	90%	6.7*	5.8	4.7	4.0	3.3	3.3	2.0	2.5	2.0	2.2
Tifway	90%	5.5	5.3	4.2	4.8	3.0	3.7	2.3	2.0	2.0	2.0
Celebration	90%	6.7*	5.3	4.5	4.3	3.3	3.3	2.2	2.0	2.0	2.0
SeaIsle Supreme	90%	6.0	6.3	4.3	4.3	3.3	3.3	2.0	2.0	2.0	2.0

Table 11 contd. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments and trinexapac-ethyl treatments from June through October 2008 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1-9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

		——Ju	ne——	——Ju	ıly——	—Au	gust—	—Sept	ember—	-October-	
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						TQ [	1-9 ——				
Champion	0%	7.3§	7.3	7.7	7.5	8.0	7.8	8.0	8.0	8.3	8.3
	60%	7.3	7.3	6.7	7.0	5.8	6.8*	5.5	7.2*	5.2	6.7*
	90%	6.2	6.5	5.2	4.3	3.5	3.3	2.3	2.3	2.0	2.1
MiniVerde	0%	7.7	7.7	7.8	7.7	7.8	7.8	8.0	8.0	8.3	8.3
	60%	7.3	7.3	6.5	6.8	6.0	6.5	5.7	7.0*	5.0	6.5*
	90%	5.7	6.8*	5.2	5.8	3.7	4.3	2.5	2.8	2.0	2.0
Diamond	0%	7.3	7.3	7.7	7.8	7.8	8.0	8.0	8.0	8.3	8.3
	60%	7.3	7.3	7.2	7.3	6.5	7.3	6.2	7.2*	6.7	6.3
	90%	7.0	7.3	7.0	7.2	6.2	5.3	5.2	4.3	2.0	2.0
Meyer	0%	7.3	7.5	7.5	8.2	7.8	8.2	8.0	8.2	8.3	8.5
	60%	7.8	7.7	7.5	7.2	7.5	7.7	7.3	7.3	7.3	7.8
	90%	7.0	6.8	6.8	6.7	6.3	6.2	5.3	5.2	2.0	3.2*

Table 12. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl treatments within reduced light environment (RLE) treatments from June through October 2008 in Clemson, South Carolina.

\*Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1-9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

		——Ju	ne——	—Ju	ıly——	—Aug	gust—	—Sept	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE <b>‡</b>	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						TQ 1	1-9 ——				
TifGrand	0%	7.2	7.0	7.5	7.2	7.8	7.5	7.8	7.5	8.0	8.2
	60%	6.8	6.8	6.5	7.2	6.8	6.8	5.5	7.2*	5.3	6.8*
	90%	6.7	5.8	4.7	4.0	3.3	3.3	2.0	2.5	2.0	2.2
Tifway	0%	7.7	7.7	7.8	8.0	7.8	8.2	7.8	8.3	8.3	8.2
	60%	6.7	7.2	6.3	6.8	6.3	6.7	5.3	6.8*	5.2	6.5*
	90%	5.5	5.3	4.2	4.8	3.0	3.7	2.3	2.0	2.0	2.0
Celebration	0%	7.3	7.2	7.7	7.8	7.9	7.8	7.8	7.7	8.2	8.2
	60%	6.8	6.8	6.2	5.3	5.8	6.3	4.7	5.3	3.3	4.2
	90%	6.7	6.2	4.5	4.3	3.3	3.3	2.2	2.0	2.0	2.0
SeaIsle Supreme	0%	7.0	7.2	7.8	7.5	7.8	7.7	7.8	7.7	8.2	8.0
	60%	6.8	7.0	6.3	6.3	6.2	6.3	5.8	6.2	4.7	5.0
	90%	6.0	6.3	4.3	4.3	3.3	3.3	2.0	2.0	2.0	2.0

Table 12 contd. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl treatments within reduced light environment (RLE) treatments from June through October 2008 in Clemson, South Carolina.

\*Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1 - 9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

			ne		—July————August—		—September—		—Oc	tober—	
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						TQ	1-9 —				
Champion	0%	7.8*	7.2	8.0	7.8*	8.0	8.2*	8.2	7.8	8.2	7.8
MiniVerde	0%	7.7	7.2	8.2	7.2	8.3*	8.3*	7.8	8.2	8.3	7.8
Diamond	0%	8.0*	7.8*	8.2	8.2*	8.5*	8.0*	8.5*	8.3	8.3	8.3*
Meyer	0%	8.2*	7.7*	8.2	8.0*	8.3*	8.5*	8.2*	8.3	8.0	8.2*
TifGrand	0%	8.0*	7.5*	8.3	7.8*	8.2*	7.7	8.0*	7.8	8.2	8.2*
Tifway	0%	7.7	7.7*	8.2	8.3*	8.5*	8.3*	8.3*	8.2	8.2	8.0*
Celebration	0%	7.8*	7.7*	8.2	7.8*	8.2*	7.8*	7.8	8.0	8.0	8.3*
SeaIsle Supreme	0%	7.5	7.0	8.0	7.2	8.2*	7.7	7.8	7.8	8.3	7.5

Table 13. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments and trinexapac-ethyl treatments from June through October 2009 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1-9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

		Ju Ju	ne	——Ju	ly——	—August—		—Septe	ember—	—Oct	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						TQ	1-9 —				
Champion	60%	7.3*	7.2	7.2	7.2*	7.2	7.2*	5.8	6.8	5.3	5.7
MiniVerde	60%	7.2	7.7*	6.7	7.7*	6.8	7.3*	5.8	6.5	5.3	5.3
Diamond	60%	7.7*	8.0*	7.5	7.7*	7.3	7.3*	7.7*	7.8*	7.0*	7.2*
Meyer	60%	8.0*	7.8*	8.2*	7.7*	8.5*	7.2*	7.5*	7.8*	7.3*	7.0*
TifGrand	60%	7.2	7.7*	5.8	7.3*	5.2	6.7	4.2	6.0	3.7	5.2
Tifway	60%	7.0	7.5*	6.2	6.7	5.3	6.5	4.2	6.3	3.8	5.2
Celebration	60%	7.3*	7.7*	6.5	7.0	5.7	6.8*	4.5	6.7	4.3	5.3
SeaIsle Supreme	60%	7.3*	7.7*	5.8	6.5	4.3	6.2	3.2	5.7	2.0	7.0

Table 13 contd. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments and trinexapac-ethyl treatments from June through October 2009 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1-9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

		Ju Ju	ne	——Ju	ly——	—Aug	gust—	—Septe	ember—	—Oc	October—	
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE	
		TQ 1-9										
Champion	90%	6.2	6.7	4.2	5.8	3.3	5.7	2.0	3.5	1.0	1.0	
MiniVerde	90%	6.2	6.8	4.7	6.5*	3.3	6.3*	2.3	3.2	1.0	1.0	
Diamond	90%	6.3*	7.2*	5.5*	6.8*	5.3*	7.0*	4.2*	4.5*	1.0	1.0	
Meyer	90%	7.2*	7.7*	6.2*	6.8*	5.3*	6.7*	4.5*	4.8*	1.0*	3.0*	
TifGrand	90%	6.7*	6.7	4.3	6.2*	3.7	6.0*	2.2	3.5	1.0	1.0	
Tifway	90%	6.0	6.8	4.5	6.3*	3.7	6.3*	2.2	4.3*	1.0	1.0	
Celebration	90%	6.7*	7.7*	5.0*	7.5*	3.7	6.7*	2.0	4.7*	1.0	1.0	
SeaIsle Supreme	90%	4.3	5.3	3.0	5.0	2.3	4.3	1.0	2.2	1.0	1.0	

Table 13 contd. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments and trinexapac-ethyl treatments from June through October 2009 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1-9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

		June		July—July—		—August—		—September—		—October—	
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						TQ	1-9 ——				
Champion	0%	7.83	7.2	8.0	7.8	8.0	8.2	8.2	7.8	8.2	7.8
	60%	7.3	7.2	7.2	7.2	7.2	7.2	5.8	6.8*	5.3	5.7
	90%	6.2	6.7	4.2	5.8*	3.3	5.7*	2.0	3.5*	1.0	1.0
MiniVerde	0%	7.7	7.2	8.2	7.2*	8.3	8.3	7.8	8.2	8.3	7.8
	60%	7.2	7.7	6.7	7.7*	6.8	7.3	5.8	6.5	5.3	5.3
	90%	6.2	6.8	4.7	6.5*	3.3	6.3*	2.3	3.2	1.0	1.0
Diamond	0%	8.0	7.8	8.2	8.2	8.5	8.0	8.5	8.3	8.3	8.3
	60%	7.7	8.0	7.5	7.7	7.3	7.3	7.7	7.8	7.0	7.2
	90%	6.3	7.2	5.5	6.8*	5.3	7.0*	4.2	4.5	1.0	1.0
Meyer	0%	8.2	7.7	8.2	8.0	8.3	8.5	8.2	8.3	8.0	8.2
	60%	8.0	7.8	8.2	7.7	8.5	7.2*	7.5	7.8	7.3	7.0
	90%	7.2	7.7	6.2	6.8	5.3	6.7*	4.5	4.8	1.0	3.0*

Table 14. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl treatments within reduced light environment (RLE) treatments from June through October 2009 in Clemson, South Carolina.

\* Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  -TE = No trinexapac-ethyl applied.

 $\ddagger +TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1 - 9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

		——June——		——July——		—August—		—September—		October		
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE	
		TQ 1-9										
TifGrand	0%	8.0	7.5	8.3	7.8	8.2	7.7	8.0	7.8	8.2	8.2	
	60%	7.2	7.7	5.8	7.3	5.2	6.7*	4.2	6.0*	3.7	5.2*	
	90%	6.7	6.7	4.3	6.2*	3.7	6.0*	2.2	3.5*	1.0	1.0	
Tifway	0%	7.7	7.7	8.2	8.3	8.5	8.3	8.3	8.2	8.2	8.0	
	60%	7.0	7.5	6.2	6.7	5.3	6.5*	4.2	6.3*	3.8	5.2*	
	90%	6.0	6.8	4.5	6.3*	3.7	6.3*	2.2	4.3*	1.0	1.0	
Celebration	0%	7.8	7.7	8.2	7.8	8.2	7.8	7.8	8.0	8.0	8.3	
	60%	7.3	7.7	6.5	7.0	5.7	6.8*	4.5	6.7*	4.3	5.3*	
	90%	6.7	7.7*	5.0	7.5*	3.7	6.7*	2.0	4.7*	1.0	1.0	
SeaIsle Supreme	0%	7.5	7.0	8.0	7.2	8.2	7.7	7.8	7.8	8.3	7.5	
	60%	7.3	7.7	5.8	6.5	4.3	6.2*	3.2	5.7*	2.0	5.0*	
	90%	4.3	5.3*	3.0	5.0*	2.3	4.3*	1.0	2.2*	1.0	1.0	

Table 14 contd. Turf quality response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl treatments within reduced light environment (RLE) treatments from June through October 2009 in Clemson, South Carolina.

\* Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  - TE = No trinexapac-ethyl applied.

 $\ddagger + TE = Trinexapac-ethyl applied at 0.57 kg ai ha<sup>-1</sup>wk<sup>-1</sup>.$ 

§ Turf quality rated from 1-9 where 9 = best turf and values  $\leq 7.0$  are unacceptable.

#### **Clipping Yield**

Analysis of clipping yields revealed interaction between shade level, TE treatments, cultivars, and years (P< 0.05); therefore, results are presented and discussed separately for each year. In June 2008, 60% reduced light environments (RLE) without TE application increased clipping yield in MiniVerde, Diamond, Meyer, TifGrand and Celebration compared to 0% RLE without TE application. Ninety percent RLE treatments without TE application increased clipping yield in Champion, MiniVerde, and Tifway bermudagrass. In treatments receiving TE, 60% RLE increased clipping yield Champion, TifGrand, Tifway, and Celebration bermudagrass compared to 0% RLE with TE application increased clipping yield in SeaIsle Supreme compared to 0% RLE with TE application (Table 15). Application of TE to 60% and 90% RLE reduced clipping yield in Celebration bermudagrass by 21% and 62%, respectively (Table 16).

In July, 60% RLE without TE application increased clipping yield in Champion bermudagrass and 90% RLE increased clipping yield in Diamond and TifGrand compared to 0% RLE without TE application. Sixty percent RLE with trinexapac-ethyl application increased clipping yield in MiniVerde and Diamond while 90% RLE with TE application increased clipping yield in TifGrand compared to 0% RLE with TE application (Table 15). Application of TE to 60% RLE reduced clipping yield in MiniVerde bermudagrass by 22%. In 90% RLE, clipping yield was reduced by TE

application in Champion, MiniVerde, Diamond, Tifway, and Celebration bermudagrass by 63%, 65%, 56%, 36% and 39%, respectively.

In August, 60% RLE without TE application increased clipping yield in Tifway compared to 0% RLE without TE application. Ninety percent RLE with TE application increased clipping yield in Champion and MiniVerde compared to 0% RLE with TE application (Table 15). Trinexapac-ethyl application decreased clipping yield in Meyer, Tifway and Celebration grown in a 90% RLE by 28%, 74% and 67%, respectively (Table 16). In September, 60% RLE with TE application increased clipping yield in Champion and SeaIsle Supreme compared to 0% RLE with TE application (Table 15). Application of TE reduced clipping yield in Meyer in 60% RLE by 21% and TifGrand in 90% RLE by 59%. In October, application to MiniVerde in a 60% RLE increased clipping yield. Increases in clipping yield during September and October after TE application can be attributed to increased plant health (Table 16).

In 2009, 60% RLE without TE application increased clipping yield in Champion and Tifway bermudagrass and 90% RLE without TE increased clipping yield in Champion bermudagrass compared to 0% RLE without TE application during June (Table 17). TE application reduced clipping yield in Tifway bermudagrass in a 60% RLE by 59% and Champion bermudagrass in a 90% RLE by 79% (Table 18). In August, 90% RLE without TE application increased clipping yield in Champion and Tifway bermudagrass compared to 0% RLE without TE. Sixty percent RLE with TE application increased clipping yield in Tifway bermudagrass and 90% RLE with TE application

increased clipping yield in MiniVerde, TifGrand and Tifway compared to 0% RLE with TE application (Table 17).

In September, 60% RLE with TE application demonstrated increased clipping yields in Champion bermudagrass and decreased clipping yields in Meyer zoysiagrass compared to 0% RLE with TE application. More prominently, 90% RLE with TE application decreased clipping yield in Meyer, Celebration, and SeaIsle Supreme compared to 0% RLE with TE application (Table 17). The reduction in clippings yields can be attributed to decreasing plant health. Application of TE increased clipping yields in TifGrand and Celebration bermudagrass in a 60% RLE by 507% and 453%, respectively, due to increase of plant health from TE application (Table 18).

In October, 60% RLE without TE application decreased clipping yield in Diamond zoysiagrass compared to 0% RLE without TE, once again due to declining plant health. In treatments receiving TE, Celebration clipping yield was increased in a 60% RLE compared to 0% RLE with TE application (Table 17). Celebration clipping yield was increased in a 60% RLE due to declining health of Celebration without TE application (Table 18).

			une——		ıly——		gust—	—Sept	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						g	m <sup>-2</sup> ——				
Champion	0%	0.3	0.5	0.6	0.9	0.9	1.2	1.7	0.4	0.6	0.7
	60%	1.7	1.5*	2.7*	2.7	1.6*	1.3	2.4*	2.5*	0.6*	1.0
	90%	1.6*	1.2*	1.6*	0.6	1.3	3.5*	4.1*	0.2	-	-
MiniVerde	0%	0.6	1.8	0.4	0.3	2.0	1.6	0.7	0.4	0.8	1.1
	60%	1.8*	1.7	2.9*	2.3*	1.4	1.9*	3.3*	1.6*	0.6	1.1
	90%	1.2*	1.4*	1.9*	0.7	1.8	4.0*	5.6*	0.3*	-	-
Diamond	0%	0.9	1.5	1.1	0.4	0.8	1.5	2.3	1.4	1.1	0.8
	60%	1.7*	1.6	2.2*	1.7*	2.4*	4.0*	2.8	4.2*	0.7	0.8
	90%	0.7	0.4*	2.4*	1.0*	2.0*	3.9*	4.3*	3.1*	-	-
Meyer	0%	0.8	1.1	1.7	2.4	1.2	4.2	2.5	0.5	0.6	0.6
	60%	1.5*	0.6*	2.1*	3.8*	3.6*	3.6*	2.3	1.8*	0.9	0.5
	90%	0.9	0.5*	0.7*	0.4*	3.0*	2.1*	2.5	0.5	-	-

Table 15. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments (RLE) and trinexapacethyl (TE) treatments from June through October 2008 in Clemson, South Carolina.

<sup>†</sup>-TE= No trinexapac-ethyl applied.

•		——Ju	ne——	——Jul	y	—Aug	gust—	—Sept	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						g	m <sup>-2</sup> ——				
TifGrand	0%	0.6	0.5	0.2	0.3	3.0	5.8	2.7	0.4	1.2	0.5
	60%	1.5*	1.8*	4.4*	4.5*	3.8*	5.6*	3.4*	3.1*	1.0	0.5
	90%	0.6	0.6	2.5*	3.0*	3.4	2.9	1.1*	0.5	-	-
Tifway	0%	0.5	0.6	3.2	1.5	3.2	4.8	4.1	0.8	0.8	1.1
	60%	1.4*	2.7*	3.0	1.0*	3.7*	3.9*	1.6*	2.3*	1.1	1.0
	90%	1.1*	0.1*	2.1*	1.4*	2.4*	0.6*	1.8*	0.1*	-	-
Celebration	0%	0.3	1.4	0.3	4.3	3.8	5.2	2.6	0.5	1.1	1.7
	60%	2.5*	2.0*	5.0*	5.4*	2.4*	4.6*	1.6*	2.1*	1.0	0.5
	90%	1.2*	0.5*	2.4*	1.5*	4.8*	1.6*	1.8*	0.6	-	-
SeaIsle Supreme	0%	0.4	0.6	2.0	2.1	0.6	1.4	2.7	0.7	2.3	0.8
	60%	0.7	1.3	1.0*	1.8	1.0	1.2	2.4*	2.5*	1.4*	1.3
	90%	0.5	1.3*	1.6*	1.2*	1.1	5.1*	3.5	1.5*	-	-

Table 15 contd. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments (RLE) and trinexapacethyl (TE) treatments from June through October 2008 in Clemson, South Carolina

 $\dagger$  -TE = No trinexapac-ethyl applied.

· · · · · · · · ·		Ju	ne——	——Ju	ıly——	—Au	gust—	—Sept	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						g	; m <sup>-2</sup> ——				
Champion	0%	0.3	0.5	0.6	0.9	0.9	1.2	1.7	0.4*	0.6	0.7
	60%	1.7	1.5	2.7	2.7	1.6	1.3	2.4	2.5	0.6	1.0
	90%	1.6	1.2	1.6	0.6*	1.3	3.5*	4.1	0.2*	-	-
MiniVerde	0%	0.6	1.9*	0.4	0.3	2.0	1.6	0.7	0.4	0.8	1.1
	60%	1.8	1.7	2.9	2.3*	1.4	1.9*	3.3	1.6*	0.6	1.1*
	90%	1.2	1.4	1.9	0.7*	1.8	4.0*	5.6	0.3*	-	-
Diamond	0%	0.9	1.5*	1.1	0.4*	0.8	1.5*	2.3	1.4*	1.1	0.8
	60%	1.7	1.6	2.2	1.7	2.4	4.1*	2.8	4.2*	0.7	0.8
	90%	0.7	0.4	2.4	1.1*	2.0	3.9*	4.3	3.1*	-	-
Meyer	0%	0.7	1.1	1.7	2.4*	1.2	4.2*	2.5	0.5*	0.6	0.6
	60%	1.5	0.6	2.1	3.8*	3.6	3.6	2.3	1.8*	0.9	0.5
	90%	0.9	0.5	0.7	0.4	3.0	2.1*	2.5	0.5*	-	-

Table 16. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to trinexapac-ethyl (TE) within reduced light environment (RLE) treatments from June through October 2008 in Clemson, South Carolina.

\* Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$ -TE = No trinexapac-ethyl applied.

		Ju	ine	——Ju	ly——	—Au	gust—	—Sept	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						g	g m <sup>-2</sup> ——				
TifGrand	0%	0.6	0.5	0.2	0.3	3.0	5.8*	2.7	0.4*	1.2	0.5*
	60%	1.5	1.8	4.4	4.5	3.8	5.6*	3.4	3.1	1.0	0.5
	90%	0.6	0.6	2.5	4.0*	3.4	2.9	1.1	0.5*	-	-
Tifway	0%	0.5	0.6	3.2	1.5*	3.2	4.8*	4.1	0.8*	0.8	1.1
	60%	1.4	2.7*	3.0	1.*	3.7	3.9	1.6	2.3*	1.1	1.0
	90%	1.1	0.1	2.1	1.4*	2.4	0.6*	1.8	0.1*	-	-
Celebration	0%	0.3	1.4*	0.3	4.3*	3.8	5.2*	2.6	0.5*	1.1	1.7*
	60%	2.5	2.0*	5.0	5.4	2.4	4.6*	1.6	2.1*	1.0	0.5
	90%	1.2	0.5*	2.4	1.5*	4.8	1.6*	1.8	0.6*	-	-
SeaIsle Supreme	0%	0.4	0.6	2.0	2.1	0.6	1.4*	2.7	0.7*	2.3	0.8*
	60%	0.7	1.3*	1.0	1.8	1.0	1.2	2.4	2.5	1.4	1.3
	90%	0.5	1.3*	1.6	1.2	1.1	5.1*	3.5	1.5*	-	-

Table 16 contd. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to trinexapac-ethyl (TE) within reduced light environment (RLE) treatments from June through October 2008 in Clemson, South Carolina.

\* Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  -TE = No trinexapac-ethyl applied.

		——Ju	ne	——Ju	ıly——	—Au	gust—	—Sept	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						g	g m <sup>-2</sup> ——				
Champion	0%	0.1	0.1	0.3	0.3	0.1	0.2	0.3	0.2	0.4	0.3
	60%	0.6*	0.4	0.5	0.5	0.5	0.4	0.5	0.8*	0.4	0.2
	90%	0.7*	0.2	0.3	0.2	0.6*	0.2	0.2	0.5	-	-
MiniVerde	0%	0.1	0.1	0.3	0.3	0.1	0.1	0.2	0.3	0.3	0.2
	60%	0.1	0.1	0.4	0.4	0.3	0.2	0.5	0.5	0.7	0.3
	90%	0.3	0.1	0.3	0.3	0.5	0.6*	0.2	0.6	-	-
Diamond	0%	0.1	0.3	0.3	0.4	0.1	0.1	0.3	0.5	1.3	0.4
	60%	0.5	0.1	0.4	0.3	0.1	0.1	0.1	0.3	0.4*	0.2
	90%	0.1	0.3	0.2	0.1	0.5	0.5	0.1	0.3	-	-
Meyer	0%	0.3	0.1	0.5	0.3	0.6	0.2	0.5	0.9	0.5	0.7
	60%	0.6	0.1	0.4	0.4	0.1	0.1	0.5	0.3*	0.7	0.4
	90%	0.7	0.3	0.2	0.2	0.7	0.4	0.1	0.1*	-	-

Table 17. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments (RLE) and trinexapacethyl (TE) treatments from June through October 2009 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

Table 17 contd. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments (RLE) and trinexapacethyl (TE) treatments from June through October 2009 in Clemson, South Carolina.

5 < 7		——Ju	ne——	——Ju	ıly——	—Aug	gust—	—Sept	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
	20101					g 1	n <sup>-2</sup> —				
TifGrand	0%	0.6	0.1	0.5	0.3	0.4	0.1	0.4	0.3	0.4	0.4
	60%	0.4	0.3	0.4	0.5	0.4	0.4	0.1	0.7	0.4	0.4
	90%	0.3	0.2	0.2	0.3	0.7	0.7*	0.2	0.2	-	-
Tifway	0%	0.6	0.5	0.4	0.3	0.1	0.1	1.7	0.7	1.1	0.7
	60%	1.3*	0.5	0.4	0.6	0.3	0.7*	0.6	0.4	0.7	0.5
	90%	0.9	0.5	0.3	0.3	0.7*	0.6	0.2	0.5	-	-
Celebration	0%	0.6	0.1	0.4	0.3	0.2	0.1	0.9	0.9	0.3	0.4
	60%	0.2	0.5	0.3	0.5	0.1	0.5	0.2	0.7	0.3	0.9*
	90%	1.0	0.3	0.3	0.3	0.5	0.6*	0.5	0.1*	-	-
SeaIsle Supreme	0%	0.1	0.1	0.3	0.4	0.1	0.2	0.6	0.8	0.5	0.4
	60%	0.3	0.5	0.5	0.4	0.4	0.2	0.6	0.5	0.7	0.7
	90%	0.2	0.1	0.2	0.3	0.3	0.4	0.1	0.1*	-	-

 $\dagger$  -TE = No trinexapac-ethyl applied.

		——Ju	ne	——Ju	ıly——	—Aug	gust—	—Septe	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						g r	m <sup>-2</sup> —				
Champion	0%	0.1	0.1	0.3	0.3	0.1	0.2	0.3	0.2	0.4	0.3
	60%	0.6	0.4	0.5	0.5	0.5	0.4	0.5	0.8	0.4	0.2
	90%	0.7	0.2*	0.3	0.2	0.6	0.2	0.2	0.5	-	-
MiniVerde	0%	0.1	0.1	0.3	0.3	0.1	0.1	0.2	0.3	0.3	0.2
	60%	0.1	0.1	0.4	0.4	0.3	0.2	0.5	0.5	0.7	0.3
	90%	0.3	0.1	0.3	0.3	0.5	0.6	0.2	0.6	-	-
Diamond	0%	0.1	0.3	0.3	0.4	0.1	0.1	0.3	0.5	1.3	0.4*
	60%	0.5	0.1	0.4	0.3	0.1	0.1	0.1	0.3	0.4	0.2
	90%	0.1	0.3	0.2	0.1	0.5	0.5	0.1	0.3	-	-
Meyer	0%	0.3	0.1	0.5	0.3	0.6	0.2	0.5	0.9	0.5	0.7
	60%	0.6	0.1	0.4	0.4	0.1	0.1	0.5	0.3	0.7	0.4
	90%	0.7	0.3	0.2	0.2	0.7	0.4	0.1	0.1	-	-

Table 18. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to trinexapac-ethyl (TE) treatment within reduced light environment (RLE) treatments from June through October 2009 in Clemson, South Carolina.

\* Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  -TE = No trinexapac-ethyl applied.

	,	——Ju	ne	Ju	ıly——	—Au	gust—	—Sept	ember—	—Oc	tober—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE	-TE	+TE
						g	g m <sup>-2</sup> ——				
TifGrand	0%	0.6	0.1*	0.5	0.3	0.4	0.1	0.4	0.3	0.4	0.4
	60%	0.4	0.3	0.4	0.5	0.4	0.4	0.1	0.7*	0.4	0.4
	90%	0.3	0.2	0.2	0.3	0.7	0.7	0.2	0.2	-	-
Tifway	0%	0.6	0.5	0.4	0.3	0.1	0.1	1.7	0.7*	1.1	0.7
	60%	1.3	0.5*	0.4	0.6	0.3	0.7	0.6	0.4	0.7	0.5
	90%	0.9	0.5	0.3	0.3	0.7	0.6	0.2	0.5	-	-
Celebration	0%	0.6	0.1*	0.4	0.3	0.2	0.1	0.9	0.9	0.3	0.4
	60%	0.2	0.5	0.3	0.5	0.1	0.5	0.2	0.7*	0.3	0.9
	90%	1.0	0.3*	0.3	0.3	0.5	0.6	0.5	0.1	-	-
SeaIsle Supreme	0%	0.1	0.1	0.3	0.4	0.1	0.2	0.6	0.8	0.5	0.4
	60%	0.3	0.5	0.5	0.4	0.4	0.2	0.6	0.5	0.7	0.7
	90%	0.2	0.1	0.2	0.3	0.3	0.4	0.1	0.1	-	-

Table 18 contd. Clipping yield response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to trinexapac-ethyl (TE) treatment within reduced light environment (RLE) treatments from June through October 2009 in Clemson, South Carolina.

\* Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  -TE = No trinexapac-ethyl applied.

#### Chlorophyll Concentration

Chlorophyll concentration was impacted by year, shade level and trinexapac-ethyl (TE) treatment. In 2008, 60% reduced light environment (RLE) without TE application significantly increased chlorophyll concentration in Champion, MiniVerde, and Celebration bermudagrass 10 weeks after shade application (WAS). Ninety percent RLE without TE application increased chlorophyll concentration in Champion, MiniVerde, Diamond, Meyer, and Celebration 10 WAS. Chlorophyll concentration was significantly reduced in TifGrand receiving 90% RLE without TE application 10 WAS due to declining plant health. Chlorophyll concentration was increased in treatments receiving 60% RLE with TE application in Champion and MiniVerde bermudagrass 10 WAS. Ninety percent RLE with TE application increased chlorophyll concentration in Champion, Mass. Ninety percent RLE with TE application in Champion and MiniVerde bermudagrass 10 WAS. Ninety percent RLE with TE application increased chlorophyll concentration in Champion, MiniVerde, Diamond, Meyer, Tifway and Celebration bermudagrass 10 WAS (Table 19).

Twenty WAS 60% RLE decreased chlorophyll concentration in Champion and Celebration bermudagrass due to declining plant health. Ninety percent RLE treatments decreased chlorophyll concentration in Champion and MiniVerde bermudagrass and increased chlorophyll concentration in Diamond and Meyer zoysiagrass (Table 19). Application of TE increased chlorophyll concentration in Meyer and TifGrand 20 WAS (Table 20). There was not plant tissue available for chlorophyll concentration analysis in any treatments in a 90% RLE 20 WAS.

69

In 2009, 60% RLE without TE application decreased chlorophyll concentration in TifGrand and Celebration bermudagrass 10 WAS. Ninety percent RLE significantly decreased chlorophyll concentration in Champion, TifGrand, Tifway and Celebration bermudagrass. In treatments receiving TE application, 60% RLE reduced chlorophyll concentration in TifGrand and Tifway bermudagrass. Ninety percent RLE treatments with TE application reduced chlorophyll concentration in Diamond, Tifway and SeaIsle Supreme 10 WAS (Table 19). Application of TE to 60% RLE increased chlorophyll concentration in Tifgrand, Celebration, and SeaIsle Supreme 10 WAS. Application of TE to a 90% RLE increased chlorophyll concentration in Champion, Diamond, Meyer, Tifway, Celebration, and SeaIsle Supreme (Table 20).

Twenty WAS no plant tissue was available for chlorophyll concentration analysis in any 60% or 90% RLE treatments, with or without TE application. Application of TE to 0% RLE increased chlorophyll concentration in Tifway bermudagrass 20 WAS (Table 20).

			20	008			20	09	
		10 V	WAS	——20 V	VAS——	——10 V	WAS——	20 V	WAS—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE
					g kg	-1			
Champion	0%	0.7	0.5	2.3	2.8	2.1	2.5	2.4	1.9
	60%	1.5*	1.7*	1.2*	2.1*	2.0	2.1	-	-
	90%	1.7*	1.6*	-	-	0.9*	2.9	-	-
MiniVerde	0%	2.6	0.3	2.0	2.4	1.7	2.2	2.4	1.7
	60%	1.7*	1.2*	1.9	1.2*	2.0	1.7	-	-
	90%	1.7*	1.6*	-	-	1.9	2.1	-	-
Diamond	0%	0.4	0.3	1.7	1.1	1.7	1.9	1.5	1.2
	60%	0.4	0.4	1.3	1.8*	1.5	1.8	-	-
	90%	1.1*	0.9*	-	-	1.2	1.1*	-	-
Meyer	0%	0.7	0.8	1.5	1.0	1.4	1.6	1.7	1.0
	60%	0.1	1.0	1.5	2.0*	1.4	1.1*	-	-
	90%	1.2*	1.4*	-	-	0.9	1.4	-	-

Table 19. Chlorophyll concentration response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

			20	008			20	)09——	
		——10 V	VAS——	—20 W	VAS—	10 V	WAS	—20 \	WAS—
Cultivar	RLE Level	NP†	P‡	NP	Р	NP	Р	NP	Р
					g k	g <sup>-1</sup>			
TifGrand	0%	1.4	1.2	1.8	3.2	3.2	2.5	2.3	1.9
	60%	1.6	1.1	1.5	2.9	2.5*	1.3*	-	-
	90%	0.5*	1.0	-	-	2.2*	2.5	-	-
Tifway	0%	1.5	1.2	1.7	1.4	2.2	2.6	1.6	2.4
	60%	1.8*	1.4	1.2	1.5	1.7	2.0*	-	-
	90%	1.7	1.9*	-	-	1.0*	1.6*	-	-
Celebration	0%	0.8	1.1	2.5	1.5	2.6	2.7	1.9	1.2*
	60%	1.4*	1.2	1.2*	1.5	1.5*	2.5	-	-
	90%	2.1*	2.4*	-	-	1.9*	2.8	-	-
SeaIsle Supreme	0%	0.7	0.6	1.1	1.7	2.9	2.2	1.6	1.5
	60%	1.2*	0.8	1.3	1.6	0.6*	1.8	-	-
	90%	1.0	1.0	-	-	0.9*	1.4*	-	-

Table 19 contd. Chlorophyll concentration response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various reduced light environments (RLE) and trinexapac-ethyl (TE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

 $\dagger$  -TE = No trinexapac-ethyl applied.

			2008				20	)09	
		——10 V	WAS	20 v	WAS	10 Y	WAS	20 V	WAS—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE
					g kg	-1			
Champion	0%	0.7	0.5	2.3	2.8*	2.1	2.5	2.4	1.9
	60%	1.5	1.7	1.2	2.1	2.0	2.1	-	-
	90%	1.7	1.6	-	-	0.9	2.9*	-	-
MiniVerde	0%	2.6	0.3*	2.0	2.4	1.7	2.2	2.4	1.7*
	60%	1.7	1.2	1.9	1.2*	2.0	1.7	-	-
	90%	1.7	1.6	-	-	1.9	2.1	-	-
Diamond	0%	0.4	0.3	1.7	1.1*	1.7	1.9	1.5	1.2
	60%	0.4	0.4	1.3	1.8	1.5	1.8	-	-
	90%	1.1	0.9	-	-	1.2	1.1*	-	-
Meyer	0%	0.7	0.8	1.5	1.0*	0.9	1.4*	1.7	1.0
	60%	0.1	1.0	1.5	2.0*	1.4	1.1	-	-
	90%	1.7	1.4	-	-	0.9	1.4	-	-

Table 20. Chlorophyll concentration response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl (TE) treatments within reduced light environment (RLE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

\*Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

 $\dagger$  -TE = No trinexapac-ethyl applied.

-			2008	8			200	9	
		——10 \	WAS	—20 Y	WAS—	—10 \	WAS—	—20 V	WAS—
Cultivar	RLE Level	-TE†	+TE‡	-TE	+TE	-TE	+TE	-TE	+TE
					g	kg <sup>-1</sup>			
TifGrand	0%	1.4	1.2	1.8	3.2*	3.2	2.5*	2.3	1.9
	60%	1.6	1.1	1.5	2.9*	2.5	1.3*	-	-
	90%	0.5	1.0	-	-	2.2	2.5	-	-
Tifway	0%	1.5	1.2	1.7	1.4	2.2	2.6	1.6	2.4*
	60%	1.8	1.4	1.2	1.5	1.7	2.0	-	-
	90%	1.7	1.9	-	-	1.0	1.6*	-	-
Celebration	0%	0.8	1.1	2.5	1.5*	2.6	2.7	1.9	1.2*
	60%	1.4	1.2	1.2	1.5	1.5	2.5*	-	-
	90%	2.1	2.4	_	-	1.9	2.8*	-	-
SeaIsle Supreme	0%	0.7	0.6	1.1	1.7*	2.9	2.2*	1.6	1.5
	60%	1.2	0.8	1.3	1.6	0.6	1.8*	-	-
	90%	1.0	1.0	-	-	0.9	1.4*	-	-

Table 20 contd. Chlorophyll concentration response means of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' bermudagrass, 'Diamond', 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum to various trinexapac-ethyl (TE) treatments within reduced light environment (RLE) treatments from June through October 2008 and 2009 in Clemson, South Carolina.

\*Denotes a significant difference between trinexapac-ethyl treatments receiving the same shade treatment according to Fisher's LSD ( $\alpha$ =0.05) test.

*†*-TE= No trinexapac-ethyl applied.

#### Summary

Reduction of light quantity is a growth limiting factor in all warm season turfgrasses. The growth of 'Champion', 'MiniVerde', 'TifGrand', 'Tifway' and 'Celebration' bermudagrass, 'Diamond' and 'Meyer' zoysiagrass, and 'SeaIsle Supreme' seashore paspalum were negativitly affected when grown in a reduced light environment (RLE). A study during the summers of 2008 and 2009 demonstrated the response of these various cultivars to three levels of RLE, 0%, 60%, and 90% and two trinexapacethyl (TE) treatments, 0.57 kg ai ha<sup>-1</sup>, and no TE application.

Plant responses such as turf quality (TQ), clipping yield, and chlorophyll concentration were measured to determine morphological and physiological responses of various cultivars to various levels of RLE. All cultivars showed negative affects to increasing levels of RLE. In 2008, all cultivars with the exception of 'Meyer' and 'Diamond' zoysiagrass demonstrated unacceptable levels of turf quality in treatments not receiving TE grown in a 90% RLE in the month of June. 'Meyer' and 'Diamond' maintained acceptable turf quality in 90% RLE without TE application through July, and August, respectively. Application of TE reduced TQ decline, however, over time turf grown in 60% and 90% RLE continued to weaken in all cultivars despite TE application.

In 2009 similar trends continued from 2008. Increasing RLE decreased TQ in all cultivars. Application of TE increased plant health in 60% and 90% RLE, however TQ eventually declined to levels close to treatments not receiving TE application.

In 2008 and 2009 clipping yield showed similar responses. All cultivars demonstrated increased clipping yields with increased RLE levels in a plant response to

75

capture more available light. However, over time the vigor of this response declined as plant health declined, and top growth became less vigorous. Application of TE first decreased clipping yield, then increased clipping yield as plant health in treatments receiving TE application became greater than those not receiving TE application.

In 2008 and 2009, increased levels of RLE initially caused an increase in chlorophyll concentration in another physiological plant response to capture more available light for photosynthesis. The application of TE allowed for some cultivars to have increased chlorophyll production in decreased amounts of plant leaf tissue due to decreased cell elongation.

Future research is needed to determine the ability of other GA synthesis inhibiting growth regulators to improve morphological and physiological responses of each cultivar. Research is also needed to determine how varying levels of nutrient application will interact with various GA synthesis inhibitors. Lastly, quantification of a daily light integral (DLI) for each cultivar would be useful in determining usefulness of each cultivar for various light conditions.

## Appendix A

# Procedure for Nelson's Assay and Glucose Standard Curves for TNC Analysis

## Aliquot preparation

- 1. Weigh 50 mg dry tissue sample in 13 x 100 mm test tube.
- 2. Add 100  $\mu$ L of 80% ethanol.

3. Add 2 mL of 0.1 M (pH 4.5) sodium acetate buffer. Prepare 3 replicates of enzyme bland as amyloglucosidase contains reducing sugars which need to be subtracted out.

4. Place test tubes in boiling water for 1 hour, allow to cool for 1 hour, and repeat. Allow solution to cool before adding enzymes.

5. Add 1.0 mL of each enzyme solution. Keep enzyme solutions on ice.

- a. Invertase (Sigma I-4743, 433 units mg<sup>-1</sup>) -5 units mL<sup>-1</sup> in 0.1 M acetate buffer
- b. Amyloglucosidase (Sigma A-9228, 23,000 units g<sup>-1</sup>) -50 units mL<sup>-1</sup> in 0.1 M acetate buffer
- 6. Incubate for 3 days at 40-45°C and vortex 3 times daily.
- 7. Allow to settle until clear.
- 8. Remove 25 µL of aliquot for TNC analysis.

### Buffers, Reagents, and Standard Glucose Curves

#### Sodium Acetate Buffer

- 1. For 2,000 mL of 0.1 M buffer, weigh 5.56 g of sodium acetate.
- 2. Dissolve in approximately 1,600 mL deionized water.
- 3. Adjust pH to 4.5 using 1 N acetic acid.
- 4. Bring to 2,000 ML volume.
- 5. Store at  $3^{\circ}$  C.

### Copper Reagent

1. Dissolve 28 g of anhydrous sodium phosphate dibasic ( $Na_2HPO_4$ ) in approximately 400 mL.

- 2. Add 21 mL of concentrated sulfuric acid.
- 3. Dissolve 3 g of sodium arsenate (Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O) in 25 mL of deionized water.
- 4. Bring to 500 mL and mix well.
- 5. Incubate at  $37^{\circ}$  C for 48 hours.
- 6. Store in brown bottle at room temperature.

## Glucose Standards

<u>Glucose Concentration</u> <u>Standard</u>	glucose (g $L^{-1}$ )	Dilution for 10 mL of
0.8 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	$1.4408 \text{ g L}^{-1}$	10.00 mL stock : 0.00 mL
0.7 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	1.2607 g L <sup>-1</sup>	8.75 mL stock : 0.00 mL
0.6 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	1.0806 g L <sup>-1</sup>	7.50 mL stock : 0.00 mL
0.5 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	0.9005 g L <sup>-1</sup>	6.25 mL stock : 0.00 mL
0.4 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	$0.7204 \text{ g L}^{-1}$	5.00 mL stock : 0.00 mL
0.3 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	$0.5403 \text{ g L}^{-1}$	3.75 mL stock : 0.00 mL
0.2 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	0.3602 g L <sup>-1</sup>	2.50 mL stock : 0.00 mL
0.1 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	0.1801 g L <sup>-1</sup>	1.25 mL stock : 0.00 mL
0.0 $\mu$ mol 100 $\mu$ L <sup>-1</sup> (stock) water	$0.0000 \text{ g L}^{-1}$	0.00 mL stock : 0.00 mL

## **Glucose Calculation**

- 1. Pipette 25  $\mu$ L of aliquot (samples and glucose standards into 13 x 100 mm test tubes.
- 2. Add 1.0 mL of copper reagent, mix, and place in boiling water bath for 20 minutes.

- 3. Remove samples and allow to cool for 5 minutes in room temperature water bath.
- 4. Read absorbance at 520 nm.
- 5. Calculate linear regression of glucose standard curve.

6. Solve for glucose concentration using linear regression equation and absorbance value.

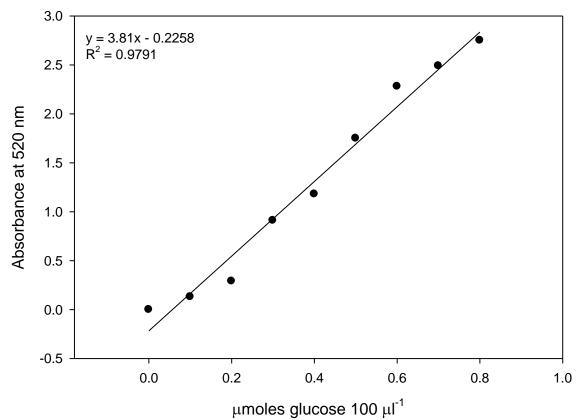


Figure A-1. Glucose standard curve for TNC calculation for 'Diamond' zoysiagrass.

### Appendix B

### Chlorophyll Extraction with DMSO

- 1. Weigh 0.1g fresh tissue into 13 x 100 mm test tubes.
- 2. Add 10 mL of Dimethyl Sulfoxide to each test tube. Cover with rubber stopper.
- 3. Incubate in  $65^{\circ}$  C water shake bath for 1.5 hour.
- 4. Transfer extract into spectrophotometer using pipetter.
- 5. Measure and record absorbance values at 645 nm and 663 nm wavelengths.
- 6. Chlorophyll content is determined by the following formula (Arnon, 1949).

 $(20.2 \text{ x } D_{645} + 8.02 \text{ x } D_{663}) \text{ x } 0.1 = \text{g chlorophyll } \text{kg}^{-1} \text{ tissue}$ 

 $D_{663 \text{ and } 645}$  = absorbance values at given wavelengths.

#### LITERATURE CITED

- Adams, R., E. Kerber, K. Pfister, E.W. Weiler. 1992. Studies on the action of the new growth retardant CGA 163'935 (Cimectacarb). P. 818-827. In C.M. Krrsenet al. (ed.) Progress in Plant Growth Regulation. Kluwer Academic Publ., Dordrecht, the Netherlands.
- Anderson, J.M. (1986), Photoregulation of the composition, function, and structure of thylakoid membranes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 44: 231-251
- Arnon, D.I., 1949. Copper enzymes isolated chloroplasts. Polyphenoloxidases in *Beta vulgaris*. Plant Physiol. 24:241-249.
- Brian, P.W. and H.G. Hemming. 1955. The effect of gibberellic acid on shoot growth of pea seedlings. Physiologia Plantarum 8:669-681.
- Bunnell, B.T., L.B. McCarty, and W.C. Bridges, Jr. 2005. Evaluation of three bermudagrass cultivars and 'Meyer' zoysiagrass grown in shade. International Turfgrass Society Research Journal. Volume 10.
- Burton, G. W., J. E. Jackson, and F. E. Knox. 1959. The influence of light reduction upon the production, persistence and chemical composition of 'Coastal' bermudagrass. Agron. J. 51:537-542.
- Cooper, J.D. 1970. Light and temperature requirements for the growth of tropical grasses. Herb. Abstr. 40:1-15.
- Duble, Richard L. 1989. Turfgrasses. Texas A&M University Press. College Station.
- Ervin, E.H., C.H. Ok, B.S. Fresenburg, and J.H. Dunn. 2002. Trinexapac-ethyl restricts shoot growth and prolongs stand density of 'Meyer' zoysiagrass fairway under shade. HortSci 37:502-505.
- Fagerness, Matthew James and D. Penner. 1998. 4C-trinexapac-ethyl absorption and translocation in Kentucky bluegrass. Crop Science. 38(4):1023-1027.
- Fry, J., and Huang, B. 2004. Applied Turfgrass Science and Physiology. John Wiley and Sons, Inc., New Jersey.
- Gawronska, H., Y-Y. Yang, K. Furukawa, R. E. Kendrick, N. Takahashi and Y. Kamiya. 1995. effects of low irradiance stress on gibberellin levels in pea seedlings. Plant Cell Physiol. 36(7):1361-1367.
- Hiscox, J.D. and G.F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1332-1334.

- Hopkins, William G. 1995. Introduction to Plant Physiology. John Wiley & Sons, Inc. New York.
- Ingram, T.M., J.B. Reid, I.C. Murfet, P. Gaskin, C.L. Willis, and J. MacMillan. 1984. Internode length in *Pisum*. Planta 160:455-463.
- Ingram, T.M., J.B. Reid, and J. MacMillan, 1986. The quantitative relationship between gibberellin A<sub>1</sub> and internode growth in *Pisum sativum* L. Planta 168:414-420.
- McCarty, L.B. 2011. Best Golf Course Management Practices. 3<sup>nd</sup> ed. Pearson Prentice Hall, Upper Saddle River, New Jersey.
- Melis, A. 1996. Excitation energy transfer: Functional and dynamic aspects of Lhc (cab) proteins. In Oxygenic Photosynthesis: The Light Reactions, D.R. Ort and C.F. Yocum, eds., Kluwer, Dordrecht, Netherlands, pp. 523-538
- Mohr, Hans, and P. Schopfer. 1995. Plant Physiology. Springer-Verlag Berlin Heidelberg. New York.
- Nelson, N. 1944. A photometric adaptation of the somogyi method for the determination of glucose. J. Biol. Chem. 153:375-380.
- Qian, Y.L., and M.C. Engelke. 1999. Influence of trinexapac-ethyl on 'Diamond' zoysiagrass in a shade environment. Crop Sci. 39:202-208.
- Qian, Y.L., and M.C. Engelke, M.J.V. Foster, and S. Reynolds. 1998. Trinexapac-ethyl restricts shoot growth and improves quality of 'Diamond' zoysiagrass under shade. HortSci. 333:1019-1022.
- Tan, Z. G., Y.L. Qian. 2003. Light intensity affects gibberellic acid content in Kentucky Bluegrass. HortSci. 38(1):113-116.
- Sage, R.F. and R.K. Monson. 1999. C<sub>4</sub> Plant Biology. Academic Press, San Diego.
- Sinha, R.K. 2004. Modern Plant Physiology. Alpha Science International Ltd. Pangbourne, England.

Somogyi, M. 1945. A new reagent for the determination of sugars. J. Biol. Chem. 160:61-68

Taiz, L. and E. Zeiger. 2006. Plant Physiology 4<sup>th</sup> Ed. Sinauer Associates, Inc. Sunderland, MA.

United States Golf Association Green Section Staff. 2004. USGA recommendations for a method of putting green construction. The 2004 revision. p. 4-7.