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**EVALUATION OF MANAGEMENT STRATEGIES AND PHYSIOLOGICAL  
MECHANISMS OF *AGROSTIS* SPECIES FOR REDUCED IRRIGATION  
ENVIRONMENTS**

A Thesis Presented

by

LISA C. GOLDEN

Submitted to the Graduate School of the  
University of Massachusetts in partial fulfillment  
of the requirements for the degree of  
MASTER OF SCIENCE

May 2014

Plant and Soil Sciences

**EVALUATION OF MANAGEMENT STRATEGIES AND PHYSIOLOGICAL  
MECHANISMS OF *AGROSTIS* SPECIES FOR REDUCED IRRIGATION  
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Lisa C. Golden

Approved as to style and content by:

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Michelle DaCosta, Chair

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J. Scott Ebdon, Member

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Patricia Vittum, Member

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Geunhwa Jung, Graduate Program  
Director

## **DEDICATION**

**To my dad, whose love and support has allows allowed me to chase down my  
dreams.**

## **ACKNOWLEDGEMENTS**

Firstly, I would like to sincerely thank my advisor, Dr. Michelle DaCosta, for all her guidance, support, and patience. I would also like to thank my graduate committee members, Dr. Scott Ebdon and Dr. Patricia Vittum, for all their advice and support. There are so many staff and students at the University of Massachusetts that made this thesis possible but in particular a heartfelt thanks to, Jennifer Albertine, Xian Guan, Lindsey Hoffman, James Poro, Teddy Norman, and Peter White, not only for their many hours of help and but for their friendship. I would also like to give a special thanks to all my friends and family, too many to name, for their encouragement and love that has helped me in ways I cannot put into words. Lastly, I would like to give a loving thanks to my sister, Tricia Downing, and to my parents, Mary Chalmers and Thomas Golden. Without their love and support, I would not have had the courage to attempt this thesis or the strength to finish it.

## ABSTRACT

# EVALUATION OF MANAGEMENT STRATEGIES AND PHYSIOLOGICAL MECHANISMS OF *AGROSTIS* SPECIES FOR REDUCED IRRIGATION ENVIRONMENTS

MAY 2014

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Water is a basic necessity for turfgrass growth and metabolic processes, with optimal levels required for the maintenance of turf quality and function. As water restrictions for irrigation of landscapes become more widespread across the United States, turfgrass managers will need to rely on management strategies to improve the performance of turfgrasses under reduced irrigation environments. Therefore, the objectives of the research were to (i) compare the performance of different *Agrostis* species and cultivars under reduced irrigation, (ii) evaluate the use of wetting agents for maintaining turf quality under reduced irrigation, (iii) and examine the physiological mechanisms associated with improved drought resistance traits of *Agrostis* species. To address our primary objectives, we conducted a two-year field study comparing cultivars of three bentgrass species, including ‘Revere’ and ‘Tiger II’ colonial bentgrasses (*Agrostis capillaris*), ‘Legendary’ and ‘Greenwich’ velvet bentgrasses (*A. canina*), and ‘13M’, ‘T-1’, ‘L-93’, and ‘Penncross’ creeping bentgrasses (*A. stolonifera*) in response to reduced irrigation with and without the use of a wetting agent. In general, the use of a

wetting agent did not result in any significant differences in turf quality or soil moisture content among treatments. There were significant differences in turf quality among bentgrass species and cultivars under reduced irrigation. Colonial bentgrass cultivars maintained high turf quality, and were found to be well suited for fairways under reduced irrigation. Due to excessive thatch accumulation in our study, velvet bentgrass cultivars exhibited significant declines in quality regardless of irrigation level. Among creeping bentgrass cultivars, T-1 exhibited improved drought tolerance compared to the older cultivars of creeping bentgrass. Based on results from the field study, we further evaluated the drought resistance and recovery characteristics among five cultivars of colonial bentgrass ('Barking', 'Tiger II', 'Revere', 'Capri', and 'Greentime'). Under moderate drought stress, Barking, Tiger II, and Revere all exhibited lower leaf relative water content levels compared to Capri and Greentime, although no significant differences in turf quality or soil water content were observed during the drought period. Following re-watering, Barking and Tiger II exhibited the most rapid recovery from drought (as measured by percent green cover), while Capri and Greentime exhibited delayed recovery. Therefore, although significant differences in turf performance during drought stress were not observed, recovery potential seems to vary among the different cultivars of colonial bentgrass.

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## CHAPTER 1

### LITERATURE REVIEW

#### Overview

In the United States, golf courses use a combined total of approximately 7.9 billion liters of water on a daily basis (Vogt, 2011). It has been reported that water is second to labor as an expense in many golf course maintenance budgets (Leslie, 2012), and this is likely because a majority of greens, tees, and fairways in the United States are irrigated (EIFG, 2007a). As many parts of the country have been subjected to extended periods of drought, water restrictions for irrigation of turf have increased (Hollister, 2010). Due to continued population growth particularly in urbanized areas, a greater strain on water supply is expected in the future. In turn, as the demand for water increases, there will be greater water restrictions particularly for low priority areas maintained as turfgrass. Given the high cost of water, the restrictions of water usage, and the need for water by turfgrass, it is critical that research is implemented to find ways to maintain turf function under reduced irrigation environments.

Golf courses have many benefits such as providing a place for the millions of golfers to get exercise. Golf courses are home to wildlife and wetlands, they can be built on former landfills, and provide income and jobs for surrounding communities. In 2011, there were approximately 30 million golfers in the United States (Amari, 2012). There are almost 16,000 golf courses throughout the United States with approximately half a million jobs created (Haydu et al., 2008). Based on a survey of the Massachusetts golf industry alone in 2006, there were a reported 31,685 jobs created and \$950 million in wages attributed to the industry (SRI International, 2008).

The average 18-hole golf course in the United States has approximately 70% of its land as managed turfgrass (greens, tees, fairways, rough, driving range, and additional grounds), which on average consists of approximately 40 hectares per golf course. Of this managed turf acreage, approximately half of that land is made up of greens, tees, and fairways (EIFG, 2007b). Although most greens, tees, and fairways are irrigated, these areas may not be irrigated on a daily basis. Moreover, it has become more common for golf course staff to hand water (rather than overhead irrigation), and to only water areas exhibiting mild symptoms of drought stress. Whether or not all of these areas are irrigated can also depend on the budget and the expectations of the golfers or members of a golf course. Many golf courses depend on natural rainfall and rarely water the rough, which can make up over 50% of managed turf areas on a golf course (EIFG, 2007b). Given that golf course superintendents are under pressure to maintain functional turfgrass with fewer inputs, it is critical that turf managers take proactive water conservation approaches.

### **Water Conservation Strategies**

Given the potential amount of acreage maintained as turfgrass on a golf course, there are several water conservation strategies that can be utilized to reduce overall irrigation use. Irrigation system efficiency can be enhanced through regular auditing, including improvements related to nozzle types and sprinkler heads, correctly spacing irrigation heads for more efficient water coverage, and ensuring proper timing and amounts of applied water (Leslie, 2012). Through the use of on-site weather stations,



golf course superintendents can irrigate according to evapotranspiration estimates to more accurately replace turfgrass water loss. In addition, superintendents are also monitoring soil moisture levels more closely using moisture sensors, and in general keeping soils drier to improve playability (EIFG, 2007a).

Turfgrass on a golf course is subject to many stresses including low mowing heights, wear, soil compaction, and environmental extremes such as high and low temperatures. In addition to abiotic stresses, turfgrasses are also exposed to weed, insect, and disease pressures. Different species and cultivars exhibit varying degrees of resistance to biotic and abiotic stresses. Therefore, a management strategy that can help to maintain the general turf quality is to select the best adapted species and cultivars for a site and its known stresses. Consequently, due to increasing issues related to water restrictions of turfgrass, additional research is necessary to identify species and cultivars with enhanced drought resistance traits under reduced irrigation.

Among cool-season grasses, *Agrostis* species (bentgrasses) are commonly found as golf turf in northern climatic regions. Although there are many different species of bentgrass, colonial bentgrass (*Agrostis capillaris* L.), velvet bentgrass (*A. canina* L.), and creeping bentgrass (*A. stolonifera* L.) are the best adapted species for golf turf, mainly related to the ability of these grasses to maintain sufficient turf quality even at low heights of cut required for green, tee, and fairway turf. Among the three species, creeping bentgrass is the most widely used turfgrass as golf turf in the northeast and north central regions of the United States (EIFG, 2007b).

There are significant inter- and intra-specific differences among bentgrass species for turf quality and performance traits, including genetic leaf color and resistance to abiotic and biotic stresses. For example, previous research has shown inter- and/or intra-specific differences in wear tolerance (Dowgiewicz et al., 2011), dollar spot (Ryan et al., 2012), and drought resistance (DaCosta and Huang 2006; DaCosta and Huang, 2007; McCann and Huang, 2008). To date, though, there is limited information available on the variability of drought resistance and water use particularly among cultivars of colonial and velvet bentgrasses.

In addition to species and cultivar selection, the use of surfactants (ie wetting agents) has also shown promise as a management strategy to improve or maintain turf quality under reduced irrigation. Wetting agents became available for use in the turfgrass industry in the 1950s (Zontek, 2012). Since then many different wetting agent chemistries have become available and have been designed to alleviate different issues. Originally wetting agent chemistry was an anionic or anionic blend. These were negatively charged formulas that have been known to disrupt soil structure by interacting with clay particles and at times be phytotoxic to turfgrass. A newer chemistry that is more commonly used today is nonionic surfactants. Nonionic surfactants can be broken down into six different classes. The first is polyoxyethylene (POE), which also became available in the 1950s and was developed to alleviate localized dry spot (LDS) but has also shown to be phytotoxic to some finer turfgrasses. The second class of non-ionic surfactants is the block co-polymer wetting agents. This surfactant class consists of straight block co-polymers which help to alleviate LDS, reverse block co-polymers which helps with hydrophobic soils, and lastly a blend of both straight and reverse co-polymer

which combines both chemistries and therefore help to alleviate both LDS and hydrophobic soils. Thirdly, there are alkyl polyglucoside surfactants that are made up of sugars and fatty acids and have been shown to help with hydrophobic soils. The fourth class is modified methyl capped block co-polymers which modified the chemistry of a block co-polymer wetting agent by adding methyl groups to the structure of the wetting agent molecules. This change in the chemistry changed the way water attaches to the soil particles in an attempt to create a more favorable growing environment with better air movement than with previous wetting agent chemistries and is used to alleviate hydrophobic soils. The fifth class is humic substance redistribution molecules, which help with LDS by altering the soil structure of the top soil and allowing for better water penetration. Lastly, the sixth class of non-ionics are multibranched regenerating wetting agents, which have a higher molecular weight than other surfactants in addition to a molecular structure that is branched, allowing for a more contact between the soil and wetting agent. Lastly, the third chemistry is cationic surfactants that are positively charged and bind to the soil. They have been shown to cause damage to plant tissues and potentially make soils more water repellent after multiple uses and therefore are not currently on the market for use on turfgrass.

Wetting agents have traditionally been used to relieve soil water repellency issues on golf course turf referred to as LDS (Fry and Huang, 2004). Localized dry spot generally occurs when the surface of the soil becomes hydrophobic, which reduces the capacity of water to evenly penetrate the soil surface and therefore cannot reach the roots. In turn, this may result in severe and premature wilting and subsequent dead areas of turf on greens, fairways, or tees. Wetting agents are surfactants that can be applied to

hydrophobic areas to break the surface tension and allow the water to penetrate into the root zone.

In addition to reduction of LDS, wetting agents have also been shown to help create more uniform soil moisture content compared with untreated soils under drought conditions (Kostka, 2000; Soldat et al., 2010). Previous research has also shown that turfgrass areas treated with wetting agents exhibited improved turf quality under drought conditions compared to untreated drought stressed turf (Oostindie et al, 2008, Aamlid et al. 2009; Soldat et al. 2010). A survey conducted by the Golf Course Superintendent Association of America (GCSAA) found that approximately 90% of 18-hole golf courses in the United States are using wetting agents as a form of water conservation. Furthermore, research done on Colorado golf courses found that 85% of courses were using wetting agents as one way to reduce irrigation quantity (Watson and Thilmany, 2011). Maintaining more uniform soil moisture can allow for less irrigation while still maintaining turf quality (EIFG, 2007a). Given the strong pressure on golf courses to reduce irrigation and water use while still maintaining high turf quality, and that a large number of superintendents are already using wetting agents, it is important that research is continued on the effectiveness of wetting agents as a management tool for water conservation.

## Research Objectives

The overall goal of this research was to evaluate different management strategies to help maintain turf quality under reduced irrigation. The specific objectives were as follows:

- (i) Compare the performance of different *Agrostis* species and cultivars under reduced irrigation.
- (ii) Evaluate the use of wetting agents for maintaining turf quality under reduced irrigation.
- (iii) Examine the physiological mechanisms associated with drought resistance and recovery traits of colonial bentgrass (*A. capillaris*).

To meet these objectives, we designed one two-year field study as well as one greenhouse study. In the field study, we evaluated eight bentgrass cultivars consisting of ‘Revere’ and ‘Tiger II’ colonial bentgrasses, ‘Legendary’ and ‘Greenwich’ velvet bentgrasses, and ‘13M’, ‘T-1’, ‘L-93’, and ‘Penncross’ creeping bentgrasses, maintained as fairway turf under reduced irrigation, and also assessed the use of wetting agents as a water conservation tool. Based on results from the field study, we then conducted a greenhouse study to evaluate the performance of five colonial bentgrass cultivars under drought stress and to examine physiological mechanisms associated with drought resistance traits for this species.

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## **CHAPTER 2**

# **EVALUATION OF *AGROSTIS* SPECIES AND CULTIVARS AND WETTING AGENTS TO REDUCE IRRIGATION REQUIREMENTS ON GOLF COURSE FAIRWAYS**

### **Introduction**

Water is necessary for growth and development of turfgrass and therefore imperative in the plant's ability to maintain high turf quality. As many parts of the country and the world are suffering from extended periods of drought, water usage is becoming more restricted. Due to continued population growth particularly in urbanized areas, a greater strain on water supply is expected in the future. In turn, as the demand for water increases, there will be greater water restrictions particularly for low priority usage such as turfgrass. This is a major concern for golf courses as the average 18-hole golf course in the United States has approximately 70% of its land as managed turfgrass (greens, tees, fairways, rough, driving range, and additional grounds), which on average consists of approximately 40 hectares of managed turf per golf course. Of these 40 hectares, approximately half of that land is made up of greens, tees, and fairways, with fairways making up approximately 30% (EIFG, 2007). Most greens, tees, and fairways are irrigated, although these areas may not be irrigated daily or always with supplemental irrigation. Given the potential amount of acreage maintained as turfgrass on a golf course, the high cost of water, and the restrictions of water usage, it is critical that research is implemented to find ways to reduce irrigation of turfgrass systems.



One management practice that can help to maintain the general health and turf quality under reduced irrigation is to select the best adapted species and cultivars. A widely used turfgrass genus on golf courses is *Agrostis*, commonly known as bentgrass. Although there are many different species of bentgrass, colonial bentgrass (*Agrostis capillaris* L.), velvet bentgrass (*Agrostis canina* L.), and creeping bentgrass (*Agrostis stolonifera* L.) are the predominant bentgrass species utilized as low-cut turf typical for golf courses in cool climatic regions. Previous research has shown that there are differences among creeping bentgrasses, colonial bentgrasses, and velvet bentgrasses in turf quality under drought stress (DaCosta and Huang 2006a; DaCosta and Huang, 2007), as well as during recovery from drought (DaCosta and Huang, 2006b; DaCosta and Huang, 2007). In addition to differences among the bentgrasses for drought resistance and recovery, there is also evidence for interspecific differences in other turf performance traits such as wear tolerance (Watkins et al., 2010; Dowgiewics et al., 2011). Watkins et al. (2010) evaluated 17 turfgrass species for low maintenance fairways on native soils in St. Paul, MN and found that colonial and velvet bentgrasses exhibited significantly higher turf quality compared to other species under the least amount of wear (0 passes per week), and with velvet bentgrass maintaining the best turf quality when exposed to the highest wear treatments. Although there was a decline in the turf quality the second year, possibly due to the lack of a fertilizer application as well as the lower levels of precipitation, colonial and velvet bentgrasses maintained better turf quality compared to many other species in the test (Watkins et al., 2010). In addition to interspecific differences among bentgrass species in turf performance under reduced irrigation, intraspecific differences in performance under drought conditions have also been

observed, although most studies have been confined to creeping bentgrass (Jordan et al., 2003; McCann and Huang, 2008).

As turf managers reduce irrigation on fairways there may be potential for increased incidence of localized dry spot (LDS), which could subsequently lead to difficulty in rewetting of the soil. Therefore, in addition to proper species and cultivar selection, another management strategy to maintain higher TQ under reduced irrigation is application of surfactants, or wetting agents. Wetting agents have been utilized to alleviate LDS in sand-based soils of creeping bentgrass (Kostka, 2000) and kikuyugrass (*Pennisetum clandestinum*) (Barton and Colmer, 2011).

In addition to aiding in reduction of LDS, wetting agents have also been shown to create more uniform moisture content within the soil compared with untreated soils under reduced irrigation conditions (Kostka, 2000; Soldat et al., 2010). Previous research has also shown that turfgrass areas treated with wetting agents exhibited improved turf quality under drought conditions compared to untreated drought stressed turf (Oostindie et al, 2008, Aamlid et al. 2009; Soldat et al. 2010). Wetting agents have also been reported to reduce water repellency in sand-based greens composed of creeping bentgrass (Aamlid et al., 2009; Soldat et al., 2010) and of bermudagrasses (*Cynodon* spp.) (Cisar et al., 2000). Oostindie et al. (2008), reported wetting agents help to alleviate LDS and prevent soil water repellency on sand-based fairways consisting of a mix of *Festuca* spp. and annual bluegrass (*Poa annua*). Increased turf quality has been linked to reduced soil water repellency compared to that of hydrophobic soils (Kostka 2000; Oostindie et al., 2008; Aamlid et al., 2009; Soldat et al., 2010). During prolonged drought events, soils will become dry and turf will show signs of drought stress resulting in lower turf quality.

If soils are allowed to dry down to the point of becoming hydrophobic, even after intense re-watering of the soils, it is common to have reduced turf quality due to the inability of the soil to re-wet (Oostindie et al., 2008; Aamild et al., 2009). Previous research has shown that the application of wetting agents can alleviate LDS and also help re-wet hydrophobic soils (Oostindie et al., 2008, Aamlid et al. 2009; Soldat et al. 2010). Localized dry spot may be found in all types of soils but is most frequent in sand-based soils, such as found on modified greens and tees. As a result, a majority of wetting agent research has been conducted on sandy soils (Kostka 2000). Fairways are rarely sand-based but do make up large areas of the irrigated turfgrass on golf courses and are susceptible to hydrophobic soil conditions.

With previous research showing that wetting agents have the ability to aid in increasing turf quality by alleviating LDS, reducing soil water repellency, and creating a more uniform wetting front in soils, and given that bentgrasses are so widely used on golf courses and previous research has shown differences among the species of bentgrasses and the cultivars of creeping bentgrass, it is important to continue research on wetting agents as well as investigate the potential of wetting agents to aid in the reduction of irrigation requirements of turfgrass and to investigate the potential for differences between multiple cultivars of different species of bentgrasses. Therefore the objectives of the study were to (i) compare turf performance of different species and cultivars of *Agrostis* under drought stress, and (ii) compare the effects of a wetting agent and its ability to improve turf quality of these species and cultivars under reduced irrigation.

## **Materials and Methods**

### **Plant Material and Growing Conditions**

Three species of bentgrass were used in this study, including colonial bentgrass, velvet bentgrass, and creeping bentgrass. The cultivars consisted of 'Revere' and 'Tiger II' colonial bentgrasses, 'Legendary' and 'Greenwich' velvet bentgrasses, and '13M', 'T-1', 'L-93', and 'Penncross' creeping bentgrasses. Cultivars were selected to represent standard and improved varieties for each species. Plants were seeded in October 2008 at  $19 \text{ g m}^{-2}$  into plots measuring  $1.3 \text{ m}^2$  onto a Hadley silt loam (coarse-silty, mixed, superactive, nonacid, mesic Typic Udifluvents) at the Joseph Troll Turf Research Center in South Deerfield, MA. The turf was mowed three times per week to a height of 0.95 cm with clippings removed. Fertilizer applications were made in May, June, September, and November in 2011 and 2012 to provide a total of  $113 \text{ kg ha}^{-1}$  of nitrogen for the growing season. Pesticides were applied preventatively to primarily control dollar spot (*Sclerotinia homoeocarpa*, F.T. Bennet), brown patch (*Rhizoctonia solani*, Kuhn), and black cutworm (*Agrotis ipsilon* (Hufnagel)).

The experiment was conducted from June through August in 2011 and 2012 under a mobile rainout shelter measuring 10.7 by 20.1 m. The rainout shelter was connected to a data logger (CR1000, Campbell Scientific, Logan, UT) and leaf wetness sensor (Model 257, Campbell Scientific, Logan, UT) to regulate the movement of the rainout shelter in response to precipitation. Consequently, all rainfall was excluded from plots to facilitate control of the irrigation treatments.

## Treatments

The experiment consisted of a split plot design, with irrigation as the main plot and cultivar as the sub-plot. There were four replicates of each irrigation and cultivar combination, for a total of 96 plots. The irrigation treatments consisted of (i) a well-watered (WW) control irrigated to maintain soil at field capacity (approximately 33 to 35% soil moisture content, v/v) and received no wetting agent treatment, (ii) a drought (DRT) treatment that received no irrigation and no wetting agent, and (iii) a drought treatment that received no irrigation plus a wetting agent (DRT+WA). An experimental wetting agent (Aquatrols Corp., Paulsboro, NJ) was mixed with tap water at a concentration of 2.175 mL L<sup>-1</sup> and was applied at a rate of 1.75 L ha<sup>-1</sup> using a CO<sub>2</sub> backpack sprayer. The wetting agent was applied twice prior to the start of the experiment, 23 June and 5 July in 2011, and 15 June and 5 July 2012. Following each application, the wetting agent was watered into the turf canopy and soil using approximately 0.64 cm of irrigation. Following the second wetting agent application, water was withheld from both the DRT and DRT+WA plots for the remainder of the experiment. Well-watered plots were individually irrigated using hand-held hose with a fan-spray nozzle, and water quantity was monitored using a digital flow meter attachment. Irrigation amounts were replaced on WW plots two to three times per week based on the evapotranspiration estimates from an onsite weather station. Additional climatic data during the experiment were also recorded, including daily temperature and rainfall (Fig. 2.1).

## Measurements

Volumetric soil moisture content was measured either weekly or bi-weekly during the study using time domain reflectometry (Trase System I, Soilmoisture Equipment Corp., Santa Barbara, CA). Probes that measured 15 cm in length were vertically placed into the soil profile at two random locations in each plot, which were then averaged together to attain one volumetric soil moisture reading per plot.

Turf quality (TQ) was measured bi-weekly using a visual rating scale based on overall color, density, and uniformity. The ratings were based on a scale from 1 to 9, with 9 being best TQ consisting of a green canopy and no wilting, 6 being acceptable with some browning, and 1 being completely brown and desiccated turf.

In addition to visual estimation of turf quality, canopy reflectance indices were measured using a Multispectral Radiometer (Cropscan, Inc. Rochester, MN) to provide additional detection of canopy color and density changes prior to visual declines in these parameters (Johnsen et al., 2009; Jiang, 2008). Measurements were taken biweekly on clear sunny days between 1100 h and 1300 h. Normalized difference vegetation index (NDVI), which is used to measure green leaf biomass or color, and leaf area index (LAI), which is used to measure turf canopy density, were calculated using near infrared, 940 nm, and red, 660 nm wavebands. The NDVI was calculated as  $((940-660)/(940+660))$ , and LAI was calculated as  $(940/660)$ .

Thatch measurements were taken twice, once at the end of each season. Cores were mechanically removed with an aerator using 0.95 cm diameter tines. Cores were collected and combined from all blocks and placed into separate paper bags based on irrigation treatment (WW and DRT) and on cultivar, giving us a total of 16 bags. All

green tissue, roots, and soil were removed from the thatch using scissors and put into an oven at 70°C for 24h. The dried thatch samples were then weighed and then put into a muffle furnace at 500°C overnight. The ashes of the thatch samples were weighed again and organic content was calculated as (dry weight – ashed weight). In 2012, thatch levels were compared between WW and DRT plots and no difference in thatch accumulation based on the irrigation treatment was detected (data not shown).

### **Experimental Design and Statistical Analysis**

The experiment was set up as a split plot design. Results were determined by analysis of variance using SAS version 9.2 (SAS Institute Inc. Cary, NC). Means were separated by the Fisher's least significant difference at the 0.05 probability level. The main effect of irrigation and cultivar as well as the interaction of the main effects were as single degree of freedom orthogonal contrasts.

## **Results**

### **Soil Moisture**

For both years of the study, soil moisture content was significantly affected by the irrigation main effect. In contrast, there were no significant cultivar or irrigation × cultivar effects on soil moisture in either year. In 2011, differences in soil moisture content between irrigation treatments were detected by 26 Jul (21 d of treatment), when the WW plots exhibited significantly higher soil moisture content compared to both the DRT and DRT+WA treatments (Fig. 2.2). These differences remained consistent

throughout the remainder of the experiment, with WW plots maintaining approximately 32.8% soil moisture, and DRT and DRT+WA plots maintaining approximately 29.8% soil moisture content. There were no statistical differences in soil moisture content in between DRT and DRT+WA treatments. Similar results were also observed for in 2012, where differences in soil moisture content between irrigation treatments were detected by 13 Jul (8 days of treatment), when WW plots exhibited significantly higher soil moisture content than the DRT and the DRT+WA treatments and remained higher throughout the study (Fig.2.2). As in 2011, there were no statistical differences in soil moisture content between the DRT and the DRT+WA treatments.

In 2011, based on single degree of freedom contrasts, there were some differences in soil moisture content detected among the cultivars. Beginning on 23 Aug (49 d of treatment) and continuing throughout the study, T-1 had overall higher soil moisture content than 13M. With the exception of 26 Jul (21 d of treatment) and 23 Aug (49 d of treatment), one or both colonial bentgrass cultivars (Tiger II and Revere) exhibited significantly higher soil moisture than Legendary velvet bentgrass. In 2012, there were no statistical differences in soil moisture content among cultivars.

### **Turf Quality**

There were significant irrigation and cultivar main effects detected for TQ in both 2011 and 2012, and only one date with significant irrigation  $\times$  cultivar interaction in either year. In 2011, WW plots exhibited significantly higher TQ compared to both the DRT and DRT+WA treatments by 2 Aug (28 days of treatment), and remained higher



throughout the study (Fig. 2.3). There were no statistical differences in TQ between the DRT and the DRT+WA treatments. Similar to 2011, WW plots exhibited significantly higher TQ compared to DRT and DRT+WA beginning 24 Jul (19 days of treatment) 2012, with no differences in TQ between the DRT and DRT+WA treatments (Fig. 2.3).

In addition to main effects for irrigation, a significant cultivar main effect was observed in 2011. By 2 Aug (28 days of treatment) and throughout the remainder of the study, TQ of Legendary velvet bentgrass was significantly lower compared to all other cultivars, with the TQ level falling below 6 (Fig. 2.4). In addition, TQ of Greenwich velvet bentgrass also declined throughout the study, but was generally not significantly different than Penncross creeping bentgrass. T-1 exhibited significantly higher in quality compared to creeping bentgrass cultivars L-93 and Penncross, while not statistically different from Tiger II and Revere colonial bentgrasses. In 2012, the colonial bentgrass cultivars were among the best in TQ by 24 July (19 days of treatment), with Tiger II having significantly better TQ than all other cultivars and equal to that of Revere (Fig. 2.4). By 9 Aug (35 days of treatment), colonial bentgrass cultivars had significantly higher TQ than all other cultivars throughout the remainder of the study. There were no significant differences in TQ among creeping and velvet bentgrasses observed throughout most of the season in 2012; however, similar to results of 2011, Legendary velvet bentgrass generally exhibited the lowest TQ among cultivars. Furthermore, other than Legendary velvet bentgrass, the average TQ of cultivars did not fall below 6 in either year.

Although only irrigation  $\times$  cultivar interaction detected was 18 Aug (44 days of treatment) in 2011, contrast interactions for both years of the study did detect differences

among the colonial and velvet bentgrasses when comparing WW and DRT plots. In 2011, beginning on 18 Aug (44 days of treatment) and continuing for the remainder of the study, Legendary under DRT treatment had lower TQ than all other colonial bentgrasses and Greenwich regardless of WW or DRT treatment. In 2012 starting 24 Jul (19 days of treatment) and continuing through 9 Aug (35 days of treatment) both Legendary and Greenwich under DRT were statistically lower than these same cultivars under WW conditions, and all colonial bentgrasses under both WW and DRT treatment. Beginning on 15 Aug (41 days of treatment) and continuing for the rest of the study, Legendary under DRT treatment was statistically lower than all colonial and velvet bentgrasses under WW and DRT with the exception of Greenwich under DRT treatment.

### **Leaf Area Index**

There were significant irrigation and cultivar main effects detected for leaf area index (LAI) in both 2011 and 2012, and no significant irrigation×cultivar interaction in either year. In 2011, WW treatments exhibited significantly higher LAI compared to both the DRT and DRT+WA treatments by 28 Jul (23 days of treatment) with no significant differences between DRT and DRT+WA treatments (Fig. 2.5). Although a similar trend was observed in 2012, statistical differences in LAI between WW and DRT and DRT+WA were only observed on 21 Aug (47 days of treatment) (Fig. 2.5).

Significant differences in LAI among cultivars were detected in both years of the study, regardless of irrigation treatment. In 2011, beginning on 28 Jul (23 days of treatment) and through 19 Aug (45 days of treatment), Tiger II and Revere colonial

bentgrasses exhibited significantly lower LAI compared to all other cultivars (Fig. 2.6). Creeping bentgrass cultivars generally exhibited the highest LAI, particularly T-1, with velvet bentgrass cultivars intermediate in LAI between creeping bentgrass and colonial bentgrass cultivars. Similar results were observed in 2012. For example, beginning on 12 Jul 2012 (7 days of treatment) and continuing throughout the study, both colonial bentgrass cultivars exhibited significantly lower LAI than all other cultivars (Fig. 2.6). Starting on 12 Jul (7 days of treatment) and throughout the study T-1, 13M, and Penncross creeping bentgrasses were among the highest in LAI, but not significantly different from one another. By 15 Aug (41 days of treatment) and throughout the remainder of the study T-1 was significantly higher than all other cultivars except for Penncross and 13M.

### **Normalized Difference Vegetation Index**

There were significant irrigation and cultivar main effects detected for normalized difference vegetation index (NDVI) in both 2011 and 2012, whereas no significant irrigation x cultivar interactions was observed. In 2011, WW plots exhibited significantly higher NDVI compared to both the DRT and the DRT+WA treatments by 12 Aug (38 d of treatment) (Fig. 2.7). There were no statistical differences in NDVI between the DRT and the DRT+WA treatments. Similarly in 2012, WW plots exhibited significantly higher NDVI than both the DRT and the DRT+WA plots (Fig. 2.7).

Beginning on 28 Jul 2011 (23 days of treatment) and continuing throughout the study, Greenwich velvet bentgrass had significantly higher NDVI compared to all other

cultivars, except on 19 Aug (45 days of treatment) when NDVI for this cultivar was not significantly different from that of Legendary velvet bentgrass (Fig. 2.8). In contrast, creeping bentgrass cultivars, particularly Penncross, exhibited the lowest NDVI. Similar results were observed in 2012, although greater separation of cultivars was detected. In general, velvet bentgrass cultivars exhibited the highest NDVI, with colonial bentgrass cultivars intermediate, and creeping bentgrass cultivars with the lowest NDVI (Fig. 2.8). For example, by 12 Aug (38 days of treatment) and continuing until 19 Aug (45 days of treatment), Penncross creeping bentgrass had lower NDVI compared to all other cultivars.

### **Thatch Accumulation**

The cultivars exhibited differences in thatch accumulation, which was consistent across both years of the study. Greenwich and Legendary velvet bentgrass cultivars accumulated significantly higher levels of thatch compared to all other cultivars (Fig. 2.9). The velvet bentgrasses averaged a dry weight of 2.5g in 2011 and 2.7g in 2012, whereas the other colonial and creeping bentgrasses collectively averaged approximately 1.8g in both years. In 2011, 13M creeping bentgrass had a higher thatch accumulation compared to other creeping bentgrass cultivars, but in 2012 there were no differences in thatch accumulation among the creeping bentgrass cultivars. In general, colonial bentgrass cultivars exhibited similar thatch accumulation to creeping bentgrass cultivars.

## Discussion

In both years of the study, withholding irrigation in the DRT and DRT+WA treatments resulted in a significant decline in soil moisture content compared to WW plots. As a result, there were also a decline in visual TQ, canopy color (NDVI), and density (LAI) for plots exposed to DRT and DRT+WA. The application of a wetting agent prior to withholding irrigation did not result in higher soil moisture content or TQ for the turfgrasses evaluated in our study. This is contrary to previous results reported on 'A-4' creeping bentgrass green, where applications of surfactants increased the TQ and soil water content as compared to untreated soils (Aamlid et al., 2009). Another study conducted on fairways consisting of mixed fescue species (*Festuca* spp.) and annual bluegrass (*Poa annua* L.) found that soil surfactants increased volumetric water content of the soil as well as TQ (Oostindie et al., 2008). One potential reason for the lack of differences between the DRT and DRT+WA treatments may be attributed to the soil type in our study, which consisted of a silt loam that showed no hydrophobic characteristics prior to the study. Much of the previous research that had shown that turfgrass areas treated with wetting agents exhibited improved TQ under drought conditions compared to untreated drought stressed turf were conducted primarily on soils exhibiting hydrophobic characteristics (Oostindie et al, 2008, Aamlid et al. 2009; Soldat et al. 2010). Our results are similar to that reported by Mobbs et al. (2012), where they observed no significant effects of four different soil surfactants on water holding capacity of two soil types, including a silt loam that was not hydrophobic (Mobbs et al., 2012). Although the DRT and DRT+WA plots received no water for over 50 days, the volumetric soil moisture content was maintained at approximately 27 to 29%. Although declines in TQ were

observed under reduced irrigation, TQ levels did not decline below 6, other than for Legendary velvet bentgrass. Given that the WW plots were maintained at approximately 33% soil moisture, it is possible that the soil moisture levels of our DRT treatment were not allowed to drop low enough to cause significant drought stress levels.

In addition to conducting the study on a generally hydrophilic soil, another potential reason for lack of significant wetting agent effect on turf performance under reduced irrigation may be attributed to pre-applications of the wetting agent rather than applying the product throughout the experiment. In other studies evaluating the effects of wetting agents on turfgrass, applications were made throughout the season on a three to four week basis (Kostka 2000, Oostindie et al. 2008, Soldat et al. 2010). Because wetting agents must be watered in and considering the amount of time it takes for the soil on our experimental site to dry down, applications after the incitation of drought treatments were avoided. It is possible that multiple applications throughout the study could have improved the capacity of the wetting agent to improve TQ under reduced irrigation, but this requires further investigation.

When comparing the responses of bentgrass cultivars to reduced irrigation, we found that velvet bentgrasses ranked among the lowest in TQ. This low level of TQ could be attributed to the excessive thatch accumulation of the velvet bentgrasses in this study. The current study was conducted on a three-year old stand of established grasses that had not been intensively managed for organic matter accumulation. This lack of cultivation is consistent with our study's objective to research low maintenance fairways but allowed for significant thatch accumulation, specifically higher levels in the velvet bentgrasses. During preliminary TQ measurements in the years prior to the study, velvet

bentgrass ranked among the highest (data not shown). Also, throughout the establishment of the plots as well as during Spring and Fall seasons of the study, the plots were fertilized with high levels of N. Velvet bentgrasses have shown to establish well under high levels of N but if these levels are maintained, high levels of thatch are accumulated (Espevig et al., 2012). Higher thatch levels result in a greater portion of a plant's roots in the thatch layer as oppose to in the soil. This section of rooting would then be more susceptible to faster dry down and be more susceptible to injury during mowing which can all lead to a decline in TQ.

In contrast to performance of velvet bentgrass, Revere and Tiger II colonial bentgrass cultivars ranked among the highest in TQ in our study. Colonial bentgrasses have been suggested for use on fairways and tees because of their preferred mowing height of 1.0 to 2.5 cm (Casler and Duncan, 2003). They have also been suggested for their use in low maintenance fairways, more so due to rapid recovery from drought stress rather than maintenance of TQ under drought stress (DaCosta et al., 2006; Watkins et al., 2010). The silt loam soil used in this study provided a very slow dry down, only allowing the non-irrigated treatments to reach a soil moisture level of 27 to 29%. This soil moisture content may not have been sufficiently low to induce drought dormancy typically seen in colonial bentgrasses, therefore allowing the colonial bentgrass cultivars to maintain high levels of TQ. We also observed that among the creeping bentgrass cultivars, T-1 generally exhibited higher TQ compared to other cultivars, particularly compared to Penncross creeping bentgrass

Based on results from previously published studies, we expected for both NDVI and LAI to estimates from canopy reflectance measurements to be associated with visual

TQ evaluations (Johnsen et al., 2009; Jiang, 2008). However, colonial bentgrass cultivars ranked among the lowest for LAI (canopy density) while still maintaining high TQ. In addition, Legendary velvet bentgrass ranked among the highest for NDVI (canopy color) but lowest in TQ. The lack of close association between TQ and canopy reflectance indices could be due to variation between species in genetic color, density, and leaf texture. Differences among species and cultivars have been shown to result in different reflectance in both red and NIR, therefore affecting the results of NDVI and LAI (Bremer et al. 2011a, b). For example, colonial bentgrasses ranked among the lowest for LAI but exhibited high TQ. Furthermore, Legendary velvet bentgrass ranked among the highest for NDVI but lowest in TQ.

Among the cultivars evaluated in the study, we found that colonial bentgrass cultivars exhibited good TQ under reduced irrigation either similar to better to that of improved creeping bentgrass cultivars. Although other studies have shown velvet bentgrasses to have high levels of drought tolerance, the high level of thatch accumulation for Greenwich and Legendary velvet bentgrasses resulted in significantly reduced TQ. When comparing the creeping bentgrasses, recently improved cultivar, T-1, showed higher TQ under drought stress as compared with older cultivars such as Penncross. Lastly, the use of a wetting agent did not result in any improvement of TQ under reduced irrigation, potentially due to the lack of hydrophobic soil in our study.



Figure 2.1. Daily average temperature (C°) (upper graph) and rainfall (mm) (lower graph) for 2011 and 2012.

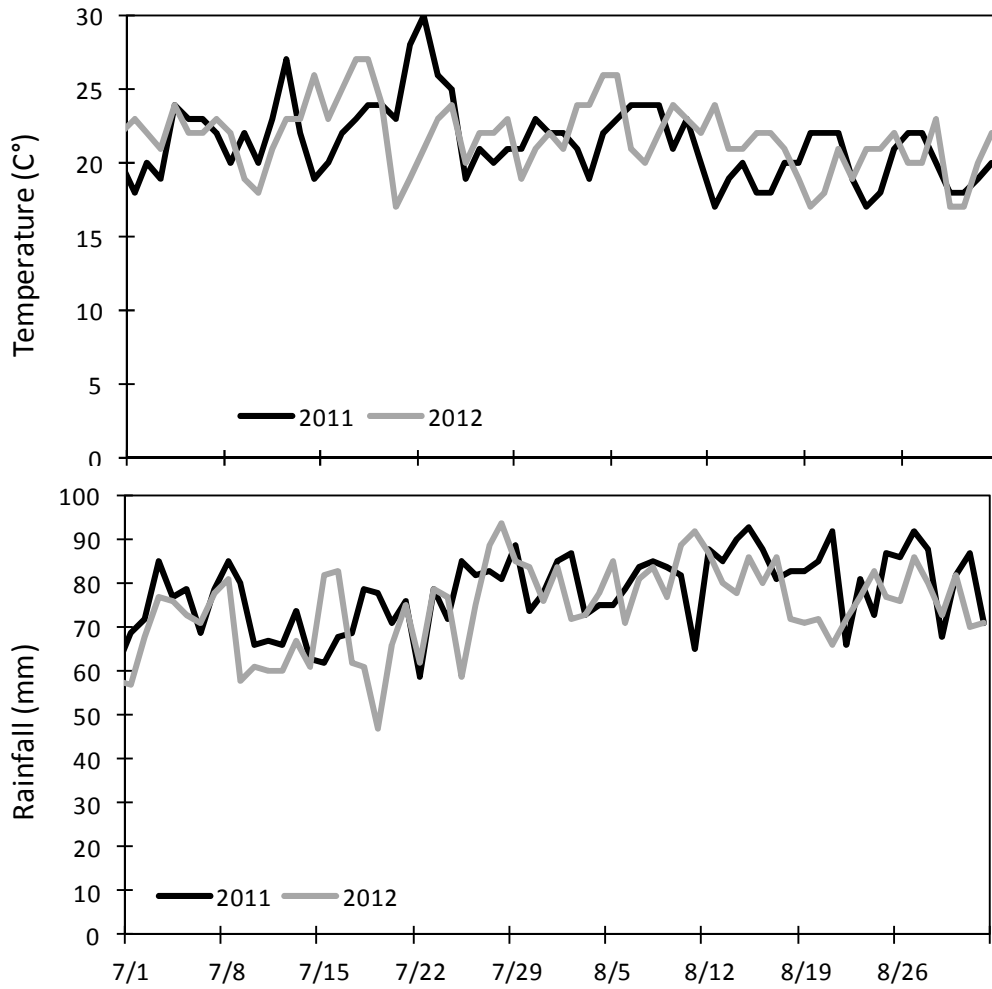


Figure 2.2. Volumetric soil moisture content of plots exposed to well-watered (WW), drought (DRT), and drought with wetting agent (DRT+WA) conditions in 2011 (upper graph) and 2012 (lower graph). Data were averaged over the eight cultivars. Vertical bars represent LSD values at the  $P \leq 0.05$  level for treatment comparisons at each day of measurement.

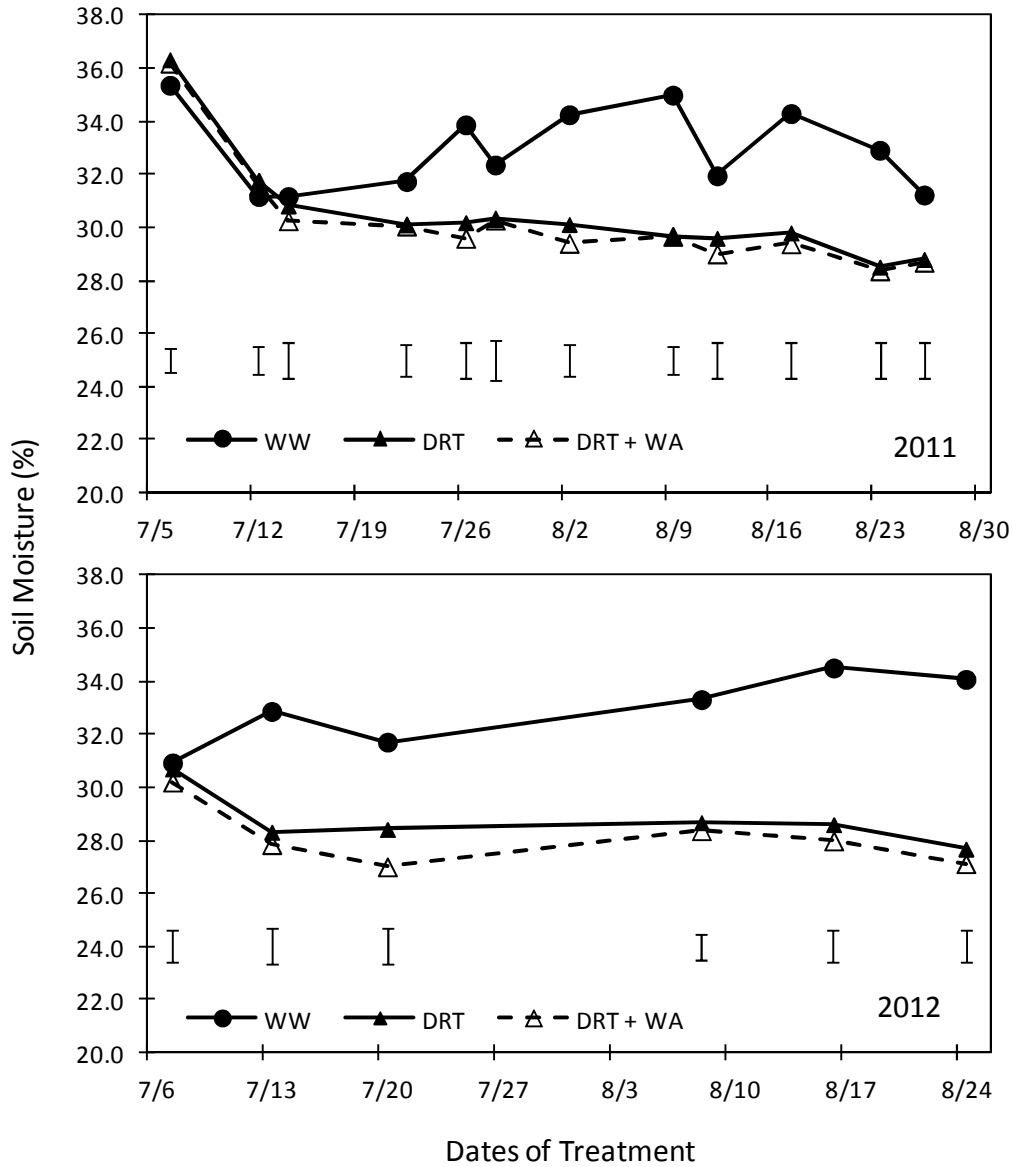


Figure 2.3. Turf quality (TQ) rating of plots exposed to well-watered (WW), drought (DRT), and drought with wetting agent (DRT+WA) conditions in 2011 (upper graph) and 2012 (lower graph). Data were averaged over the eight cultivars. Turf quality was rated on a 1 to 9 scale, with 9 representing the best TQ comprised of green color and no wilting, 6 being acceptable TQ but with some browning, 1 being completely brown and desiccated turf. Vertical bars represent LSD values at the  $P \leq 0.05$  level for treatment comparisons at each day of measurement.

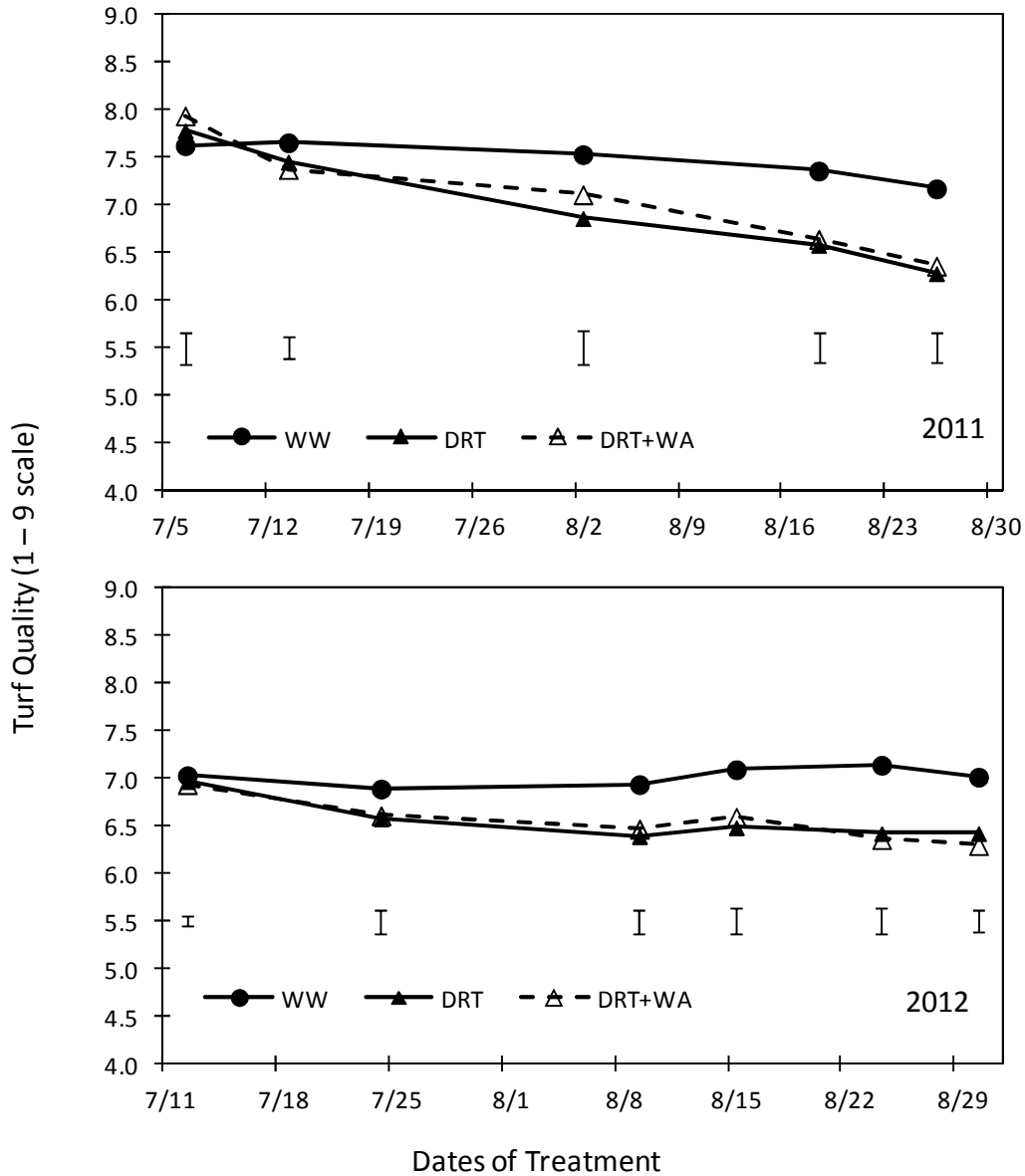


Figure 2.4. Turf quality (TQ) rating of eight bentgrass cultivars in 2011 (upper graph) and 2012 (lower graph), including Penncross, L-93, 13M, and T-1 creeping bentgrasses, Tiger II and Revere colonial bentgrasses, and Legendary and Greenwich velvet bentgrasses. Data were averaged over three irrigation treatments. Turf quality was rated on a 1 to 9 scale, with 9 representing the best TQ comprised of green color and no wilting, 6 being acceptable TQ but with some browning, 1 being completely brown and desiccated turf. Vertical bars represent LSD values at the  $P \leq 0.05$  level for treatment comparisons at each day of measurement.

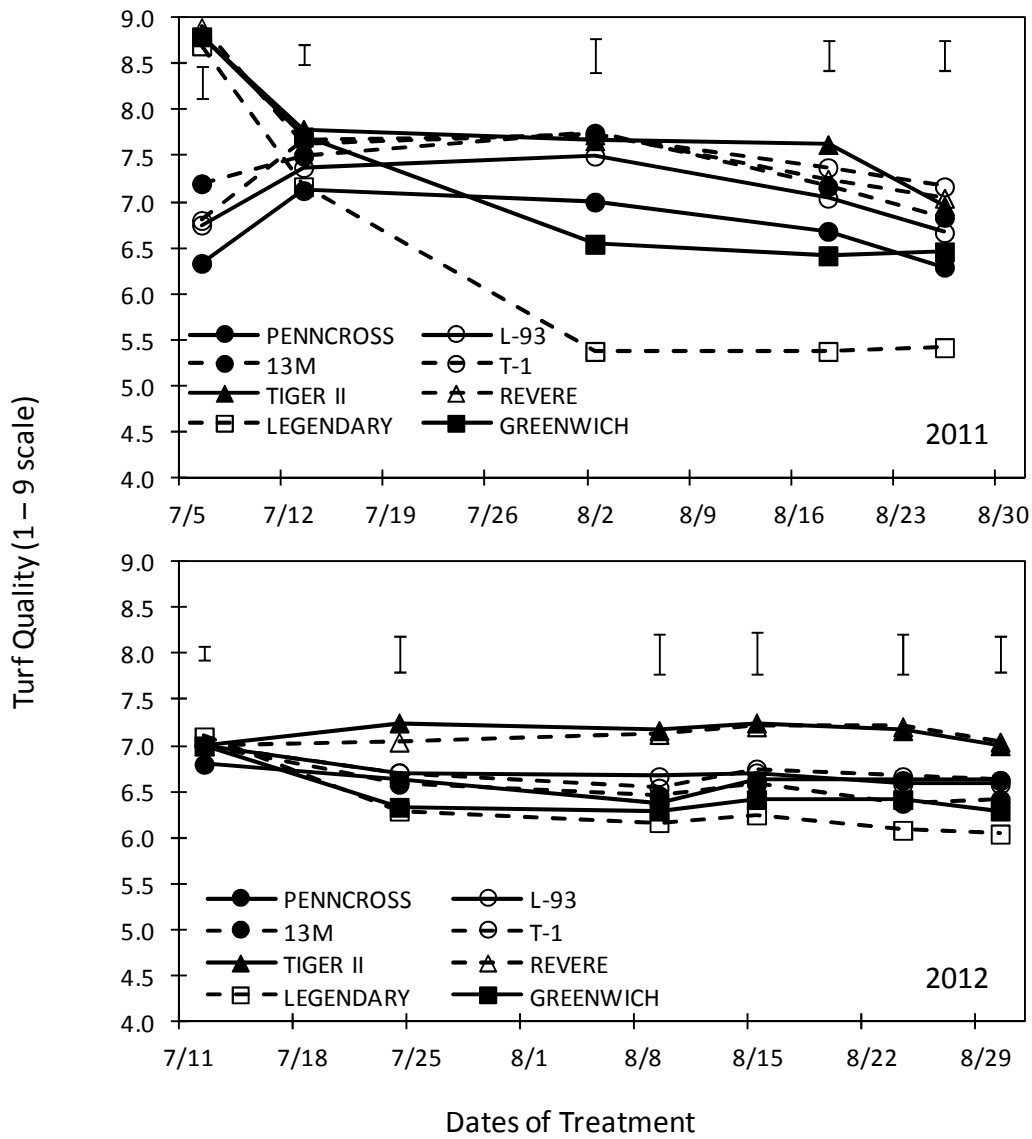


Figure 2.5. Changes in leaf area index (LAI), used as a measure of canopy density, of plots exposed to well-watered (WW), drought (DRT), and drought with wetting agent (DRT+WA) conditions in 2011 (upper graph) and 2012 (lower graph). Data were averaged over the eight cultivars. The LAI was calculated based on the spectral reflectance ratio of infrared, 940 nm, and red, 660 nm wavebands. Vertical bars represent LSD values at the  $P \leq 0.05$  level for treatment comparisons at each day of measurement.

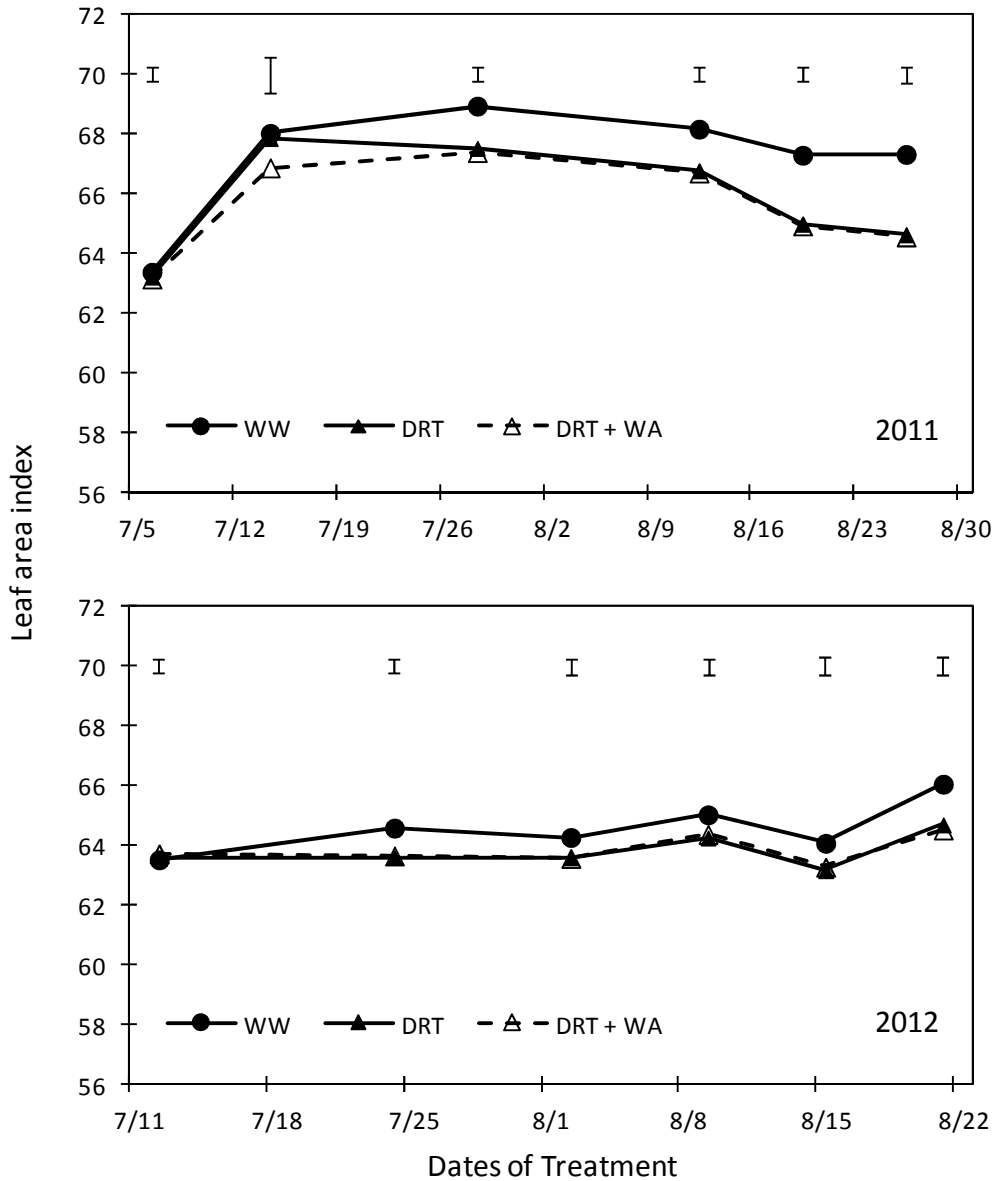


Figure 2.6. Changes in leaf area index (LAI) of eight bentgrass cultivars in 2011 (upper graph) and 2012 (lower graph), including Pennncross, L-93, 13M, and T-1 creeping bentgrasses, Tiger II and Revere colonial bentgrasses, and Legendary and Greenwich velvet bentgrasses. Data were averaged over three irrigation treatments. The LAI was calculated based on the spectral reflectance ratio of infrared, 940 nm, and red, 660 nm wavebands. Vertical bars represent LSD values at the  $P \leq 0.05$  level for treatment comparisons at each day of measurement.

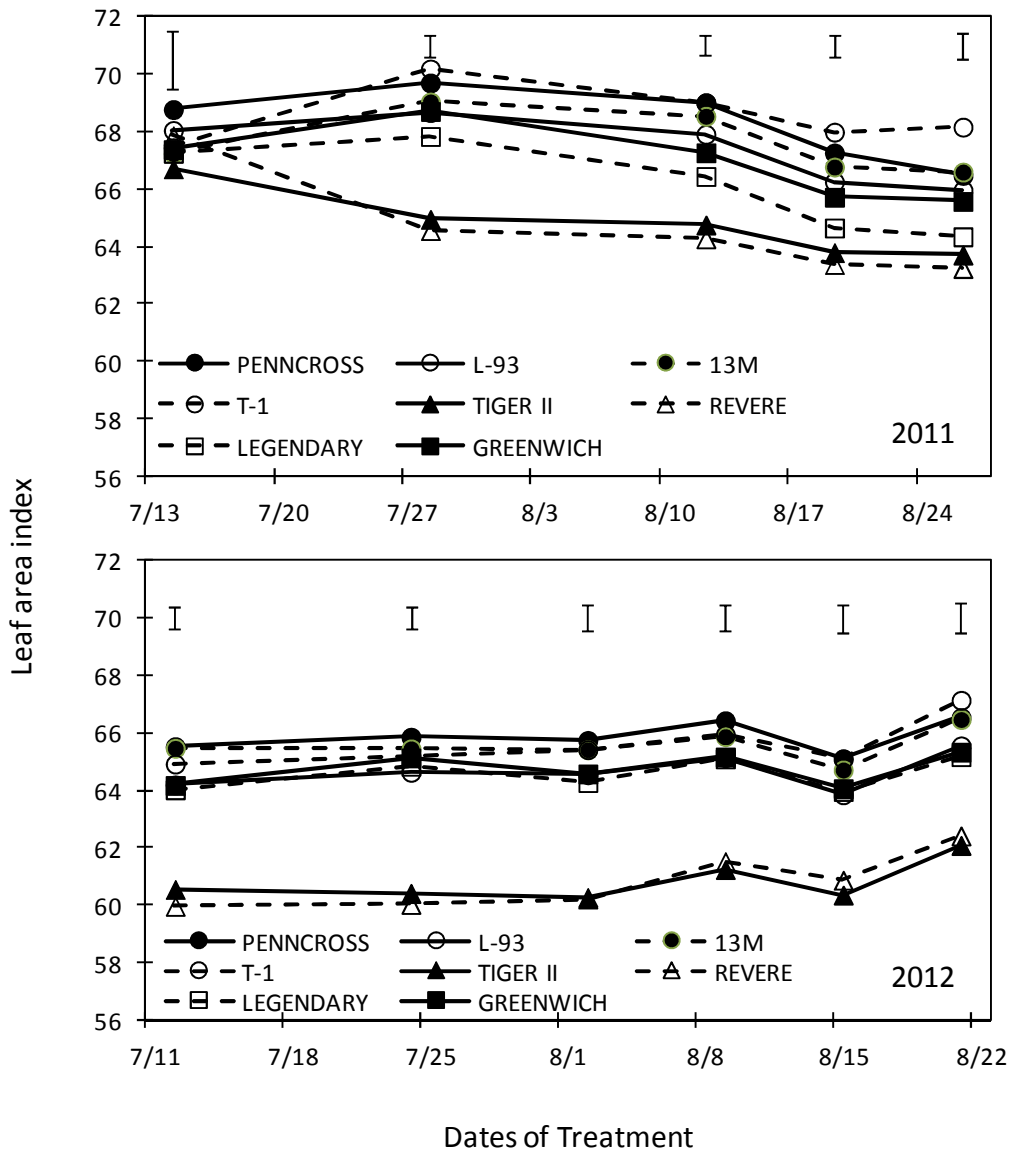


Figure 2.7. Changes in normalized difference vegetation index (NDVI), used as a measure of canopy color, of plots exposed to well-watered (WW), drought (DRT), and drought with wetting agent (DRT+WA) conditions in 2011 (upper graph) and 2012 (lower graph). Data were averaged over the eight cultivars. The NDVI was calculated based on the spectral reflectance of near infrared, 940 nm, and red, 660 nm wavebands. Vertical bars represent LSD values at the  $P \leq 0.05$  level for treatment comparisons at each day of measurement.

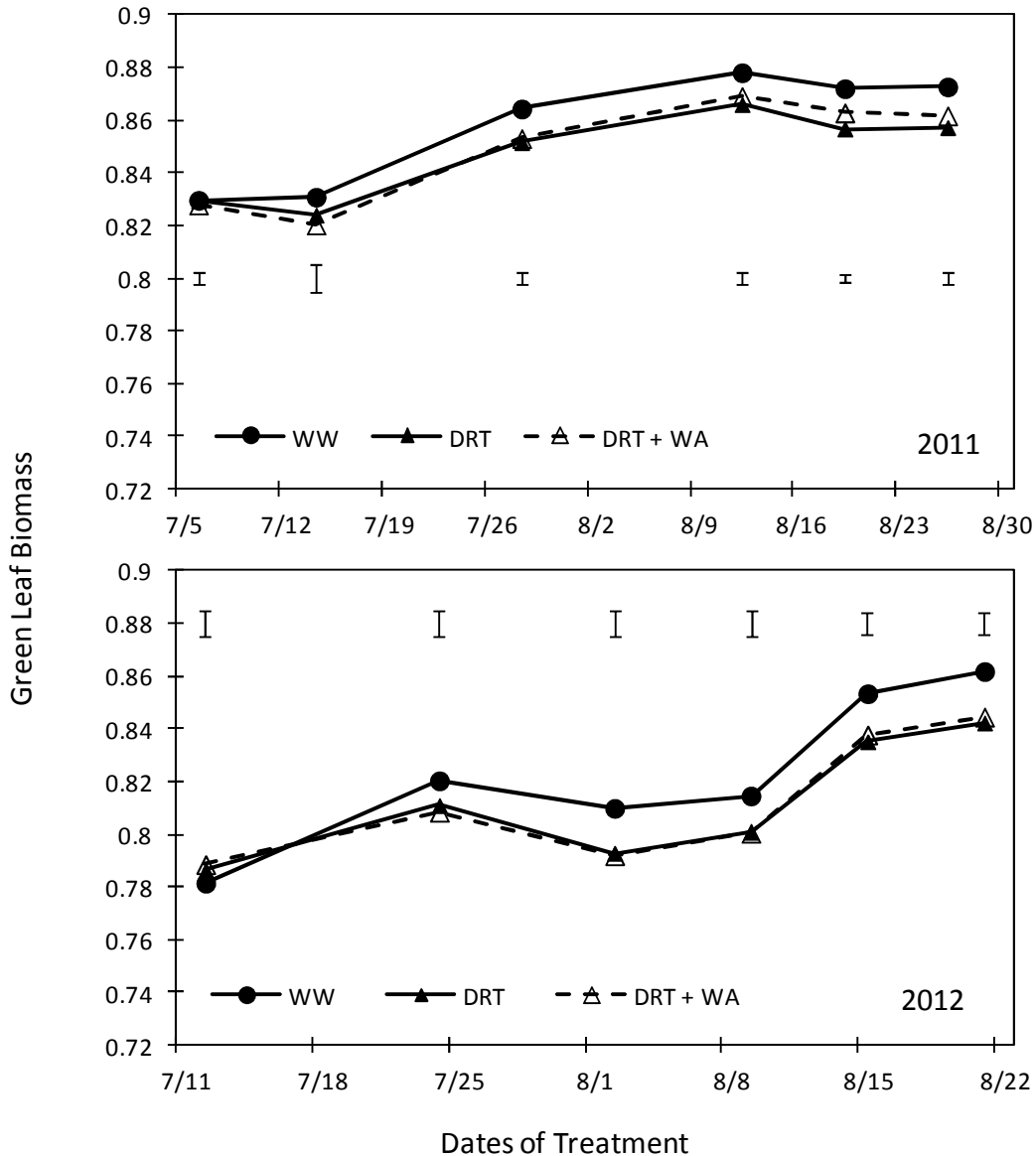


Figure 2.8. Changes in normalized difference vegetation index (NDVI), used as a measure of canopy color, of eight bentgrass cultivars in 2011 (upper graph) and 2012 (lower graph), including Pennncross, L-93, 13M, and T-1 creeping bentgrasses, Tiger II and Revere colonial bentgrasses, and Legendary and Greenwich velvet bentgrasses. Data were averaged over three irrigation treatments. The NDVI was calculated based on the spectral reflectance of near infrared, 940 nm, and red, 660 nm wavebands. Vertical bars represent LSD values at the  $P \leq 0.05$  level for treatment comparisons at each day of measurement.

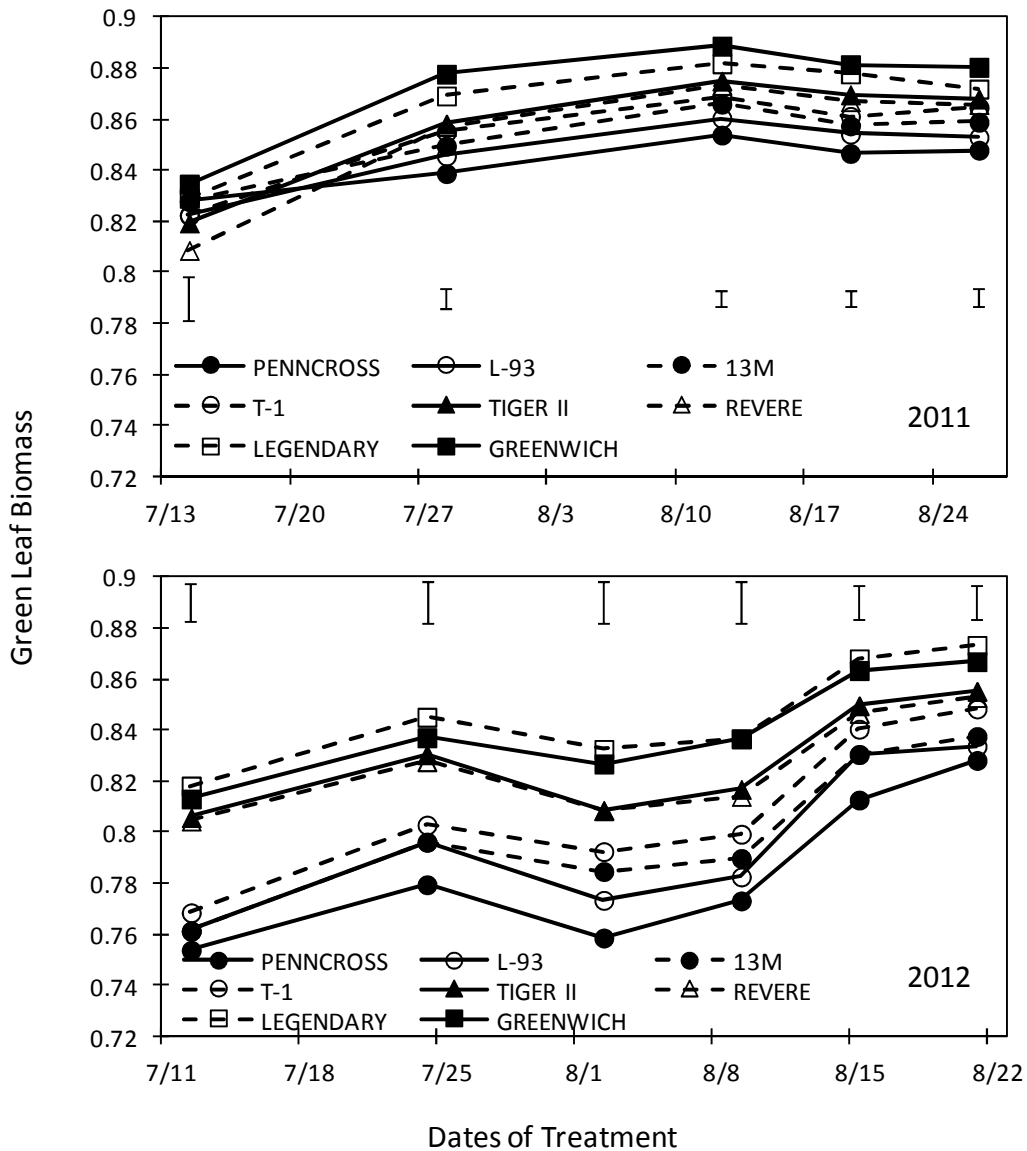
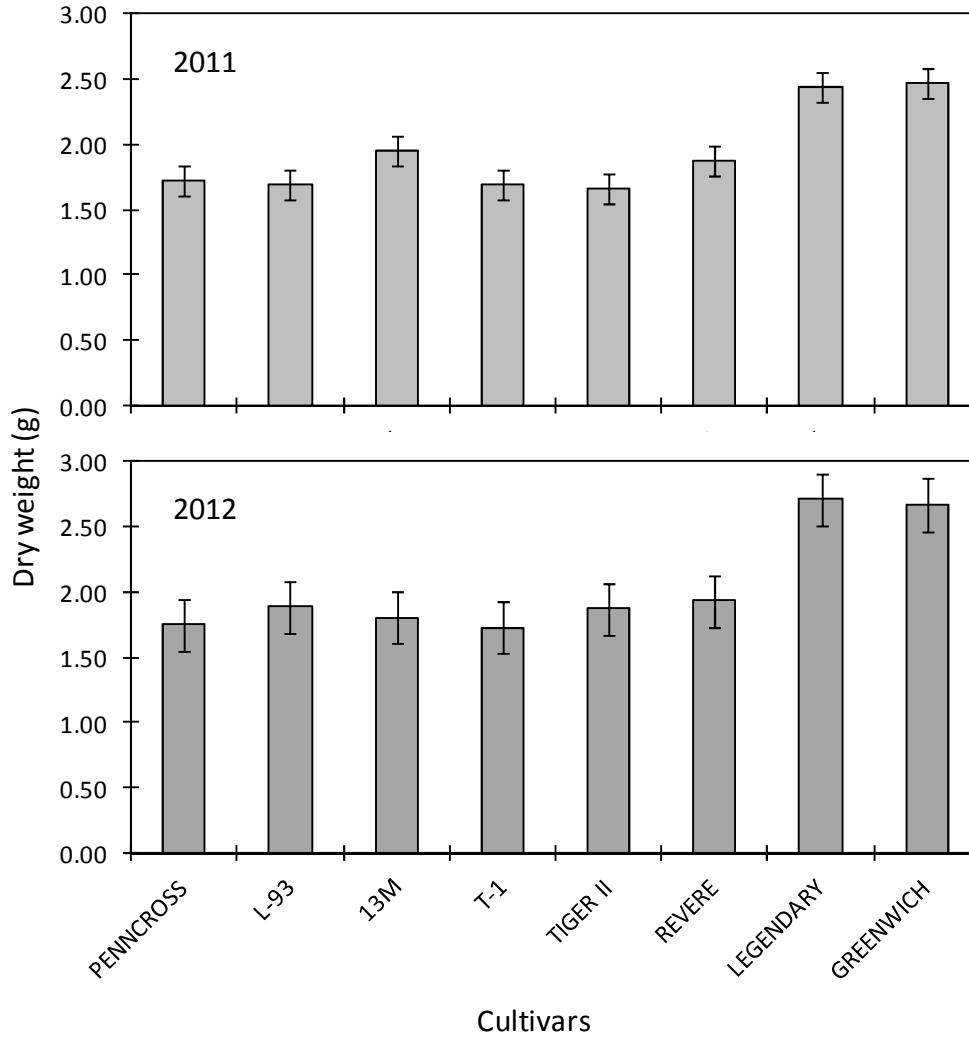




Figure 2.9. Thatch dry weight for the cultivar main effect for both 2011 and 2012. Bars represent LSD at the  $P \leq 0.05$  level for each day of measurement.



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## CHAPTER 3

# EVALUATION OF DROUGHT RESISTANCE AND RECOVERY POTENTIAL AMONG COLONIAL BENTGRASS CULTIVARS

### Introduction

As many parts of the country and the world are suffering from extended periods of drought, water usage is becoming more restricted (Hollister, 2010). Due to continued population growth, particularly in urbanized areas, a greater strain on water supply is expected in the future. As the demand for water increases, there will be greater water restrictions particularly for low priority usage such as turfgrass. As water usage becomes more restricted, it is important that research is done on turf grasses that have potential for good drought resistance as well as potential for good recovery from drought stress.

A widely used turfgrass genus on golf courses in the northeast is *Agrostis*, commonly known as bentgrass. Although there are many different species of bentgrass, colonial bentgrass (*Agrostis capillaris* L.), velvet bentgrass (*Agrostis canina* L.), and creeping bentgrass (*Agrostis stolonifera* L.) are the predominant bentgrass species utilized as golf course turf. Within the species there are differences among the cultivars, such as genetic leaf color and resistance to both abiotic and biotic stresses. For example, previous research has shown inter- and intra-specific differences in wear tolerance among bentgrasses (Dowgiewicz et al., 2011), as well as differences in susceptibility to diseases such as dollar spot (Ryan et al., 2012). Previous research has also shown that there are differences in drought resistance among the three bentgrass species (DaCosta and Huang

2006a; DaCosta and Huang, 2007a), as well as within cultivars of creeping bentgrass (McCann and Huang, 2008). To date, though, there is limited information available on the variability of drought resistance and water use among cultivars of colonial bentgrass.

Colonial bentgrasses (*Agrostis capillaris* L.) are rarely used on greens, given the preferred height of cut range is 0.8 to 2 cm, and therefore are better adapted for tees and fairways (Turgeon, 2005). Although creeping bentgrass is the most widely used turfgrass on fairways and tees in the northeastern United States (EIFG, 2007), colonial bentgrasses are well adapted to the cool and humid regions of New England (Turgeon, 2005). In addition, previous research has shown colonial bentgrass may be better suited for low input fairways compared to creeping bentgrass (Watkins et al., 2010). Watkins et al. (2010) reported that although colonial bentgrass exhibited drought-induced dormancy, resulting in a lower turf quality under summer conditions compared to other species of bentgrasses, the species also recovered rapidly at the end of the summer stress period. Similarly, colonial bentgrass maintained as a fairway turf exhibited more rapid recovery from drought compared to other bentgrass species (DaCosta and Huang, 2006b; DaCosta and Huang, 2007b).

Earlier research comparing colonial bentgrasses to other bentgrasses has been limited in the number of cultivars evaluated (only one cultivar in the research published by DaCosta et al., 2006, and one cultivar in the Watkins et al., 2010). Therefore investigation of additional cultivars of colonial bentgrasses and their potential for reduced irrigation environments should be studied. Little is known about the differences among cultivars of colonial bentgrasses under drought stress or their comparative ability to recover from drought stress. Therefore, the objectives of this study were to compare the

performance of different colonial bentgrass cultivars under reduced irrigation, and to examine the physiological mechanisms associated with drought resistance and recovery traits of colonial bentgrass.

## **Materials and Methods**

### **Plant Materials and Growing Conditions**

The study was conducted in the CNS Research and Education Greenhouse at the University of Massachusetts, Amherst. There were five cultivars of colonial bentgrass selected for evaluation, including ‘Barking’, ‘Capri’, ‘Greentime’, ‘Revere’, and ‘Tiger II’. The grasses were planted into pots (10 cm in diameter, 36 cm in height) containing USGA sand comprised of 90% sand, 8% silt, and 2%. Grasses were seeded on 28 August 2012 at a rate of 19 g m<sup>-2</sup>. The plants were watered on a daily basis during establishment and fertilized once per week with Hoagland solution (Hoagland and Arnon, 1950). The greenhouse was maintained at 18/15°C (day/night temperatures), 12 h of photoperiod, and photosynthetic photon flux density of 600 μmol m<sup>-2</sup> s<sup>-1</sup>.

### **Treatments**

The experiment consisted of two treatments, including (i) well-watered control (WW) which was irrigated three times per week to maintain plants at pot capacity, and (ii) drought (DRT) which received no irrigation once the study began. At the start of the experiment all plants were watered to pot capacity (approximately 14% volumetric moisture content) prior to commencing treatments. Fertilizer was withheld from all plants during the experiment.

## Measurements

Soil moisture measurements were taken weekly during the study. Within each pot, a 20 cm buriable probe was installed vertically and measurements were taken with the MiniTrase System (Soilmoisture Equipment Corp., Santa Barbara, CA) using time domain reflectometry (TDR) and expressed as volumetric soil moisture content (v/v).

Turf quality (TQ) was measured throughout the study using a visual rating scale. Turf quality was rated on uniformity, density, and color based on a scale from 1 to 9, with 9 representing the highest TQ (green canopy and no wilting), 6 being the minimal acceptable TQ with some browning, and 1 representing completely brown and desiccated turf.

Leaf relative water content (RWC) was measured weekly during the study based on the method previously described by Barrs and Weatherley (1962). Clippings from each pot were removed (approximately 300 mg) and recorded as fresh weight (FW). The clippings were then placed in a covered Petri dish and floated in distilled water for 18 to 24 hours. The clippings were then removed from the water, blotted dry and weighed, which was recorded as the turgid weight (TW). The clippings were then transferred into coin envelopes and placed in the oven at 70°C and dried for a minimum of 3 days prior to recording the tissue dry weight (DW). The leaf RWC was then calculated as  $[(FW - DW) / (TW - DW)] * 100$ .

Plants were destructively harvested for root biomass determination at three times throughout the study representing pre-stress, moderate, and severe drought stress periods. Levels of drought stress were visually assessed based on turf quality, with moderate

drought stress defined as turf leaves showing early symptoms of wilting and loss in color and severe drought stress as leaf firing, browning and permanent wilting. Roots were washed free of soil and placed in an oven at 70°C. After drying for a minimum of 3 d, roots were weighed for dry weight determination. At severe drought stress, crowns were also harvested for carbohydrate analysis. Individual crowns were harvested (stems and roots removed) and dried in an oven at 70°C. Crowns were then ground and sieved through a 20 mesh sieve. These samples were then used to determine the total soluble sugars according to the methods previously described by Fu and Dernoeden (2009).

To examine the extent of recovery of grasses following severe drought stress, an additional group of plants subjected to DRT treatment were trimmed down to leaf bases and then re-watered to pot capacity as needed at the end of the study. Pots were evaluated on a weekly basis for percent recovery based on leaf regrowth capacity for three weeks.

### **Experimental Design and Statistical Analysis**

Plants were arranged in a randomized split-block design with irrigation level as the main plot and cultivar as the sub-plot. There were four blocks, each containing WW and DRT treatments. The effects of water treatment, cultivar, and corresponding interactions were determined by analysis of variance according to the general linear model procedure of the Statistical Analysis System version 9.2 (SAS Institute, Cary, N.C.). Differences between treatment means were separated by Fisher's least significance (LSD) test at the 0.05 probability level.



## **Results and Discussion**

There was a significant main effect for both irrigation level and cultivar for soil moisture content, but no irrigation x cultivar interaction. Following 6 d of treatment, WW plants exhibited significantly higher soil moisture content compared to plants exposed to DRT treatment (Fig. 3.1). These differences were maintained throughout the remainder of the study. The WW treatments, on average, had a soil moisture content of 13.7% throughout the study, whereas soil moisture of plants exposed to DRT declined to approximately 2% by 35 d of treatment.

When comparing soil moisture of cultivars averaged over irrigation treatment, Barking and Revere generally maintained higher soil moisture content compared to Greentime (Fig. 3.2). Although soil moisture content of plants exposed to DRT treatment began to decline as early as 6 days of the treatment, declines in TQ were only observed following 16 d of treatment, coinciding with an average soil moisture content of 6% (Fig. 3.3). Although there were significant differences in TQ between the DRT and WW treatments beginning on day 16, the DRT treatment TQ did not drop below 6 until 31 days of treatment, when the soil moisture content for DRT plants was approximately 3%. All five cultivars responded similarly to treatments, with differences in TQ being primarily observed at 31 days of treatment, when Revere exhibited lower TQ compared to other cultivars (Fig. 3.4). There were no interactions between irrigation level x cultivar for TQ.

Under drought stress, higher levels of RWC are associated with the ability of the plant to stay turgid and maintain higher levels of photosynthetic activity. Based on the

significant irrigation main effect, differences in RWC between WW and DRT treatments were not detected until 27 days of treatment (Fig. 3.5). This difference in RWC coincided with a decline in TQ of the DRT treatment below the acceptable rating of 6 at 31 d of treatment. The only day we observed differences in RWC for the main effect of cultivar was at 22 d of treatment, when Barking exhibited a lower RWC compared to Tiger II, Greentime, and Revere. This difference in RWC is similar to the decline in TQ for Barking where on day 16 days of treatment, Barking had significantly higher TQ than all other cultivars but by day 22 Barking had dropped in TQ from an 8.6 to a 7.6 and there were no longer significant differences among cultivars. This is similar to what we have seen in other studies where higher measurements of RWC have been positively linked to higher TQ under drought conditions (DaCosta and Huang, 2007b; McCann and Huang, 2008; Jiang et al., 2009). When comparing the means of irrigation x cultivar interaction at 27 d of treatment, Revere, Barking, and Tiger II exposed to DRT all had significantly lower leaf RWC compared to their respective WW plants. In contrast, Capri and Greentime exposed to DRT treatment did not significantly differ in leaf RWC compared to under WW conditions (Fig. 3.6).

Turfgrasses utilize different physiological mechanisms in order to maintain high water content in their tissues, such as increases in root mass and distribution to help increase water uptake. For example, deeper rooting in lower portions of the soil profile has been positively correlated with improved drought avoidance in tall fescue (*Festuca arundinaceae* Shreb.) cultivars (Huang and Gao, 2000). In this study, prior to the onset of drought stress there were no significant differences in rooting dry weight among treatments (Fig.3.7). However, at moderate and severe drought stress (27 and 36 days of

treatment, respectively) plants exposed to DRT had significantly lower root dry weight compared to WW plants. When comparing cultivars for root dry weight, Barking had significantly greater rooting under moderate stress (27 days of treatment) compared to other cultivars, whereas Capri had the lowest root dry weight (Fig. 3.8). By day 27 there were no differences between the main effect of cultivar for TQ or RWC. Although, Barking had the highest soil moisture content on day 20, it was only significantly different from Capri; there were no differences in soil moisture as of day 27. We also observed a significant interaction for irrigation x cultivar, where Barking and Tiger II under WW conditions had significantly higher dry root weight than other treatment combinations (Fig. 3.9).

We did not observe differences in total soluble sugars levels of the crown tissue based on the main effects of irrigation, cultivar, or the interaction of irrigation x cultivar. When comparing treatment means we did observe an overall trend for a higher soluble sugar content for DRT compared to WW pots; however this was not statistically significant except for Greentime (Fig. 3.10).

Recovery levels have been shown to vary among cultivars of tall fescue (Karcher et al., 2008), as well as *Poa* species (Abraham et al., 2004). In both these studies turfgrasses that maintained green leaf tissue longer under drought stress also had faster green up and recovery levels after re-watering. In contrast, previous research comparing the responses of three bentgrass species to reduced irrigation reported that colonial bentgrass did not maintain the highest TQ under drought stress, but exhibited the most rapid recovery (DaCosta and Huang, 2006b; DaCosta and Huang, 2007a). In the current study we observed differences in recovery of colonial bentgrass cultivars from drought

stress. Capri exhibited significantly lower recovery levels compared to all other cultivars at 3, 14, 17, and 21 d of rewatering (Fig. 3.10). In contrast, Tiger II had significantly higher recovery than all other cultivars by 3 d of re-watering, with the exception of Barking from which it was not significantly different. By 17 days of re-watering, Tiger II and Barking exhibited significantly higher recovery compared to Greentime.

In conclusion, when exposing five colonial bentgrass cultivars to drought stress, Barking initially maintained higher level of soil moisture and a higher TQ compared to other cultivars. At moderate periods of drought stress (22 to 27 d of treatment), Tiger II, Revere, and Barking exhibited significantly lower RWC compared to Capri and Greentime. These physiological differences in responses during DRT treatments may subsequently impact recovery potential, as Tiger II and Barking ranking among the highest in recovery levels and Capri and Greentime among the lowest. Therefore, it is suggested that drought-induced dormancy in colonial bentgrass aids in more rapid recovery from water stress.

Figure 3.1. Volumetric soil moisture content of the irrigation main effect of well watered (WW) and drought (DRT) treatments. Bars represent LSD at the  $P \leq 0.05$  level for each day of measurement.

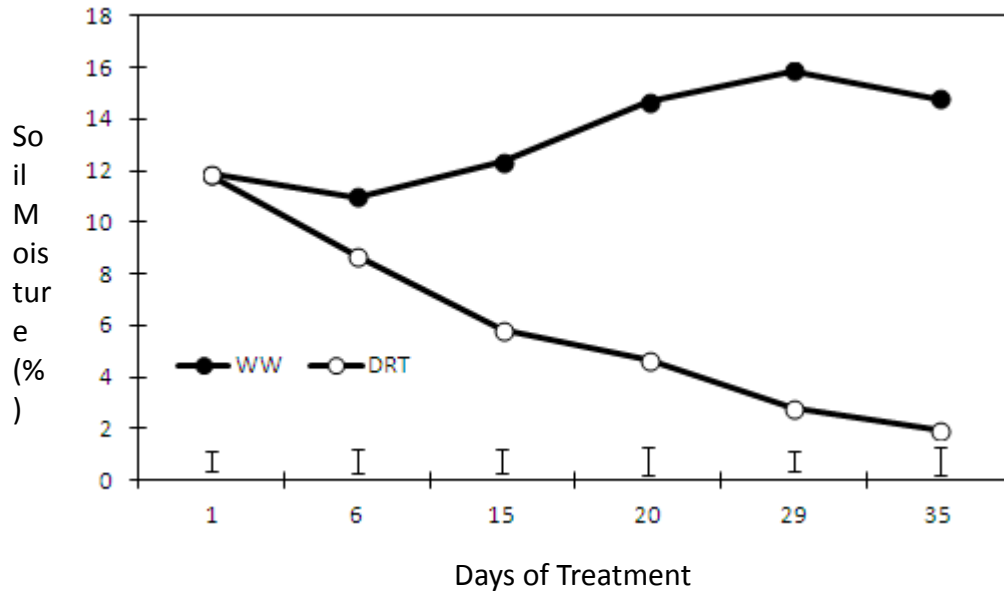


Figure 3.2. Volumetric soil moisture content of the cultivar main effect consisting of five Colonial bentgrasses cultivars. Bars represent LSD at the  $P \leq 0.05$  level for each day of measurement.

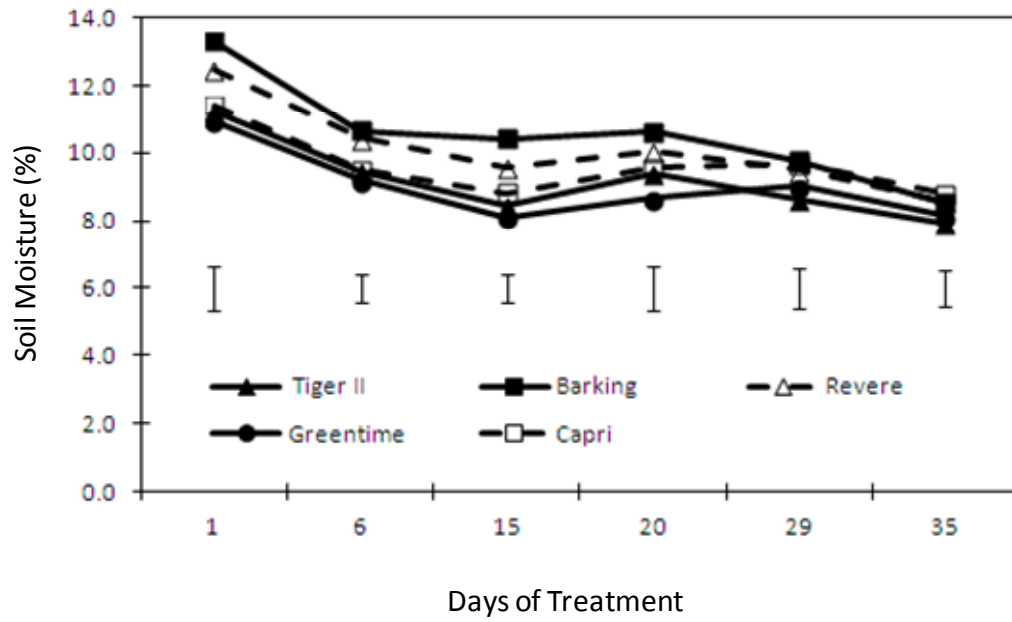


Figure 3.3. Turf quality rating of the irrigation main effect of well watered (WW) and drought (DRT) treatments. Turf quality rating is done visually based of uniformity, density, and color. The measurements are on a scale from 1 to 9, 9 being best with a turf stand of all green color and no wilting, 6 being acceptable but with some browning, and below 6 is not acceptable quality with 1 being completely dead. Bars represent LSD at the  $P \leq 0.05$  level for each day of measurement.

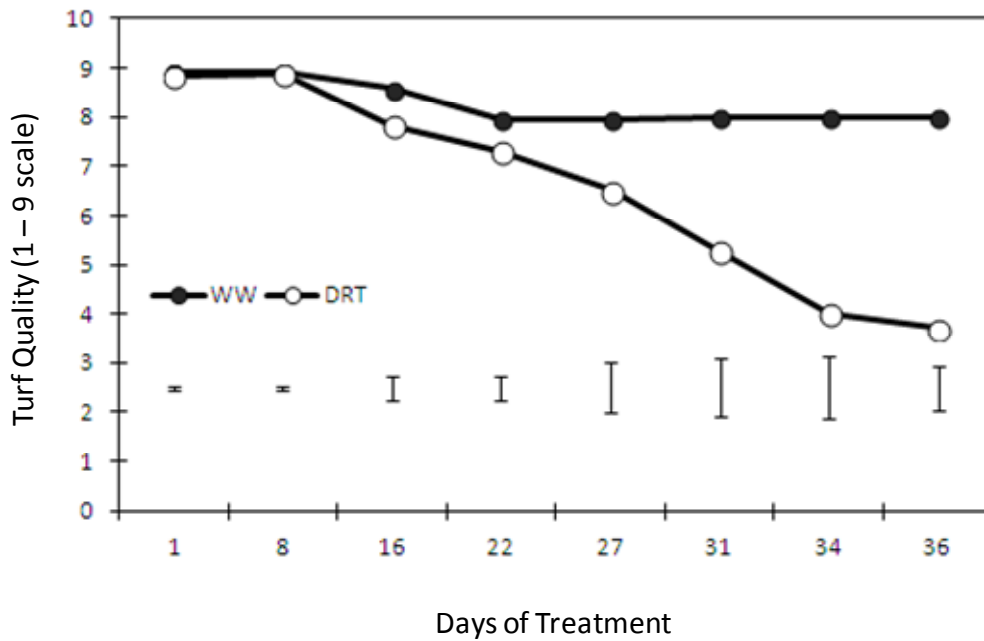


Figure 3.4. Turf quality rating of the cultivar main effect consisting of five colonial bentgrass cultivars. Turf quality rating is done visually based of uniformity, density, and color. The measurements are on a scale from 1 to 9, 9 being best with a turf stand of all green color and no wilting, 6 being acceptable but with some browning, and below 6 is not acceptable quality with 1 being completely dead. Bars represent LSD at the  $P \leq 0.05$  level for each day of measurement.

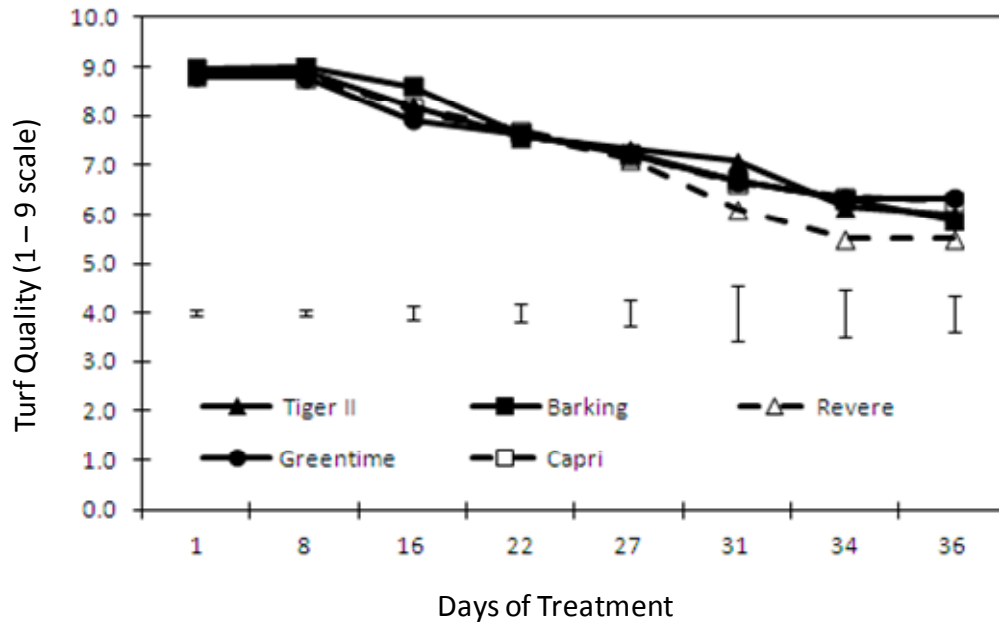




Figure 3.5. Leaf relative water content of the irrigation main effect of well watered (WW) and drought (DRT) treatments. Bars represent LSD at the  $P \leq 0.05$  level for each day of measurement.

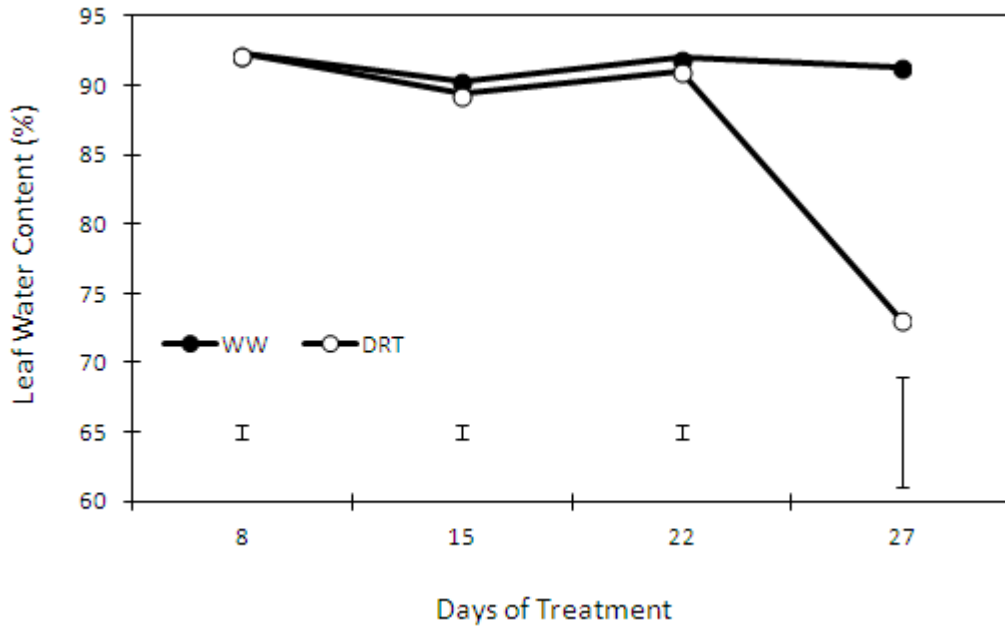


Figure 3.6. Leaf relative water content of the cultivar x irrigation main effect consisting of five colonial bentgrass cultivars and two irrigation treatments (i) well watered (WW) and (ii) drought (DRT) on day 27 of treatment. Bars represent LSD at the  $P \leq 0.05$  level.

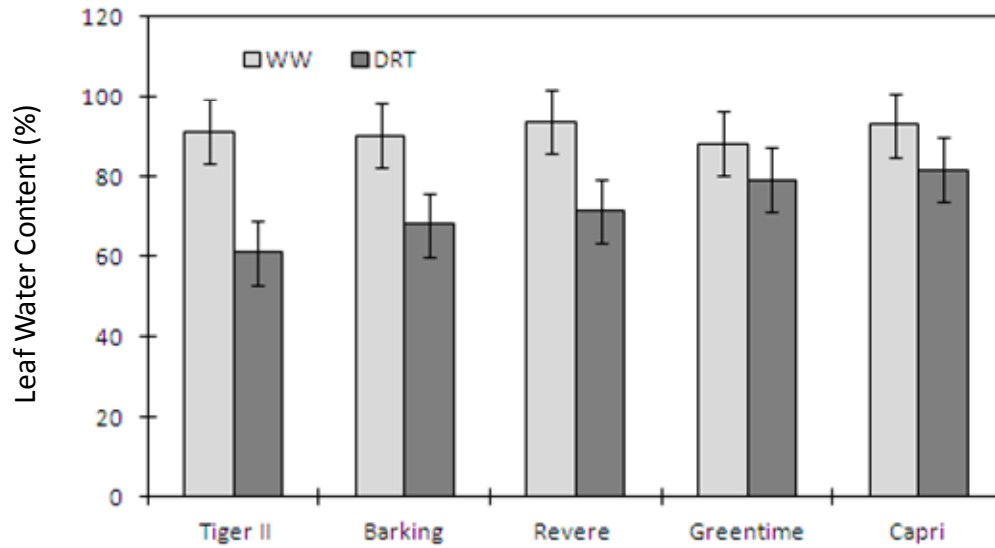


Figure 3.7. Root dry weight (g) for the main effect of irrigation (i) well watered (WW) and (ii) drought (DRT) under three drought levels (i) initial (day 1 of drought treatment), (ii) moderate (27 d), and (iii) severe (36 d) . Bars represent LSD at the  $P \leq 0.05$  level.

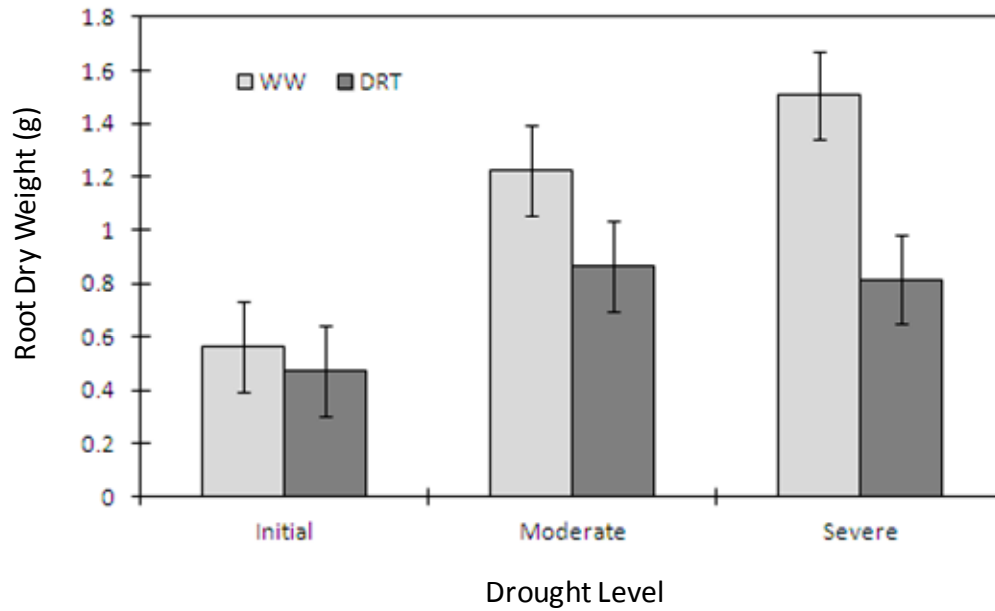


Figure 3.8. Root dry weight (g) for the main effect of cultivar under moderate drought stress on day 27 of treatment. Bars represent LSD at the  $P \leq 0.05$  level.

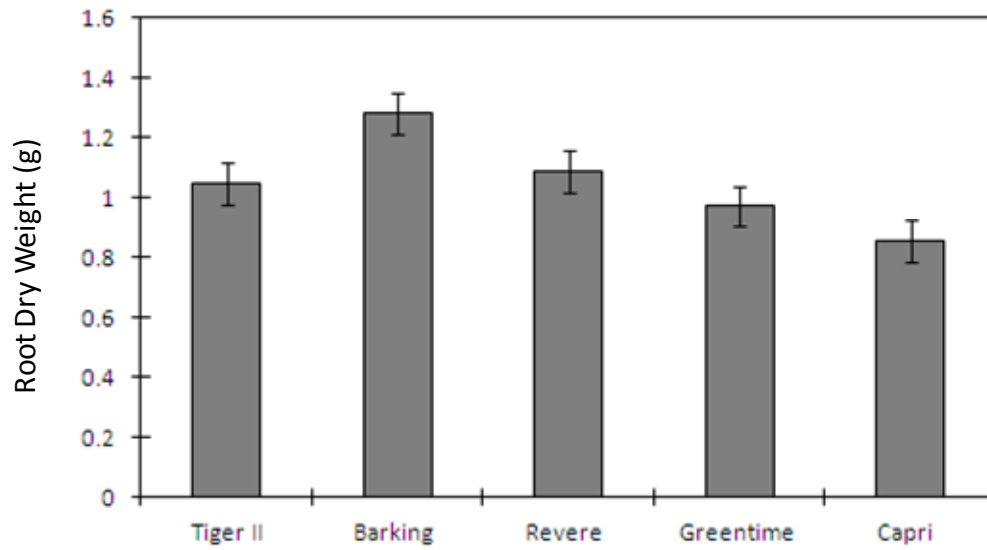


Figure 3.9. Root dry weight (g) for the interaction of the main effect of irrigation x cultivar on day 27 of treatment under moderate drought stress consisting of five colonial bentgrass cultivars and two irrigation treatments (i) well watered (WW) and (ii) drought (DRT). Bars represent LSD at the  $P \leq 0.05$  level.

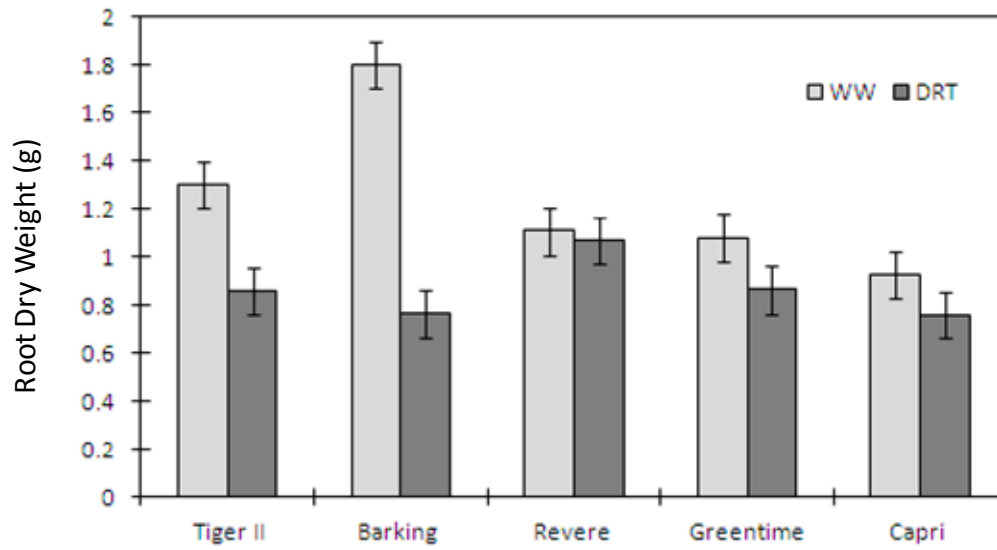


Figure 3.10. Total soluble sugar content of the crowns on day 27 of treatment of the cultivar x irrigation main effect consisting of five colonial bentgrass cultivars and two irrigation treatments (i) well watered (WW) and (ii) drought (DRT). Bars represent LSD at the  $P \leq 0.05$  level.

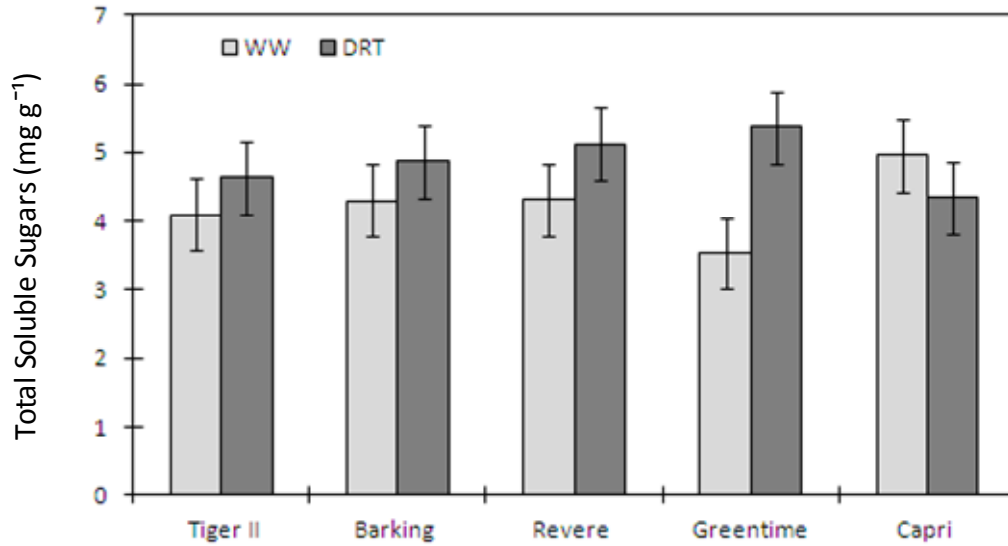
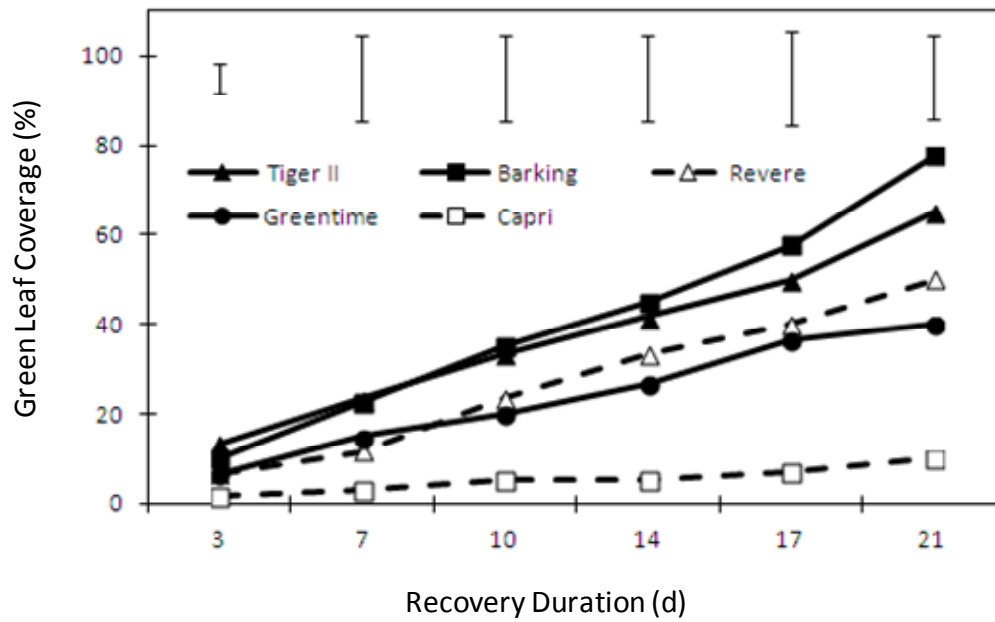


Figure 3.11. Green leaf coverage (%) of the main effect of cultivar during recovery. Bars represent LSD at the  $P \leq 0.05$  level for each day of measurement.



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## CONCLUSIONS

Under the conditions of our field study, the use of a wetting agent did not result in any significant differences in turf quality or soil moisture retention among treatments. However, there were significant differences in turf quality among bentgrass species and cultivars under reduced irrigation (Revere and Tiger II colonial bentgrasses, Legendary and Greenwich velvet bentgrasses, and 13M, T-1, L-93 and Penncross creeping bentgrasses). Colonial bentgrass cultivars maintained higher turf quality among the cultivars evaluated, and were found to be as fairway turf under reduced irrigation. Due to excessive thatch accumulation in our study, velvet bentgrass cultivars exhibited significant declines in quality regardless of irrigation level. Among creeping bentgrass cultivars, T-1 exhibited the highest turf quality compared to the older cultivars of creeping bentgrass. Based on results from the field study, we further evaluated the drought resistance and recovery characteristics among five cultivars of colonial bentgrass ('Barking', 'Tiger II', 'Revere', 'Capri', and 'Greentime'). Under moderate drought stress, Barking, Tiger II, and Revere all exhibited lower leaf relative water content levels compared to Capri and Greentime, although no significant differences in turf quality or soil water content were observed during the drought period. Following re-watering, Barking and Tiger II exhibited the most rapid recovery from drought (as measured by percent green cover), while Capri and Greentime exhibited delayed recovery. Therefore, although significant differences in turf performance during drought stress were not observed, recovery potential seems to vary among the different cultivars of colonial bentgrass and should be evaluated in future studies.

**APPENDIX A**  
**RAINOUT SHELTER PROGRAM**

'Program Name: RAINOUT PROGRAM\_LISA.CR1 = CONTROLS THE SHADE  
SYSYTEM

'Written by: Ali Farsad, Univ. of Mass, Jun 2009

'Revised by: Lisa Golden University of Massachusetts, June 2011

'VARIABLE DISCRPTION:

' Shade Open: means shade is aside

' Shade Close: means shade is over

' VS: sensor value. Greater umber means more rain

' V: V is 1000 times bigger than VS (for convenient use)

'  $V \geq 5$  means it is raining

'  $V < 5$  means it is not raining

' P1=1: electric motor is working (closing the shade): relay1 is close (on)

' P1=0: electric motor is not working: relay1 is off (open)

' P2=1: electric motor is working (opening the shade): relay2 is close (on)

' P2=0: electric motor is not working: relay2 is off (open)

' S=0: shade is open (aside)

' S=1: shade is close (over)

' D=0: Program is working

' D=1: program is in Delay time

' R=0: it has not been raining for a while (Delay time, say 10 min).

' R=1: it has been raining for a while (Delay time, say 10 min).

Public VS, P1, P2, S, D, R, V

DataTable (Test,1,-1)

OPENINTERVAL

DATAINTERVAL(0,1,MIN,10)

SAMPLE(1,VS,FP2)

HISTOGRAM(VS,FP2,0,1,011,1,0,150)

EndTable

'Main Program

BeginProg

Scan (1,sec,30,0)

BrHalf(VS,1,mV25,1,VX1,1,2500,1,0,250,1,0) ' reads the sensor attached to (black=ex1;  
red=h1; clear=ground; purple=ground) and stores it in VS

V=VS \* 1000

IF V>=5 AND R=0 THEN ' If rain begins (after a period of not raining)

D=1

R=1

DELAY(1,1,SEC) ' Wait for a while (Say 1 sec).

D=0

ELSEIF V>=5 AND R=1 AND S=0 THEN ' If it is already raining for a while and the  
shade is open (aside), then

S=1

PORTSET(3,1)

P1=1

D=1

DELAY(1,70,SEC)

D=0

```
PORTSET(3,0)
P1=0
ENDIF
IF V<5 AND R=1 THEN ' If rain stops (after it was raining for a while)
D=1
R=0
DELAY(1,10,MIN) ' Wait for a while (say 10 min).
D=0
ELSEIF V<5 AND R=0 AND S=1 THEN ' If it is not raining for a while and the shade
is close, then
S=0
PORTSET(4,1)
P2=1
D=1
DELAY(1,70,SEC)
D=0
PORTSET(4,0)
P2=0
ENDIF
CALLTABLE TEST
NEXTSCAN
ENDPROG
```

## **APPENDIX B**

### **PRELIMINARY GREENHOUSE STUDY**

#### **Introduction**

This greenhouse study was designed as a preliminary study in preparation for an additional greenhouse study and was based on the results of a two year field study. We selected two creeping bentgrass cultivars; ‘T-1’ which performed well in our field trials and ‘Penncross’ which did not perform as well. We also selected the two colonial bentgrasses used in our field study, which both performed well, ‘Tiger II’ and ‘Revere’ and added two additional colonial bentgrasses, ‘Greentime’ and ‘Barking’. This study was designed to not only compare the visual response of these different species and cultivars of bentgrasses but to also better understand the physiological mechanisms being used by these turfgrasses under drought stress. Therefore the objectives of this study was to (i) compare different species and cultivars of bentgrasses in a controlled environment and differentiate between the levels of turf quality of these bentgrasses under drought stress and (ii) understand the physiological mechanisms used by the different species and cultivars which contribute to the ability of certain turfgrasses to maintain higher turf quality during drought stress.

#### **Materials and Methods**

##### **Plant Materials and Growing Conditions**

The study was conducted in the French Hall Greenhouses at the University of Massachusetts, Amherst. There were two species of bentgrass: creeping bentgrass and

colonial bentgrass, with a total of six cultivars being studied. Creeping bentgrass cultivars included 'T-1' and 'Penncross'; colonial bentgrass cultivars included 'Greentime', 'Barking', 'Revere', and 'Tiger 2'. The grasses were seeded at a rate of  $19 \text{ g m}^{-2}$  into pots (10 cm in diameter and 36 cm tall) filled with USGA sand from Holliston Sand Company containing  $89.8 \pm 0.2\%$  sand,  $7.8 \pm 0.2\%$  silt, and  $2.4 \pm 0.2\%$  clay on 18 April 2011. The plants were grown in the greenhouse and watered as needed to prevent wilt, fertilized with Hoagland's solution (Hoagland and Arnon, 1950) once per week, and maintained at a height of cut of 1 to 1.5 cm. The greenhouse was maintained at  $18^{\circ}\text{C}$  during the day and  $13^{\circ}\text{C}$  at night throughout the establishment and treatment periods.

### **Treatments**

The treatments consisted of (i) a well-watered control that was irrigated to maintain soil at container capacity ( $\approx 19\%$  soil moisture content, v/v) and (ii) a drought treatment that received no water. Container capacity was achieved by watering containers until water was draining from the bottom of the containers and then left for 24 hours to assure all gravitational water had drained and pots were at container capacity. On 27 October 2011, the plants were all watered to container capacity and 28 October was Day 1 of the study. Once the study began the plants were no longer fertilized and the well watered plants were watered as needed to be kept at pot capacity ( $\approx 19\%$  soil moisture content v/v) and the drought plants received no water.

### **Measurements**

Soil moisture measurements were taken at least once per week during the study. Within each pot, a 20 cm probe was installed vertically and measurements were taken

with the MiniTrase System (Soilmoisture Equipment Corp., Santa Barbara, CA) using time domain reflectometry (TDR) and were expressed as volumetric soil moisture content.

Turf quality (TQ) was measured throughout the study by using a visual rating scale. Turf quality was rated on uniformity, density, and color. The measurements were based on a scale from 1 to 9; with 9 being best with a turf stand of all green color and no wilting, 6 being acceptable but with some browning, and below 6 is not acceptable quality with 1 being completely dead.

Leaf relative water content (RWC) was measured weekly during the study according to the method of Barrs and Weatherley (1962). Clippings from each pot were taken and weighed ( $\approx 0.3$  g) and that number was recorded as fresh weight (FW). The clippings were then wrapped in a Kimwipe and placed in a covered Petri dish and floated in distilled water for 18 to 24 hours. The clipping were then removed from the water, blotted dry and weighed. That number was recorded as the turgid weight (TW). The clippings were then transferred into coin envelopes and placed in the oven at 60°C and dried. Once the clippings were dry, they were removed from the oven and weighed again for a dry weight (DW) measurement. Based on these measurements RWC was calculated as:  $[(FW-DW)/(TW-DW)]*100$ .

Leaf clippings were taken weekly during this study for osmotic adjustment (OA) and were assessed according to the rehydration method (Babu et al., 1999). Each week fresh clippings were taken from each pot and placed in a covered Petri dish of distilled water for 18 to 24 hours in order to become fully rehydrated. The clippings were then



blotted dry and wrapped in aluminum foil and are currently stored in a freezer at  $-80^{\circ}\text{C}$ . The samples were then removed from the freezer and allowed to thaw. Once thawed, the fluid was expressed from the leaves and the osmolality of the liquid was measured using the VAPRO model 5600 Vapor Pressure Osmometer (WESCOR, Inc. Logan, Utah). Osmolality of the leaf sap ( $\text{mmol kg}^{-1}$ ) was then converted into osmotic pressure (MPa) using the van't Hoff equation:  $\Psi_s = C_sRT$ , where  $C_s$  is the osmolality ( $\text{mol kg}^{-1}$ ),  $R$  is a gas constant of 0.0821, and  $T$  is a temperature constant of 298 K.

### **Experiential Design and Statistical Analysis**

The experiment was arranged as a randomized complete block design with 4 replicates. Results were determined by analysis of variance using SAS version 9.2 (SAS Institute Inc. Cary, NC). Means were separated by the least significant difference at 0.05 probability level.

Table 1. Results from the analysis of variance (ANOVA) for the preliminary greenhouse study, including measurements for turf quality, soil moisture, relative water content, and osmotic potential.

|                           |                    | Weeks of treatment |    |    |     |    |     |     |    |
|---------------------------|--------------------|--------------------|----|----|-----|----|-----|-----|----|
|                           |                    | 0                  | 1  | 2  | 3   | 4  | 5   | 6   | 7  |
| Turf Quality              | Block              | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
|                           | Irrigation         | NS                 | NS | NS | NS  | NS | *   | *   | ** |
|                           | Irrigation × Block | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
|                           | Cultivar           | NS                 | NS | NS | NS  | NS | *** | *** | ** |
|                           | Irrigation ×       | NS                 | NS | NS | NS  | NS | *   | **  | *  |
| Soil Moisture             | Block              | NS                 | NS | NS | *   | NS | *   | NS  | NS |
|                           | Irrigation         | NS                 | *  | ** | *** | ** | *** | **  | ** |
|                           | Irrigation × Block | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
|                           | Cultivar           | NS                 | *  | *  | NS  | *  | *   | NS  | NS |
|                           | Irrigation ×       | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
| Relative Water<br>Content | Block              | NS                 | NS | *  | NS  | NS | NS  | *   | NS |
|                           | Irrigation         | NS                 | *  | NS | NS  | NS | NS  | *   | NS |
|                           | Irrigation × Block | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
|                           | Cultivar           | *                  | *  | NS | NS  | NS | NS  | NS  | NS |
|                           | Irrigation ×       | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
| Osmotic Potential         | Block              | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
|                           | Irrigation         | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
|                           | Irrigation × Block | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |
|                           | Cultivar           | *                  | *  | *  | NS  | NS | NS  | NS  | NS |
|                           | Irrigation ×       | NS                 | NS | NS | NS  | NS | NS  | NS  | NS |

Table 2. Results from the analysis of variance (ANOVA) for the main effect of irrigation for the preliminary greenhouse study.

|                           |     | Weeks of treatment |        |        |        |        |        |        |        |
|---------------------------|-----|--------------------|--------|--------|--------|--------|--------|--------|--------|
|                           |     | 0                  | 1      | 2      | 3      | 4      | 5      | 6      | 7      |
| Turf Quality              | WW  | NS                 | NS     | NS     | NS     | NS     | 7.3 a  | 7.0 a  | 6.9 a  |
|                           | DRT | NS                 | NS     | NS     | NS     | NS     | 6.1 b  | 5.5 b  | 5.3 b  |
| Soil Moisture             | WW  | NS                 | 17.2 a | 19.0 a | 19.0 a | 19.2 a | 20.0 a | 21.1 a | 19.2 a |
|                           | DRT | NS                 | 12.7 b | 10.4 b | 8.9 b  | 7.8 b  | 6.2 b  | 5.4 b  | 4.3 b  |
| Relative Water<br>Content | WW  | NS                 | 96.2 a | NS     | NS     | NS     | NS     | 97.3 a | NS     |
|                           | DRT | NS                 | 91.7 b | NS     | NS     | NS     | NS     | 90.7 b | NS     |
| Osmotic Potential         | WW  | NS                 | NS     | NS     | NS     | NS     | NS     | NS     | NS     |
|                           | DRT | NS                 | NS     | NS     | NS     | NS     | NS     | NS     | NS     |

Table 3. Results from the analysis of variance (ANOVA) for the main effect of cultivar for the preliminary greenhouse study.

|                        |           | Weeks of treatment |           |           |    |         |         |        |       |
|------------------------|-----------|--------------------|-----------|-----------|----|---------|---------|--------|-------|
|                        |           | 0                  | 1         | 2         | 3  | 4       | 5       | 6      | 7     |
| Turf Quality           | Barking   | NS                 | NS        | NS        | NS | NS      | 7.3 a   | 6.3 b  | 6.1 a |
|                        | Greentime | NS                 | NS        | NS        | NS | NS      | 6.8 ab  | 6.8 a  | 6.3 a |
|                        | Revere    | NS                 | NS        | NS        | NS | NS      | 5.5 c   | 5.0 c  | 5.3 b |
|                        | Tiger II  | NS                 | NS        | NS        | NS | NS      | 6.8 ab  | 6.5 ab | 6.2 a |
|                        | Penncross | NS                 | NS        | NS        | NS | NS      | 7.1 a   | 6.6 ab | 6.4 a |
|                        | T-1       | NS                 | NS        | NS        | NS | NS      | 6.5 b   | 6.5 ab | 6.3 a |
| Soil Moisture          | Barking   | NS                 | 12.5 c    | 12.8 bc   | NS | 12.0 bc | 12.1 bc | NS     | NS    |
|                        | Greentime | NS                 | 15.6 ab   | 15.1 ab   | NS | 14.0 ab | 13.5 ab | NS     | NS    |
|                        | Revere    | NS                 | 12.8 bc   | 12.2 c    | NS | 11.0 c  | 12.0 c  | NS     | NS    |
|                        | Tiger II  | NS                 | 16.4 a    | 16.0 a    | NS | 15.4 a  | 13.6 a  | NS     | NS    |
|                        | Penncross | NS                 | 15.9 ab   | 16.2 a    | NS | 14.4 ab | 13.9 a  | NS     | NS    |
|                        | T-1       | NS                 | 16.4 a    | 16.0 a    | NS | 14.5 a  | 13.6 a  | NS     | NS    |
| Relative Water Content | Barking   | 92.7 c             | 95.9 ab   | NS        | NS | NS      | NS      | NS     | NS    |
|                        | Greentime | 96.0 abc           | 96.6 a    | NS        | NS | NS      | NS      | NS     | NS    |
|                        | Revere    | 99 a               | 91.6 b    | NS        | NS | NS      | NS      | NS     | NS    |
|                        | Tiger II  | 96.3 ab            | 94.8 ab   | NS        | NS | NS      | NS      | NS     | NS    |
|                        | Penncross | 96 abc             | 91.4 b    | NS        | NS | NS      | NS      | NS     | NS    |
|                        | T-1       | 95 bc              | 93.4 ab   | NS        | NS | NS      | NS      | NS     | NS    |
| Osmotic Potential      | Barking   | -1.724 bc          | -1.349 bc | -1.114 a  | NS | NS      | NS      | NS     | NS    |
|                        | Greentime | -1.661abc          | -1.241 ab | -1.424 c  | NS | NS      | NS      | NS     | NS    |
|                        | Revere    | -1.838 c           | -1.431 c  | -1.077 a  | NS | NS      | NS      | NS     | NS    |
|                        | Tiger II  | -1.625             | -1.230 ab | -1.249abc | NS | NS      | NS      | NS     | NS    |
|                        | Penncross | -1.491 ab          | -1.139 a  | -1.295 bc | NS | NS      | NS      | NS     | NS    |
|                        | T-1       | -1.427 a           | -1.136 a  | -1.169abc | NS | NS      | NS      | NS     | NS    |

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