WATER USE AND SUMMER STRESS TOLERANCE MECHANISMS FOR
CREEPING BENTGRASS AND KENTUCKY BLUEGRASS

by

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ABSTRACT OF THE DISSERTATION

Creeping bentgrass (*Agrostis stolonifera*) and Kentucky bluegrass (*Poa Pratensis* L.) are two widely-used cool-season grasses grown extensively in northern regions of the United States. Creeping bentgrass is primarily used on golf courses, while Kentucky bluegrass has extensive uses in both home lawns and sports fields. Each of these areas of use are coming under increased scrutiny in regards to water management and conservation. Communities are increasingly putting greater restrictions on both the amount and type of water that can be used for irrigating golf courses, sports fields, and home lawns. The focus of our project was to develop a greater understanding of these grasses’ water use, as a way to establish ideal water management practices. The field portion of the project focused on irrigation frequency as applied to three bentgrass species maintained as golf course fairways. Results suggested that reducing frequency and watering one or two times per week, while still replacing 100% of evapotranspiration (ET), produced equivalent, if not improved turf quality, and actually held water deeper in the soil profile, reducing evaporation losses to the atmosphere. In addition to the field portion of the project, experiments were conducted in growth chambers evaluating both creeping bentgrass and Kentucky bluegrass physiological responses to drought and summer stress. Specific findings suggested that exfoliar application of abscisic acid (ABA) or trinexapac-ethyl, a gibberelin inhibitor, significantly improved plant drought tolerance. Further research evaluated genotypic variation between varieties of these species, suggesting that both tolerance and avoidance traits are employed to protect plant physiological function under water deficit.
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# TABLE OF CONTENTS

ABSTRACT OF THE DISSERTATION.........................................................................................ii

ACKNOWLEDGEMENTS.....................................................................................................iii

TABLE OF CONTENTS.......................................................................................................iv

LIST OF TABLES.............................................................................................................vii

LIST OF FIGURES...........................................................................................................x

LITERATURE REVIEW..................................................................................................1

  Introduction..................................................................................................................1

  Mechanisms of Drought Resistance........................................................................3

    Drought Tolerance..................................................................................................3

    Drought Avoidance...............................................................................................8

    Drought Escape....................................................................................................13

  Genetic Variation in Drought Resistance.............................................................14

Growth Regulation and Drought Stress Responses.............................................17

Water-saving Irrigation Management.................................................................20

Conclusions...............................................................................................................26

References.................................................................................................................29

CHAPTER 1. Irrigation Frequency Impact on Three Bentgrass Species Maintained as

  Fairways....................................................................................................................40

  Introduction.............................................................................................................40

  Materials and Methods.........................................................................................42

  Results.....................................................................................................................44
CHAPTER 2. Effects of Trinexapac-ethyl Foliar Application on Creeping Bentgrass

Responses to Combined Drought and Heat Stress..................................................80
Introduction...........................................................................................................80
Materials and Methods..........................................................................................82
Results....................................................................................................................86
Discussion..............................................................................................................88
References..............................................................................................................92

CHAPTER 3. Drought Responses of Kentucky Bluegrass and Creeping Bentgrass as
Affected by Abscisic Acid and Trinexapac-ethyl....................................................107
Introduction..........................................................................................................107
Materials and Methods.........................................................................................109
Results..................................................................................................................113
Discussion............................................................................................................116
References............................................................................................................120

CHAPTER 4. Physiological Indices Associated with Drought Responses for Kentucky
Bluegrass Cultivars.................................................................................................133
Introduction..........................................................................................................133
Materials and Methods.........................................................................................134
Results..................................................................................................................137
Discussion............................................................................................................139
References............................................................................................................143
CHAPTER 5. Evaluation of Drought Tolerance and Avoidance Traits for Six Creeping Bentgrass Cultivars Drought Stress

Introduction

Materials and Methods

Results

Discussion

References

CURRICULUM VITA
LIST OF TABLES

Literature Review:

Table 1. Drought resistant\(^a\) varieties of commonly used turfgrass species (based on NTEP data between 1996 to 2003). The top four commercially-available cultivars are listed for each species (not including experimental cultivars).

Chapter 1:

Table 1. Soil volumetric water content (VWC) (%) for creeping, colonial, and velvet bentgrasses maintained at 9.5 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments \((P=0.05)\) based on Fischer’s protected LSD test.

Table 2. Soil volumetric water content (VWC) (%) for creeping, colonial, and velvet bentgrasses maintained at 6.4 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments \((P=0.05)\) based on Fischer’s protected LSD test.

Table 3. Leaf relative water content (RWC) (%) for creeping, colonial, and velvet bentgrasses maintained at 9.5 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments \((P=0.05)\) based on Fischer’s protected LSD test.
Table 4. Leaf relative water content (RWC) (%) for creeping, colonial, and velvet bentgrasses maintained at 6.4 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.

Table 5. Evapotranspiration (ET) (%) for creeping, colonial, and velvet bentgrasses maintained at 9.5 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.

Table 6. Evapotranspiration (ET) (%) for creeping, colonial, and velvet bentgrasses maintained at 6.4 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.

Table 7. Crop Water Stress Index (CWSI) for creeping, colonial, and velvet bentgrasses maintained at 9.5 and 6.4 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant
differences between irrigation treatments \((P=0.05)\) based on Fischer’s protected LSD test.

Table 8. Meteorological parameters from an on-site weather station located approximately 130 m from the rainout shelter in North Brunswick, NJ, in 2004 and 2005. Data presented include bi-monthly means for maximum air temperature, relative humidity, daily solar radiation, and wind speed during the study period. Sensors for the climatic variables were located approximately 2 m off the ground.

Chapter 3:

Table 1. Soil volumetric water content (%) in treatments with abscisic acid or trinexapac-ethyl during drought stress of Kentucky bluegrass var. Brilliant and creeping bentgrass var. L-93.

Table 2. Vertical shoot growth as affected by application of abscisic acid or trinexapac-ethyl during drought stress of Kentucky bluegrass var. Brilliant and creeping bentgrass var. L-93.

Chapter 4:

Table 1. Cultivar variation in physiological traits, leaf relative water content (RWC), electrolyte leakage (EL), net photosynthetic rate \((P_n)\), transpiration \((\Psi_{\text{leaf}})\), and stomatal conductance \((g_s)\), under well-watered (control) conditions and at 14 d of drought stress.
LIST OF FIGURES

Chapter 1:

Fig. 1. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on turf quality of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 2. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on turf quality of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 3. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on turf quality of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 4. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on turf quality of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich
maintained at 6.4 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 5. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on green leaf biomass of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 6. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on green leaf biomass of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 7. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on green leaf biomass of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 8. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on green leaf biomass of (A) creeping bentgrass var. L-
93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 9. Effects of four different irrigation frequencies (3x week$^{-1}$, 2x week$^{-1}$, 1x week$^{-1}$, or 14 d interval) on leaf area index of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 10. Effects of four different irrigation frequencies (3x week$^{-1}$, 2x week$^{-1}$, 1x week$^{-1}$, or 14 d interval) on leaf area index of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 11. Effects of four different irrigation frequencies (3x week$^{-1}$, 2x week$^{-1}$, 1x week$^{-1}$, or 14 d interval) on leaf area index of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.
Fig. 12. Effects of four different irrigation frequencies (3x week⁻¹, 2x week⁻¹, 1x week⁻¹, or 14 d interval) on leaf area index of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 13. Scatter-plot graph of turf quality and crop water stress index (CWSI) of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich in 2004.

Fig. 14. Scatter-plot graph of turf quality and crop water stress index (CWSI) of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich in 2005.

Fig. 15. Scatter-plot graph of leaf relative water content (RWC) and crop water stress index (CWSI) of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich in 2004.

Fig. 16. Scatter-plot graph of leaf relative water content (RWC) and crop water stress index (CWSI) of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich in 2005.
Chapter 2:

Fig. 1. Effects of foliar application of trinexapac-ethyl (TE) on turf quality during combined heat and drought stress (stress). Turf quality was expressed on a scale of 1-9 based on turf color, density, and uniformity. Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 2. Effects of foliar application of trinexapac-ethyl (TE) on relative water content (RWC) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 3. Changes in soil volumetric water content (%) in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (p=0.05) for comparison of changes over treatment period.

Fig. 4. Changes in vertical shoot growth in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Vertical bars
indicate LSD values (p=0.05) for comparison of changes over the treatment period.

Fig. 5. Effects of foliar application of trinexapac-ethyl (TE) on canopy photosynthesis rate (CO₂ μmol m⁻² s⁻¹) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Fig. 6. Effects of foliar application of trinexapac-ethyl (TE) on canopy ET (H₂O mmol m⁻² s⁻¹) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Fig. 7. Changes in photochemical efficiency (Fv/Fm) in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (p=0.05) for comparison of changes over treatment period.

Fig. 8. Effects of foliar application of trinexapac-ethyl (TE) on leaf chlorophyll content (mg gram⁻¹ of dry weight) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).
Fig. 9. Effects of foliar application of trinexapac-ethyl (TE) on total nonstructural carbohydrates (TNC) (mg gram⁻¹ of dry leaf tissue) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Chapter 3:

Fig. 1. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on leaf relative water content during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 2. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on evapotranspiration (mm.day⁻¹) during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Treatments with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Fig. 3. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on osmotic potential during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).
Fig. 4. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on turf quality during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Turf quality was expressed on a scale of 1-9 based on turf color, density, and uniformity. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 5. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on photochemical efficiency (Fv/Fm) during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Chapter 4:

Fig. 1. Scatter-plot graph of turf quality and soil volumetric water content (VWC) of eight Kentucky bluegrass cultivars exposed to drought for 19 d with data collected at 0, 7, 14, and 19 d of drought stress.

Fig. 2. Scatter-plot graph of turf quality and leaf relative water content (RWC) of eight Kentucky bluegrass cultivars exposed to drought for 19 d with data collected at 0, 7, 14, and 19 d of drought stress.

Fig. 3. Scatter-plot graph of turf quality and leaf electrolyte leakage (EL) of eight Kentucky bluegrass cultivars exposed to drought for 19 d with data collected at 0, 7, 14, and 19 d of drought stress.
Fig. 4. Scatter-plot graph of leaf electrolyte leakage (EL) and leaf relative water content (RWC) of eight Kentucky bluegrass cultivars exposed to drought for 19 d with data collected at 0, 7, 14, and 19 d of drought stress.

Fig. 5. Scatter-plot graph of turf quality and stomatal conductance (mmol H₂O m⁻² s⁻¹) (A), transpiration rate (mmol H₂O m⁻² s⁻¹) (B), and net leaf photosynthetic rate (mmol CO₂ m⁻² s⁻¹) (C) for eight Kentucky bluegrass cultivars exposed to drought for 19 d. Data were collected at 0, 7, 14, and 19 d of drought stress.

Fig. 6. Scatter-plot graph of leaf relative water content (RWC) and stomatal conductance (mmol H₂O m⁻² s⁻¹) (A), transpiration rate (mmol H₂O m⁻² s⁻¹) (B), and net leaf photosynthetic rate (mmol CO₂ m⁻² s⁻¹) (C) for eight Kentucky bluegrass cultivars exposed to drought for 19 d. Data were collected at 0, 7, 14, and 19 d of drought stress.

Chapter 5:

Fig. 1. Creeping bentgrass cultivar variation in turf quality under well-watered (dotted line) and drought stress (solid line). Vertical bars indicate LSD values (p=0.05) for cultivar and treatment comparisons at a given day of treatment.

Fig. 2. Creeping bentgrass cultivar variation in leaf photochemical efficiency (Fv/Fm) under well-watered (dotted lines) and drought stress (solid lines).
Vertical bars indicate LSD values (p=0.05) for cultivars and treatment comparisons at a given day of treatment.

Fig. 3. Creeping bentgrass cultivar variation in leaf relative water content under well-watered (dotted lines) and drought stress (solid lines). Vertical bars indicate LSD values (p=0.05) for cultivar and treatment comparisons at a given day of treatment.

Fig. 4. Creeping bentgrass cultivar variation in osmotic adjustment under drought stress. Columns with the same lowercase letters are not significantly different based on LSD values (p=0.05).

Fig. 5. Creeping bentgrass cultivar variation in evapotranspiration rate under well-watered (dotted lines) and drought stress (solid lines). Vertical bars indicate LSD values (p=0.05) for cultivar and treatment comparisons at a given day of treatment.

Fig. 6. Creeping bentgrass cultivar variation in carbon isotope discrimination ($\delta^{13}C$) under well-watered and drought stress at 14 d of treatment. Columns with the same lowercase letters are not significantly different based on LSD values (p=0.05).

Fig. 7. Creeping bentgrass cultivar variation in root viability, expressed as TTC Reduction (Absorbance 490 nm mg$^{-1}$), at 17 d of drought stress. Columns with the
same lowercase letters are not significantly different based on LSD values (p=0.05).

Fig. 8. Creeping bentgrass cultivar variation in total root length under well-watered (A) and drought stress (B) at 17 d of treatment. Columns with the same lowercase letters are not significantly different based on LSD values (p=0.05).

Fig. 9. Creeping bentgrass cultivar variation in total number of roots under well-watered (A) and drought stress (B) at 17 d of treatment. Columns with the same lowercase letters are not significantly different based on LSD values (p=0.05).
LITERATURE REVIEW

INTRODUCTION

Water for use in turfgrass irrigation is becoming increasingly limited, which often leads to decline in turf quality, particularly in semi-arid and arid regions. Therefore, water conservation becomes a prime concern of turfgrass managers and growers as communities place greater restriction on the use of portable water for irrigating turfgrass in home lawns, parks, athletic fields, and golf courses (Kenney et al., 2004; Carbone and Dow, 2005; Lyon et al., 2005). This situation is complicated by weather patterns that lead to extreme weather such as extended drought and heat. The combination of these factors puts great demand on water conservation strategies in turfgrass management that decrease the overall quantity of irrigation water required to maintain healthy turf, as well as decreasing fresh water use by increasing the use of reclaimed or recycled water.

Developing efficient, water-saving management practices for any particular site, whether it is a golf course or an athletic field, requires an understanding of environmental factors that influence turfgrass growth and water use, water use characteristics of plants, and how plants respond to water deficit. Extensive research studies have investigated drought-resistance mechanisms for various turfgrass species. During the process of plant adaptation to drought stress, plants have developed various mechanisms, including drought avoidance, drought tolerance, and drought escape. Many water-conservation practices are based on physiological responses of plants to water stress, including the use of plant growth regulators (PGRs) to improve stress tolerance and reallocate plant water use, applying anti-transpirants to reduce evaporative losses, applying osmo-regulants to
improve osmotic adjustment, and adjusting fertilizer applications to improve drought tolerance. Plant water absorption can also be increased by avoiding establishment in heavy soils, limiting soil compaction, irrigating to avoid overwatering, as well as raising mowing heights to increase root systems. Other water conservation strategies may be developed based on soil and climatic conditions, including irrigation delivery systems that have reduced evaporative losses, application of surfactants to evenly distribute soil water and reduce surface water runoff, and irrigation at minimum frequency and quantity and at times when evapotranspirational demand is low.

As potable water supply for irrigation decreases, the use of reclaimed water to irrigate turf has been increasing over the past 30 years. The use of recycled water for irrigation increased from 8% to 21% over the period of 1974-1994 in Florida, which has the greatest number and acreage golf courses in the United States (Haydu et al., 1997). Harivandi (2000) suggests that recycled water has application in golf courses, parks, cemeteries, and other venues of non-food urban horticulture. Irrigation with reclaimed water would greatly reduce potable water consumption in turfgrass management, although turf quality may be adversely affected. Reclaimed water can have a high salt content, which can result in soil salinity and induce osmotic stress in plants. Such problems may be intensified by abiotic factors leading to high evapotranspirational demand, such as high temperature, wind, and low humidity. Water-conservation strategies that are developed with a comprehensive understanding of interaction between plants, soils, and climates is therefore of great importance in turfgrass management, whether potable or reclaimed water is used for irrigation.
This chapter provides a review of recent literature on mechanisms of drought resistance in various turfgrass species, including drought tolerance, avoidance, and escape. Physiological factors and management practices that can reduce water use or improve turfgrass drought resistance, including use of plant growth regulators, osmo-regulants, and anti-transpirants will also be discussed. Efficient irrigation management practices that could be implemented for water conservation and improving plant drought resistance will conclude the chapter.

**MECHANISMS OF DROUGHT RESISTANCE**

Generally, all plants including turfgrasses that are growing in water-limiting environments utilize various adaptive mechanisms. Plants that are capable of growing and surviving under drought stress conditions are broadly considered drought resistant (Turner, 1986). The literature suggests classification of drought resistance into three separate categories: drought avoidance, drought tolerance, and drought escape. These drought-resistance strategies are not mutually exclusive. A plant may exhibit more than one of these strategies to cope with drought stress. For an orderly discussion, three drought-resistance strategies are discussed separately in this section.

**Drought Tolerance**

Drought tolerance can be defined as a plant’s ability to maintain physiological functions when little or no water is available to the plant. One of the important factors controlling cell tolerance to desiccation or dehydration is the cell’s capability to maintain adequate turgor pressure during drought stress. Generally, this is done by increasing the
concentration of compatible solutes within the cell. Compatible solutes or osmoregulants include inorganic solutes such as potassium (K\(^+\)), calcium (Ca\(^{2+}\)), and sodium (Na\(^+\)) and organic solutes such as soluble sugars (e.g. sucrose), polyols (e.g. mannitol), organic acids (e.g. malate), ammonium compounds (e.g. glycine betaine), and non-protein amino acids (e.g. proline) (Nilsen et al., 1996). Compatible solutes accumulate in cells subjected to water deficit serving to lower the osmotic potential of the cell without causing toxic effects. The accumulation of compatible solutes triggered by dehydration differs from the accumulation of solutes simply due to the concentration effect resulting from water loss in that it is an active accumulation rather than a passive one, respectively (Blum, 1988). This active accumulation of solutes is known as osmotic adjustment (OA), and has been shown to be positively correlated to drought tolerance. By increasing compatible solutes, a plant cell can lower its osmotic potential and thus, water potential, which prevents water loss to intercellular spaces or can increase water movement into cells with low water potential due to OA.

There are various compounds and pathways that plants can utilize to adjust osmolality, each having differing energy requirements. Compatible solutes or osmoregulants include inorganic solutes such as potassium (K\(^+\)), calcium (Ca\(^{2+}\)), and sodium (Na\(^+\)) and organic solutes such as soluble sugars (e.g. sucrose), polyols (e.g. mannitol), organic acids (e.g. malate), ammonium compounds (e.g. glycine betaine), and non-protein amino acids (e.g. proline) (Nilsen et al., 1996).

Many studies have demonstrated the importance of OA in drought tolerance in turfgrass species. For example, DaCosta and Huang (2006) have shown the importance of OA in creeping bentgrass (Agrostis stolonifera L.) and velvet bentgrass (A. canina L.).
Compared to creeping bentgrass, velvet bentgrass showed a 50% to 60% higher magnitude of OA under water deficit. The increase in OA was associated with better drought tolerance in velvet bentgrass, as determined by higher visual turf quality (TQ) and leaf relative water content (RWC) under drought stress conditions, compared to bentgrass. In another study, Kentucky bluegrass (Poa pretensis L.) plants that were pre-exposed to moderate drought stress maintained higher relative water content and turf quality during subsequent stress exposure, compared to plants with no prior exposure to drought; the improved stress tolerance following pre-conditioning was at least partially attributed to increases in OA (Jiang and Huang, 2001). Qian and Fry (1997) have shown a positive correlation between OA and recuperative ability from drought stress for buffalograss (Buchloe dactyloides), zoysiagrass (Zoysia japonica), bermudagrass (Cynodon dactylon [Nut.] Engelm.), and tall fescue (Festuca arundinacea (Schreb.). However, studies with non-turfgrass species have shown that increased accumulation of osmotic solutes is not always correlated with increased stress tolerance (Maggio et al., 1997), and the degree of OA can vary with species and with genotypes of the same species (Zhang et al., 1999; Morgan, 1984; Rhodes and Samars, 1994). Genetic variation in OA capacity has been reported in four turfgrass species, with the magnitude of osmotic adjustment ranking as buffalograss = zoysiagrass > bermudagrass > tall fescue (Qian and Fry, 1997).

In addition to OA, other mechanisms such as cell wall elasticity also play important roles in maintenance of cell turgor pressure and desiccation tolerance. The general response of plants to drought stress is to increase cell wall elasticity. Cells that are more elastic can maintain turgor pressure at low water potential. As a result of
increased cell wall elasticity, plants can maintain turgor for a longer period of time under drought stress. Research investigating the drought response of buffalograss (‘Biloela’) has shown that cell wall elasticity, along with OA, function to maintain turgor under water deficit (Wilson and Ludlow, 1983). White et al. (2001) reported that zoysiagrass genotypes with high relative water content at zero turgor and tissue elasticity recovered better from stress and required less supplemental irrigation. This result implies that the improvement in biophysical properties is also an important factor contributing to drought tolerance in turfgrass. Some plant species may rely solely on increasing cell wall elasticity without OA involvement in turgor maintenance (Auge et al., 1987). Research conducted by Barker et al. (1993) of five forage grasses showed that C_3 grasses tended to have more elastic cell walls and were less reliant on OA for maintenance of turgor pressure.

Drought tolerance mechanisms also involve changes in various metabolic processes, which help protect cells from further injury and help maintain metabolic functioning. A relatively drought sensitive metabolic function is photosynthesis; drought stress inhibits carbon fixation. While light absorption continues under drought stress, reduced electron transport to carbon fixation leads to an accumulation of excessive energy that can be dissipated by reducing molecular oxygen, thus generating active oxygen species. Active oxygen species include singlet oxygen (\(^1\)O_2), superoxide (O_2 \(-\)), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH \(-\)). These species can interact with lipids, nucleic acids, and proteins, and thus cause cellular damage. It has also been suggested that oxidative stress resulting from drought can negatively impact turf quality and physiological functions (Zhang and Schmidt, 1999; Huang and Fu, 2001).
Many plant species have antioxidant defense systems to protect cells from oxidative stress induced by drought. One of the defense mechanisms against oxidative stress is to increase the activity of superoxide dismutase (SOD), catalase (CAT), hydrogen peroxidase (POD), and ascorbate peroxidase (APX). Through the increase of antioxidant enzyme activity, active oxygen species are scavenged thereby reducing the amount of oxidative damage done to cells. Zhang and Schmidt (1999) reported that Kentucky bluegrass plants that had higher antioxidant levels performed better under drought stress. Fu and Huang (2001) found that tolerance of tall fescue and Kentucky bluegrass to soil surface drying was associated with the maintenance or enhancement of antioxidant enzyme activity. Exogenous application of plant growth regulators to creeping bentgrass increased SOD activity and improved turf performance under drought stress (Zhang and Schmidt, 2000).

In response to drought stress, plants exhibit significant changes in protein composition, protein synthesis, and expression, including synthesis of stress-inducible proteins (Ouvraud et al., 1996; Riccardi et al., 1998). Synthesis of stress-induced proteins such as late embryogenesis abundant (LEA) proteins in response to drought stress has been associated with increased adaptive ability and tolerance to drought stress in various non-turfgrass species (Riccardi et al., 1998; Bewley and Oliver, 1992; Han and Kermode, 1996). Limited information is available on the association of protein induction with drought tolerance in turfgrass (Jiang and Huang, 2002). A class of stress-induced LEA proteins, known as dehydrins, play a positive role in drought tolerance. Dehydrins have been shown to accumulate under drought stress and in response to increased abscisic acid concentrations in tall fescue (Jiang and Huang, 2002). The function of these
proteins involved in drought tolerance remains unclear. However, some studies suggest that these proteins may act in the stabilization of membranes and as molecular chaperones, which prevent denaturation of other proteins (Close, 1996; Dure, 1993). Dehydrins may also act as osmotic adjustment regulators thereby enhancing desiccation tolerance (Nylander et al., 2001). Transgenic plants over-expressing the genes that code for these proteins have increased tolerance to drought and high salinity (Brini, 2005). The authors suggest that the known physical properties of dehydrins make them candidates for participation in stabilizing nuclear or cytoplasmic macromolecules, thus preserving structural integrity.

**Drought Avoidance**

Drought avoidance is the ability of a plant to maintain normal physiological function by postponing tissue dehydration. This mechanism may be achieved by increasing water uptake of the root system and/or reducing water loss from transpiring leaves.

Plants have developed multiple ways of procuring water, including developing extensive, deep root systems, increasing root branching and surface areas to exploit large soil volumes for water absorption. Research in turfgrass has shown that a shallow-rooted species, *Poa supina* Schrad, had lower drought tolerance than species with well-developed root systems, such as *A. stolonifera* and *Festuca rubra* (Leinauer et al., 1997). Huang and Gao (2000) reported that drought-resistant tall fescue cultivars had deeper root systems than sensitive cultivars. A study evaluating *Zoysia japonica* (cv. Meyer) showed that turfgrass plants exposed to drought developed more extensive rooting at
deeper soil depths than well-watered turf (Qian, 1996). A study done by Marcum et al. (1998) also indicates the importance of rooting depth, weight, and branching at lower depths in zoysiagrass adaptation to drought stress. Tall fescue is an excellent example of a drought avoider by developing deep root system. It exhibited maximum root extension of 33 to 60% greater than buffalograss, zoysiagrass, or bermudagrass in a greenhouse study, and extracted over 50% more water at a 90 cm depth than zoysiagrass (Qian et al., 1997). Various studies of tall fescue cultivars investigating the relationship between root development and drought avoidance have shown a strong correlations between the two, with greater total root length and density delaying water deficit and prolonging plant survival in drying soil (White et al., 1992; Carrow, 1996). Additional research has shown that cultivars of tall fescue with extensive root systems are capable of delivering more water to the plant, and as such avoid desiccation and wilting (Sheffer et al., 1987; Qian et al, 1997; Bonos and Murphy, 1999). Highly plastic root systems were found to contribute to the persistence of tall fescue under prolonged periods of drought stress (Duncan and Carrow, 1997; Huang et al., 1997; Huang et al., 1997). Huang et al. (1997) reported that drought resistance of tall fescue is more closely related to root plasticity and root viability than total root mass or length.

Some grasses are able to access more water through deeper root systems, which can result in water reallocation through the root zone. Water deep in the profile can be transported at night by roots to dry, shallow areas where water leaks from roots into the drying surface soil. This phenomenon is referred to as hydraulic lift. Huang (1999) showed that in buffalograss, water absorbed by roots deep in the profile at night helped to support root growth and function in nutrient uptake in upper soil profile, resulting in
delaying drought stress injury. This reallocation of water in the soil profile can lead to efficient use of water under surface soil drying conditions.

Differences in root distribution during drought stress may be due to carbon reallocation from shoots to roots for formation of a more extensive root system into deep soil. In a study investigating carbon metabolic responses under surface soil drying, both Kentucky bluegrass and tall fescue exhibited decreased respiration rates in shoots and roots in the upper drying layer. In contrast, there was enhanced carbon allocation to those roots in the lower, wet soil layer (Huang and Fu, 2000). In general, there may be more carbon allocated to roots, with a greater proportion of newly fixed carbon for roots in soil layers where water may be more available (Huang and Gao, 2000).

Another important avoidance mechanism is the ability of plants to reduce water loss through transpiration. Most transpirational water loss is through stomata in leaf epidermal surfaces. Stomatal closure is one of the most sensitive responses to drought stress, which increases resistance for water diffusion out of leaves, and thus results in water conservation.

Stomatal closure can be affected by many factors. It has been found to be induced by increasing leaf concentration of abscisic acid (ABA) that is transported from roots exposed to drying soils (Davies et al., 1994; Davies et al., 2002). Roots are important sites for the synthesis of ABA, which is transported to shoots and initiates a signal cascade in guard cells that alters the membrane transport of several ions, and as a result, guard cells lose their turgor and stomata close. This results in changes of stomatal conductance, transpiration rate, and photosynthesis (Zhang and Davies, 1987; Ludewig et al., 1988; Bray, 1993; Bohnert and Jensen, 1996). The importance of ABA as a
metabolic factor in the regulation of plant tolerance to stresses has received great
attention in recent years in other species (Chen et al., 1998; Quarrie, 1993). However,
limited information is available on the association of ABA signaling and drought
tolerance in turfgrass. Wang et al. (2003) reported that Kentucky bluegrass cultivars
tolerant of drought exhibited slower ABA accumulation rate than drought sensitive
cultivars during short-term drought stress, suggesting that low accumulation rate of ABA
in leaves would be beneficial for the maintenance of photosynthesis during short-term
drought, and allow dry matter to accumulate to support plant survival during prolonged
drought. Drought- tolerant cultivars of Kentucky bluegrass were characterized by lower
ABA accumulation and less severe decline in $\psi_{\text{leaf}}$, $P_n$, $g_s$, and turf quality during drought stress. The stomates of drought tolerant cultivars were more sensitive to changes in ABA
level in leaves during drought (Wang and Huang, 2003).

In addition to stomatal regulation of water loss, modification of shoot
c characteristics such as leaf shedding or folding, or the development of a thick cuticle
reduces leaf transpiration. Research in both tall fescue and *Eragrostis curvula* (Schrad.)
Nees. complex cv. Consol., a temperate-zone C4 grass, has shown that transpiration can
also be reduced by decreasing light intensity via rolling leaves (Renard and Francois,
1985; Johnston et al., 2002). Better tall fescue performance during drought stress was
positively related to leaf thickness, epicuticular wax content, and tissue density but
negatively related to stomatal density and leaf width (Fu and Huang, 2004). Beard and
Sifers (1997) reported that better dehydration avoidance among *Cynodon* species
compared to *Zoysia* species was attributed to lower water use rate due to a faster rate of
wax formation over the stomata during progressive drought stress.
Drought-avoidant plants control water loss to maintain growth under drought stress, which, therefore, may lead to increases in water use efficiency (WUE). WUE is often used to assess the expense of water loss or consumption in terms of carbon gain. WUE can be defined as the ratio between the amount of CO₂ assimilated and the amount of water lost. A key issue limiting WUE is an inescapable trade-off between controlling water use and carbon assimilation. The opposing need of the plant to both assimilate CO₂ and avoid excessive water loss must ultimately be resolved in the control of leaf gas exchange by the stomata, water loss and efficient expenditure of carbon, especially under drought conditions. Turfgrass species that performed better under drought stress maintained higher WUE than drought-sensitive species (DaCosta and Huang, 2006).

Low-water-use turfgrass species and cultivars may postpone tissue desiccation or survive longer when water supply is limited. Turfgrass water use can be estimated by a measurement of the evapotranspiration rate (ET), the sum of the amount of water transpired from leaves and evaporated from soil under the turf canopy within a given period of time. Water use rate varies with plant species and cultivars within a species, depending largely on shoot growth characteristics in addition to stomatal behaviors. For example, Beard (1994) summarized that tall fescue, Kentucky bluegrass, annual bluegrass (*Poa annua* L.), and creeping bentgrass had the highest ET rates; rough bluegrass (*Poa trivialis* L.) and perennial ryegrass (*Lolium perenne* L.) ranked intermediate; and chewsings (*Festuca rubra* L. subsp. *fallax* (Thuill.) Nyman), hard (Festuca brevipila R. Tracey), and red fescues (*Festuca rubra* L. subsp. *rubra*) had the lowest ET rates. Among warm-season grasses, bermudagrass (*Cynodon* spp.), zoysiagrass (*Zoysia* spp.), and buffalograss have relatively low water use rates while centipedegrass...
(Eremochloa ophiuroides [Munro] Hack.), seashore paspalum (Paspalum vaginatum Swartz.), and St. Augustinegrass (Stenotaphrum secundatum [walt.] Kuntze.) have relatively high water use rates (Beard, 1994). Within a species, cultivars also vary in water use rate, and the difference may range from 20 to 60% (Sherman, 1986; Kopec et al., 1988; Shearman, 1989; Bowman and Macaulay, 1991; Salaiz et al., 1991; Ebdon and Petrovic, 1998; Kjelgren et al., 2000). It is important to point out that the comparative water use rankings for different species and cultivars may change across different environments, climatic conditions, and cultural regimes. Thus, one strategy for reducing turfgrass irrigation requirements is to utilize grasses with reduced ET rates that perform well in a given environment. Available water supplies could be extended for longer periods of time to maintain favorable water status and also decrease the irrigation requirements with use of the appropriate species or cultivar.

Drought Escape

Drought escape, the third category of drought mechanisms discussed here, is when a plant completes its life cycle prior to drought exposure or becomes dormant during drought stress. An example of early reproduction to escape drought stress would be of an annual plant that germinates in the fall, over-winters, quickly matures through the spring and bears seed prior to summer stress. The plant then dies, and seeds germinate after the summer heat and drought, or germination possibly results from such stresses. Evolution of such a life cycle is simple and avoids the need to incorporate more complicated systems into its overall functioning.

Drought escape is used by some weedy annual grasses as a drought resistance approach. Annual bluegrass is a winter annual that germinates in autumn and grows
vegetatively until late spring when seeds are produced and the mother plants die. Although efficient in nature, grasses that escape drought are not reliable selections for perennial stands. However, this mechanism has been exploited by crop breeders to develop lines that mature early or that are planted earlier before drought occurs (Gimeno et al., 1989; Rose et al., 1992).

When not supplied with water for an extended period, turfgrass may stop growing and leaves may become desiccated, but crowns, stolons, or rhizomes are still alive. This is often referred to as dormancy, which preserve the vital parts of the plant for regeneration of shoots and roots when water becomes available. By becoming dormant, turfgrasses can escape drought stress by reducing demand on water until soil water is replenished. The length of turfgrass survival in a dormant condition depends on many factors, including soil water availability, plant growth rate, and the health of the plants at the onset of dormancy. Turfgrasses generally can maintain dormancy for weeks with limited damage.

**Genetic Variation in Drought Resistance**

There are large genetic variations in drought resistance and water use characteristics among turfgrass species and cultivars. A particular species or cultivar may possess multiple drought-resistance traits (Fry and Huang, 2004). In a summarized literature ranking, Fry and Huang (2004) reported that overall drought-resistance for warm-season turfgrass species is ranked from excellent to fair in the following order: buffalograss, bermudagrass, zoysiagrass, seashore paspalum (*Paspalum vaginatum*), St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze), and centipedegrass
(Eremochloa ophiuroides (Munro) Hack). For cool-season grass, overall drought resistance range from excellent to poor level in the order as tall fescue, fine fescue, Kentucky bluegrass, perennial ryegrass, creeping bentgrass, and annual bluegrass.

Cultivars within a species also vary in drought resistance. The National Turfgrass Evaluation Program (NTEP) was developed to evaluate various turfgrasses, including both existing and new varieties, grown in various climates across the United States. Full reports are based on four or five years of field data, and summary reports are generated each year. Recent NTEP data comparing drought resistance are summarized in Table 1.
Table 1. Drought resistant\textsuperscript{a} varieties of commonly used turfgrass species (based on NTEP data between 1996 to 2003). The top four commercially-available cultivars are listed for each species (not including experimental cultivars).

<table>
<thead>
<tr>
<th>Bentgrass (Fairway/Tee)\textsuperscript{b}</th>
<th>Tall Fescue\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seaside</td>
<td>Lion</td>
</tr>
<tr>
<td>SR 7200</td>
<td>Kitty Hawk S.S.T.</td>
</tr>
<tr>
<td>Glory</td>
<td>Marksman</td>
</tr>
<tr>
<td>Golfstar</td>
<td>Rembrandt</td>
</tr>
<tr>
<td>Chewings Fescue\textsuperscript{b}</td>
<td>Bermudagrass\textsuperscript{b}</td>
</tr>
<tr>
<td>Ambrose</td>
<td>Riviera</td>
</tr>
<tr>
<td>Ambassador</td>
<td>Shanghai</td>
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<tr>
<td>Treazure</td>
<td>Blackjack</td>
</tr>
<tr>
<td>Bridgeport</td>
<td>Blue-muda</td>
</tr>
<tr>
<td>Kentucky Bluegrass\textsuperscript{b}</td>
<td>Buffalograss\textsuperscript{b}</td>
</tr>
<tr>
<td>Unique</td>
<td>Cody</td>
</tr>
<tr>
<td>Apollo</td>
<td>Tatanka</td>
</tr>
<tr>
<td>Brilliant</td>
<td>Bison</td>
</tr>
<tr>
<td>Showcase</td>
<td>BAM-1000</td>
</tr>
<tr>
<td>Perennial Ryegrass\textsuperscript{c}</td>
<td>Zoysiagrass\textsuperscript{b}</td>
</tr>
<tr>
<td>Passport</td>
<td>Emerald</td>
</tr>
<tr>
<td>Affinity</td>
<td>Victoria</td>
</tr>
<tr>
<td>Calypso II</td>
<td>Zeon</td>
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<tr>
<td>Edge</td>
<td>El Toro</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Drought resistance was assessed as either wilting, leaf firing, dormancy or recovery. A 1 to 9 visual rating scale is used with 1 being complete wilting, 100% leaf firing, complete dormancy or no plant recovery; and 9 being no wilting, no leaf firing, 100% green-no dormancy, or 100% recovery.

\textsuperscript{b} Assessed via dormancy

\textsuperscript{c} Assessed via wilting
Among different water-conservation practices, use of grass species and cultivars that are capable of tolerating, avoiding or escaping drought stress, as well as utilizing different strategies is critical for water conservation and maintenance of high quality turfgrass in water-limiting environments. Planting drought-resistant grasses can result in significant water saving and reduce the overhead costs of irrigation water.

GROWTH REGULATION AND DROUGHT STRESS RESPONSES

Various chemical and hormonal compounds have been shown to impact plant drought resistance. These compounds can be divided into three broad classifications that correspond to plant drought response mechanisms: (1) plant growth regulators (PGR), (2) osmo-regulants, and (3) anti-transpirants. Growth regulators are synthetic compounds with hormone-like functions, which may affect plant growth, morphology, and metabolic activities, or can have a combined effect and can result in either drought avoidance or tolerance. Osmo-regulants influence the osmotic potential of a cell by modifying the production of various solutes, which can change a cell’s water potential. Cells with the ability to adjust osmotically can maintain positive turgor pressure by increasing concentration of various solutes in the vacuole or cytosol. Maintenance of turgor pressure has been shown to correlate to maintenance of physiological functions and overall plant health, as discussed in the above section. Anti-transpirants are chemicals that function in inhibiting transpiration by causing stomatal closure or by forming a film covering the leaf epidermal surface. Use of anti-transpirants helps to reduce water loss through transpiration and may enhance drought avoidance.
Turfgrasses with rapid vertical shoot extension rate tend to have higher water use rates than slower growing or dwarf-type grasses, because of increasing growth demand on water. Shearman (1986) reported that shoot vertical extension rate was positively correlated with water use rate for 20 Kentucky bluegrass cultivars with upright growth pattern. While many compounds can influence plant growth and morphology, possibly the most widely used PGR in turfgrass management are growth inhibitors, such as trinexapac-ethyl (TE). Trinexapac-ethyl inhibits vertical shoot extension by inhibiting synthesis of gibberilic acid (GA), a hormone produced by plants. Numerous studies in turfgrass have shown that trinexapac-ethyl not only reduces vertical shoot growth rate, but also increases cell density in leaves, promotes darker green leaves, and in some cases increases tiller density (Heckman et al., 2001; Stier and Rogers, 2001; Pannacci et al., 2004). This growth inhibition effect may result in reduction of water consumption, and thus may enhance plant drought resistance. Exploration of the specific mechanisms related to improved stress tolerance has suggested involvement of multiple mechanisms. Application of trinexapac-ethyl, which blocks GA formation, has improved drought stress tolerance by reducing the rate of evapotranspiration resulting in available water supply for an extended duration (Ervin and Koski, 2001; McCann and Huang, 2007). Trinexapac-ethyl has also been shown to reduce sod heating during storage (Heckman et al., 2001) and to reduce disease severity (Maxson and Jones, 2002; Costa et al., 2004; Pannacci et al., 2004).

Numerous studies have cited the importance of maintenance of cell turgor pressure in plants under drought stress (Gaxiola et al., 2001; Wang et al., 2003; DaCosta and Huang, 2006). Plants can maintain cell turgor pressure through osmotic adjustment,
which is accomplished by increasing solute concentration, such as sugars and carbohydrates, in the cytosol. Increases in cell solute concentration decrease the cells osmotic potential, which greatly reduces cell water loss. Many studies with agronomic crops have suggested that exogenous foliar application of glycine betaine (GB) on crop varieties that accumulate GB can maintain cell turgor pressure under water deficit (Agboma et al., 1997). GB application has been shown to enhance osmotic adjustment, net photosynthesis, photochemical efficiency of PS II (Fv/Fm), and yield in various crops (Makela et al., 1996; Agboma et al., 1997; Diaz-Zorata et al., 2001). There is limited information available on the use of GB in turfgrass management for improving drought tolerance. A study with creeping bentgrass by Saneoka et al. (2004) has also shown that GB levels increased when the plant was exposed to drought stress, suggesting possible link of GB and drought adaptation.

Strategies that can reduce water loss through transpiration can have significant impact on water conservation and promoting plants to avoid drought stress. Among the amount of water absorbed by plants, the majority (more than 90%) is transpired and only less than 3% of absorbed water is used in photosynthesis and other metabolic activities. If transpirational water loss through stomata can be controlled to any extent, it is possible to maintain plant growth with the use of much less water. Anti-transpirants, such as abscisic acid (ABA) have been shown to reduce transpiration rates more than photosynthesis, causing significant increases in water use efficiency and helping plants to survive for an extended period of drought. Several controlled-environment studies with turfgrasses demonstrated reduced water use with foliar spray of ABA (Stahnke, 1981; Wand and Huang, 2003; Wang et al., 2003; DaCosta and Huang, 2006). Stahnke (1981) reported
ABA application reduced transpiration in creeping bentgrass by 59% by inducing stomatal closure.

Recent research conducted by Wang et al (2003) showed that exogenous ABA application to Kentucky bluegrass in drought conditions resulted in higher turf quality, higher cell membrane stability, as indicated by less electrolyte leakage, and higher photochemical efficiency (Fv/Fm) when compared to untreated turf. ABA has been widely used in field crops and vegetables for reducing water loss from plants, but little has been done on the practical use of ABA to reduce water requirements and enhance drought tolerance in turfgrasses under natural field conditions. This could be largely due to the unavailability of a synthetic form of ABA. Nevertheless, available data suggest use of anti-transpirants, including ABA, in combination with routine cultural practices would be beneficial in water conservation for turfgrass in water-limiting environments.

**WATER-SAVING IRRIGATION MANAGEMENT**

As discussed in the previous sections, turfgrass water use is a function of plant growth characteristics and environmental conditions. Therefore, a sound, efficient irrigation program should be developed based on plant needs and environmental conditions. Factors such as water quality, soil type, local weather, and turf type, all play a role in developing a water-conservation irrigation program.

The most important consideration in developing an irrigation program for any particular sites is the frequency and amount of water that should be applied. Monitoring weather conditions, daily water use rate, and soil water availability is critical for determining the appropriate quantity and timing of watering. In the case of using
reclaimed water for irrigation, knowledge of dissolved salt content in irrigation water is also important. In order to design and manage an efficient irrigation program, it is imperative to determine the minimum water requirements of plants. Irrigation requirement may be estimated through visual evaluation of both the canopy and soil cores extracted with a soil probe. Leaf wilting or firing indicates water deficit, which is a good indicator of when to water. However, visual assessment can be highly subjective and may lead to excess irrigation or inadequate irrigation without knowing how much to water. Soil dry-down is also a good indication of drought stress and when and how much to irrigate to maintain turfgrass growth. The availability of soil water can be estimated using two parameters, field capacity and permanent wilting point. Field capacity is the maximum water holding capacity of a soil when drainage ceases. Permanent wilting point is the level of soil water content at which plants become permanently wilted. The amount of water available for plant growth in the soil is the difference between field capacity and wilting point, which varies with soil types and plant species. Fine-textured soils, such as clay, have a greater water holding capacity, and thus irrigation may be applied less frequently. In contrast, light, frequent irrigation may be required to replenish water reservoir in sandy soils due to low field capacity. Various methods are used to measure soil water content, including time domain reflectometry, tensiometers, and gypsum blocks (Fry and Huang, 2004). Daily ET measurement enables precise determination of how much water has been lost within a day, and the ET rate can be used to decide how much to irrigate in the next irrigation cycle. Canopy ET rate can be estimated using tools such as lysimeters, weather station data, and evapotranspiration pans (Fry and Huang, 2004). Weather stations gather data such as air temperature, humidity, barometric
pressure, level of photosynthetically active radiation, and wind speed to calculate ET rates.

Over the past 20 years, scientists have been studying the theory between using light and frequent water applications versus heavy and infrequent application to turfgrass systems. It is now generally accepted that deep and infrequent irrigation promotes plant tolerance to drought stress and may result in water savings. Fry and Huang (2004) reviewed extensive literature of previous research that supported the benefits of deep, infrequent irrigation in various turfgrass species. Decreasing irrigation frequency resulted in a 6 to 18% reduction in water use for some warm-season grasses, and a 24 to 34% reduction in water use for some cool-season grasses (Biran et al., 1981). However, optimum irrigation frequency varies with plant species, climatic conditions, and soil types. Research conducted by Jordan et al. (2003) evaluating various cultivars of creeping bentgrass maintained under putting green conditions concluded that an irrigation frequency of every 4 days produced a larger and deeper root system, higher turf quality and shoot density when compared to treatments irrigated every 1 or 2 days. Zoysiagrass irrigated at the onset of leaf wilting resulted in 40% reduction in shoot vertical growth rate compared to that watered daily, which decreases water demand (Qian and Fry, 1996). Tall fescue with an irrigation frequency of two times a week produced higher quality turf than that watered three or four times a week (Richie et al., 2002).

Further proof of the benefits of deep and infrequent irrigation in turfgrasses was identified in our most recent study of three bentgrass species maintained under golf course fairway conditions and replacing 100% ET. Our study showed that turf generally performed best when irrigated twice a week in the summer, and one time a week in the
spring and fall, when compared to plots watered three times a week (Richie et al., 2002). The experiment showed that ideal soil volumetric water content for turf growing in a native soil is approximately 25%. However, under light and frequent irrigation (watering three times per week and replacing 100% ET), soil water content averaged 30% or greater. By increasing the amount of water in the soil there is a reduction in the amount of air. For example, a loam-type soil at field capacity generally consists of 50% soil, 25% water, and 25% air. If water content is increased to 30%, then air content is decreased to 20%. This can create an imbalance, where the plant has more than enough water for physiological functioning, but begins to experience limited O₂ supply to roots because of the decreased air content. Problems that have generally been associated with light and frequent applications in turf are increased moss and algae, increased disease pressure, decreased O₂ availability in the rootzone, shallow rooting, and thinning turf canopy.

The quantity of irrigation applied should also be determined through the evaluation of water needs of a particular turfgrass species and soil water content. Various studies have demonstrated that it may not be necessary, or beneficial, to replace 100% of ET water loss, and many turfgrass species such as Kentucky bluegrass, perennial ryegrass, tall fescue, creeping bentgrass, velvet bentgrass, colonial bentgrass, zoysiagrass, and bermuagrass are able to maintain acceptable turf quality when irrigated in an amount below that of the plant's maximum water demand (deficit irrigation) (reference?). Deficit irrigation practice maintains soil water content below field capacity but above permanent wilting points, and thus limits water use by plants. Deficit irrigation can result in overall water savings and increases in water use efficiency without loss of turf quality in various
turfgrass species. Fu et al. (2002) found that tall fescue and bermudagrass maintained similar quality levels irrigated to replace 60 and 40% of actual ET compared to the same species under well-watered (replace 100% ET) conditions. Irrigating bermudagrass, buffalograss, and St. Augustinegrass at rates greater than 55% of actual water loss did not result in higher turf quality (Qian and Engelke, 1999). In a two-year field project conducted by DaCosta and Huang (2006) evaluating three bentgrass species, data showed that turf receiving 80% of ET had similar or higher quality than turf replaced with 100% ET. An explanation for these results is that in replacing 100% of ET, the top few centimeters of the soil become saturated for a period long enough to prevent O₂ diffusion, increasing algae and moss content, and creating an environment favorable for disease development. Other research exploring irrigation quantity has been conducted in sod establishment and has shown that spring establishment requires less water (45 mm week⁻¹) than sod established in summer months (63 mm week⁻¹) during the first seven weeks (Peacock, 2001). The research also showed that increasing irrigation volume did not improve turf quality.

Reducing irrigation quantity and frequency can also be accomplished by incorporating PGRs and anti-transpirants into management programs. As previously discussed, application of such compounds can moderate ET losses of plants. Measures that modify soil physical properties, such as use of surfactants, to improve water holding capacity or infiltration could also be practiced for water conservation. Soil surfactants decrease surface tension in soil and reduce water runoff, and create matrix water flow, making water more uniformly and consistently distributed throughout the soil profile. In cases of practicing deep, infrequent irrigation that allows soil to dry down between
irrigation events, surfactants may be required to minimize localized dry spots, particularly on sandy soils.

Effluent or reclaimed water is a potential source of irrigation water for turfgrass during periods of drought or restrictions on potable water. Use of reclaimed water can result in significant reduction of potable water consumption. In addition, reclaimed water may contain more nutrients than potable water and can enhance plant growth. Mujeriego et al. (1996) reported that reclaimed water at high application rates can maintain turf quality comparable to potable water with N application up to 25 kg ha\(^{-1}\) month\(^{-1}\). Reduced N requirement has been shown in a study evaluating reclaimed water use in bermudagrass and seashore paspalum (Beltrao et al., 1999). A study evaluating two common turf grasses, *Cynodon dactylon* and *Lolium perenne*, showed that effluent-treated turf could be grown at a high quality level, with reduced fertilizer needs, when compared to treatments only irrigated with tap water (Hayes et al., 1990). A wide range of ornamental plants (including *Cupressus sempervirens*, *Asparagus densiflorus*, *Pococarpus macrophyllus*, *Strelitzia reginae*, *Juniperus procumbens*, *Philodendron williamsii*, *Ilex vomitoria* cv. Schellings, chrysanthemums, *Hibiscus rosa-sinensis* and its hybrids, and various Rhododendron hybrids) irrigated with reclaimed water showed no detrimental effect and were generally determined to respond best to irrigation rates of 1.0-1.5 inches/week (Parnell, 1988).

The practical application of this research can be evaluated at a site like Meadow Lakes Golf Course in Prineville, Oregon. The golf course covers hundreds of acres and has used treated wastewater in management of the facility to much success (Boss, 1994). Mujeriego et al. (1996) evaluated agronomic and human health impact of using reclaimed
water on a golf course in Spain (Mas Nou). They concluded that reclaimed water containing fecal coliforms and fecal streptococci concentrations below 100 c.f.u./100 ml had no detrimental effects to human health, while use of reclaimed water resulted in significant reductions in potable water use and also achieved management savings through reduced need for fertilizer inputs.

While use of reclaimed water can be a viable strategy for water conservation, care should be taken in areas irrigated frequently with large quantities. In addition to beneficial nutrients, all reclaimed water also contains dissolved mineral salts such as sodium, which may have adverse effects when accumulated in large amount in the soil. Water quality should be monitored closely during the course of reclaimed water irrigation. Soils should be irrigated periodically with potable water to leach out excess salt and prevent toxic effects. If soil salinity is expected to be a problem with reclaimed water irrigation, salt tolerant grasses, such as seashore paspalum and weeping alkaligrass (Puccinellia distans (Jacq.) Parl.) may be used in order to maintain quality turfgrass in salt-affected areas.

**CONCLUSIONS**

The 21st century will present numerous challenges in turfgrass management, including limited water resources for irrigation. Increased human demand on water consumption, declines in potable water supply, and extreme weather resulting in drought stress are putting great demand on water conservation in all sectors of the turfgrass industry. Developing drought-resistant turfgrass species and efficient irrigation practices has become imperative to growing turfgrass in water-limiting environments.
As discussed throughout this chapter, turfgrass water use is a function of plant growth characteristics and environmental conditions. Therefore, an effective conservation program should be developed based on water use characteristics of plants, soil, and climatic conditions. Turfgrass developed various survival mechanisms in response to drought stress, including tolerance, avoidance, and escape of drought stress. These mechanisms are not mutually exclusive, and in fact, one species may possess multiple mechanisms. Turfgrass species and cultivars vary in drought resistance. Using low water use or drought-resistant turfgrass species and cultivars is a primary means of reducing water needs, as well as improving water use efficiencies. Growing warm-season turfgrass in arid and semi-arid regions may provide a better turf quality and more water savings than using cool-season turfgrasses. In addition to utilization of genetic variation, a myriad of other possibilities exist to maintain acceptable quality turf with reduced water consumption. Monitoring actual water use by measuring evapotranspiration rate under different environmental conditions and different times of the year is important for developing efficient, plant-based irrigation programs under changing environmental conditions. Knowledge of plant growth conditions and soil moisture status or available water content of different types of soils is necessary to decide when and how much to irrigate. Deficit irrigation can be effective in water savings without resulting in significant loss of turfgrass quality. Deep, infrequent irrigation can be practiced to promote drought-resistance of plants and reduce water use. Management programs that incorporate surfactants and plant growth regulators, osmoregulants, and anti-transpirants can be effective for water conservation in low-maintenance turf or water-limiting environments. In dry regions where potable water use is restricted, there is increasing use
of reclaimed water as an alternative water source. Irrigation with reclaimed water has been proved to be a viable approach in dealing with potable water shortage.

Implementing such programs and designing programs based on the numerous scientific studies evaluating drought-resistance mechanisms and various water conservation irrigation strategies, such as those referenced in this chapter, would greatly benefit the turfgrass industry and the sustainability of water resources.
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CHAPTER 1

IMPACT OF IRRIGATION FREQUENCY ON THREE BENTGRASS SPECIES MAINTAINED AS FAIRWAYS

INTRODUCTION

Drought is certainly not an uncommon malady facing superintendents across the nation, especially in recent years. For example, the drought in 1999 and 2002 in Northeast and Mid-Atlantic states was so devastating that some courses were unplayable and many superintendents lost jobs. The situation became even worse when the most stricken states began implementing water restrictions. Golf courses region-wide were required to reduce water usage by 75 to 90%. Some courses in New Jersey had turf losses of 30-50% during the driest period in 2002. However, golfers at private and public facilities express their displeasure when fairways and tees are no longer of the quality they’ve come to expect when irrigation is restricted. Over the past two decades, there has been an increase in the use of bentgrass on golf course fairways because of disease epidemics in perennial ryegrass fairways. Fairways represent the greatest acreage of high quality turf on a golf course, and often receive the greatest proportion of water. However, there is a lack of water-saving irrigation programs for managing bentgrass fairways with limited water resources.

DaCosta and Huang (2006) investigated minimum irrigation quantity required to maintain acceptable quality of bentgrasses under fairway conditions. In both 2002 and 2003, their results showed that replacement of 100% daily water loss (expressed as evapotranspiration rate or ET) was not necessary for maintaining plant growth and
physiological processes for creeping bentgrass, velvet bentgrass, and colonial bentgrass. Irrigation to replace 80% ET three times per week maintained best turf quality for all three species during spring, summer, and fall. There was also less disease (mainly brown patch) with 80% ET irrigation, compared to 100% ET irrigation. Irrigation at 60% was sufficient to maintain minimum acceptable quality for velvet bentgrass in summer. During the cooler months of spring and fall, 40% ET irrigation was adequate to maintain acceptable quality for all three species. The results demonstrated that practicing deficit irrigation would result in 20%-60% water saving to maintain equal or better turf quality of bentgrasses under fairway conditions than complete ET replacement. Similarly, deficit irrigation has been found to result in water savings and no loss in turf quality in various other turfgrass species. Johnson (2003) reported that a 6-day interval of 70% ET irrigation is a good water saving program for Kentucky bluegrass and tall fescue. While previous studies determined irrigation quantity for managing fairway bentgrass at different seasons, optimum irrigation frequency for bentgrass fairways has not been determined. The current study evaluated the irrigation frequency that can maintain turf quality with 100% ET replacement for three bentgrass species mowed at two different heights and at different seasons (spring, summer, and fall). Such information is needed for developing water-saving irrigation programs for bentgrass fairways. Therefore, the objectives of this study were (1) to determine optimum irrigation frequency for creeping bentgrass, colonial bentgrass and velvet bentgrass under fairway management regimes at different times of the year and under different mowing heights; and (2) to establish CWSI threshold values for developing water-saving irrigation scheduling for three bentgrass species at different times of the year and under different mowing heights.
MATERIALS AND METHODS

Plant Materials

‘L-93’ creeping bentgrass, ‘Tiger II’ colonial bentgrass, and ‘Greenwich’ velvet bentgrass were examined to compare species variation in irrigation frequency requirements and CWSI values. Grasses were established in 2001 in 16 plots (5’ x 8’ each plot) with four replicates. These plots were used to determine irrigation quantity in our study conducted in 2004 and 2005. Turf was managed following typical cultural practices for fairways in the Northeast.

Treatments and Experimental Design

The project was conducted in a fully automated, mobile rainout shelter (35’ x 60’) at Rutgers University in North Brunswick. The study was designed to determine optimum irrigation frequency using 100% ET replacement. Treatments include irrigation at four intervals: 1) three times per week (Monday, Wednesday, Friday); 2) two times per week (Monday and Friday); 3) once per week (Friday); and 4) biweekly (every other Friday). Grasses were mowed at two heights: 0.250 inch and 0.375 inch, to compare irrigation frequency requirements and develop CWSI under different mowing heights.

All treatments were imposed from June to September. Each treatment was replicated in four plots. Plots with different irrigation treatments are separated using 30-cm deep plastic edging to prevent lateral movement of water. This has been found to be effective in separating irrigation treatments in the previous study by DaCosta and Huang (2006). Each plot was irrigated manually based on actual ET rate. The amount of water
applied to each plot was controlled with a flow meter connected to the hose. Actual ET rate was measured daily using microlysimeters installed in each plot.

**Measurements**

All measurements were made periodically from June to September in four plots for each treatment. To determine optimum irrigation frequency for maintaining healthy and water-saving turf, various physiological parameters were determined in each irrigation treatment. Canopy photosynthesis (carbon fixation rate) and transpiration (water loss rate) was determined with a gas exchange analyzer (Licor 6400) to examine physiological function and water use of turf. Turf quality was rated visually to evaluate overall turf performance. Leaf relative water content (RWC) was measured to evaluate water status of plants. Soil volumetric water content (VWC) in 0-20 cm soil depth was measured with time domain reflectometry method (TDR) using probes inserted in the soil to determine water depletion rate and dryness of the soil.

General turf performance was evaluated by visual rating of turf quality on a 0-9 scale (0=worst and 9=best) and by determining canopy characteristics (green leaf biomass and leaf area index) using a multispectral radiometer.

**RESULTS**

**Turf Growth**

Visual quality is one of the most important characteristics that govern many management practices, including irrigation frequency. Using ‘6’ as the minimal
acceptable turf quality, it was possible to monitor how long each of the species could maintain general turf performance when irrigation frequency was reduced. Throughout both seasons, all three grasses generally performed well when watered two or three times a week (Figs. 1-4). Watering at the 14 day interval resulted in poor turf quality for the majority of the season, and should not be considered a practical irrigation frequency for any of the evaluated species at either mowing height.

During July and August of 2004, creeping bentgrass performed best when watered three times a week, as opposed to twice a week or less (Figs. 1A, 2A). Similar results were not observed in 2005 (Figs. 3A, 4A). This difference in results may be due to temperature differences between the two seasons. The first year of the study, 2004, had more moderate temperatures through the summer months, while 2005 had a considerable number of days over 90° Fahrenheit (Table 8). It is reasonable to assume that these excessive temperatures produced higher soil temperatures, which had a negative impact on roots, especially in areas with high soil volumetric content. Additionally, high temperatures would have triggered the plant to close stomata, limiting water uptake and gas exchange. Soils with high volumetric water content (30% or greater) can also limit gas exchange. The combined effect in this situation may have resulted in detrimental plant health of creeping bentgrass.

In 2005, watering one, two or three times a week was sufficient to maintain good turf quality at the 3/8” cutting height. In the previous year, when creeping and colonial bentgrasses were watered once a week, marginal quality was maintained in August and September. However, this frequency was sufficient to maintain higher quality levels both earlier and later in the 2004 season.
Another notable difference between the two seasons of study was the performance of velvet bentgrass. In 2004, velvet bentgrass maintained good color and density when watered one, two or three times a week and was not significantly different from the other grasses at any watering frequency (Figs. 1,2). In the following year, during mid-summer, velvet showed an overall lower turf quality when less frequently watered (Figs. 3,4). Significant differences were recorded between velvet and colonial bentgrass on multiple dates in late July and August or 2005. Again, this may result from atmospheric differences between the two years.

The data indicate that the lower mowing height generally produced lower quality turf in all plots regardless of species or mowing height. While differences were not always significant, there was a strong trend toward turf quality of the ¼” cutting height being a point lower than the higher height of cut. The figures shown below are fairly representative of the other grass species evaluated.

A multispectral radiometer was used to record and analyze reflected light from the turf canopy. Particular wavelengths have been shown to correspond to turf color and density, allowing this equipment to be used as an objective supplement to the overall evaluation of turf quality. The data collected was overwhelmingly consistent with data collected for visual turf quality.

Photosynthesis and transpiration measurements were taken utilizing the Licor 6400 gas exchange analyzer. Only one date was measured in the 2004 season (9/23/04), while five measurements were taken in the 2005 season. The data collected from the 2004 season show significant drops in photosynthetic rates for both creeping and colonial bentgrass when irrigation frequency is once a week or less. This is consistent between
the two cutting heights. Also in 2004, photosynthetic rates of velvet bentgrass were more consistent across all irrigation frequencies, with a slight trend toward lower rates in less frequently irrigated plots. Water use efficiency (WUE), as calculated by the ratio of the amount of carbon fixed during photosynthesis to the amount of water lost through transpiration, showed results similar to photosynthesis (data not shown). In 2004, WUE is lower when plants are watered once a week or less. When compared to the other grasses in that same year, velvet bentgrass maintains higher efficiency rates as watering frequency drops, though some efficiency is lost.

Data collected from 2005 give a broader context of WUE through the season. While there are no significant differences between any watering frequencies in July or September, some differences are significant on the two dates recorded in August. The 3x week\(^{-1}\) and 2x week\(^{-1}\) frequencies were significantly higher than the 14D frequency. Additionally, the 3x week\(^{-1}\) and 2x week\(^{-1}\) frequencies were also significantly higher than 1x week\(^{-1}\) on the second date measurements were taken.

There were generally no difference in WUE between species. The only exception in 2005 was with velvet bentgrass maintained at the 14 day watering frequency. This treatment produced very low WUE, which was reflected in the quality data results.

**Soil and Plant Water Relations**

Two weeks after withholding irrigation (14d), declines in soil moisture content were observed in all three bentgrass species (high and low cutting height) compared to control plots watered three times per week (3x) and plots watered twice a week (2x) (Tables 1,2). There was a similar trend for all three species through the season: plots
watered three times per week retained the highest soil moisture, and those watered every 14 days showed the most decline in moisture.

Differences in water depletion rate were seen between the bentgrass species. For all treatments, velvet bentgrass had the lowest rate of soil moisture depletion (thus highest soil moisture content), while colonial bentgrass plots depleted soil water supply the fastest (Fig. 2). During late summer and early fall, there were multiple dates when soil moisture content fell below 20% (v/v) in plots watered once a week and every 14 days. While this finding was consistent in both heights of cut, soil moisture contents of velvet bentgrass watered every 14 days only fell into that critical range twice during the same period. Lowest soil moisture content was generally recorded from the beginning to mid September. In 2004, the study ran until late October with all plots having consistently higher readings through the month, indicating lower water use.

RWC is the standard unit for determining a plant’s water status. It is determined via collection and weighing of leaf tissue samples, using the following formula:

\[
RWC = \left(\frac{fw - dw}{tw - dw}\right) \times 100
\]

where \(fw\) is fresh weight, \(dw\) is dry weight, and \(tw\) is turgid weight. Leaf relative water content was determined once every two weeks. Clippings were collected from plots watered 3x week\(^{-1}\), 1x week\(^{-1}\) and every 14 days.

The data show that watering every 14 days, during July and August, was not sufficient to keep adequate plant RWC (Tables 3,4). Values during this period dropped to between 40% and 20% RWC. Plant health generally declines when RWC values fall below 50%. This is the only treatment effect that had any significant influence on RWC. Data show that neither height of cut or species had significant impact on RWC. The only
exception to this is in 2005 when velvet bentgrass at the 14 day watering interval was significantly lower than the other two species at both the high and the low cutting height during July and August. This was not observed in the previous year. However, these findings coincide with the 2005 quality data which also showed significantly lower values for velvet bentgrass under this treatment.

Lysimeter were installed in the field plots to calculate rates of evapotranspiration (ET). Lysimeters were designed using 20 cm lengths of PVC pipe that had the bottom covered and secured with a screen mesh and were filled with soil and established with each of the grasses evaluated. These PVC tubes (lysimeters) were inserted into precisely cut 20 cm holes cut in the plots and which maintained the turf under the same conditions as the turf surrounding it in the plot. Lysimeters were pulled from the ground and weighed on a daily basis to determine water loss via difference in weight in a 24 hour period. These weight differences were then converted into water loss for each plot.

Plots watered three times a week had the greatest rates of ET (Tables 5,6). These ET rates were significantly higher than plots watered every 14 days, and during a number of dates in the summer were significantly higher than the 2x and 1x watering treatments. There was generally no difference in ET rates between the 2x and 1x watering treatments.

While irrigation frequency seems to have significant influence on potential ET, mowing height and species type do not seem significantly impact ET.

The CWSI is a technique that uses the measurement of canopy surface temperature and air temperature to infer water stress status and whether irrigation is needed. CWSI is expressed as the ratio of actual canopy and air temperature difference to the maximum canopy and air temperature difference of a plant:
\[
\text{CWSI} = \frac{(T_c - T_a)_{\text{a}} - (T_c - T_a)_{\text{p}}}{(T_c - T_a)_{\text{u}} - (T_c - T_a)_{\text{p}}}
\]

where \(T_c\) = canopy temperature, \(T_a\) = air temperature, \(a\) = actual measurement, \(p\) = fully transpiring plant (lower limit), and \(u\) = nontranspiring plant (upper limit). After calculating CWSI, the data is then paired with data collected measuring turf quality and green leaf biomass. Plotting the data points can express CWSI values that are necessary for maintaining acceptable turf quality (a rating of 6 or higher). A low CWSI value indicates there is minimal plant water stress. Additionally, green leaf biomass (GLB) data has been correlated to turf quality, with results showing that a GLB value of 0.80 is equivalent to a turf quality rating of ‘6.’

The data show that CWSI values below 0.40 result in acceptable turf quality (Figures 13, 14). These figures plot turf quality with CWSI values. While there is some variation in this value between treatments, the result is generally reflected through all data collected. Instances where low turf quality and low CWSI values were observed may be explained by disease occurrence.

**DISCUSSION**

Our results demonstrate that replacing 100% of ET, three times a week may not be necessary to sustain plant growth and physiological processes, and that this depended on species and time of year. Generally, irrigating once or twice a week and replacing 100% of ET was adequate to maintain acceptable turf quality during summer months for all grass species tested. The exception to this finding was seen in 2004 where it was necessary to water colonial and creeping bentgrass more frequently (2x week\(^{-1}\)) during July and August to maintain acceptable quality. Thus, while environmental conditions
may allow for watering once a week during summer month, it is advisable to adjust
frequency to irrigating twice a week during periods of summer stress, particularly from
mid-July to late August.

Lysimeter readings conclude that the greatest rates of ET are from plots irrigated
three times a week. These rates of ET have been shown to be significantly higher than
plots watered once or twice a week during summer months. Considering the previous
conclusion that adequate turf quality can be maintained when irrigated less frequently (2x
or 1x week⁻¹), it may be possible to schedule irrigation to reduce ET and result in water
savings.

Using hand-held infrared thermometers, it is possible for turf managers to monitor
canopy temperature levels, and thus determine crop water stress index (CWSI) values for
the turf they are managing. Using the results presented in this report, it is possible for
these values to be used to scheduling irrigation programs. For the three grasses evaluated
here, it is advisable to irrigate turf when CWSI levels approach 0.40.

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Table 1. Soil volumetric water content (VWC) (%) for creeping, colonial, and velvet bentgrasses maintained at 9.5 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.
<table>
<thead>
<tr>
<th>Species</th>
<th>Irrigation Treatment</th>
<th>Soil Volumetric Water Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2004</td>
</tr>
<tr>
<td></td>
<td>2-Jul</td>
<td>13-Jul</td>
</tr>
<tr>
<td>Creeping</td>
<td>3x week⁻¹</td>
<td>29.6 a</td>
</tr>
<tr>
<td></td>
<td>2x week⁻¹</td>
<td>29.0 a</td>
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<td></td>
<td>1x week⁻¹</td>
<td>28.2 a</td>
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<tr>
<td></td>
<td>14 days</td>
<td>28.3 a</td>
</tr>
<tr>
<td>Colonial</td>
<td>3x week⁻¹</td>
<td>28.5 a</td>
</tr>
<tr>
<td></td>
<td>2x week⁻¹</td>
<td>28.7 a</td>
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<td>27.5 a</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>28.8 a</td>
</tr>
<tr>
<td>Velvet</td>
<td>3x week⁻¹</td>
<td>30.5 a</td>
</tr>
<tr>
<td></td>
<td>2x week⁻¹</td>
<td>30.1 a</td>
</tr>
<tr>
<td></td>
<td>1x week⁻¹</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>2005</td>
<td>30-Jun</td>
</tr>
<tr>
<td>Creeping</td>
<td>3x week⁻¹</td>
<td>32.2 a</td>
</tr>
<tr>
<td></td>
<td>2x week⁻¹</td>
<td>27.1 b</td>
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Table 2. Soil volumetric water content (VWC) (%) for creeping, colonial, and velvet bentgrasses maintained at 6.4 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.
Table 3. Leaf relative water content (RWC) (%) for creeping, colonial, and velvet bentgrasses maintained at 9.5 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.
Table 4. Leaf relative water content (RWC) (%) for creeping, colonial, and velvet bentgrasses maintained at 6.4 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.

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<th>Species</th>
<th>Irrigation Treatment</th>
<th>Relative Water Content (%)</th>
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<td>Creeping</td>
<td>3x week$^{-1}$</td>
<td>85 a</td>
<td>80 a</td>
<td>97 a</td>
</tr>
<tr>
<td></td>
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<td>83 ab</td>
<td>80 a</td>
<td>90 b</td>
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<tr>
<td></td>
<td>14 days</td>
<td>81 b</td>
<td>78 a</td>
<td>88 b</td>
</tr>
<tr>
<td>Colonial</td>
<td>3x week$^{-1}$</td>
<td>78 a</td>
<td>77 a</td>
<td>96 a</td>
</tr>
<tr>
<td></td>
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<td>83 a</td>
<td>89 a</td>
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<tr>
<td></td>
<td>14 days</td>
<td>84 a</td>
<td>82 a</td>
<td>87 a</td>
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<td>Irrigation Treatment</td>
<td>Relative Water Content (%)</td>
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<td>2004</td>
<td>2005</td>
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<tr>
<td>Creeping</td>
<td>3x week⁻¹</td>
<td>71 a  79 a  93 a  88 a</td>
<td>74 a  70 a  64 a  69 a  64 a  58 a</td>
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<tr>
<td></td>
<td>1x week⁻¹</td>
<td>77 a  72 b  85 b  72 b</td>
<td>72 a  66 a  65 a  69 a  53 b  55 a</td>
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</tr>
<tr>
<td></td>
<td>14 days</td>
<td>72 a  73 b  77 c  65 b</td>
<td>67 a  66 a  51 b  43 b  49 b  51 a</td>
<td></td>
</tr>
<tr>
<td>Colonial</td>
<td>3x week⁻¹</td>
<td>78 a  76 a  95 a  89 a</td>
<td>73 a  66 a  62 a  73 a  68 a  65 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1x week⁻¹</td>
<td>75 a  81 a  87 ab  76 a</td>
<td>70 ab  64 a  64 a  72 a  62 ab 65 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>79 a  69 a  78 b  59 b</td>
<td>64 b  53 b  42 b  48 b  51 b  61 a</td>
<td></td>
</tr>
<tr>
<td>Velvet</td>
<td>3x week⁻¹</td>
<td>85 a  74 b  92 a  92 a</td>
<td>74 a  70 a  66 a  75 a  68 a  65 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1x week⁻¹</td>
<td>80 a  82 a  89 ab  81 b</td>
<td>72 a  69 a  64 a  71 a  63 a  63 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>79 a  77 ab  72 c  56 c</td>
<td>65 a  53 b  26 b  23 b  38 b  45 b</td>
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</tr>
</tbody>
</table>

Table 5. Evapotranspiration (ET) (%) for creeping, colonial, and velvet bentgrasses maintained at 9.5 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.
Table 6. Evapotranspiration (ET) (%) for creeping, colonial, and velvet bentgrasses maintained at 6.4 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments ($P=0.05$) based on Fischer’s protected LSD test.

| Species | Irrigation Treatment | 2004 |  |  |  |  |  |  |  |
|---------|----------------------|------|---|---|---|---|---|---|
|         |                      | 2-Jul| 21-Jul| 4-Aug| 9-Aug| 30-Aug| 7-Sep| 15-Sep| 20-Sep |
| Creeping| 3x week$^{-1}$       | 5.4 a| 4.2 b| 4.2 ab| 4.5 a| 3.8 a| 2.1 a| 3.3 a| 2.5 a |
|         | 2x week$^{-1}$       | 5.6 a| 5.8 a| 5.0 a| 5.1 a| 4.2 a| 1.5 a| 2.6 a| 0.6 b |
|         | 1x week$^{-1}$       | 5.9 a| 3.5 b| 5.0 a| 4.7 a| 3.6 a| 1.2 a| 1.8 a| 0.1 b |
|         | 14 days              | 4.8 a| 4.1 b| 2.2 b| 1.4 a| 2.4 b| 0.5 a| 1.7 a| 0.0 b |
| Colonial| 3x week$^{-1}$       | 5.5 a| 5.1 a| 5.2 a| 4.3 a| 2.7 a| 3.5 a| 4.8 a| 1.6 a |
|         | 2x week$^{-1}$       | 5.7 a| 7.7 a| 5.7 a| 4.8 a| 1.8 a| 2.6 b| 3.4 a| 0.5 ab|
|         | 1x week$^{-1}$       | 5.6 a| 6.0 a| 5.5 a| 3.8 ab| 1.4 ab| 2.4 b| 1.6 a| 0.0 b |
|         | 14 days              | -    | -    | -    | -    | -    | -    | -    | -    |
| Velvet  | 3x week$^{-1}$       | 2.8 a| 4.8 a| 5.1 a| 4.6 a| 3.0 a| 3.5 a| 4.8 a| 1.9 a |
|         | 2x week$^{-1}$       | 2.9 a| 5.6 a| 4.9 a| 4.4 a| 1.8 ab| 2.7 ab| 1.1 a| 0.2 b |
|         | 1x week$^{-1}$       | 3.0 a| 6.3 a| 5.6 a| 3.8 a| 1.3 b| 2.4 ab| 0.6 a| -    |
|         | 14 days              | 3.1 a| 2.9 a| 3.2 a| 2.8 a| 0.4 b| 1.9 b| 0.0 a| 0.4 b |

| Species | Irrigation Treatment | 2005 |  |  |  |  |  |  |  |
|---------|----------------------|------|---|---|---|---|---|---|
|         |                      | 1-Jul| 22-Jul| 3-Aug| 5-Aug| 31-Aug| 6-Sep| 16-Sep| 19-Sep |
| Creeping| 3x week$^{-1}$       | 3.9 a| 2.8 a| 4.7 a| 4.5 a| 6.3 a| 4.1 a| 6.7 a| 3.7 a |
|         | 2x week$^{-1}$       | 4.2 a| 2.8 a| 3.4 ab| 3.7 ab| 5.1 ab| 4.3 a| 4.3 ab| 2.3 ab|
|         | 1x week$^{-1}$       | 3.5 ab| 1.8 b| 3.0 ab| 1.2 bc| 3.5 bc| 2.1 b| 2.7 bc| 0.8 bc|
|         | 14 days              | 2.7 b| 1.6 b| 2.1 b| 0.4 c| 1.1 c| 0.8 c| 0.9 c| 0.0 c |
| Colonial| 3x week$^{-1}$       | 5.5 a| 7.0 a| 5.6 a| 4.6 a| 6.2 a| 4.3 a| 5.3 a| 3.5 a |
|         | 2x week$^{-1}$       | 3.9 ab| 5.2 ab| 4.9 a| 4.0 a| 4.3 b| 2.4 b| 5.4 a| 2.9 a |
|         | 1x week$^{-1}$       | 2.3 b| 4.4 b| 3.6 a| 2.8 b| 3.1 b| 1.2 b| 4.9 a| 2.3 a |
|         | 14 days              | -    | -    | -    | -    | -    | -    | -    | -    |
| Velvet  | 3x week$^{-1}$       | 4.0 ab| 4.4 a| 5.0 a| 4.8 a| 7.4 a| 4.8 a| 6.3 a| 3.6 a |
|         | 2x week$^{-1}$       | 4.6 a| 4.3 a| 3.6 ab| 4.0 a| 5.3 b| 4.4 a| 4.9 b| 2.7 ab|
|         | 1x week$^{-1}$       | 3.6 bc| 4.0 b| 3.8 ab| 2.4 b| 4.3 b| 2.5 b| 3.1 c| 2.0 bc|
|         | 14 days              | 2.9 c| 2.4 c| 3.0 b| 0.8 c| 1.5 c| 1.3 b| 1.3 d| 1.4 c |
Table 7. Crop Water Stress Index (CWSI) for creeping, colonial, and velvet bentgrasses maintained at 9.5 and 6.4 cm in 2004 and 2005. Values followed by the same letter within a column for each bentgrass species indicate no significant differences between irrigation treatments (P=0.05) based on Fischer’s protected LSD test.

<table>
<thead>
<tr>
<th>Species</th>
<th>Irrigation Treatment</th>
<th>Evapotranspiration (mm day⁻¹)</th>
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<td>6.6 a</td>
</tr>
<tr>
<td>2x week⁻¹</td>
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<td>14 days</td>
<td>5.2 b</td>
<td>5.7 a</td>
</tr>
<tr>
<td>Colonial</td>
<td></td>
<td></td>
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<tr>
<td>3x week⁻¹</td>
<td>5.9 a</td>
<td>7.2 a</td>
</tr>
<tr>
<td>2x week⁻¹</td>
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<td>6.7 ab</td>
</tr>
<tr>
<td>1x week⁻¹</td>
<td>-</td>
<td>-</td>
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<tr>
<td>14 days</td>
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<td>6.0 b</td>
</tr>
<tr>
<td>Velvet</td>
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<td></td>
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<tr>
<td>3x week⁻¹</td>
<td>2.5 a</td>
<td>4.2 a</td>
</tr>
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<td>5.1 a</td>
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<tr>
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Table 8. Meteorological parameters from an on-site weather station located approximately 130 m from the rainout shelter in North Brunswick, NJ, in 2004 and 2005. Data presented include bi-monthly means for maximum air temperature, sea level.
pressure, and wind speed during the study period. Sensors for the climatic variables were located approximately 2 m off the ground.

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<th>Month</th>
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FIGURE LEGENDS
Fig. 1. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on turf quality of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 2. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on turf quality of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 3. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on turf quality of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 4. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on turf quality of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.
Fig. 5. Effects of four different irrigation frequencies (3x week$^{-1}$, 2x week$^{-1}$, 1x week$^{-1}$, or 14 d interval) on green leaf biomass of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 6. Effects of four different irrigation frequencies (3x week$^{-1}$, 2x week$^{-1}$, 1x week$^{-1}$, or 14 d interval) on green leaf biomass of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 7. Effects of four different irrigation frequencies (3x week$^{-1}$, 2x week$^{-1}$, 1x week$^{-1}$, or 14 d interval) on green leaf biomass of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 8. Effects of four different irrigation frequencies (3x week$^{-1}$, 2x week$^{-1}$, 1x week$^{-1}$, or 14 d interval) on green leaf biomass of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in
2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 9. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on leaf area index of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 10. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on leaf area index of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2004. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 11. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on leaf area index of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 9.5 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 12. Effects of four different irrigation frequencies (3x week\(^{-1}\), 2x week\(^{-1}\), 1x week\(^{-1}\), or 14 d interval) on leaf area index of (A) creeping bentgrass var. L-93, (B) colonial
bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich maintained at 6.4 mm in 2005. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 13. Scatter-plot graph of turf quality and crop water stress index (CWSI) of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich in 2004.

Fig. 14. Scatter-plot graph of turf quality and crop water stress index (CWSI) of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich in 2005.

Fig. 15. Scatter-plot graph of leaf relative water content (RWC) and crop water stress index (CWSI) of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich in 2004.

Fig. 16. Scatter-plot graph of leaf relative water content (RWC) and crop water stress index (CWSI) of (A) creeping bentgrass var. L-93, (B) colonial bentgrass var. Tiger II, and (C) velvet bentgrass var. Greenwich in 2005.
Turf quality

A - Creeping bentgrass, 9.5 mm

B - Colonial bentgrass, 9.5 mm

C - Velvet bentgrass, 9.5 mm

Fig. 1
Fig. 2.

A - Creeping bentgrass, 6.4 mm

B - Colonial bentgrass, 6.4 mm

C - Velvet bentgrass, 6.4 mm
Fig. 3.

A - Creeping bentgrass, 9.5 mm

B - Colonial bentgrass, 9.5 mm

C - Velvet bentgrass, 9.5 cm

Turf quality

- 3x/week  - 2x/week  - 1x/week  - 14 days

2005 year

6/23  7/7  7/21  8/4  8/18  9/1  9/15
Fig. 5

A - Creeping bentgrass, 9.5 mm

B - Colonial bentgrass, 9.5 mm

C - Velvet bentgrass, 9.5 mm

Green leaf biomass

Year 2004

6/30 7/14 7/28 8/11 8/25 9/8
**Fig. 6**

A - Creeping bentgrass, 6.4 mm

B - Colonial bentgrass, 6.4 mm

C - Velvet bentgrass, 6.4 mm

Green leaf biomass

*2004 year*
Fig. 7

A - Creeping bentgrass, 9.5 mm

B - Colonial bentgrass, 9.5 mm

C - Velvet bentgrass, 9.5 mm

Green leaf biomass over the 2005 year.
Fig. 8

A - Creeping bentgrass, 6.4 mm

B - Colonial bentgrass, 6.4 mm

C - Velvet bentgrass, 6.4 mm

Green leaf biomass

2005 year

6/24 7/8 7/22 8/5 8/19 9/2 9/16
Fig. 9

A - Creeping bentgrass, 9.5 mm

B - Colonial bentgrass, 9.5 mm

C - Velvet bentgrass, 9.5 mm
Fig. 10

A - Creeping bentgrass, 6.4 mm

B - Colonial bentgrass, 6.4 mm

C - Velvet bentgrass, 6.4 mm

Leaf area index over the 2004 year.
Fig. 11

A - Creeping bentgrass, 9.5 mm

B - Colonial bentgrass, 9.5 mm

C - Velvet bentgrass, 9.5 mm

Leaf area index

2005 year

6/24 7/8 7/22 8/5 8/19 9/2 9/16
Fig. 12

A - Creeping bentgrass, 6.4 mm

B - Colonial bentgrass, 6.4 mm

C - Velvet bentgrass, 6.4 mm
Fig. 13

A - Creeping, 2004

Minimal acceptable turf quality

B - Colonial, 2004

Minimal acceptable turf quality

C - Velvet, 2004

Minimal acceptable turf quality

Turf quality (1-9)

Crop water stress index
Fig. 15

A - Creeping, 2004

B - Colonial, 2004

C - Velvet, 2004

Crop water stress index

Relative water content (%)

0.00 0.20 0.40 0.60 0.80 1.00
Fig. 16

A - Creeping, 2005

B - Colonial, 2005

C - Velvet, 2005

Relative water content (%) vs. Crop water stress index.
CHAPTER 2
EFFECTS OF TRINEXAPAC-ETHYL ON CREEPING BENTGRASS RESPONSES TO COMBINED DROUGHT AND HEAT STRESS

INTRODUCTION

Drought and heat stress often occur simultaneously during summer months in warm, arid- or semi-arid regions. Combined drought and heat stress is detrimental to turfgrasses, particularly for cool-season grasses during summer months (Jiang and Huang, 2002; Wang and Huang, 2005). As such, it is important to understand methods of improving plant tolerance to summer stress and to develop management programs that both reduce overall water consumption and that minimize turf loss during periods of extended summer stress.

Plants have developed various mechanisms to adapt to drought stress, including avoiding stress by reducing water loss or consumption and slowing plant growth (Kang, 2002). Plant growth regulators (PGRs), such as growth inhibitors, are widely used in turfgrass management to suppress shoot growth and inflorescences (Turgeon, 2002). A growth inhibitor, trinexapac-ethyl (TE), blocks the final step in the biosynthesis of the biologically active forms of gibberellins, resulting in slower shoot growth (King et al., 1997). The effects of TE on growth inhibition are well documented in various turfgrass species (Ervin et al., 2002; Ervin and Koski, 1998; McCarty et al., 2004; Pannacci et al., 2004), however, effects of TE on plant responses to environmental stresses are less understood. Heckman et al. (2001a, b) have shown that TE application significantly reduced sod heating and injury during storage of Kentucky bluegrass (Poa pratensis L.).
Jiang and Fry (1998) demonstrated that foliar TE treatments increased turf quality of perennial ryegrass (*Lolium perenne* L.) during dry-down, suggesting that TE may enhance drought tolerance. The application of TE during exposure of plants to salinity enhanced root growth in two cultivars of bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* Burtt-Davy) (Baldwin et al., 2006). Creeping bentgrass (*Agrostis stolonifera* L.) quality and color were improved by TE application under shaded conditions (Goss et al., 2002). Richardson (2002) reported that TE enhanced fall green color retention, but had no consistent effect on growth and development of rhizomes or stolons, and did not improve freeze tolerance of rhizomes in bermudagrass.

Limited available data, as reported in the above referred studies, suggest that TE application may be beneficial for plant tolerance to stresses, but the effectiveness varies with turfgrass species, dose and duration of TE treatment, and type of stress. In addition, most studies examined TE effects on plant tolerance to a single stress factor. Whether pre-treating plants with TE enhances turfgrass tolerance to subsequent exposure to multiple stresses, such as the combination of drought and heat stress, is unknown. Furthermore, physiological factors involved in changes in turfgrass stress tolerance with TE application are not well understood. Most studies conducted under non-stressed conditions have found that TE application increased chlorophyll content, turf quality, turf density, and reduced shoot extension rate (Beasley, 2005; Bingaman, 2001; Ervin and Koski, 1998; Ervin and Koski, 2001a; Heckman, 2001b; Pannacci, 2004). We hypothesized that TE may influence plant tolerance to combined drought and heat stress by regulating photosynthesis and water use. The objectives of this study were (1) to assess the impact of pre-treatment of TE on creeping bentgrass responses to subsequent
exposure to combined drought and heat stress; and (2) to examine physiological factors involved in the combined stress tolerance associated with TE application. The information would provide further insight into plant growth regulation of turfgrass tolerance to drought and heat stress.

MATERIALS AND METHODS

Growth Conditions

Sod pieces of creeping bentgrass (A. stolonifera cv. ‘L-93’) were transplanted from mature field plots into polyvinyl chloride (PVC) tubes (40 cm length, 10 cm diameter) filled with sterilized sandy loam soil (fine-loamy, mixed mesic Typic Hapludult). Plants were maintained in a greenhouse under seasonal daylight conditions and approximately 20°C for four months, and then moved to growth chambers where treatments were imposed. Growth chambers were set at 20 °C (day and night), 12-h photoperiod, with photosynthetically active radiation (PAR) level of 400-450 μmol m⁻² s⁻¹ at the canopy level. Grasses were maintained at approximately 2-cm canopy height. Plants were watered three times per week to maintain soil moisture at field capacity. A 100 mL of a soluble 20-20-20 (N-P-K) fertilizer was applied weekly at a concentration of 5 g L⁻¹ to each container.

Treatments and Experimental Design

Plants were pre-treated with 0.8 L ha⁻¹ trinexapac-ethyl (Primo Maxx, Syngenta Professional Products, Greensboro, NC) [1.95 ml L⁻¹ (v:v); a.i. TE = 0.113] every 14 d for 42 d and then exposed to drought and heat stress. Final TE treatment was made just
prior to initiation of stresses, with no additional applications of TE after stress initiation. The experiment consisted of three treatments: (i) Stress-TE: plants that were not pre-treated with TE were exposed to drought stress and heat stress (35°C, day/night); (ii) Stress + TE: plants pre-treated with TE were then exposed to drought and heat stress (35°C, day/night); and (iii) non-stressed control: plants that were not pre-treated with TE and maintained under well-watered and optimum temperature (20°C) conditions.

Drought stress was imposed by withholding irrigation. Well-watered plants were irrigated to soil moisture reaching field capacity. No TE, fertilizers, or irrigation were applied after initiation of the stress treatments. Plants were re-watered at the end of stress treatment to examine for recuperative ability from drought stress.

High temperature treatments (35°C) were maintained in four growth chambers, containing both TE-treated and untreated plants. A separate growth chamber was used to maintain non-treated control plants at a nominal temperature (20°C). Four replicated plants of TE-treated and untreated plants were arranged randomly inside each growth chamber. The experiment was considered as a randomized block design. Statistical significance of data was tested using the analysis of variance procedure (SAS Institute Inc., Cary, NC). Differences between treatment means were separated by Fisher’s protected least significance (LSD) test at the 0.05 probability level.

**Measurements**

Turf quality was visually rated on a scale of 1 to 9 based on color, density, and uniformity (Turgeon, 2002). Plants rated 1 were completely desiccated with a completely necrotic turf canopy. A rating of 9 represented healthy plants with dark green, turgid leaf
blades, and a full turf canopy. A rating of 6 was considered the minimal acceptable turf quality. Shoot vertical growth rate was determined by measuring the difference in average canopy height between measurement dates using a ruler.

Water use characteristics were evaluated by measuring leaf relative water content (RWC), osmotic adjustment (data not shown), and soil volumetric water content (VWC). Measurements were taken on a weekly basis. Soil water content in 0-20 cm soil depth was measured with the time domain reflectometry method (Soil Moisture Equipment, Santa Barbara, CA) using a 20 cm long probe inserted in the soil. Relative water content (RWC) was calculated using the formula: 100 * [(FW – DW) / (TW – DW)] where FW is fresh weight, TW is turgid weight, and DW is dry weight following oven-drying leaf samples for 72 h at 100°C. Turgid weight was determined as the weight of fully turgid leaves after having soaked in distilled water for 24 h.

Canopy net photosynthetic rate (Pn) was measured as CO₂ μmol m⁻² s⁻¹ using the Licor 6400 gas exchange analyzer (Licor Biosciences, Lincoln, NE). The unit also measured canopy evapotranspiration (ET) as H₂O mmol m⁻² s⁻¹. A clear plexiglass chamber was designed for canopy Pn and ET measurement, which was fitted tightly over the top of the PVC pot, creating a seal from the surrounding atmosphere. Turf canopy was provided constantly with 400 μL L⁻¹ CO₂ during the measurement. This design allowed for gas exchange measurement of intact leaves for the entire canopy area with constant CO₂ supply.

Leaf photochemical efficiency was estimated by measuring variable to maximum fluorescence ratio (Fᵥ/Fₘ) in the non-energized state accomplished by exposure to
darkness. Measurements were made of intact leaves with a fluorometer (ADC BioScientific, Hoddedson, UK) after plants were adapted to dark for 30 min.

Chlorophyll was extracted by placing 0.1 g of fresh leaf tissues in a test tube containing 20 mL dimethyl sulphoxide and left in the dark for 48 h (Hiscox and Israelstam, 1979). The absorbance of the resulting solution was measured at 663 and 645 nm with a spectrophotometer (Spectronic Instruments, Rochester, NY) and total chlorophyll concentration was calculated as described by Arnon (1949).

For carbohydrate analysis, 200 mg leaf tissue was oven-dried for 72 h at 100°C. The tissue was finely ground and hydrolyzed with 2.5 mL amylase for 24 h at 37°C. A 0.5 mL of 0.6 N HCL (1:1 v/v) was added and slowly shaken for 18 h at 37°C. The solution was neutralized with approximately 0.31 mL of 10 N NaOH to a pH between 5-7, diluted to 50 mL, and filtered through No. 40 Whatman filter paper. One mL of the filtered solution was transferred to a 20-mL volumetric tube and 1.5 mL of ferricyanide reagent was added. The solution was boiled in a water bath for 10 min and then cooled in running water. A 3.0 mL of 2 N sulfuric acid was added to partially neutralize the solution. After neutralization, the solution in the tube was shaken until gas evolution ceased, and 1.2 mL of arsimonolybate solution was added. The solution was again shaken and diluted to a volume of 25 mL. The absorbance of the solution was measured at 515 nm using a spectrophotometer (Spectronic Genesys 2, Spectronic Instruments Inc., Rochester, NY). The reducing sugar content was calculated as mg TNC g⁻¹ dry tissue.

At the study’s conclusion, plants were carefully removed from PVC columns and gently washed with water to remove all soil material but to keep roots intact. Intact roots were measured to determine overall length and were then cut from the turf crowns and
dried. Root dry weight was measured to determine root mass.

**RESULTS**

Prior to initiation of stress (0 d), TE-treated plants had significantly higher quality ratings than other treatments following 42 d of TE application (Fig.1). During 21 d of stress exposure, quality ratings for non-treated plants dropped while TE-treated plants maintained significantly higher turf quality than the non-treated plants, and was still equivalent to the non-stressed control plants. After 10 d of re-watering (irrigated, 35°C), turf quality of TE-treated plants fully recovered, while that of non-treated turf improved to some extent, but was still below minimal acceptable turf quality (quality rating 6).

Both TE-treated plants exposed to stress and non-stressed control treatment maintained leaf relative water content (RWC) between 85% and 90% during the entire treatment period (Fig. 2). RWC dropped significantly during stress in the non-treated turf, and was significantly lower than TE-treated turf at 10, 18 and 21 d of stress. RWC of non-treated turf was below 50% by 21 d of stress. RWC quickly recovered in non-treated plants and was similar to other treatments after 10 d of re-watering.

Soil volumetric water content (VWC) remained at field capacity (approximately 30%) in the non-stressed control treatment (Fig. 3). Soil VWC in both TE-treated and non-treated treatments decreased during the stress, beginning at 10 d. The decline in VWC was 3% in the TE treatment and 21% in the non-TE treatment at 10 d of stress. After 18 d of stress, decline in VWC was 42% in the TE treatment and 59% in the non-TE treatment.

Vertical shoot growth (VSG) was significantly reduced following 42 d of TE
treatment before stress was imposed (0 d) (Fig. 4). During the stress period, TE-treated plants exhibited increasing VSG from 0.12 cm day$^{-1}$ at 0 d to 0.21 cm day$^{-1}$ at 21 d of stress, whereas the rate of VSG in non-treated plants steadily decreased from 0.25 cm day$^{-1}$ at 0 cm day$^{-1}$ to 0.08 cm day$^{-1}$ at 21 d of stress and fell below TE-treated plants at 18 and 21 d of stress. Control plots maintained a rate of growth at about 0.25 cm day$^{-1}$.

Canopy net photosynthetic rates (Pn) were not significantly different between TE-treated and non-treated plants at 0 and 10 d of stress (Fig. 5). At 21 d of stress exposure, TE-treated turf had significantly higher Pn than non-treated turf. Pn of TE-treated plants remained unchanged throughout the stress period while that of non-treated plants declined by 60% by 21 d of stress.

TE-treated turf had similar rates of canopy ET prior to initiation of stress (0 d) when compared to non-treated turf (Fig. 6). However, at 10 d of stress, non-treated plants had significantly (11%) higher ET rates than TE-treated plants. At 21 d of stress, ET of non-treated plants dropped and was significantly lower (53%) than TE treatments. TE-treated plants maintained a consistent rate of ET (4.9 mmol H$_2$O m$^{-2}$ s$^{-1}$) throughout the stress period (10 and 21 d).

Non-treated plants showed a steady decline in F$_{v}$/F$_{m}$ values, while TE-treated plants exhibited a constant F$_{v}$/F$_{m}$ during the stress period, which was at levels similar to the non-stressed control plants (Fig. 7). However, F$_{v}$/F$_{m}$ data were not statistically different between treatments at a given day of stress due to large variation between replicated plants. Measurements taken 10 d after re-watering showed that F$_{v}$/F$_{m}$ of non-treated plants increased to the control level.

Chlorophyll (Chl) content of TE-treated plants was significantly higher than both
non-treated plants and control plants at 0 and 10 d of stress (Fig. 8). At 21 d of stress, Chl in TE treatments (16.3 mg g⁻¹ dwt) was still maintained at the level of the non-stressed control, and was significantly higher than that of non-treated turf (5.0 mg g⁻¹ dwt).

Prior to stress initiation, no differences existed in levels of total nonstructural carbohydrates (TNC) between treatments following 42 d of TE application. However, TNC in TE-treated plants was significantly lower than non-treated plants at 10 and 21 d of stress (Fig. 9).

Average root length of TE-treated and non-TE treated stressed plants at the study’s conclusion was 13.1 cm and 13.0 cm, respectively. Similar to root length, root mass showed no statistically significant difference between the two stress treatments (data not shown).

**DISCUSSION**

Pre-conditioning creeping bentgrass with TE prior to exposure of plants to the combined drought and heat stress helped maintain higher turf quality, representing overall turf performance, during the exposure to the combined stress for a period of 21 d. Plants treated with TE also had better recuperative ability from the combined stress than non-treated plants. Our data suggested that pre-conditioning of plants with TE had a positive impact on creeping bentgrass survival of combined drought and heat stress.

The mechanisms of TE-regulation of stress tolerance are not well understood. Some studies have demonstrated that TE application promotes tiller production
(Bingaman et al., 2001; Beasley et al., 2005; Ervin and Koski, 1998) and increases in chlorophyll content (Heckman et al., 2001c; Ervin and Koski, 2001c). Improved turf color and chlorophyll content have also been observed in different turfgrass species exposed to shade (Goss et al., 2002; Stier and Rogers, 2001). In the present study, TE application increased canopy Pn and leaf chlorophyll content and suppressed the decline in Fv/Fm ratio during the stress period, as compared to non-TE treatment. These results indicated that TE application may improve canopy photosynthesis capacity and single-leaf photochemical efficiency, which could lead to increased turf quality during summer stress. Increased tiller density with TE treatment (Bingaman et al., 2001; Beasley et al., 2005; Ervin and Koski, 1998) may also contribute to the higher canopy Pn due to increased leaf area available for light absorption. In the present study, turf canopy treated with TE appeared to be denser than untreated turf, but this parameter was not quantified.

The accumulation of total nonstructural carbohydrates (TNC) has been associated with a plant’s ability to tolerate stresses and assists in recuperation following stress damage (Kang, 2002). In the present study, the positive effects of TE on Pn and turf quality, however, did not seem to be related to leaf TNC accumulation associated with TE application. Plants treated with TE for 42 d had a similar amount of TNC as non-treated plants prior to stress treatment. Furthermore, TE-treated plants had lower TNC content than non-treated plants at 10 and 23 d of stress. TNC accumulation is the result of the balance between carbohydrate production and consumption (Kang, 2002). The lower TNC content may be the result of active shoot growth (VSGR increased from 0.12 to 0.2 cm day\(^{-1}\) during 21 d of stress) for TE-treated plants that continued carbohydrate consumption during prolonged period of summer stress. In contrast, growth rate of non-
treated plants declined from 0.25 to 0.08 cm day\(^{-1}\) during 21 d of stress, which had lower demand on carbohydrates. The growth decline of non-treated plants may have resulted in TNC accumulation during the stress period. Previous research has also shown that TE applications had no effects on TNC accumulation or reduced TNC content (Han et al., 2004; Richie et al., 2001). It is also possible that carbohydrates (CHO) are partitioned from leaves to crowns due to TE application. Our measurement of leaf TNC does not account for a possible reallocation of CHO from leaves to crowns, but could help to explain why leaf TNC was lower in TE-treated plants even when Pn rates were higher. If more CHO were allocated to the crown, it would also explain increased tillering that has been observed in previous research. Regardless, the current results suggest that TE effects on creeping bentgrass tolerance to the combined stress was not associated with leaf TNC accumulation, but could be mainly due to growth regulation and water conservation.

Research has shown that application of TE may reduce water consumption of plants due to its growth inhibiting effects. Studies evaluating both Kentucky bluegrass (*Poa pratensis*) and tall fescue (*Festuca arundinacea*) have found lower rates of ET when TE was applied under non-stressed conditions (Ervin and Koski, 2001b; Marcum and Jiang, 1997). In the present study, under non-stressed conditions (0 d of stress), TE-treated turf had similar canopy ET, but by 10 d stress exposure TE treatments were significantly lower (11\%) than non-treated plants. However, following an extended period of stress (21 d), TE-treated plants maintained higher canopy ET and higher shoot growth rate than non-treated plants. These data suggested that TE-treated plants continued transpiring following a prolonged period of stress, which may facilitate
transpirational cooling and shoot growth under combined drought and heat conditions, and, therefore, the maintenance of higher canopy ET with TE application may help plants survive prolonged period of heat stress. In addition, TE-treated plants did not exhibit water deficit during the entire stress period, while non-treated plants had severe water deficit as shown by lower RWC. High leaf RWC may be the result of low water loss from leaves through transpiration and/or water retention through osmotic adjustment (Nilsen and Orcutt, 1996). In the present study, TE application had no significant effects on osmotic adjustment and root growth (data not shown), suggesting that TE effects on the maintenance of cellular hydration was not due to osmotic regulation.

Maintenance of turf quality and physiological functions in TE-treated plants could have also resulted from increased production of antioxidants, and reduced levels of reactive oxygen species (ROS). Increased levels of antioxidants (such as superoxide dismutase (SOD)) have been associated with increased drought tolerance in grasses (Price and Hendry, 1989). Previous studies have shown TE application to be partially associated with increased levels of endogenous SOD (Zhang and Schmidt, 2000). It has also been shown to improve cell membrane thermostability of Kentucky bluegrass leaf tissue (Heckman et al., 2002). As such, TE application could increase SOD, decrease ROS, and subsequently maintain cell membrane thermostability, all of which could result in the observed maintenance of Pn, RWC, and quality through the stress period.

In summary, pre-conditioning plants with TE was beneficial for plant survival of extended period of combined drought and heat stress, as manifested by improved turf quality and shoot growth rate under stress conditions. The effects of TE on plant tolerance to combined drought and heat stress could be related to its effect on the
promotion of photosynthetic capacity associated with increased chlorophyll content and
dehydrogenase activity, and on the maintenance of cellular hydration. Our results
imply that TE application could be utilized as a preconditioning treatment for turf that
seasonally experiences heat and drought stress. Such a management program could result
in reduced turf loss, quicker recovery, and reduced need for irrigation. However, the use
of TE in alleviating stress injury under field conditions deserves investigation.

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FIGURE LEGENDS

Fig. 1. Effects of foliar application of trinexapac-ethyl (TE) on turf quality during combined heat and drought stress (stress). Turf quality was expressed on a scale of 1-9 based on turf color, density, and uniformity. Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 2. Effects of foliar application of trinexapac-ethyl (TE) on relative water content (RWC) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 3. Changes in soil volumetric water content (%) in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (p=0.05) for comparison of changes over treatment period.

Fig. 4. Changes in vertical shoot growth in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Vertical bars indicate LSD values (p=0.05) for comparison of changes over the treatment period.

Fig. 5. Effects of foliar application of trinexapac-ethyl (TE) on canopy photosynthesis rate (CO$_2$ μmol m$^{-2}$ s$^{-1}$) during combined heat and drought stress (stress). Columns with
the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Fig. 6. Effects of foliar application of trinexapac-ethyl (TE) on canopy ET (H₂O mmol m⁻² s⁻¹) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Fig. 7. Changes in photochemical efficiency (Fₜ/Fₘ) in treatments with or without trinexapac-ethyl (TE) during combined heat and drought stress (stress). Unconnected symbols on the right are data for recovery (Rec.) following rewatering. Vertical bars indicate LSD values (p=0.05) for comparison of changes over treatment period.

Fig. 8. Effects of foliar application of trinexapac-ethyl (TE) on leaf chlorophyll content (mg gram⁻¹ of dry weight) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Fig. 9. Effects of foliar application of trinexapac-ethyl (TE) on total nonstructural carbohydrates (TNC) (mg gram⁻¹ of dry leaf tissue) during combined heat and drought stress (stress). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).
Fig. 1

![Graph showing turf quality over days of treatment.]

- Stress
- Stress + TE
- No stress

Turf quality (1-9)

Days of treatment

Rec.
Fig. 2

- Stress
- Stress + TE
- No stress

RWC (%)

Days of treatment

Stress + TE and No stress show a decrease in RWC (%) over time compared to Stress.
Fig. 3

Days of treatment

Volumetric water content (%)
Fig. 4

Shoot growth rate (cm day\(^{-1}\))

- Stress-TE
- Stress+TE
- Non-stress Control

Days of treatment
Fig. 5

Canopy $P_n$ (CO$_2$ $\mu$mol m$^{-2}$ s$^{-1}$)

Days of treatment

Stress-TE  Stress+TE

Canopy $P_n$ (CO$_2$ $\mu$mol m$^{-2}$ s$^{-1}$)
Fig. 6

Days of treatment

Canopy ET (H2O mmol m⁻² s⁻¹)

<table>
<thead>
<tr>
<th>Days</th>
<th>Stress-TE</th>
<th>Stress+TE</th>
</tr>
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<tr>
<td>0</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>10</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>21</td>
<td>b</td>
<td>a</td>
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</tbody>
</table>
Fig. 7

Photochemical efficiency (Fv/Fm)

Days of treatment

Stress-TE, Stress+TE, Non-stress control
Fig. 8

Chl content (mg g⁻¹ dwt)

Days of treatment

Stress-TE □ Stress+TE □ Non-stress control
Fig. 9

The graph shows the TNC content (mg g⁻¹ dwt) over different days of treatment. The treatments include Stress-TE, Stress+TE, and Non-stress control. The data points are marked with letters to indicate statistical significance. The x-axis represents the days of treatment, with values at 0, 10, and 23 days. The y-axis represents the TNC content, ranging from 0 to 100 mg g⁻¹ dwt.
CHAPTER 3

DROUGHT RESPONSES OF KENTUCKY BLUEGRASS AND CREEPING BENTGRASS AS AFFECTED BY ABScisIC ACID AND TRINEXAPAC-ETHYL

INTRODUCTION

Drought is one of the most detrimental abiotic stresses for turfgrass growth across a wide range of geographic locations. Most cool-season grass species are not well adapted to extended periods of drought, particularly during summer months. Decline in turf quality caused by drought stress is a major concern in turfgrass culture. Therefore, developing management practices for improving drought resistance of turfgrasses has become imperative in arid and semi-arid regions, especially during periods of water use restriction. One strategy to improve plant drought resistance is to promote drought avoidance by reducing water loss during drought, which may be achieved by slowing growth rate of shoots and lowering canopy leaf area to reduce demand for water (Nilsen and Orcutt, 1996). Another mechanism serving to increase drought tolerance is through osmotic adjustment, which allows plants to maintain leaf cellular hydration and sustain metabolic activities during drought (Nilsen and Orcutt, 1996).

Previous studies have reported that plants with slow-growing shoots may survive more extended periods of drought than faster-growing plants (Kondoh et al., 2006; O’Reagan et al., 1993; Simane et al., 1993). Slow growth may reduce the adverse impact of drought by conserving water and carbon energy, such that plants can use limited water to survive drought for an extended period of time (Kang, 2002). Plant growth regulators, such as TE, are traditionally used to suppress vertical shoot growth for reducing mowing
frequency of turfgrasses (Turgeon, 1999). TE blocks the final step in the biosynthesis pathway of the biologically active forms of gibberellins, results in slower vertical shoot growth (King et al., 1997), enhances superoxide dismutase and photochemical activity (Zhang and Schmidt, 2000), and has no negative impact on root growth (Fagerness and Yelverton, 2001). Reduction in vertical shoot growth may reduce the demand for water, and thus, may reduce water requirement for plant survival in water-limiting conditions for a prolonged period of time. Jiang and Fry (1998) have shown that foliar TE treatments increased turf quality of perennial ryegrass (*Lolium perenne* L.) during soil dry-down. In our previous work, exogenous application of TE prior to plant exposure to stress significantly improved growth and physiological activities of creeping bentgrass (*Agrostis stolonfera* L.) subjected to combined heat and drought for 21 d (McCann and Huang, 2007).

Abscisic acid is a plant hormone and growth regulator known to be involved in plant adaptation to drought stress. Exogenous application of ABA has been reported to improve drought tolerance in various plant species, such as maize (*Zea mays* L.) (Bochicchio et al., 1991), pepper (*Capsicum annuum* L.) (Leskovar and Cantliffe, 1992), old jack pine (*Pinus banksiana* L.) (Rajasekaran and Blake, 1999), and *Tradescantia virginiana* L. (Franks and Farquhar, 2001). Foliar application of ABA improved growth of Kentucky bluegrass (*Poa pratensis* L.) (Wang et al., 2003) and tall fescue (*Festuca arundinacea* Schreb.) (Jiang and Huang, 2002) under drought stress. ABA-induced plant tolerance to water deficit has been associated with changes in various physiological processes, including inhibition of leaf growth or transpirational area for water loss (Alves and Setter, 2000; Bacon et al., 1998), induction of stomatal closure (Kirkham, 1983;
Wilkinson and Davies, 2002), and enhancement of osmotic adjustment (Kirkham, 1983; LaRosa et al., 1987).

While there is evidence that TE or ABA application may promote drought tolerance of turfgrass plants (Jiang and Fry, 1998; Jiang and Huang, 2002; McCann and Huang, 2007; Wang et al., 2003), the information on how TE and ABA may regulate turfgrass responses to drought stress is still limited. In addition, the relative effects of TE and ABA on drought tolerance for different turfgrass species are not well documented. We have hypothesized that treatment of turfgrass plants with TE or ABA may allow plants to survive a prolonged period of drought stress with greater tolerance than controls by regulating shoot growth and water relations. Therefore, the objectives of this study were (1) to investigate the effects of exogenous application of TE and ABA on the responses of two cool-season turfgrass species, Kentucky bluegrass and creeping bentgrass, to drought stress; and (2) to examine changes in water relations associated with improved drought tolerance due to TE or ABA treatment.

**MATERIALS AND METHODS**

**Plant Materials**

Sod pieces of creeping bentgrass (cv. L-93) and Kentucky bluegrass (cv. Brilliant) were transplanted from field plots into polyvinyl chloride (PVC) tubes (10 cm diameter and 40 cm length) filled with sterilized sandy loam soil (fine-loamy, mixed mesic Typic Hapludult). Plants were maintained in a greenhouse under natural light conditions with temperatures of approximately 21/13 °C (day/night) for 2 months in the autumn of 2006 (approximate 12-h photoperiod), and then moved to a walk-in growth chamber where
treatments were imposed. The growth chamber (3 x 2.5 m) was set at 20/15 °C (day/night), 12 hour photoperiod, with a photosynthetically active radiation level of 450 \( \mu \text{mol m}^{-2} \cdot \text{s}^{-1} \) at the canopy level. Plants in each container were watered three times per week to maintain soil moisture at field capacity and fertilized weekly with 100 mL of a soluble fertilizer of 20N·8.8P·16.6K (Peter’s General Purpose 20-20-20, Grace-Sierra Horticultural Products Company, Milpitas, CA; including micronutrients: Mg 0.05%, B 0.0068, Cu 0.0036, Fe 0.05, Mn 0.025, Mo 0.0009, Zn 0.0025) at a concentration of 5 g·L\(^{-1}\) prior to exposure to drought. Grasses were maintained at approximately 4 cm height.

**Treatments and Experimental Design**

The experiment consisted of three treatments: (i) Drought stress without TE or ABA treatment (control); (ii) Drought stress with TE treatment (Stress+TE); and (iii) drought stress with ABA treatment (Stress+ABA). For TE treatment, turf was foliar sprayed with a hand-sprayer at 0.8 L·ha\(^{-1}\) Primo Maxx (Syngenta Professional Products, Greensboro, NC) [1.95 mL·L\(^{-1}\) v:v; a.i. TE = 0.113%] every two weeks for 9 weeks prior to drought stress treatment. This treatment was recommended by the manufacturer for growth inhibition in *Agrostis* and *Poa* species. A total of five TE applications were made with the final application made one week before the initiation of drought stress. For ABA treatment, plants were foliar sprayed with 6.75 mL of a 100 \( \mu \text{M} \) ABA, a rate of 54.7 g·ha\(^{-1}\), weekly, beginning nine weeks prior to stress exposure and then weekly during drought treatment. This rate of ABA was previously found to be effective in promoting drought tolerance in Kentucky bluegrass (Wang et al., 2003). A spray mist of
water, matching the ABA rate, was also applied to control and TE-treated plants during drought. Treated and untreated plants were exposed to drought stress by withholding irrigation for 28 days. No TE, fertilizers, or irrigation were applied to the plants during the drought stress period. Treatments in each turfgrass species had four replicates (4 containers). Treatments and species were arranged as a randomized complete block design in the growth chamber (a total of 24 containers). Statistical significance of data was tested using the analysis of variance procedure (SAS Institute Inc., Cary, NC). Differences between treatment means were separated by Fisher’s protected least significance difference (LSD) test at the 0.05 probability level.

**Measurements**

Turf quality was visually rated on a scale of 1 to 9, with a rating of 1 being a completely desiccated brown turf canopy, a rating of 9 representing healthy plants with dark green, turgid leaf blades, and a dense turf canopy (Turgeon, 1999). A rating of 6 was considered the minimal acceptable turf quality level.

Vertical shoot growth rate was determined by measuring the difference in average canopy height between measurement dates using a ruler. The ruler was placed in three different areas of the canopy and brought to rest on the soil surface. Canopy height was measured as the average height of plants in each container.

Leaf photochemical efficiency was estimated by measuring the variable to maximum fluorescence ratio ($F_v/F_m$) in the non-energized state accomplished by exposure to darkness. Measurements were made of intact leaves with a chlorophyll fluorescence meter (ADC BioScientific, Hoddesdon, UK) after plants were adapted to
darkness for 30 min.

Water use characteristics were evaluated by measuring soil VWC, leaf RWC, evapotranspiration (ET), and osmotic potential at full turgor ($\psi_{\pi 100}$). Measurements were taken on a weekly basis. Soil volumetric water content in 0-20 cm soil depth (where most roots are located in turfgrass) was measured with the time domain reflectometry method (Soil Moisture Equipment, Santa Barbara, CA) using a 20-cm long probe inserted in the soil. Relative water content (RWC) was calculated using the formula: $100 \times [(FW \ - \ DW) / (TW \ - \ DW)]$ where FW is leaf fresh weight, TW is leaf turgid weight, and DW is leaf dry weight following oven-drying leaf samples for 72 h at 100 °C. Turgid weight was determined as fresh weight of fully turgid leaves after soaking leaves in distilled, refrigerated water for 24 h. Leaves for RWC measurement were a random mix of old and new leaves and were cut from uniform and representative areas of the canopy.

Evapotranspiration was determined by the gravimetric mass balance method. This was accomplished by weighing pots to calculate the total amount of water lost by comparing differences in pot weight between two measurement dates.

Osmotic potential ($\psi_{\pi 100}$) was determined according to the rehydration method, where $\psi_{\pi 100}$ of leaves was determined after soaking in water for full rehydration (Blum, 1989; Blum and Sullivan, 1986). Turgid leaf samples were frozen in liquid nitrogen and subsequently stored at –20° C until analysis of leaf osmotic potential. Frozen tissue samples were thawed and cell sap was pressed from leaves, which was subsequently analyzed for osmolality (C) (mmol kg$^{-1}$) using a vapor pressure osmometer (Vapro© Model 5520, Wescor, Inc., Logan, Utah). Osmolarity of cell sap was converted from mmol kg$^{-1}$ to osmotic potential (MPa) using the formula: MPa = -C x 2.58 x 10$^{-3}$. 
RESULTS

Soil and Plant Water Relations

The initial soil volumetric water content (VWC) under well-watered conditions averaged 27.4% in all treatments for both grass species (Table 1). During the 26-d period of drought, soil VWC of all treatments decreased to between 2.8 to 4.4%. Kentucky bluegrass with TE-treatment had significantly higher soil VWC than the untreated control at 13 d of drought, but no differences in VWC were observed between treatments at 26 d of drought stress. For creeping bentgrass, TE and ABA treatments maintained significantly higher soil VWC than the untreated control by 13 and 26 d of drought. Compared to ABA treatment, TE treatment had the same VWC at 0 and 26 d of drought, but higher VWC at 13 d of drought.

Leaf relative water content (RWC) of Kentucky bluegrass averaged 82% within 14 d of drought, and then declined rapidly after 14 d of drought in all treatments (Fig. 1A). The decline in RWC was more severe in untreated control plants than in TE or ABA-treated plants. Significantly higher leaf RWC was observed in ABA-treated Kentucky bluegrass than in the untreated control at 20 and 27 d of drought stress. For creeping bentgrass, leaf RWC of untreated plants dropped sharply after 7 d of drought and was significantly lower than TE- or ABA-treated plants at 14 and 20 d of drought stress (Fig. 1B). No differences in RWC were observed between TE- and ABA-treated plants for either Kentucky bluegrass or creeping bentgrass under well-watered conditions (0 d) or during drought stress.

Evapotranspiration (ET) rates of Kentucky bluegrass did not differ among treatments within 15 d of drought, but were significantly higher in TE-treated plants than
the untreated control during 15-19 d of drought and were significantly higher in ABA-
treated plants than the untreated control during 19-26 d of drought (Fig. 2A). TE or
ABA-treated creeping bentgrass had significantly lower rates of ET than the untreated
control from 0 to 13 d of stress, but higher ET rates from 13 to 26 d of drought (Fig. 2B).
No differences in ET rates were detected between TE and ABA treatments during the
entire drought period.

Osmotic potential at full turgor (\(\Psi_{\pi_{100}}\)) was not impacted by TE or ABA
treatment in Kentucky bluegrass prior to exposure to drought (0 d) (Fig. 3A). However, \(\Psi_{\pi_{100}}\) in Kentucky bluegrass was significantly lower in ABA treatment at 21 d of drought
and in both TE and ABA treatments at 28 d of drought, compared to the untreated
control. For creeping bentgrass, \(\Psi_{\pi_{100}}\) was significantly lower in TE treatment at 21 d of
drought and in both TE and ABA treatments at 28 d compared to the untreated control
(Fig. 3B).

**Turf Growth**

Kentucky bluegrass treated with ABA or TE maintained turf quality at the same
level as the untreated control under well-watered conditions (0 d of drought) (Fig. 4A).
However, TE-treated Kentucky bluegrass exhibited significantly higher turf quality than
untreated turf at 7, 21, and 28 d of drought stress. ABA-treated Kentucky bluegrass had
significantly higher turf quality than untreated turf at 28 d of drought stress. At 21 and 28
d of drought, Kentucky bluegrass treated with TE had higher turf quality than turf treated
with ABA. For creeping bentgrass, TE-treated plants had lower turf quality than the
untreated control at 0 and 7 d of drought, but maintained turf quality above the minimum
acceptable level (6.0) and the untreated control level from 14 to 28 d of drought (Fig. 4B). No difference in turf quality was detected between ABA and untreated bentgrass at 0 and 7 d of drought, but ABA-treated plants had significantly higher turf quality than untreated control from 14 and 28 d of drought. ABA-treated creeping bentgrass had higher turf quality than TE-treated plants at 0 and 7 d of drought, but the difference diminished after 14 d of drought.

TE-treated plants had a significantly lower vertical shoot growth rate (VSG) than the respective untreated control for Kentucky bluegrass and creeping bentgrass during 1-4 d of drought treatment (Table 2). Creeping bentgrass treated with ABA also had lower VSG than the untreated control within the first 4 d of drought. During 4-15 d of drought, VSG of the untreated Kentucky bluegrass decreased by 87% while TE-treated plants maintained the same level of VSG as well-watered plants, and the VSG of TE-treated plants was 3 times greater than that of the untreated control and ABA-treated plants. For creeping bentgrass, a 91% reduction in VSG was detected in the untreated control plants from 4 to 15 d of drought compared to well-watered conditions, whereas TE- or ABA-treated plants did not exhibit a significant decline in VSG when comparing well-water conditions to 4-15 d of drought. In addition, the VSG for ABA- and TE-treated creeping bentgrass was 2 and 3 times greater, respectively, than the untreated control during 4-15 d of drought. TE treatment in Kentucky bluegrass regulated greater growth inhibition than ABA treatment under well-watered conditions, but maintained higher VSG than the ABA treatment during 15 d of drought. TE and ABA treatments had similar effects on VSG for creeping bentgrass under well-watered or drought conditions.
Photochemical Efficiency

Untreated plants showed a more rapid decline in leaf photochemical efficiency (Fv/Fm) than TE or ABA-treated plants during 27 d of drought for both species (Fig. 5). Higher Fv/Fm ratios were detected in ABA- and TE-treated Kentucky bluegrass at 21 d of drought and in ABA-treated bluegrass at 27 d of drought, compared to the untreated control (Fig. 5A). Both TE- and ABA-treated creeping bentgrass exhibited higher Fv/Fm than the untreated control at 15, 21, and 27 d of drought (Fig. 5B). TE treatment and ABA treatment had similar effects on Fv/Fm ratio for both species during the entire drought period.

DISCUSSION

TE or ABA application prior to turf exposure to drought stress helped maintain higher turf quality and shoot growth rate for both Kentucky bluegrass and creeping bentgrass during prolonged periods of drought compared to the respective untreated controls. TE application at the manufacturer’s recommended rate was more effective than ABA application in improving turf quality and maintaining active shoot growth rate for Kentucky bluegrass, but was equally effective as ABA treatment for creeping bentgrass. Maintenance of higher turf quality and active shoot growth of TE or ABA-treated plants under long-term drought stress could be associated with the modification of morphological and physiological traits associated with drought avoidance and tolerance. These included slower shoot growth rate and reduced ET during early phase of drought and improved plant water status and photochemical efficiency during prolonged drought in TE- or ABA-treated plants compared to untreated plants.
Foliar application of TE or ABA may promote drought avoidance in both species at the beginning of drought stress. The slower shoot growth rate during the early phase of drought may reduce demand for water, and thus sustain plant growth for a longer period of drought. In fact, creeping bentgrass treated with TE or ABA exhibited a significantly lower ET rate than the untreated control during the first 13 d of drought. Soil water content was also higher under TE-treated Kentucky bluegrass and TE-and ABA-treated creeping bentgrass at 13 d of drought, suggesting that TE or ABA treatment may result in lower water depletion rates due to growth inhibition during the early phase of drought. Previous studies evaluating both Kentucky bluegrass (*Poa pratensis* L.) and tall fescue (*Festuca arundinacea* Schreb.) have found lower rates of ET when TE was applied under non-stress conditions, possibly resulting from effects on growth inhibition (Ervin and Koski, 2001; Marcum and Jiang, 1997). ABA is also known to induce stomatal closure, leading to reduction in water loss (Finkelstein et al., 2002; Nambara and Marion-Poll, 2005). Stahnke and Beard (1981) reported that exogenous application of ABA reduced transpiration of creeping bentgrass by 59% compared to untreated plants under well-watered conditions. However, following a prolonged period of drought (19 d), ET rates in Kentucky bluegrass and creeping bentgrass treated with TE or ABA were significantly higher than the untreated control. This could be related to the maintenance of higher turf quality and normal shoot growth in TE- and ABA-treated plants at this time, and thus greater demand for water.

Improved water relations by TE or ABA treatment during prolonged periods of drought suggest that both treatments may also promote drought tolerance, which could contribute to the higher turf quality under prolonged periods of drought for both
Kentucky bluegrass and creeping bentgrass. Leaf RWC of the untreated control for both Kentucky bluegrass and creeping bentgrass dropped sharply during drought stress, whereas RWC of TE- and ABA-treated plants exhibited less severe decline and was significantly higher in ABA-treated Kentucky bluegrass and TE or ABA-treated creeping bentgrass after 14 d of drought. High leaf RWC may be the result of water retention through osmotic adjustment (Nilsen and Orcutt, 1996). Osmotic adjustment facilitates the maintenance of water retention and cell turgor through accumulation of organic or inorganic solutes, thus protecting tissues from desiccation as water potentials decrease (Bohnert et al., 1995). In the current study, osmotic potential at full turgor or full hydration of TE or ABA-treated turf was significantly lower than the untreated control, suggesting TE or ABA application may help maintain cell hydration by increasing the accumulation of osmotic solutes, as reflected by lower osmotic potential, and thus improved drought tolerance. Increases in cellular osmotic adjustment associated with ABA treatment have been attributed to ABA-induced accumulation of sucrose, reducing sugars, and proline under water stress conditions (LaRosa et al., 1987). How foliar application of TE may affect osmotic potential of plant cells is not clear.

Maintaining active photosynthetic activities is a critical factor controlling plant growth under stressful conditions, serving to provide carbohydrates for growth and maintenance (Woolhouse, 1986). TE- or ABA-treated Kentucky bluegrass were able to maintain a significantly higher photochemical efficiency than the untreated plants during prolonged periods of drought stress. This finding is consistent with our previous results of improved photochemical efficiency with TE treatment for creeping bentgrass exposed to combined heat and drought (McCann and Huang, 2007) and with ABA treatment for
Kentucky bluegrass exposed to drought stress (Wang et al., 2003). TE or ABA treatment helped maintain the integrity of PSII for photosynthesis. Rajasekaran and Blake (1999) reported that old jack pine seedlings treated with ABA maintained higher photosynthetic rate, which was related to the protective action of ABA on membrane integrity during drought stress.

While the results here are encouraging for development of a field trial, the complex interactions of environmental factors can diminish or negate findings from controlled-environment experiments. Jiang and Fry (1998) have previously found that improved drought tolerance of ryegrass treated with ethephon or TE in greenhouse trials did not result in improved drought tolerance under field conditions. Regardless, the data warrant further research to evaluate plant impact under field conditions.

In summary, our data suggested that foliar application of TE or ABA improved turf quality and growth for both creeping bentgrass and Kentucky bluegrass exposed to drought stress. TE and ABA treatments promoted drought avoidance during the early phase of stress, as suggested by an initial reduction in shoot growth rate and ET rate, and improved drought tolerance during prolonged stress, as manifested by the maintenance of higher RWC, lower osmotic potential, and higher photochemical efficiency later in the process of drought stress. The combination of avoidance and tolerance mechanisms could result in the improvement in turf performance following TE or ABA application.
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Table 1. Soil volumetric water content (%) in treatments with abscisic acid or trinexapac-ethyl during drought stress of Kentucky bluegrass var. Brilliant and creeping bentgrass var. L-93.

<table>
<thead>
<tr>
<th>Species</th>
<th>Compound</th>
<th>Soil volumetric content (%)</th>
<th>Days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
<td>Control</td>
<td>26.1a</td>
<td>5.3b</td>
</tr>
<tr>
<td></td>
<td>Abscisic acid</td>
<td>28.0a</td>
<td>6.1b</td>
</tr>
<tr>
<td></td>
<td>Trinexapac-ethyl</td>
<td>27.2a</td>
<td>7.9a</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
<td>Control</td>
<td>28.0a</td>
<td>3.8c</td>
</tr>
<tr>
<td></td>
<td>Abscisic acid</td>
<td>27.4a</td>
<td>6.4b</td>
</tr>
<tr>
<td></td>
<td>Trinexapac-ethyl</td>
<td>27.7a</td>
<td>8.3a</td>
</tr>
</tbody>
</table>

* Any two means within a column and plant species not followed by the same letter are significantly different by Duncan's multiple range test at $P \leq 0.05$. 
Table 2. Vertical shoot growth as affected by application of abscisic acid or trinexapac-ethyl during drought stress of Kentucky bluegrass var. Brilliant and creeping bentgrass var. L-93.

<table>
<thead>
<tr>
<th>Species</th>
<th>Compound</th>
<th>Vertical shoot growth</th>
<th>Days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Kentucky bluegrass</td>
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<td></td>
<td>0.375 aA</td>
</tr>
<tr>
<td>Control</td>
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<td>0.406 aA</td>
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<tr>
<td>Abscisic acid</td>
<td></td>
<td></td>
<td>0.167 bA</td>
</tr>
<tr>
<td>Trinexapac-ethyl</td>
<td></td>
<td></td>
<td>0.331 aA</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>0.156 bA</td>
</tr>
<tr>
<td>Creeping bentgrass</td>
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<td></td>
<td>0.125 bA</td>
</tr>
<tr>
<td>Control</td>
<td></td>
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<tr>
<td>Abscisic acid</td>
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<tr>
<td>Trinexapac-ethyl</td>
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<td>0.331 aA</td>
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</tr>
<tr>
<td>Trinexapac-ethyl</td>
<td></td>
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<td>0.125 bA</td>
</tr>
</tbody>
</table>

Any two means within a column and plant species not followed by the same lower-case letter are significantly different by Duncan's multiple range test at $P \leq 0.05$. Any two means within a row not followed by the same upper-case letter are significantly different by Duncan's multiple range test at $P \leq 0.05$. 

FIGURE LEGENDS

Fig. 1. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on leaf relative water content during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.

Fig. 2. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on evapotranspiration (mm.day\(^{-1}\)) during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Treatments with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Fig. 3. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on osmotic potential during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Columns with the same lowercase letters were not significantly different at a given day of treatment based on LSD values (p=0.05).

Fig. 4. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on turf quality during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Turf quality was expressed on a scale of 1-9 based on turf color, density, and uniformity. Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.
Fig. 5. Effects of foliar application of abscisic acid (ABA) or trinexapac-ethyl (TE) on photochemical efficiency ($F_v/F_m$) during drought stress for Kentucky bluegrass var. Brilliant (A) and creeping bentgrass var. L-93 (B). Vertical bars indicate LSD values (p=0.05) for treatment comparisons at a given day of treatment.
Fig 1.

A - Kentucky bluegrass

B - Creeping bentgrass
A - Kentucky bluegrass

B - Creeping bentgrass

Fig 2.
Fig 3.

A - Kentucky bluegrass

B - Creeping bentgrass
Fig 4.

![Graph A: Kentucky bluegrass](image)

![Graph B: Creeping bentgrass](image)
Fig 5.

A - Kentucky bluegrass

B - Creeping bentgrass
CHAPTER 4
PHYSIOLOGICAL INDICES ASSOCIATED WITH DROUGHT RESPONSES FOR KENTUCKY BLUEGRASS CULTIVARS

INTRODUCTION

Drought stress is detrimental to plant growth, especially for temperate plants species such as cool-season turfgrasses. Kentucky bluegrass (KBG) is widely used in cool season climates for home lawns and sports fields because of its good turf quality when supplied with adequate water and its superior recuperative ability from stress damages via rhizomes (Turgeon, 1999). Various studies that evaluated drought responses for various KBG cultivars have shown a wide range of genetic variations in drought resistance for this species (Keeley and Koski, 2001; McKernan et al., 2001; Wang and Huang, 2004; Chai et al., 2006; Ebdon and Kopp, 2004). Intraspecific variations in turf responses to drought stress have also been reported in many other turfgrass species, such as tall fescue (*Festuca arundinacea*) (Richie et al., 2002; Jiang and Carrow, 2005), creeping bentgrass (*Agrostis stolonifera*) (Lehman et al., 1993), and bermudagrass (*Cynodon dactylon* L. × *C. transvaalensis*), and zoysiagrass (*Zoysia japonica*) (Jiang and Carrow, 2005). However, most studies examined only turf quality or other visual symptoms, such as leaf wilting or firing as the criteria to compare cultivar variation in drought resistance or evaluate severity of drought stress damages in turfgrass. In addition, turf quality or leaf wilting is also often used to decide on irrigation quantity or schedule. Various physiological processes, such as photosynthesis, are very sensitive to drought stress and physiological damages often precede the changes in turf quality or visual
symptoms of leaf desiccation (Fry and Huang, 2004). Therefore, taking measures when physiological damages occur may be more effective for alleviating drought damage or for stress recovery than using practices when visual symptoms appear.

Several physiological parameters, including leaf relative water content, cell membrane stability as measured by electrolyte leakage and photosynthetic activities have been used to evaluate severity of drought injury in various plant species (Kirkham, 1983; O’Reagan, 1993) and in several turfgrass species (Dernodoen and Butler, 1979; Huang and Wang, 2005; McCann and Huang, 2007; Su et al., 2007). However, limited studies evaluated the quantitative relationships among physiological traits associated with drought resistance. Understanding the critical values of different physiological traits is important for the evaluation of severity of stress damage on plants, developing efficient irrigation scheduling, as well as screening stress-tolerant species and cultivars. Huang and Wang (2005) suggested that knowledge of critical values of physiological traits is important for determining irrigation timing that allows plants to recover from drought stress and for the evaluation of the recuperative ability of previously stressed plants.

The objectives of this study were 1) to examine differential responses of Kentucky bluegrass cultivars to drought stress; 2) to identify the critical values of physiological parameters in relation to overall turf performance under drought stress; and 3) to evaluate critical level of leaf water deficit contributing to drought-induced decline in turf quality and physiological activities.

MATERIALS AND METHODS

Plant Materials
Eight cultivars were examined, including ‘Midnight,’ ‘Eagleton,’ ‘Langara,’ ‘Cabernet,’ ‘Kenblue,’ ‘Baron,’ ‘Julia,’ and ‘Lakeshore’. Sod pieces of Kentucky bluegrass cultivars were transplanted from mature plants from three year old Kentucky bluegrass trials at Horticultural Farm II and the Adelphia Research Farm, Rutgers University, New Brunswick, NJ. Plants were established in polyvinyl chloride (PVC) tubes (10 cm diameter and 40 cm length) filled with sterilized sandy loam soil (fine-loamy, mixed mesic Typic Hapludult). Plants were maintained in a greenhouse under 12-14 hours natural light conditions with temperatures of approximately 23/17 °C (day/night) for one month, and then moved to a walk-in growth chamber where treatments were imposed. The growth chamber (3 x 2.5 m) was set at 20/15 °C (day/night), 12-h photoperiod, with a photosynthetically active radiation level of 450 μmol m\(^{-2}\) s\(^{-1}\) at the canopy level. Plants in each container were watered three times per week to maintain soil moisture at field capacity and fertilized weekly with 100 mL of a soluble fertilizer with 20N-20P-20K (Peter’s General Purpose 20-20-20, Grace-Sierra Horticultural Products Company, Milpitas, CA; including micronutrients: Mg 0.05%, B 0.0068, Cu 0.0036, Fe 0.05, Mn 0.025, Mo 0.0009, Zn 0.0025) at a concentration of 5 g L\(^{-1}\) prior to exposure to drought. Grasses were maintained at 5-6 cm in height.  

**Treatments and Experimental Design**

The eight cultivars were exposed to two soil water treatments: (i) Well-watered control: plants were watered every other day to soil reaching field capacity; (ii) Drought stress: irrigation was withheld for 19 d. Other environmental conditions, including temperature, relative humidity, and light intensity were maintained constant and uniform.
in the growth chamber during the entire experimental period. Each treatment was repeated in four containers randomly arranged inside the growth chamber. Containers for all treatments were rearranged in space inside the growth chamber every week to minimize location effects. The cultivars and treatments were arranged as a randomized complete block design in the growth chamber (a total of 48 containers). Data were analyzed using the analysis of variance procedure (SAS Institute Inc., Cary, NC). Differences between treatment means were separated by Fisher’s protected least significance difference (LSD) test at the 0.05 probability level.

**Measurements**

Measurements were taken on a weekly basis. Turf quality was visually rated on a scale of 1 to 9, with a rating of 1 being a completely desiccated brown turf canopy, a rating of 9 representing healthy plants with dark green, turgid leaf blades, and a dense turf canopy (Turgeon, 1999). A rating of 6 was considered the minimal acceptable turf quality level, which had approximately 30% of yellow and wilting leaves.

Water use characteristics were evaluated by measuring soil volumetric water content (VWC) and leaf relative water content (RWC). Soil volumetric water content in 0-20 cm soil depth (where most roots were located in turfgrass) was measured with the time domain reflectometry method (Soil Moisture Equipment, Santa Barbara, CA) using a 20-cm long probe inserted in the top-20 cm soil. This measurement was taken once at the initiation of treatments to confirm that all pots had equal amounts of water available to their root systems. All pots were found to have similar volumetric water contents. Last measurements were not taken until the end of the treatment period as to avoid soil
disruption caused by the probe to soil and plant that can result from the limited pot
surface area. Relative water content (RWC) was calculated using the formula: 100 *
[(FW – DW) / (TW – DW)], where FW is leaf fresh weight, TW is leaf turgid weight,
and DW is leaf dry weight following oven-drying leaf samples for 72 h at 100 °C. Turgid
weight was determined as fresh weight of fully turgid leaves after soaking leaves in
distilled, refrigerated water for 24 h.

Electrolyte leakage (EL) was determined to evaluate cell membrane stability.
Electrical conductance of a water solution incubated with 1 gram of fresh leaf samples for
24 h on a shaker was determined as the initial level of tissue leakage (Ci). The same
samples were then killed in an autoclave, and the maximum conductance (Cm) was
measured following 24 h incubation on the shaker. The percent of membrane damage was
expressed as EL = Ci/Cm X 100.

Leaf gas exchange measurements were made on individual fully-expanded leaves
using the Licor 6400 gas exchange analyzer (Licor Biosciences, Lincoln, NE). Two sub-
samples of 5 leaves from each container were measured for net photosynthetic rate (Pn),
transpiration rate, and stomatal conductance. Leaves inside the leaf chamber were
supplied with 400 μL L⁻¹ CO₂ and exposed to constant light of 500 μmol m⁻² s⁻¹.

RESULTS

Cultivar Variation in Drought Responses

Under well-watered conditions, no significant cultivar variation was observed for
any of the physiological traits examined in this study. Turf quality for all cultivars was
maintained at 8-9 at soil moisture above 15% (Fig. 1). Leaf relative water content (RWC)
was maintained at approximately 90% and electrolyte leakage was averaged 15% in well-watered control plants (Table 1).

Turf quality declined with decreasing soil moisture content during drought stress for all cultivars (Fig. 1). Under severe drought with soil moisture content below 10%, turf quality varied from 2 to 8 among cultivars. Significant difference in RWC was observed among drought-stressed cultivars at 14 day of treatment, with highest RWC in ‘Julia’ and lowest RWC in ‘Eagleton’ (Table 1). Leaf EL varied between 48.2% for ‘Julia’ and 78.5% for ‘Langara’ under drought stress. Net photosynthetic rate ($P_n$) decreased significantly at 14 d of drought in all cultivars, but least severe decline was observed in ‘Julia’ and the greatest decline was found in ‘Cabernet’ and ‘Langara’ (Table 1). Transpiration rate and stomatal conductance ($g_s$) also differed significantly among cultivars, with highest level of transpiration rate and $g_s$ detected in ‘Julia’ under drought stress (Table 1).

### Physiological Traits Associated with Drought Stress Injury

Data for turf quality and physiological parameters plants of all cultivars exposed to drought stress were pooled and the relationship between turf quality and physiological parameters, RWC, EL, $P_n$, $g_s$ and transpiration rate, was examined to determine the critical values of different physiological parameters at which turf quality became unacceptable for Kentucky bluegrass and when severe physiological damage occurred during drought stress (Fig. 2 - 5A, B, C). The critical levels of RWC, at which transpiration and $P_n$ reached to the minimal level or stomatal were closed with a zero $g_s$, were also determined (Fig. 6A, B, C).
Turf quality declined linearly with decreases in leaf RWC (Fig. 2) and increases in EL (Fig. 3). When RWC dropped below 60%, turf quality declined to below the minimal acceptable level of 6.0 (Fig. 2). As EL increased to above 60%, turf quality dropped below the minimal acceptable level of 6.0 (Fig. 3). A negative linear correlation between RWC and EL was observed (Fig. 4). EL reached to the highest level of 90% when RWC was below 20%. When RWC was maintained above 80%, leaves had minimal EL, less than 20%.

Turf quality increased in a non-linear pattern with increasing level of stomatal conductance ($g_s$), and transpiration rate, and single-leaf net photosynthetic rate ($P_n$), (Fig. 5A,B,C). Turf quality dropped below the minimal acceptable level when $g_s$ declined to below 0.1, but increased steadily with $g_s$ increasing above 0.1 mmol H$_2$O m$^{-2}$ s$^{-1}$ (Fig. 5A). A minimal acceptable turf quality occurred when transpiration rate declined to below 2.0 mmol H$_2$O m$^{-2}$ s$^{-1}$ (Fig. 5B) or when $P_n$ declined to below 1.0 mmol CO$_2$ m$^{-2}$ s$^{-1}$ (Fig. 5C).

All three gas exchange parameters also exhibited non-linear relationships with RWC for plants exhibiting water deficit under drought stress (Fig. 6A, B, C). Leaf conductance and $P_n$ dropped to near zero when RWC decreased to below 40% (Fig. 6A, C). Leaf transpiration rate decreased with the decline in RWC under drought stress, reaching the lowest level when RWC was below 20% (Fig. 6B).

**DISCUSSION**

Our study revealed the critical values of different physiological traits, leaf relative water content (RWC), electrolyte leakage (EL), single-leaf net photosynthetic rate ($P_n$),
stomatal conductance ($g_s$), and transpiration rate, associated with variation in turf quality and water status for Kentucky bluegrass exposed to drought stress. Turf quality declined in a non-linear fashion as soil VWC decreases, with the rapid decline at soil VWC below 10% (Fig. 1). Turf quality fell below the minimal acceptable value (6) when soil VWC reached 5%. The soil VWC for the active growth of KBG in sandy loam soils ranged from 15-20%. These specific values relate to KBG grown in a sandy loam soil in our study. The critical soil VWC values may vary with soil type and root-zone mixes. While there were variation in drought resistance among KBG cultivars, as indicated by changes in turf quality, RWC, EL, $P_n$, $g_s$, and transpiration under drought stress, all KBG cultivars examined in this study were able to maintain a minimal acceptable turf quality as long as soil VWC was at or above 10%. Among all cultivar examined, ‘Julia’ was shown to be the top performer under the specific drought conditions in our study. The maintenance of high turf quality of KBG cultivars under drought stress was positively associated with RWC, $P_n$, $g_s$, and transpiration rate, and negatively correlated to EL, but it depended on the critical level of each physiological parameter, as discussed in the following.

Leaf RWC is one of the most commonly used indicators for the evaluation of plant international water status and the severity of drought injury (Rachmilevitch et al., 2006). A close relationship between RWC and other parameters (turf quality, EL, $P_n$, $g_s$, and transpiration rate) for KBG cultivars subjected to drought stress suggested the importance of cellular hydration of individual leaves in maintaining overall turf quality and physiological functions under drought stress. For most plant species, 85 to 95% of RWC was optimal for maintaining active growth whereas physiological damages occurs when RWC declines to below approximately 50%, varying among plant species and
tissue types (Taiz and Zeiger, 1998). Our data showed that maintaining RWC above 60% was critical for maintaining high quality turf for KBG. Similar critical level of RWC was reported for drought-resistant Texas bluegrass cultivars and hybrids with KBG (Abraham and Huang, 2004). The maintenance of RWC above 25% was reported to be required for complete recovery of turf quality from drought stress following a period of re-watering (Huang and Wang, 2005). Our results, combined with previous studies in KBG, suggest that a minimum RWC of 25% was critical for whole-plant survival of drought stress and 60% RWC was important for maintaining high quality turf for Kentucky bluegrass.

Cellular injury of drought stress is often reflected by changes in cell membrane stability, which is expressed as increased leakage of electrolytes. Quantifying electrolyte leakage of different tissues has been widely used as a tool for the evaluation of stress severity or tolerance (Blum and Ebercon, 1981; Rachmilevitch et al., 2006). In the present study, turf quality decline to below the minimal acceptable level when EL increased to above 60% EL. Similar critical EL level was reported in a previous study with different cultivars of KBG, in which an EL level of below 70% was critical for plants to recover from drought stress (Huang and Wang, 2005). Therefore, maintaining EL below 60% or membrane integrity was important for maintaining high quality KBG under drought stress.

Numerous studies have shown that Pn maintenance is of critical importance in both plant survival under various environmental stresses and recovery from the stresses, including drought (Baker et al., 2007; Galle et al., 2007, Xu and Zhou, 2007). The current study showed a logarithmic relationship between gas exchange parameters (Pn, gs, and transpiration rate) and turf quality, demonstrating the positive impact of maintaining
\( P_{\text{n}}, g_{\text{s}}, \) and transpiration rate on turf quality for KBG subjected to drought stress. When gas exchange parameters were correlated to leaf RWC of plants exposed to drought stress (0-85% RWC), an exponential relationship was detected, exhibiting a rapid decline in \( P_{\text{n}}, g_{\text{s}}, \) and transpiration at RWC between 0-80%. The data indicated that complete stomatal closure and inhibition of photosynthesis occurred when RWC dropped to below 40% during drought stress while transpiration rate reached to the minimum level when RWC decreased to below 20%. The results suggest that maintaining RWC above 40% is critical for single-leaf photosynthesis and RWC below 20% lead to termination of water loss from KBG plants during drought stress.

Proper maintenance of turf under drought stress is challenging, especially in situations where water quantity or quality is an issue. Various measures may be taken prior to drought stress that can prevent drought damages or help in prolonging survival when poor conditions arise. Such measures include design of efficient irrigation practices in terms of irrigation quantity (DaCosta and Huang, 2006) and optimal irrigation scheduling (McCann and Huang, 2007), and appropriate selection of species or cultivar for the specific site of management (Turgeon, 1999). The critical values of physiological traits developed in this study may be used as a criterion for drought resistance evaluation and to optimize irrigation scheduling for Kentucky bluegrass grown on home lawns or sports field in water limiting environments in cool climates. However, the absolute value for each physiological parameter may vary with turfgrass species, cultural practices such as mowing height and under different environmental conditions such as changes in temperature.
REFERENCES


Table 1. Cultivar variation in physiological traits, leaf relative water content (RWC), electrolyte leakage (EL), net photosynthetic rate ($P_n$), transpiration ($\Psi_{\text{leaf}}$), and stomatal conductance ($g_s$), under well-watered (control) conditions and at 14 d of drought stress.

<table>
<thead>
<tr>
<th></th>
<th>RWC (%)</th>
<th>EL (%)</th>
<th>$P_n$ (µmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>$\Psi_{\text{leaf}}$ (mmol H$_2$O m$^{-2}$ s$^{-1}$)</th>
<th>$g_s$ (mmol H$_2$O m$^{-2}$ s$^{-1}$)</th>
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<td>Control</td>
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FIGURE LEGENDS

Fig. 1. Scatter-plot graph of turf quality and soil volumetric water content (VWC) of eight Kentucky bluegrass cultivars exposed to drought for 19 d with data collected at 0, 7, 14, and 19 d of drought stress.

Fig. 2. Scatter-plot graph of turf quality and leaf relative water content (RWC) of eight Kentucky bluegrass cultivars exposed to drought for 19 d with data collected at 0, 7, 14, and 19 d of drought stress.

Fig. 3. Scatter-plot graph of turf quality and leaf electrolyte leakage (EL) of eight Kentucky bluegrass cultivars exposed to drought for 19 d with data collected at 0, 7, 14, and 19 d of drought stress.

Fig. 4. Scatter-plot graph of leaf electrolyte leakage (EL) and leaf relative water content (RWC) of eight Kentucky bluegrass cultivars exposed to drought for 19 d with data collected at 0, 7, 14, and 19 d of drought stress.

Fig. 5. Scatter-plot graph of turf quality and stomatal conductance (mmol H₂O m⁻² s⁻¹) (A), transpiration rate (mmol H₂O m⁻² s⁻¹) (B), and net leaf photosynthetic rate (mmol CO₂ m⁻² s⁻¹) (C) for eight Kentucky bluegrass cultivars exposed to drought for 19 d. Data were collected at 0, 7, 14, and 19 d of drought stress.
Fig. 6. Scatter-plot graph of leaf relative water content (RWC) and stomatal conductance (mmol H$_2$O m$^{-2}$ s$^{-1}$) (A), transpiration rate (mmol H$_2$O m$^{-2}$ s$^{-1}$) (B), and net leaf photosynthetic rate (mmol CO$_2$ m$^{-2}$ s$^{-1}$) (C) for eight Kentucky bluegrass cultivars exposed to drought for 19 d. Data were collected at 0, 7, 14, and 19 d of drought stress.
Fig. 2

\[ y = 0.0764x + 1 \]

\[ R^2 = 0.7726 \]

Turf quality (1-9) vs. Relative water content (%)

Minimal acceptable turf quality
Fig. 3

- Equation: $y = -0.0494x + 8.8977$
- $R^2 = 0.6424$

Plot showing the relationship between electrolyte leakage (%) and turf quality (1-9). The minimal acceptable turf quality is indicated by a dashed line at a turf quality of 6.
Fig. 4

Electrolyte leakage (%) vs. Relative water content (%)

\[ y = -0.9711x + 100 \]

\[ R^2 = 0.6312 \]
Fig. 6

A. Leaf conductance rate (mmol H₂O m⁻² s⁻¹) vs. Relative water content (%)

B. Leaf transpiration rate (mmol H₂O m⁻² s⁻¹) vs. Relative water content (%)

C. Leaf photosynthesis rate (μmol CO₂ m⁻² s⁻¹) vs. Relative water content (%)

CHAPTER 5
EVALUATION OF DROUGHT TOLERANCE AND AVOIDANCE TRAITS FOR SIX CREEPING BENTGRASS CULTIVARS

INTRODUCTION

In response to drought stress, plants develop various adaptive mechanisms, including drought tolerance and avoidance strategies (Nilsen and Orcutt, 1996). Plants may avoid drought stress by maintaining favorable water status under drought either by increasing the capacity for water uptake of roots and/or reducing water loss from leaves. Previous studies with turfgrass species have shown that extensive root systems and root viability contribute positively to water uptake and thus, plant survival of drought through avoiding water deficit (Sheffer et al., 1987; Huang et al., 1997; Bonos and Murphy, 1999; Jiang and Huang, 2001). Some plant species are able to tolerate low water content in plant tissues, exhibiting growth and maintenance of metabolic processes even under cellular water deficit. Drought tolerance may be accomplished through various mechanisms, such as osmotic adjustment (OA), which involves accumulation of solutes to maintain cellular turgidity. Drought tolerance has been positively correlated with OA in many species, including turfgrasses (White et al., 1992; Qian and Fry, 1997; DaCosta and Huang, 2006).

Interspecific variation in drought survival strategies have been demonstrated in previous studies. Some turfgrass species, such as buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.), seashore paspalum (*Paspalum vaginatum* Swartz), and tall fescue (*Festuca arundinacea* Schreb.) have been found to exhibit drought avoidance
characteristics, associated with enhanced root extension deeper in the soil profile for
greater extraction of water (Carrow, 1996; Huang et al., 1997; Ervin and Koski, 1998;
Huang, 1999). Drought tolerance traits, such as osmotic adjustment, have been exhibited
in grass species like creeping bentgrass (*Agrostis stolonifera* L.) (DaCosta and Huang,
2006), Kentucky bluegrass (*Poa pratensis* L.) (Perdomo et al., 1996), and zoysiagrass
(*Zoysia japonica* Steud.) (Qian and Fry, 1997). Regardless, adaptation of specific plant
species to periods of drought stress is not limited to the use of a single mechanism, but
can employ both avoidance and tolerance traits as a means to survival (Nilsen and Orcutt,
1996). Any specific mechanism may be influenced by a variety of factors, including both
the amount and length that water is withheld, as well as specific genetic variation in both
species and cultivar. While differential drought resistance strategies have been studied
extensively among species, limited research has been conducted to examine cultivar
variation in drought tolerance and avoidance characteristics. Genetic differences among
cultivars in drought tolerance or avoidance traits could be exploited by turf breeders as
selection criterion in breeding programs working toward improved drought resistance.

Creeping bentgrass is a widely used cool-season turfgrass species on golf courses.
Breeding efforts in recent years have led to the development of creeping bentgrass
cultivars with improved turfgrass quality characteristics and greater biotic and abiotic
stress tolerance (Engelke et al., 1995; Bonos et al., 2004). Newer cultivars, such as
‘Declaration’, ‘Independence’, ‘Penn A-4’ and ‘L-93’, have been utilized widely on golf
courses. However, little is known of the improvement in drought performance of these
newer cultivars relative to older standard cultivars, such as ‘Penncross’, and the
mechanisms which they employ in the adaptation to drought stress. The objectives of
this study were: (1) to compare drought tolerance among the more recently developed creeping bentgrass cultivars with other standard cultivars and (2) to determine differential drought tolerance and avoidance characteristics associated with cultivar variation in drought resistance.

**MATERIALS AND METHODS**

**Plant Materials**

Sod pieces of six cultivars of creeping bentgrass (*Agrostis stolonifera* L) (‘Penn A-4,’ ‘Declaration,’ ‘Independence,’ ‘L-93,’ ‘Penncross,’ and ‘Putter’) were transplanted from field plots into polyvinyl chloride (PVC) tubes (10 cm diameter and 40 cm length) filled with sterilized sandy loam soil (50% fine-loamy, mixed mesic Typic Hapludult; 50% sand). Plants were maintained in a greenhouse under 10-12 hours natural light conditions and temperatures of approximately 21/13 °C (day/night) for three months in the winter of 2006-2007, and then moved to a walk-in growth chamber where treatments were imposed. The growth chamber was maintained at 20/15 °C (day/night), 70% humidity, 12-h photoperiod, and photosynthetically active radiation of 450 μmol m⁻² s⁻¹ at canopy height. Plants tubes were watered three times per week to maintain soil moisture at field capacity. Tubes were watered until collection containers underneath each tube had water fill into them, giving us the reasonable assumption that field capacity was reached. Plants were fertilized weekly with 100 mL of a soluble 20-20-20 (N-P₂O₅-K₂O) fertilizer (Peter’s General Purpose 20-20-20, Grace-Sierra Horticultural Products Company, Milpitas, CA; including micronutrients: Mg 0.05%, B 0.0068, Cu 0.0036, Fe 0.05, Mn 0.025, Mo 0.0009, Zn 0.0025) at a concentration of 5 g L⁻¹ prior to exposure to
drought. Actual N applied at 123.35 kg ha$^{-1}$. Grasses were cut every two days with scissors, with clippings removed, and maintained at approximately 4-cm height.

**Treatments and Experimental Design**

The six cultivars were exposed to two soil water treatments: (i) Well-watered control: plants were watered every other day to soil reaching field capacity; (ii) Drought stress: irrigation was withheld for 17 d. Each treatment was replicated four times in space (different containers). The cultivars and treatments were arranged as a randomized complete block design in the growth chamber (a total of 48 containers). Repeated measurements were made on four replicates for each treatment. Data were analyzed using the analysis of variance procedure (SAS Institute Inc., Cary, NC). Differences between treatment means were separated by Fisher’s protected least significance difference (LSD) test at the 0.05 probability level.

**Measurements**

Water use characteristics were evaluated by measuring soil volumetric water content (VWC), leaf relative water content (RWC), evapotranspiration (ET), and osmotic potential when plants were well watered ($\psi_{s100}$). Measurements were taken on a weekly basis. Soil volumetric water content in 0-20 cm soil depth (where most roots are located in turfgrass) was measured with the time domain reflectometry method (Soil Moisture Equipment, Santa Barbara, CA) using a 20-cm long probe inserted in the top-20 cm soil. Relative water content (RWC) was calculated using the formula: $100 \times [(FW – DW) / (TW – DW)]$, where FW is leaf fresh weight, TW is leaf turgid weight, and DW is leaf
dry weight following oven-drying leaf samples for 72 h at 100 °C. Turgid weight was determined as weight of fully turgid leaves after soaking leaves in distilled water in the refrigerator for 24 h. Evapotranspiration rate (ET) was determined by the gravimetric mass balance method. Pots were weighed every 24 hours to calculate the total water lost through comparison of differences in weight between the two measurements.

Osmotic adjustment (\(\psi_{\pi100}\)) was determined according to the rehydration method, where \(\psi_{\pi100}\) of leaves was determined after soaking in water for full rehydration (Blum and Sullivan, 1986; Blum, 1989). Turgid leaf samples were frozen in liquid nitrogen and subsequently stored at –20°C until analysis of leaf osmotic potential. Frozen tissue samples were thawed and cell sap was pressed from leaves, which was subsequently analyzed for osmolality (C) (mmol kg\(^{-1}\)) using a vapor pressure osmometer (Vapro© Model 5520, Wescor, Inc., Logan, Utah). Osmolality of cell sap was converted from mmol kg\(^{-1}\) to osmotic potential (MPa) using the formula: MPa = -C x 2.58 x 10\(^{-3}\). Osmotic adjustment was determined as the difference in osmotic potential between well-watered and drought-exposed plants.

Plant tissue was sampled for carbon isotope analysis at 14 d of treatment. Leaf tissue was dried at 80°C and ground to a powder to pass through a 40-mesh screen. Carbon isotope composition (\(\delta^{13}C\)) was analyzed by Augustana College, Biology Department, Sioux City, South Dakota. A more detailed description on carbon isotope analysis and theory were reported in Smedley et al. (1991) and Ebdon et al. (1998).

Turf quality was visually rated on a scale of 1 to 9, with a rating of 1 being a completely desiccated brown turf canopy, a rating of 9 representing healthy plants with dark green, turgid leaf blades, and a dense turf canopy (Turgeon, 1999). A rating of 6
was considered the minimal acceptable turf quality level.

Leaf photochemical efficiency was estimated by measuring the variable to maximum fluorescence ratio ($F_v/F_m$) in the non-energized state accomplished by exposure to darkness. Measurements were made of intact leaves with a chlorophyll fluorescence meter (ADC BioScientific, Hoddesdon, UK) after plants were adapted to darkness for 30 min.

Following the treatment period, all roots in each container were washed free of soil. Root viability was determined using a representative sample from each container and measuring dehydrogenase activity with the triphenyltetrazolium chloride (TTC) reduction technique (Knievel, 1973; McMichael and Burke, 1994). A different representative sample from each container was digitally imaged using WinRhizo 2002 computer software (Regent Instruments Inc., Quebec, Canada). Total root length and total number of roots were determined using the WinRhizo 2002 program.

RESULTS

Turf Quality and Leaf Photochemical Efficiency

Turf quality was maintained at approximately 8.0 throughout the treatment period in all cultivars, with no cultivar variations under well-watered conditions (Fig. 1). Under drought stress, turf quality exhibited a steady decline, and the rate of decline varied between cultivars. All cultivars exposed to drought maintained acceptable turf quality (6.0 or higher) within 7 d of drought stress. By 14 d of drought, only ‘Penn A-4,’ ‘L-93,’ and ‘Penncross’ maintained acceptable quality, and ‘Penn A-4’ had significantly higher turf quality (7.0) than all other cultivars except ‘L-93’ (6.5); ‘L-93’ had significantly
higher turf quality than ‘Declaration’ (5.75), ‘Independence’ (5.75), and ‘Putter’ (5.25). By 17 d of drought stress, turf quality of all cultivars declined to below the acceptable turf quality level; ‘Penn A-4’ and ‘Independence’ had significantly higher turf quality than ‘Declaration,’ ‘Penncross,’ and ‘Putter,’ but did not differ from ‘L-93.’

Well-watered plants maintained constant leaf photochemical efficiency ($F_{v}/F_{m}$) throughout the duration of the study, with no significant differences between cultivars on any days of treatment (Fig. 2). In drought-stressed plants, $F_{v}/F_{m}$ was maintained at the well-watered control level during 7 d of treatment, but declined to below their respective control level by 14 d of treatment in all cultivars, with the exception of ‘Penn A-4’ which $F_{v}/F_{m}$ was not different from the control. At 7 and 14 d of drought, ‘Penn A-4’ had a significantly higher $F_{v}/F_{m}$ than all other cultivars. ‘Independence’ and ‘L-93’ had a significantly higher $F_{v}/F_{m}$ than ‘Putter’ at 14 d of drought stress. At 17 d of drought, no significant differences in $F_{v}/F_{m}$ were observed between cultivars.

**Plant Water Relations**

Soil volumetric water content (VWC) of pots were approximately 20% at study initiation, with no differences between treatments (data not shown). At the study’s conclusion (17d), soil volumetric in well-watered plants remained approximately 20%, while all drought-exposed plants had VWC below 5%.

Leaf relative water content (RWC) of well-watered plants averaged approximately 90% throughout the treatment period, with no significant differences among cultivars (Fig. 3). Under drought stress, significant declines in RWC were observed by 14 d, dropping to below 50% in all cultivars. At 14 d of drought stress,
‘Penn A-4’ and ‘Independence’ had significantly higher RWC than ‘Penncross’ and ‘Putter’. At 17 d of drought stress, ‘Independence’ had significantly higher RWC than ‘Declaration,’ ‘Penncross,’ and ‘Putter.’

Significant differences in osmotic adjustment (OA) were detected among six cultivars exposed to drought stress for 14 d (Fig. 4). OA of ‘Penn A-4’ and ‘L-93’ was significantly lower than ‘Declaration’ and ‘Penncross.’ No significant differences in OA were detected among ‘Declaration,’ ‘Independence,’ ‘Penncross,’ and ‘Putter.’

Evapotranspiration (ET) rates of well-watered plants varied among cultivars (Fig. 5). ‘L-93’ generally had the lowest ET rate among the six cultivars, lower than ‘Penncross’ at 5-7 d, ‘Penn A-4’ at 10-11 d, and ‘Declaration’ at 10-11 d, 12-14 d, and 16-18 d. Significant declines in ET rates of drought-stressed plants were observed by 10-11 d in all cultivars. At 12-14 d of drought stress, ‘Penn A-4’ maintained significantly greater ET than ‘Putter.’ No cultivar difference in ET was observed on other treatment days.

Carbon isotope discrimination ratio ($\delta^{13}$C) was lowest in ‘Penncross,’ highest in ‘Declaration,’ and intermediate in the other four cultivars under well-watered conditions (Fig. 6). $\delta^{13}$C of plants exposed to drought stress significantly increased in all cultivars, with the exception of ‘Penn A-4,’ compared to well-watered plants. Cultivars also varied in $\delta^{13}$C under drought stress, which ranked as: ‘Penn A-4’ < ‘Penncross’ = ‘L-93’ ≤ ‘Independence’ = ‘Putter’ = ‘Declaration.’

**Root Characteristics**
Root viability, expressed as TTC reduction, did not vary significantly among cultivars under well-watered conditions (data not shown). However, root viability did vary between cultivars exposed to drought stress (Fig. 7). ‘Independence’ maintained significantly higher root viability in the upper 20-cm soil layer compared to ‘Penn A-4,’ ‘Declaration,’ ‘L-93,’ and ‘Penncross,’ and in the lower 20-cm soil than all five other cultivars. No difference in root viability was detected among ‘Penn A-4,’ ‘Declaration,’ ‘L-93,’ ‘Putter,’ and ‘Penncross’ at either soil depth.

Total root length varied among cultivars under well-watered conditions, with ‘Declaration,’ ‘Penncross,’ and ‘Putter’ having longest root systems and ‘L-93’ had the shortest root system. Total root length of drought-stressed plants decreased significantly for ‘Declaration’ and ‘Putter,’ which was 46% and 40% lower than the well-watered control plants, respectively (Fig. 8). ‘Penn A-4,’ ‘Independence,’ and ‘L-93’ showed no significant decline in total root length under drought stress, compared to their respective well-watered control.

No cultivar variation in the number of roots was observed under well-watered conditions (Fig. 9). Under drought stress, ‘Penn A-4’ had the most number of roots, which was significantly higher than ‘Declaration’ but not different from other cultivars. ‘Declaration’ exhibited significant decline in the number of roots under drought stress while other cultivars maintained the number of roots at their respective control level (Fig. 9).

**DISCUSSION**

Quality ratings and leaf photochemical efficiency results demonstrated that ‘Penn
A-4,’ ‘Independence,’ and ‘L-93’ were generally better able to maintain growth and metabolic activity under drought stress than the other three cultivars evaluated in the current study. The better drought resistance of the three newer bentgrass cultivars could be primarily attributed to drought avoidance traits, such as reduced water use and improved rooting characteristics, as discussed in details in the following section.

Leaf relative water content (RWC) is a widely used parameter to determine the level of internal water status. During a prolonged period of drought (14 d), drought-exposed ‘Penn A-4,’ ‘Independence,’ and ‘L-93’ had higher RWC than the other cultivars, with ‘Penn A-4’ and ‘Independence’ being significantly higher than ‘Penncross’ and ‘Putter.’ The maintenance of leaf water status is essential for continuation of physiological and biochemical functioning. Plants that can maintain adequate RWC for a longer period of time under drought exposure will have the greatest likelihood of continued metabolic functioning and survival. In the current study, higher RWC was associated positively with higher turf quality ($R^2 = 0.8238$) and leaf photochemical efficiency ($R^2 = 0.7945$), with ‘Penn A-4’ and ‘Independence’ rating highest in both parameters. This suggested that cultivars with greater drought resistance were able to maintain higher cellular hydration under drought conditions.

Osmotic adjustment has been identified as a drought tolerance mechanism in many species (LaRosa et al., 1987; Bohnert et al., 1995). Increasing osmotic adjustment facilitates the maintenance of cell turgor under conditions of limited water availability. In the present study, the two cultivars, ‘Penn A-4’ and ‘L-93,’ that maintained higher RWC, turf quality, and leaf photochemical efficiency under drought stress, had low osmotic adjustment. In contrast, ‘Declaration’ and ‘Penncross,’ that did not perform well
under drought stress, exhibited high levels of osmotic adjustment. These results suggest that osmotic adjustment was not a major mechanism for ‘Penn A-4’ and ‘L-93’ to tolerate drought stress, but was important for the survival of ‘Declaration’ and ‘Penncross’ under drought stress. The overall implication is that a particular species may employ an important attribute, such as OA, but that one mechanism, alone, will not necessarily predict comparative performance between cultivars.

The ability to maintain low ET rates has long been considered a trait for water conservation (Kirkham, 1983; Bacon et al., 1998; Alves and Setter, 2000). Salaiz et al. (1991) found significant variability in ET rates among different cultivars when comparing water use of different creeping bentgrass cultivars under well-watered field conditions. Our study also found cultivar variation in ET under well-watered conditions, with ‘L-93’ being the lowest water user. These results indicated there was a potential for developing creeping bentgrass cultivars with reduced water use under non-limiting water environments. However, under drought stress, cultivar variations in ET diminished, suggesting that cultivar variation in drought resistance was not necessarily related to water use rates. Previous studies have also reported the lack of correlation between ET and drought resistance in creeping bentgrass (McCann and Huang, 2007) and other turfgrass species. Fernandez and Love (1993) compared cultivars of tall fescue and perennial ryegrass (Lolium perenne L.) and reported that some tall fescue cultivars with higher water use maintained higher turf quality under drought stress than some perennial ryegrass cultivars with lower water use rates. Another study has shown better performance of drought stress-tolerant Kentucky bluegrass cultivars with higher water use compared to poorer performing cultivars that adapted to drought by decreasing water
Carbon isotope discrimination ($\delta^{13}C$) has been associated with plant water use efficiency (WUE). Plants under water stress have been shown to discriminate less against $C^{13}$ than $C^{12}$ in the photosynthetic reaction (Farquhar et al., 1989; Lambers et al., 1998). As such, greater WUE is achieved when less carbon discrimination occurs or lower $\delta^{13}C$ is correlated with higher WUE. Negative correlations between $\delta^{13}C$ and WUE have been reported in Kentucky bluegrass (Ebdon et al., 1998; Ebdon and Kopp, 2004), tall fescue (Johnson and Bassett, 1991; Johnson, 1993), and perennial ryegrass (Johnson and Bassett, 1991). In the present study, cultivar variations in $\delta^{13}C$ were observed under both well-watered and drought stress conditions. Under well-watered conditions, ‘Penncross’ had the lowest $\delta^{13}C$ and ‘Declaration’ had the highest $\delta^{13}C$, suggesting that ‘Penncross’ had lower WUE than ‘Declaration’ under non-water limiting conditions. Under drought stress, however, ‘Penn A-4’ had the highest $\delta^{13}C$, suggesting that ‘Penn A-4’ may have maintained better growth and physiological functioning via other pathways, such as cell membrane stability or by increasing antioxidant activity.

Rooting characteristics, including root viability, root length, and number, are important factors controlling water uptake of a root system. No cultivar variation in root viability was observed under well-watered conditions; however, ‘Independence’ maintained significantly higher root viability at the 20- to 40-cm soil depth than other cultivars, suggesting that ‘Independence’ may be better able to maintain higher turf quality, $F_v/F_m$, and RWC under drought conditions by avoiding drought stress through maintaining more active roots for water uptake. These results also suggest the importance of maintaining higher root viability in deeper soil profiles where water may be more
available than the surface soil.

The variation in drought avoidance between different turfgrass species has also been associated with the total amount of roots in deeper soil profiles under drought stress (Ervin and Koski, 1998). In our study, among the six cultivars compared, the three cultivars (‘Penn A-4’, ‘Independence’, and ‘L-93’) that showed the best turf quality performance had lower total root length than the other three cultivars under well-watered conditions. These same three cultivars showed only slight differences in total root length between well-watered and drought stressed plants. However, ‘Declaration,’ ‘Penncross,’ and ‘Putter’ exhibited significant decline in total root length under drought conditions, compared to their respective control. The data suggest an extensive root system under well-watered conditions does not necessarily correlate to greater drought resistance. In addition, ‘Penn A-4,’ the cultivar with the highest turf quality under drought stress, showed no significant change in root number between well-watered and drought stress conditions while other cultivars exhibited some extent of decline in root number under drought stress, particularly ‘Declaration.’ It seems that those cultivars with greater drought sensitivity are the first to lose roots when water becomes limited. In contrast, drought resistant cultivars, particularly ‘Penn A-4,’ had the ability to maintain root elongation and production even when drought was imposed. These results further suggested that ‘Penn A-4’ may sustain growth under drought through avoiding drought by developing persistent, extensive root systems.

In summary, our results demonstrated genetic variation in drought tolerance and drought avoidance characteristics in creeping bentgrass cultivars. ‘Penn A-4,’ ‘Independence,’ and ‘L-93’ performed better than other three cultivars under drought
conditions. The majority of physiological parameters evaluated suggested that creeping bentgrass cultivars that were better able to survive drought stress was mainly through some avoidance mechanisms, such as maintaining higher WUE, root viability, root elongation, and production under drought stress. These parameters could be used as criterion to select for drought resistant bentgrass cultivars.
REFERENCES


FIGURE LEGENDS

Fig. 1. Creeping bentgrass cultivar variation in turf quality under well-watered (dotted line) and drought stress (solid line). Vertical bars indicate LSD values ($p=0.05$) for cultivar and treatment comparisons at a given day of treatment.

Fig. 2. Creeping bentgrass cultivar variation in leaf photochemical efficiency ($Fv/Fm$) under well-watered (dotted lines) and drought stress (solid lines). Vertical bars indicate LSD values ($p=0.05$) for cultivars and treatment comparisons at a given day of treatment.

Fig. 3. Creeping bentgrass cultivar variation in leaf relative water content under well-watered (dotted lines) and drought stress (solid lines). Vertical bars indicate LSD values ($p=0.05$) for cultivar and treatment comparisons at a given day of treatment.

Fig. 4. Creeping bentgrass cultivar variation in osmotic adjustment under drought stress. Columns with the same lowercase letters are not significantly different based on LSD values ($p=0.05$).

Fig. 5. Creeping bentgrass cultivar variation in evapotranspiration rate under well-watered (dotted lines) and drought stress (solid lines). Vertical bars indicate LSD values ($p=0.05$) for cultivar and treatment comparisons at a given day of treatment.
Fig. 6. Creeping bentgrass cultivar variation in carbon isotope discrimination ($\delta^{13}$C) under well-watered and drought stress at 14 d of treatment. Columns with the same lowercase letters are not significantly different based on LSD values ($p=0.05$).

Fig. 7. Creeping bentgrass cultivar variation in root viability, expressed as TTC Reduction (Absorbance 490 nm mg$^{-1}$), at 17 d of drought stress. Columns with the same lowercase letters are not significantly different based on LSD values ($p=0.05$).

Fig. 8. Creeping bentgrass cultivar variation in total root length under well-watered (A) and drought stress (B) at 17 d of treatment. Columns with the same lowercase letters are not significantly different based on LSD values ($p=0.05$).

Fig. 9. Creeping bentgrass cultivar variation in total number of roots under well-watered (A) and drought stress (B) at 17 d of treatment. Columns with the same lowercase letters are not significantly different based on LSD values ($p=0.05$).
Fig. 1

Days of treatment vs. turf quality for different treatments. The graph shows the decline in turf quality over time for various treatments labeled as A-4W, DecW, IndW, L-93W, PenW, PutW, A-4D, DecD, IndD, L-93D, PenD, and PutD. The dashed line indicates the acceptable turf quality level.
Fig. 2

Photochemical efficiency (Fv/Fm)

Days of treatment
Fig. 3

The graph shows the relative water content (%) over days of treatment, with different treatments represented by various symbols and lines. The x-axis indicates days of treatment, ranging from 0 to 17, while the y-axis represents the relative water content, ranging from 0 to 100%.
Fig. 4

The graph shows the osmotic adjustment (MPa) for different cultivars: A-4, Dec, Ind, L-93, Pen, and Put. The bars are labeled with letters indicating significant differences among the cultivars. The osmotic adjustment ranges from 0 to 0.4 MPa.
Fig. 5

![Graph showing evapotranspiration (mm day\(^{-1}\)) over different days of treatment.](image-url)
Fig. 6

The diagram illustrates the differences in δ¹³C values between Watered and Drought conditions. Different symbols and patterns represent various treatments or conditions (A-4, Dec, Ind, L-93, Penn, Put). The δ¹³C values range from -32.50 to -36.00.

Key:
- A-4
- Dec
- Ind
- L-93
- Penn
- Put

Legend:
- Watered
- Drought

Note: Letters (a, b, c, d, e, f, g) indicate statistical differences.
Fig. 7

TTC reduction (absorbance 490 nm mg\(^{-1}\))

<table>
<thead>
<tr>
<th>Bentgrass Variety</th>
<th>D (0-20cm)</th>
<th>D (21-40cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ind.</td>
<td>a</td>
<td>ab</td>
</tr>
<tr>
<td>Putter</td>
<td>bc</td>
<td>c</td>
</tr>
<tr>
<td>A4</td>
<td>abc</td>
<td>c</td>
</tr>
<tr>
<td>Dec.</td>
<td>bc</td>
<td>bc</td>
</tr>
<tr>
<td>L93</td>
<td>bc</td>
<td>bc</td>
</tr>
<tr>
<td>Penn</td>
<td>c</td>
<td>bc</td>
</tr>
</tbody>
</table>
Fig. 8

The bar chart illustrates the total root length (cm) for different treatments under watered and drought conditions. The treatments include A-4, Dec, Ind, L-93, Pen, and Put. The chart shows significant differences between treatments, with specific letters indicating statistical significance. For instance, in the watered condition, the treatment labeled 'a' is significantly different from 'abcd' and 'cd'. In the drought condition, 'abc' shows a distinct pattern compared to other groups.
Fig. 9

![Bar chart showing the number of roots for different conditions and species.](image-url)

- **Watered**
  - A-4: a
  - Dec: a
  - Ind: a
  - L-93: ab
  - Pen: a
  - Put: ab

- **Drought**
  - A-4: a
  - Dec: b
  - Ind: ab
  - L-93: ab
  - Pen: ab
  - Put: ab
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