PERFORMANCE OF TEXAS BLUEGRASS HYBRIDS IN THE TRANSITION ZONE

by

KEMIN SU

B.S., Beijing Agricultural University, P. R. China, 1991

M.S., China Agricultural University, P.R. China, 1997

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture, Forestry, and Recreation Resources
College of Agriculture

KANSAS STATE UNIVERSITY
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Abstract

High temperature and drought may reduce quality in cool-season turfgrasses during summer in the transition zone. Texas bluegrass hybrids (HBG) are genetic crosses between native Texas bluegrass (*Poa arachnifera* Torr.) and Kentucky bluegrass (*Poa pratensis* L.) (KBG) that resemble KBG but may have greater drought and heat resistance than other cool-season grasses. The objectives of four studies were to evaluate high temperature, drought, and lower mowing height effects on HBG compared with KBG ('Apollo') and tall fescue (*Festuca arundincea* Schreb.)(TF, 'Dynasty'), compare their rooting characteristics, and investigate their membrane lipid molecular species compositional differences.

Under high temperature (35/25°C, 14-h light/10-h darkness), HBG ('Thermal Blue') had greater quality and gross photosynthesis (Pg), and lower electrolyte leakage than KBG and TF in a growth chamber study. The combination of high temperature and drought (60% ET replacement) caused rapid declines in quality, but HBG generally performed better.

In field and greenhouse studies, TF had more roots deeper in the profile than two HBG ('Reveille' and Thermal Blue) and KBG under well-watered conditions. In the field, quality and P_g were greatest in TF among turfgrasses. Performances in quality and P_g generally ranked: TF > Reveille >= Thermal Blue = KBG.

In a separate mowing height and drought field study, HBG (Thermal Blue) generally had lower quality and P_g than KBG at both high (7.62 cm) and low (3.81 cm) heights but particularly at the low height. Drought resistance and tolerance to low mowing in Thermal Blue was similar to or poorer than in KBG.

Under supra-optimum temperature (35/25°C and 40/30°C, 14-h light/10-h darkness), the ratio of digalactosyldiacylglycerol (DGDG) to monogalactosyldiacylglycerol (MGDG) was highest in HBG (Thermal Blue) and lowest in TF. Heat tolerance was also associated with higher phosphatidylethanolamine (PE) content, higher phosphatidylglycerol (PG) content, and reduced overall unsaturation compared with heat-sensitive. Results suggest that 40 membrane lipid molecules are potential biomarkers for heat tolerance and that compositional changes in lipids in response to heat may contribute to differences in heat tolerance among cool-season grasses.

Generally, studies indicated greater heat resistance, but not drought resistance, in HBG than in KBG or TF.

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CHAPTER 1 - Effects of High Temperature and Drought on a Hybrid Bluegrass Compared with Kentucky Bluegrass and Tall Fescue

Abstract

High temperature and drought stresses may reduce quality in cool-season turfgrasses during summer months in the transition zone. This growth chamber study was conducted to evaluate effects of high temperature and drought on physiology and growth of 'Apollo' Kentucky bluegrass (*Poa pratensis* L.) (KBG), 'Dynasty' tall fescue (*Festuca arundincea* Schreb.) (TF), and 'Thermal Blue', a hybrid (HBG) between KBG and Texas bluegrass (*Poa arachnifera* Torr.). Turfgrasses were exposed for 48 days to supra-optimal (high temperature; 35/25°C, 14-h day/10-h night) and optimal (control; 22/15°C, 14-h day/10-h night) temperatures under well-watered (100% evapotranspiration [ET] replacement) and deficit (60% ET replacement) irrigation. Heat resistance was greater in HBG, which had greater visual quality, gross photosynthesis (Pg), dry matter production, and lower electrolyte leakage and soil-surface temperatures than KBG and TF under high temperature. Cumulative Pg during the study was 16% and 24% greater in HBG than in KBG and TF, respectively. Green leaf area index (LAI) in HBG was not affected by high temperature, but LAI was reduced by 29 % in KBG and 38% in TF. Differences in drought resistance were negligible among species. The combination of high temperature and drought caused rapid declines in visual quality and dry matter production, but HBG generally performed better. Results indicated greater heat resistance, but not drought resistance, in HBG than in KBG or TF.

Abbreviations: DOT, days of treatment; EL, electrolyte leakage; ET, evapotranspiration; HBG, a hybrid between Kentucky and Texas bluegrass; KBG, Kentucky bluegrass; LAI, leaf area index; Pg, gross photosynthesis; TF, tall fescue.

Introduction

High temperature and drought stresses are significant problems in cool-season turfgrasses during summer months in the U.S. transition zone, which covers 480 to 1120 km north to south between the northern regions where cool-season grasses are adapted and the southern regions where warm-season grasses are adapted (Dunn and Diesburg, 2004). High temperature and drought stresses often occur simultaneously during summer months and may limit growth and cause a severe decline in the visual quality of cool-season turfgrasses (Perdomo et al., 1996; Bonos and Murphy, 1999; Jiang and Huang, 2000; Wang and Huang, 2004). Recent increases in competition for water have resulted in restrictions in water use for irrigation of turfgrasses (EIFG, 2004), which further exacerbates the problem of drought stress in cool-season turfgrasses. Predictions of higher temperatures from global warming also suggest that heat stress in cool-season turfgrasses may become more common in some regions, including the transition zone (National Assessment Synthesis Team, 2000).

Hybrid bluegrasses (HBG), which are genetic crosses between native Texas bluegrass and KBG, may have greater heat and drought resistance than other cool-season grasses. Hybrid bluegrasses have similar visual qualities to KBG, which is a fine-textured, cool-season turfgrass commonly used in lawns and golf courses in the U.S. (Read et al., 1999; Turgeon, 2002). Consequently, new cultivars of HBG are being investigated as potential water-saving, heat-resistant alternatives to current cool-season turfgrasses.

Abraham et al. (2004) evaluated, in a growth chamber study, the drought resistance of thirty HBG and their genetic parents and concluded that their drought resistance varied significantly. Those researchers also determined that highly drought resistant hybrids could be achieved by selecting first generation hybrids with good drought resistance and backcrossing them with elite drought resistant genotypes of KBG. In field tests in Colorado, USA, 'Reveille' HBG used significantly less water while maintaining higher quality than 'Bensun's A-34' KBG (Suplick-Ploense and Qian, 2005). Bremer et al. (2006) reported little difference in the general performance or drought resistance among two HBG (Thermal Blue and 'Dura Blue') and a KBG (Apollo) in a field study in Kansas, USA. Dura Blue HBG and Apollo KBG were the most desirable among five cool-season turfgrasses under different nitrogen rate levels in Tennessee, USA (Teuton et al., 2005). Thermal Blue and Dura Blue HBG may be acceptable replacements for KBG in the Upper Midwest, USA because they had similar quality and earlier spring greenup

than KBG (Stier et al, 2005). However, little information is available about the effects of both high temperature and drought on HBG.

The objectives of this study were to evaluate the effects of high temperature and drought stress on the growth, appearance, and physiology of HBG, KBG, and TF.

Materials and Methods

Preparation and maintenance of turfgrasses in lysimeters

Lysimeters (10 cm diam. by 60 cm deep) were filled with a mixture of sand and topsoil (1:1, v:v) and bulk densities inside lysimeters ranged from 1.62 to 1.70 g cm^{-3}. Apollo KBG and Thermal Blue HBG were seeded at 153 kg ha^{-1} and Dynasty TF was seeded at 395 kg ha^{-1} in lysimeters. They were kept under a mist system after seeding for about 30 d in a greenhouse. The mist system was automatically turned on 4 times per day for 5 min each. After 30 d, lysimeters were moved away from the mist system but continued to be maintained in the greenhouse. Average day/night air temperature was 22°C /15°C and supplemental light was 12-h/day. Turfgrasses were well-watered, mowed twice weekly at 6.5 cm, and fertilized weekly at the rate of 15 kg N ha^{-1} (15N-13P-12K). Totally, turfgrasses were planted and maintained in the greenhouse for about 135 d.

Lysimeters were then transferred to growth chambers at 22°C /15°C, 14-h day/10-h night with photosynthetically active radiation of 580 µmol m^{-2} s^{-1} during the daylight period. The turfgrasses were kept well-watered in the growth chambers for 14 d to allow for acclimation to growth chambers before treatments. Turfgrasses were mowed and watered every 3 d and fertilized every 6 d with a solution supplying 20 kg N ha^{-1} (15N-13P-12K) during the treatments.

Treatments and Experimental Design

Thermal Blue HBG, Apollo KBG, and Dynasty TF were exposed to the following treatments in growth chambers: 1) supra-optimal temperature (high temperature; 35°C /25°C, 100% ET); 2) drought (22°C /15°C, 60%ET); 3) high temperature and drought (35°C /25°C, 60%ET); and 4) optimal temperature (control; 22°C /15°C, 100%ET).

Six whole plots (2 temperature treatments x 3 replications) and six subplots per whole plot (3 species x 2 irrigation treatments), for a total of 36 subplots, were arranged in a split-plot

design. Whole plots were the growth chambers, where the supra-optimal and optimal temperature treatments were applied in separate chambers in a randomized complete block design. Subplots, which were the lysimeters, were arranged in a completely randomized design within each whole plot. Because only two growth chambers were available, whole plots could not be replicated concurrently and had to be replicated sequentially in time. The first whole-plot replication was from March to May 2004, the second replication from May to July 2004, and the third from July to September 2004. To ensure that turf age was similar in each replication, turfgrasses were seeded in lysimeters five months before treatments were applied. Therefore, lysimeters for the first, second, and third replications (blocks) were seeded in October 2003, December 2003, and January 2004, respectively.

Evapotranspiration (100%) was measured gravimetrically with the well-watered lysimeters (Bremer, 2003). Using this method, lysimeters were irrigated, allowed to drain until free drainage ceased, sealed, and weighed. Lysimeters were then weighed after 3 d and the water loss was attributed to ET. Lysimeters were irrigated every 3 d according to their ET losses. Cumulative ET (mm) for each treatment was determined as the sum of all ET during the study.

Turfgrass quality, photosynthesis, electrolyte leakage, and canopy temperature

Turf visual quality was rated on a scale of 1 to 9 (1=poorest quality, 6=minimally acceptable, and 9=highest quality) according to color, texture, density, and uniformity (Emmons, 2000). Quality ratings were recorded every 6 d by the same individual during the entire study.

Photosynthesis was measured every 6 d at about 8 h into the daily light cycle, with a LI-6400 portable gas exchange system (LI-COR Inc., Lincoln, NE) using a custom surface chamber described by Bremer and Ham (2005). According to their Equations 5 and 6, sunlit chamber measurements determine $Pg - (Rc + Rs')$ and shaded chamber measurements determine $Rc + Rs'$, where Pg is gross photosynthesis, Rc is canopy respiration, and Rs' is residual soil respiration in a pressurized chamber; all values are positive and units are μmol m^{-2}s^{-1}. Shaded chamber measurements were obtained by covering the chamber with a black cloth that completely blocked solar radiation from the chamber. Gross photosynthesis was calculated using equation 8 in Bremer and Ham (2005): Pg = sunlit chamber + shaded chamber. Cumulative Pg for each treatment was calculated by summing the products of mean Pg on each measurement

day and the number of hours between samples when lights were turned on (i.e., 14 h daily period when plants were photosynthesizing).

Leaf electrolyte leakage (EL) was determined by the method of Blum and Ebercon (1981) and Marcum (1998) with modifications. Five living leaves about the same age were collected from each lysimeter at 0, 3, 15, 27, 39, and 45 days of treatment (DOT). Each leaf was cut into two to three 2-cm segments and rinsed 3 times with distilled deionized water. All rinsed leaf segments from each lysimeter were placed in a test tube containing 20 ml deionized water. Test tubes were shaken on a shaker table at 120 RPM (Lab-Line Instruments Inc., Melrose Park, Illinois) for 24 h to dissolve electrolytes that had leaked from cells (e.g., due to membranes damaged by heat or drought stress treatments). After measuring conductivity (C_1) with a conductivity meter (Model 32, Yellow Spring Instrumental Inc., Yellow Spring, Ohio), the test tubes with leaf samples were placed in an autoclave at 140°C for 20 min to destroy all cell membranes, shaken for 24 h to extract all electrolytes from the cells, and then the conductivity (C_2) was measured again. The percentage of the total electrolytes that had leaked from cells during treatments was calculated as (C_1/ C_2)*100. Lower EL indicated greater resistance to stresses.

Canopy temperature was measured every 6 d with three infrared thermometers (model IRTP5, Apogee, Logan, UT). The infrared thermometers were positioned at 0.2 m above the turf canopy. Measurements were automatically logged every 5 sec and averaged and recorded every 30 min with a micrologger (CR10x, Campbell Scientific, Logan, UT). Measurements for each lysimeter were conducted for about 1.5 to 2.5 h.

Dry matter production, soil-surface temperature, and volumetric soil water content

Turfgrasses were mowed every 3 d, and all clippings were collected. Clippings were dried in a forced-air oven for 48 h at 70°C and then weighed. Cumulative dry matter production for each treatment was determined by summing the dry weights of all clippings during the 48 d study. Daily dry matter production was calculated as the clipping weight at each mowing divided by the number of days since the previous mowing.

Soil surface temperature was measured with soil-encapsulated thermocouples using the method of Ham and Senock (1992). To evaluate potential cumulative heat effects among treatments during the most stressful periods, heat units (degree-hours) were calculated as the sum

of soil surface temperatures during the final 8 h of each daily light cycle. Our data indicated that this was the period of maximum soil surface temperatures, which may have had important physiological impacts on the turfgrasses (e.g., on meristematic activity in the crowns).

Volumetric soil water content (θ_v) and temperature inside the lysimeters at 5, 25, and 45 cm were measured automatically using the dual-probe heat-pulse technique (Campbell et al., 1991; Tarara and Ham, 1997; Song et al., 1998). Sensors were fabricated in the laboratory as described by Basinger et al. (2003) and Bremer (2003). Measurements of θ_v were logged twice daily at 0800 and 2000 CST and soil temperatures were logged every 60 min. All data acquisition and control were accomplished with a micrologger and accessories (CR10x, two AM16/32's, and one AM25T, Campbell Scientific, Logan, UT).

Green LAI, aboveground biomass, and root mass density

At the end of each 48 d replication, aboveground biomass was harvested from each lysimeter and separated into living and dead components. Green leaves were separated from green shoots and the area of the leaves was measured with an area meter (LI-3100, LI-COR, Lincoln, NE). All green and dead tissue was then dried in a forced-air oven for 48 h at 70°C and weighed separately. Green LAI was calculated as the ratio of the green leaf area to ground surface, and total aboveground biomass for each treatment was calculated as the sum of the dry weights of all living and dead tissue.

After aboveground biomass was harvested, lysimeters were laid horizontally and cut into three sections (0 to 15, 15 to 35, and 35 to 57.5 cm). The soil was washed from the roots in each section and roots were dried in a forced-air oven for 48 h at 70°C and then weighed. Root mass density of each section was calculated as dry root mass divided by the volume of soil inside each respective section of lysimeter.

All data were analyzed with the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Variation was partitioned into grass species, temperature treatment, and irrigation level. Interactions among species, temperature, and irrigation level were not significantly different on a given day after treatment initiation (Days of treatment). Therefore, the comparison of species under temperature and / or drought treatments was statistically analyzed. Differences between means were separated by the least significance difference (LSD) test at P=0.05.

Results and Discussion

Visual quality

In the high temperature treatment, HBG consistently had the highest visual quality of all three species, always greater than or equal to a relative quality rating of six (Fig. 1-1A). Visual quality of HBG was significantly greater than TF and KBG starting on 24 and 42 DOT, respectively, and differences remained significant thereafter. Visual quality of KBG was also significantly greater than TF during the last week of the study (i.e., 42 and 48 DOT).

Under drought stress at optimal temperatures, visual quality of all turfgrasses began to decline after about two weeks (Fig. 1-1B). Although the quality of HBG was greater than TF on 6 and 12 DOT, this was consistent with the control, in which HBG was also higher than TF on those dates under well-watered conditions (Fig. 1-1D). As the severity of drought effects intensified, differences in quality between HBG and TF diminished. Visual quality was similar among species after 12 DOT, which indicates no differences in drought resistance among species. These results contradict Supplick-Ploense and Qian (2005), who found greater drought resistance in HBG (Reveille) because of its greater root length density and root mass. Our results also contradict Bremer et al. (2006), who reported that TF had greater drought resistance than HBG in the field. In contrast to the previous studies in which soils were deep, the development of roots in our study was probably more restricted because of limited soil volumes and soil water reservoirs inside lysimeters. Thus, our results may be more comparable to turfgrasses grown in shallow or poor soils such as is typical of home lawns, where soils are often disturbed, layered, and compacted during home construction (Hamilton and Waddington, 1999).

The combination of high temperature and drought stresses caused a more rapid decline in visual quality among species than individual treatments of high temperature or drought (Figs. 1-1A, 1-1B, and 1-1C). For example, visual quality of TF fell below six by 6 DOT, and HBG and KBG fell below six by 18 DOT. Nevertheless, the visual quality of HBG was consistently higher among species late in the study (i.e., after 18 DOT), albeit significantly higher only than TF.

In the control, visual quality was consistently higher in HBG and KBG than in TF although differences were not always significant; visual qualities were similar between HBG and KBG (Fig. 1-1D). Tall fescue had a lower visual quality than HBG and KBG in the control primarily because of its coarser texture.

Photosynthesis

High temperature reduced cumulative Pg by 21% in HBG, 30% in KBG, and 27% in TF, compared with the control (Fig. 1-2). In the high temperature treatment, however, cumulative Pg of HBG was 16% greater than KBG and 24% greater than TF. During the study, high temperature caused a general decline in daily Pg among species although Pg was consistently higher in HBG than in TF and KBG late in the study (Fig. 1-3). Daily Pg was significantly higher in HBG than in TF from 24 through 42 DOT, and higher in HBG than in KBG on 42 DOT. These data indicate greater resistance to heat in HBG than in TF and KBG.

Drought stress reduced cumulative Pg by 36% in HBG, 33% in KBG, and 29% in TF, compared with the control (Fig. 1-2). Late in the study, the deleterious effects of sustained drought on daily Pg surpassed that of well-watered turfgrasses experiencing only high temperature stress (data not shown). Cumulative Pg under drought stress was similar among species, however, indicating no differences in drought resistance among the three species as related to photosynthetic capacity.

Under combined high temperature and drought stress, cumulative Pg of HBG, KBG, and TF was reduced by 51%, 56%, and 60%, respectively, compared with the control (Fig. 1-2). The combination of high temperature and drought stresses reduced cumulative Pg more than individual treatments of high temperature or drought. This result is similar to other reports where the combined stresses of heat and drought caused photosynthesis to decline in Kentucky bluegrass (Jiang and Huang, 2000) and in tall fescue (Jiang and Huang, 2001). Wang and Huang (2004) reported that the combination of high temperature and drought stresses decreased leaf photochemical efficiency and chlorophyll content of two Kentucky bluegrass cultivars ('Midnight' and 'Brilliant') and had a detrimental effect on the photosynthesis system for Kentucky bluegrass. Cumulative Pg in this study was greater in HBG than in TF under the combined stresses of high temperature and drought.

Electrolyte leakage (EL)

High temperature had no effect on EL in well-watered HBG, but EL increased in well-watered KBG on 27 DOT, and remained higher than HBG thereafter (Fig. 1-4A). Mean EL also increased in TF on 27 DOT and remained higher than HBG thereafter, but differences were significant only on 39 DOT. Drought stress under optimal temperatures had no significant effect

on EL among species, although mean EL was consistently higher in TF among species beginning on 15 DOT (Fig. 1-4B). The combination of high temperature and drought caused EL to increase in all species (Fig. 1-4C). Interestingly, EL decreased in HBG after 27 DOT and was significantly lower than in KBG and TF thereafter. In the control, EL remained consistently low and was similar among the three turfgrasses throughout the study (Fig. 1-4D).

The EL results from our study agree with Wang and Huang (2004), who reported that EL increased rapidly during the combined stresses of heat and drought in Kentucky bluegrass. Haldimann and Feller (2005) reported that improved thermal stability of thylakoid membranes preserved the functional potential of the photosynthetic apparatus at high temperature and significantly minimized the effect of heat stress in pea (*Pisum sativum* L.) leaves. Our results suggest that cell membrane stability is higher in HBG than in KBG and TF under high temperature stress.

Dry matter production

In all treatments and the control, cumulative dry matter production was greater in HBG than in KBG and TF (albeit not significantly in the combination high temperature and drought treatment) and similar between KBG and TF (Fig. 1-5). Within each species, however, cumulative dry matter production was significantly reduced by high temperature, drought, and the combination of high temperature and drought. High temperature reduced cumulative dry matter production in KBG, HBG, and TF by 88%, 74%, and 91% respectively, which was greater than the reduction under drought; drought reduced cumulative dry matter production in KB, HBG, and TF by 49%, 48%, and 52% respectively. Lower cumulative dry matter production in high temperature than in drought was a result, in part, of a dramatic decrease in clippings after the initial three days of treatments compared with the more gradual decline in daily dry matter production under drought as soils dried (data not shown). The combination of high temperature and drought reduced cumulative dry matter production in KBG, HBG, and TF by 95%, 86%, and 93% respectively. Within each species, the combination of heat and drought had no additional effect on cumulative dry matter production compared to high heat only.

Evapotranspiration and green leaf area index

High temperature increased cumulative ET during the study by 57, 67, and 37% in KBG, HBG, and TF, respectively, compared with the control (Fig. 1-6A). Interestingly, ET also increased in HBG when exposed to high temperature and drought (i.e., despite reduced irrigation) compared with the control. Presumably, higher vapor pressure deficits contributed to increases in ET in the well-watered, high temperature treatment and to increased ET in HBG or sustained ET in KBG and TF in high temperature combined with drought.

Within the high temperature treatment, cumulative ET in HBG was 13% greater than in KBG and 22% greater than in TF (Fig. 1-6A). Within the drought and high temperature treatment, cumulative ET was 20% greater in HBG than in TF but was similar between HBG and KBG. In both of the latter treatments, greater ET in HBG may have been caused, in part, by its greater membrane integrity in transpiring leaves than in KBG and TF, as indicated by the lower EL in HBG in those treatments (Figs. 1-4A and 1-4C). Greater dry matter production (i.e., clippings) in HBG (Fig. 1-5) may also have contributed to its greater ET compared with KBG and TF. For example, during the study HBG consistently grew faster and was visibly taller than KBG and TF between mowing. Greater amounts of new growth between mowing indicate a greater surface area of young, actively transpiring leaves in HBG. Green LAI harvested from lysimeters at the end of the study was also higher in HBG than in TF under high temperature (Fig. 1-6B) indicating a greater surface area of green, transpiring leaves in HBG compared with TF.

Drought alone reduced ET compared with the control, but similar ET among species indicated no differences in drought resistances (Fig. 1-6A). Similarly, no differences in ET were observed among species in the well-watered, optimal temperature of the control. Our results contradict those of Suplick-Ploense and Qian (2005), who found that a HBG (Reveille) used less water and exhibited greater drought resistance in the field than a KBG (Bensun's A-34). Our results also differ from Minner and Butler (1985), who found that tall fescue used significantly more water than Kentucky bluegrass by using lysimeters filled with sand to measure ET under field conditions. High variability in water use among individual HBG and cultivars of KBG and TF may partially explain the contrasts between results from those studies and ours; the specific hybrids and cultivars used in their studies were different from those used in ours. Research by others has indicated significant variability in water use among individual cultivars of KBG and

TF, respectively (Shearman, 1986; Bowman and Macaulay, 1991). Furthermore, considerable variability in relative water content and drought resistance, which suggests variability in water use, was found among 30 HBG, and some HBG exhibited less drought resistance than KBG (Abraham et al., 2004).

Green LAI in HBG was not affected by high temperature compared with the control, but LAI in HBG was reduced (i.e., 56 to 64%) by drought and by the combination of high temperature and drought (Fig. 1-6B). High temperature reduced green LAI in KBG compared with the control, and high temperature combined with drought caused further reductions in LAI in KBG compared with high temperature only. Green LAI in KBG was 29%, 55%, and 61% lower in high temperature, drought, and combined high temperature and drought treatments, respectively, than in the control. Green LAI in TF was reduced by 38 to 68% among treatments compared with the control, but differences in green LAI in TF were not significant among stress treatments. Green LAI was similar among all three species in the control.

Canopy and soil-surface temperature and volumetric soil water content

In the drought and the combination drought and high temperature treatments, canopy temperature increased steadily by 4.1 to 4.8^{o}C during the study (data not shown). Presumably, decreasing transpiration as the soils dried caused a corresponding decrease in evaporative cooling of leaves and increased drought stress on leaf tissue (Sifers and Beard, 1993). Within each treatment, canopy temperature was generally similar among species (data not shown).

In the high temperature treatment, mean soil-surface temperature was consistently cooler in HBG than in KBG and TF, by about 1°C (data not shown). Cumulative heat (degree-hours) at the soil surface was significantly lower in HBG than in KBG and TF under high temperature (Fig. 1-6C), which suggests that long-term heat impacts on meristematic activity may have been reduced in HBG compared with KBG and TF. Under combined high temperature and drought stress, the soil-surface also was consistently cooler in HBG than in KBG and TF on a daily basis (data not shown). Cumulative heat at the soil surface was lower in HBG than in TF, but was statistically similar to KBG. Cooler soil-surface temperature in HBG exposed to high temperature may have resulted from greater shading of the soil surface, caused by faster growth in HBG between mowing as discussed earlier. Faster growth between mowing may have increased transpiration of the canopy and thus, cooled the air above the soil surface in HBG

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(Bonos and Murphy, 1999). Under drought alone, there was no difference in soil-surface temperature among species, which was similar to the control.

Volumetric soil water content inside the lysimeters was similar among species under all treatments and the control (data not shown). At lower depths (i.e., 25 and 45 cm) under the combination of high temperature and drought, however, volumetric soil water content was consistently lower in TF and HBG than in KBG, which indicated that TF and HBG were using water from deeper in the profile than KBG.

Aboveground biomass and root biomass density

In all treatments and the control, total aboveground biomass at the end of the study was greatest in TF, except under high temperature, although more than half was dead (Table 1-1). In all treatments and the control, dead aboveground biomass was greater in TF than in HBG. Conversely, in HBG, the living biomass as a percentage of the total was greater than in TF under high temperature, indicating that HBG has a greater resistance to heat than TF.

High temperature and high temperature combined with drought reduced total root biomass compared with the control and drought treatments (Table 1-2). These results are similar to other studies where root biomass production was reduced by high temperature (Howard and Watschke, 1991; Huang et al., 1997; Huang et al, 1998; Xu and Huang 2000a,b; Huang and Liu, 2003). In our drought treatment, total root biomass density was not different among species from the control, indicating drought alone did not reduce total root biomass production of HBG, KBG, or TF. This is contrary to reports by other researchers who observed declines in total root biomass production under drought (Howard and Watschke, 1991; Huang et al., 1997). In our study, 60% ET replacement (drought treatment) may have stimulated root growth during the early periods of treatment before inhibiting root growth as severity of the drought effects intensified. Thus, forty eight days may be not long enough to show the decline in total root biomass production under drought (60%ET replacement).

In the lower profile (i.e., 35 to 57.5 cm), root mass density was greater in TF than in HBG and KBG under all treatments (Table 1-2), which indicates TF may be able to extract soil water from deeper depths than HBG and KBG. A deep extensive root system, which is an important drought-avoidance mechanism, has been observed in TF in other studies (White et al., 1993; Carrow, 1996; Qian et al., 1997, Jiang and Huang, 2001). In a growth chamber study,

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however, the amount of extractable water by turfgrass roots is limited to the available reservoir inside the lysimeters, whereas in field soils the roots may extract water from lower in the profile. Therefore, TF was probably not able to benefit substantially from its deeper root system in our study.

At shallower depths (i.e., 0 to 15 and 15 to 35 cm), root biomass was generally similar among species within each treatment (Table 1-2). Root biomass was greater in TF than in KBG, however, in the middle profile (15-35 cm) of the control and under the combination of high temperature and drought. Conversely, root biomass was greater in KBG than in TF in the surface layer under the combination of high temperature and drought. Total root biomass at all depths was similar among species within each treatment with the exception of the control, where total root biomass was greater in TF than in KBG.

Conclusions

In the high temperature treatment, HBG generally exhibited higher visual quality, cumulative Pg, dry matter production, green LAI, and cumulative ET and lower EL and soil-surface temperature than KBG and TF (P=0.05). High temperature did not affect green LAI in HBG but reduced green LAI in KBG and TF by 29 and 38%, respectively. Under drought stress, the performance of all species declined and differences among species were negligible. The only exception was in cumulative dry matter production, which was higher in HBG than in KBG and TF under drought. The combination of high temperature and drought caused reductions in visual quality, cumulative Pg, cumulative dry matter production, and living aboveground biomass among species although mean values were generally greater in HBG late in the study. High temperature and drought combined caused EL and dead aboveground biomass to increase among species although EL was significantly higher in KBG and TF than in HBG late in the study.

In this growth chamber study, HBG exhibited a higher resistance to heat than KBG and TF, which suggests that HBG may be better suited for areas where frequent high temperature may damage other cool-season turfgrasses. Differences in drought resistance were negligible among HBG, KBG, and TF, however, indicating no advantage of this HBG over KBG and TF under dry conditions. Field studies are needed to further investigate the heat and drought resistance of HBG, including additional cultivars of HBG, under the actual dynamic and stressful environments where turfgrasses are grown.

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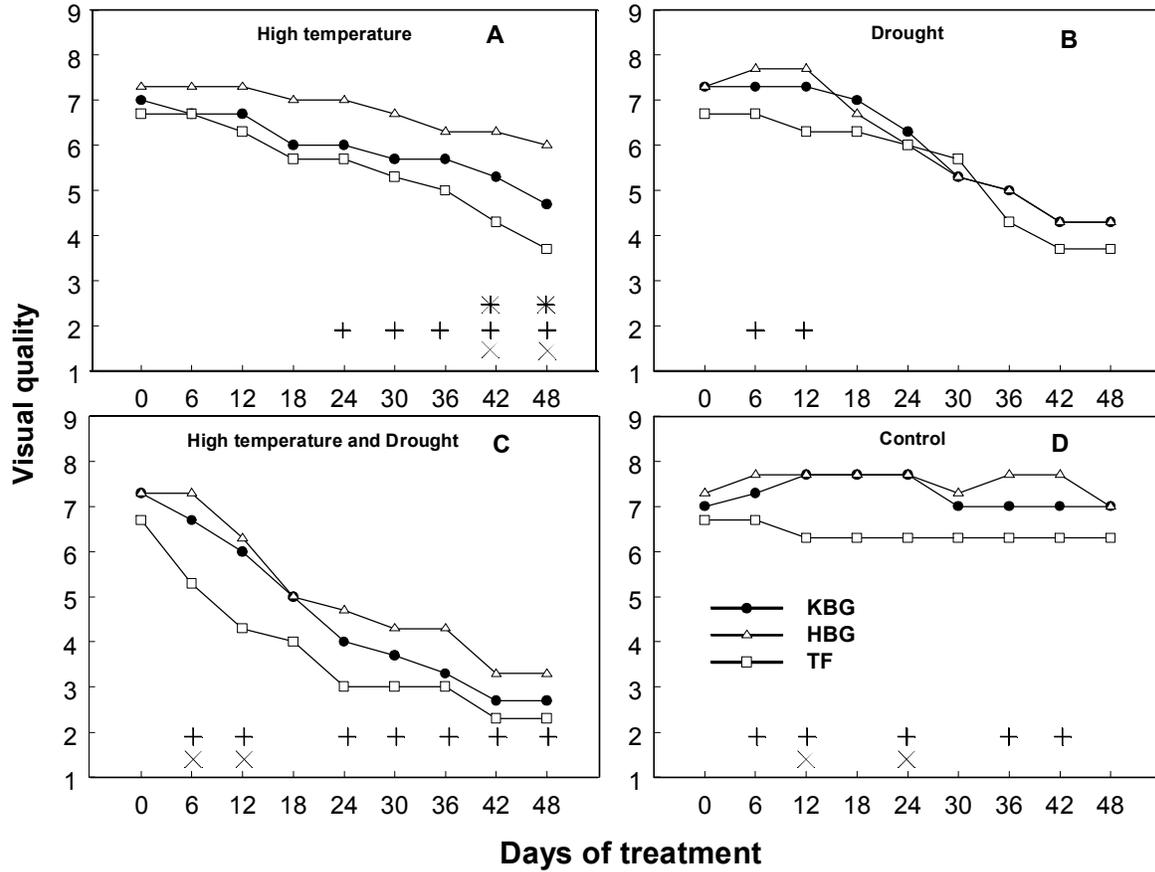
Figure 1-1 Effects on visual quality rated on a scale of 1 to 9 (1=poorest and 9=highest) of: high temperature (A), drought (B), high temperature and drought (C), and control (D) in KBG, HBG, and TF. Symbols along the abscissa of each graph indicate significant differences (P=0.05) between: HBG and KBG (*); HBG and TF (+); and KBG and TF (X); on a given day after treatment initiation (Days of treatment).
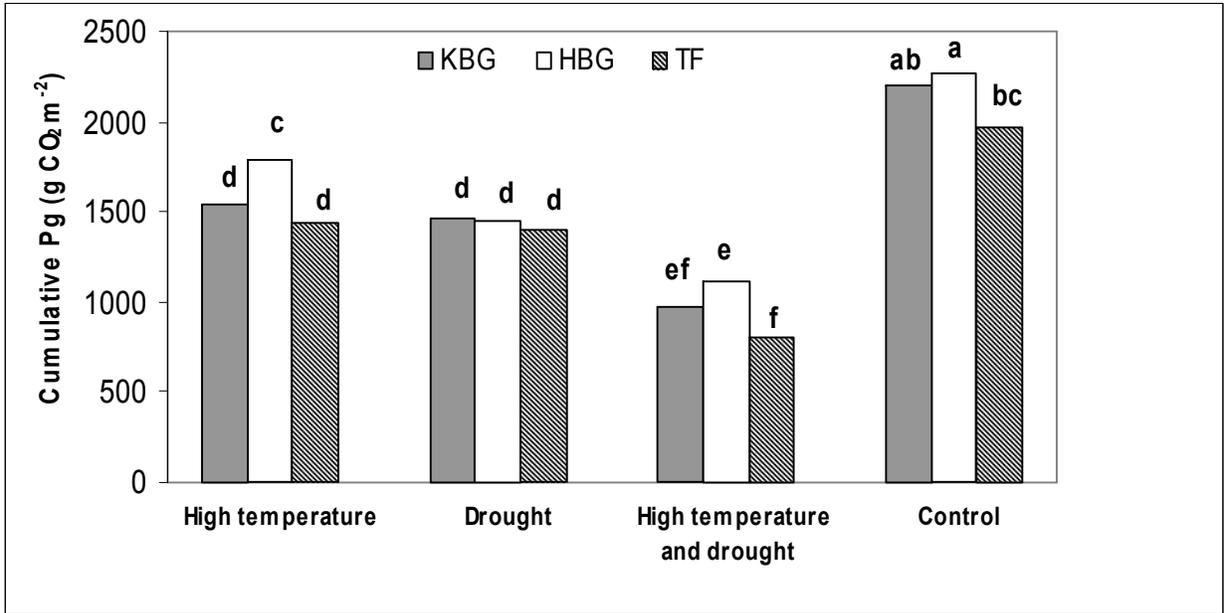
Figure 1-2 Effects on cumulative Pg of: high temperature, drought, high temperature and drought, and control in KBG, HBG, and TF. Means with the same letters were not significantly different (P=0.05).
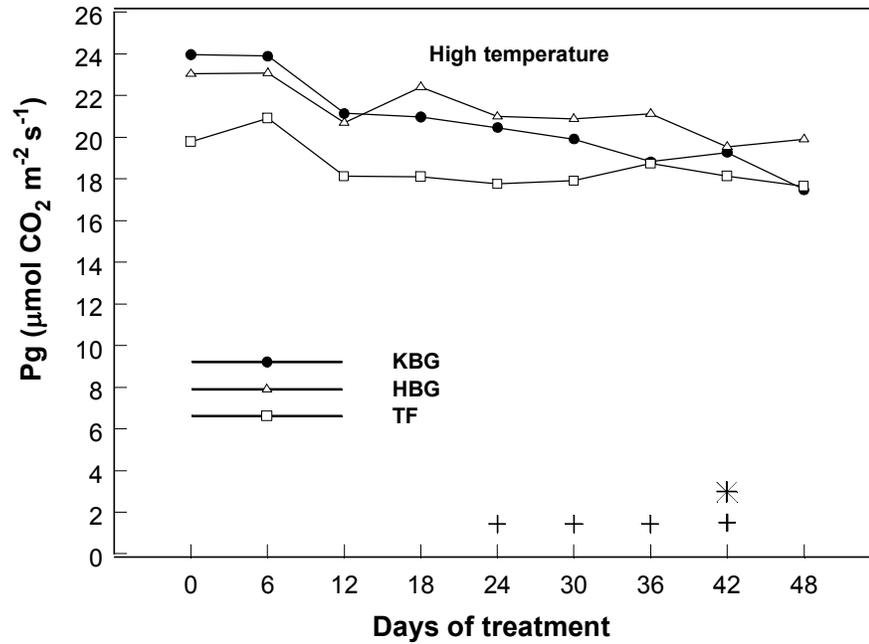
Figure 1-3 Effects on gross photosynthesis (Pg) of high temperature in KBG, HBG, and TF. Symbols along the abscissa indicate significant differences (P=0.05) between: HBG and KBG (*); and HBG and TF (+); on a given day after treatment initiation (Days of treatment).
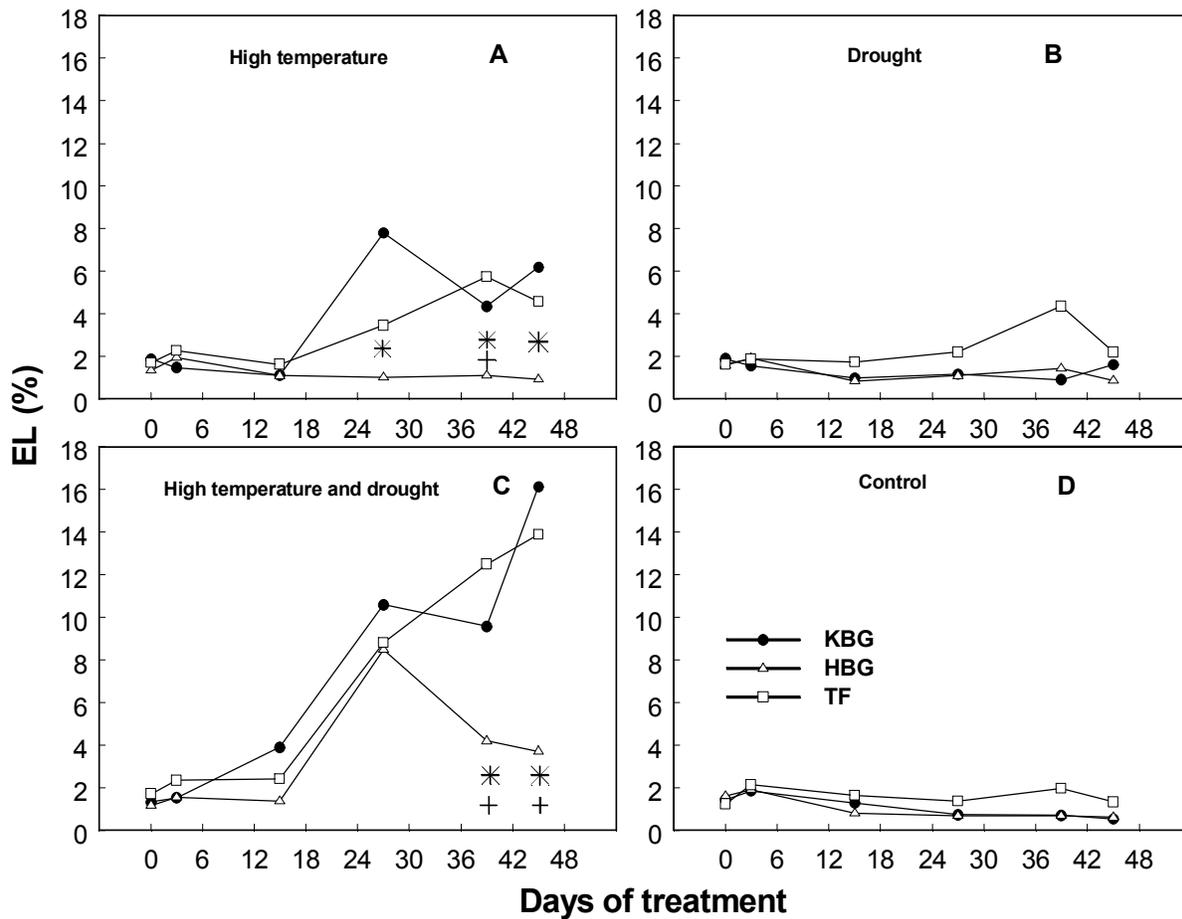
Figure 1-4 Effects on electrolyte leakage (*EL*) of: high temperature (A), drought (B), high temperature and drought (C), and control (D) in KBG, HBG, and TF. Symbols along the abscissa of each graph indicate significant differences (P=0.05) between: HBG and KBG (*); and HBG and TF (+); on a given day after treatment initiation (Days of treatment).
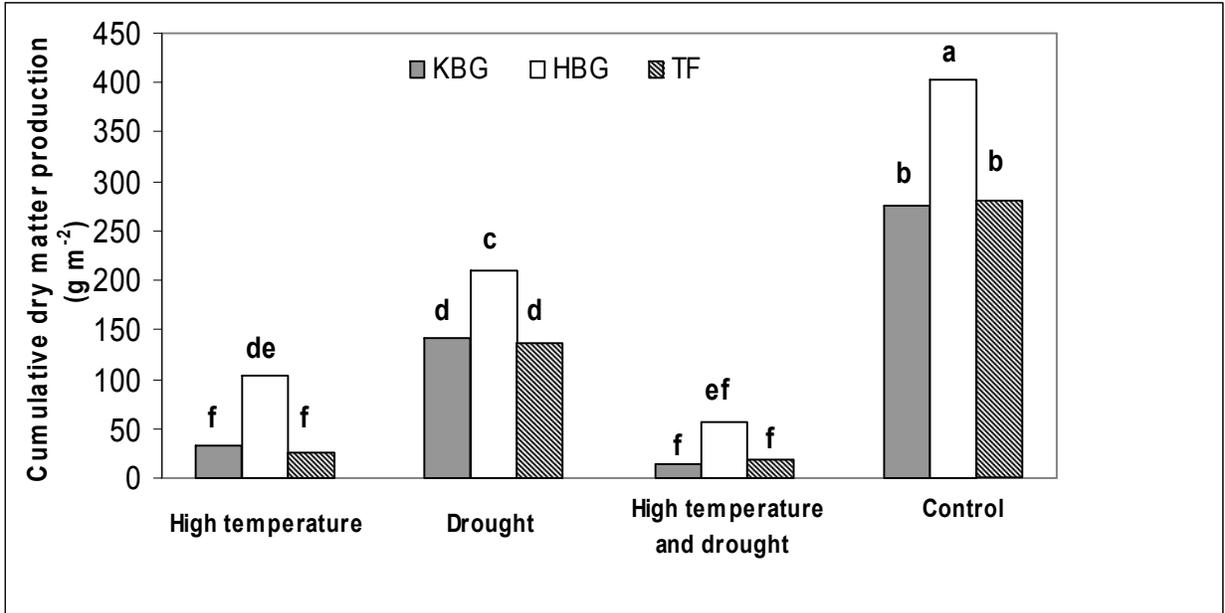
Figure 1-5 Effects on cumulative dry matter production of: high temperature, drought, high temperature and drought, and control in KBG, HBG, and TF. Means with the same letters were not significantly different (P=0.05).
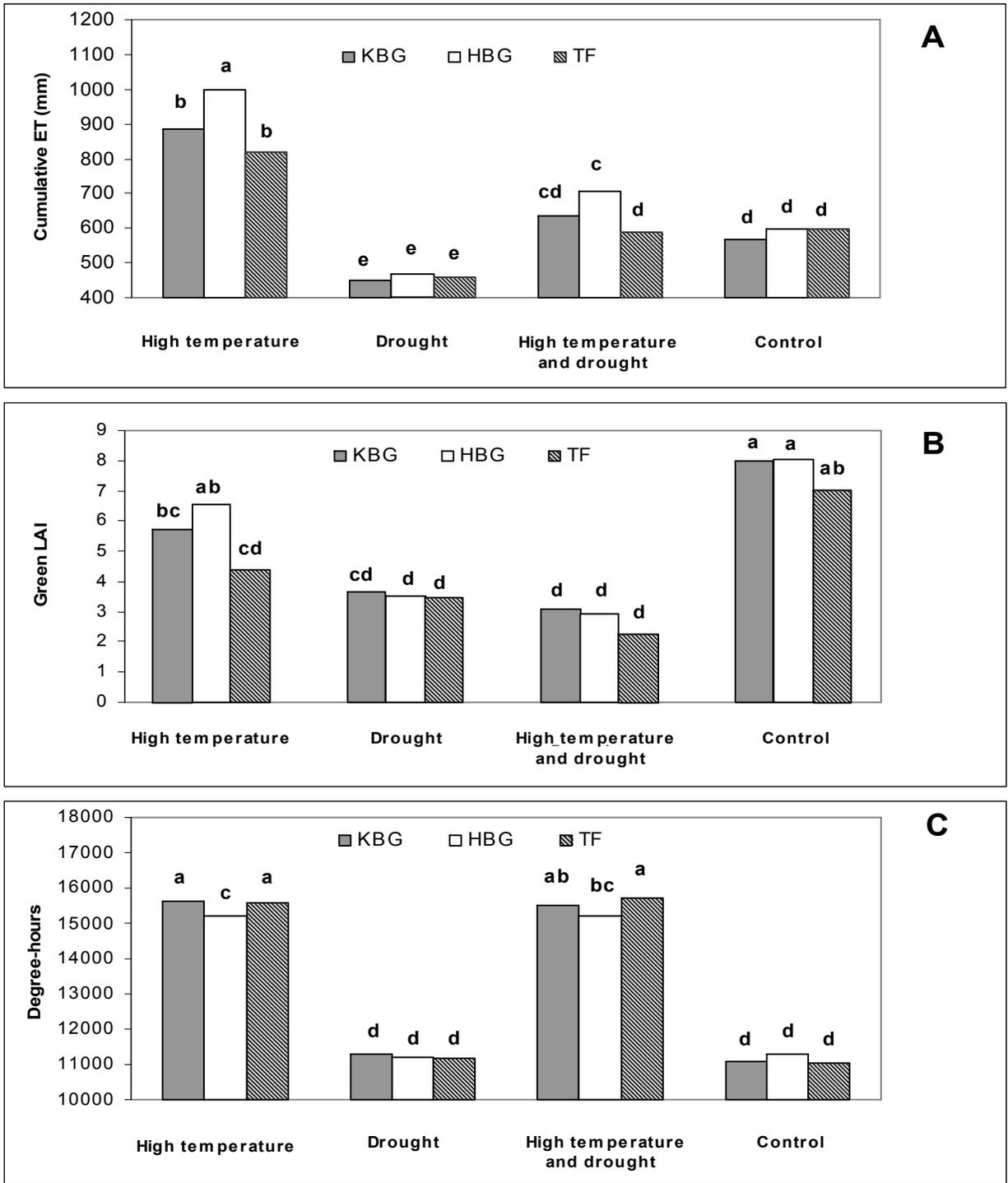
Figure 1-6 Effects on cumulative ET (mm) (A), green LAI (B), and degree-hours (C) at the soil surface in: high temperature, drought, high temperature and drought, and control treatments in KBG, HBG, and TF. Means with the same letters in each graph were not significantly different (P=0.05).

Table 1-1 Effects on dry aboveground biomass of high temperature, drought, high temperature and drought, and control in KBG, HBG, and TF.

Treatments	Species	Aboveground biomass(g m^{-2})			Percentage of living biomass in total (%)
		Total	Dead	Living	
High temperature	KBG	1411.8 ef[†]	776.4 de	635.4 bcdef	45 abcd
	HBG	1430.6 def	606.5 de	824.2 abc	58 a
	TF	1706.7 bcd	1092.6 abc	614.1 cdef	36 cde
Drought	KBG	1596.4 cde	869.5 bcde	726.9 abcd	46 abcd
	HBG	1338.7 ef	687.2 de	651.5 bcde	49 abc
	TF	1932.6 ab	1153.6 ab	779.0 abc	40 bcde
High temperature and drought	KBG	1266.4 f	903.1 abcd	363.3 f	29 de
	HBG	1179.7 f	718.0 de	461.7 def	39 bcde
	TF	1581.3 cde	1205.0 a	376.3 ef	24 e
Control	KBG	1717.6 bc	802.2 cde	915.5 ab	53 ab
	HBG	1444.8 cdef	571.4 e	873.4 abc	60 a
	TF	2151.1 a	1207.4 a	943.6 a	44 abcd

[†] Means followed by the same letter within a column were not significantly different (P=0.05)

Table 1-2 Effects on root mass density of high temperature, drought, high temperature and drought, and control in KBG, HBG, and TF.

Treatments	Species	Root mass density			
		Total	0 to 15 cm	15 to 35 cm	35 to 57.5 cm
		---------------- kg dry root mass m^{-3} soil -----------------			
High temperature	KBG	2.01 d[†]	6.30 cd	0.59 e	0.40 de
	HBG	2.16 d	6.58 cd	0.84 de	0.38 de
	TF	2.23 d	5.71 cd	0.95 cde	1.06 bc
Drought	KBG	3.01 b	8.82 a	1.13 bcd	0.80 bcde
	HBG	3.11 ab	8.72 a	1.37 ab	0.92 bcd
	TF	3.31 ab	8.15 ab	1.51 ab	1.69 a
High temperature and drought	KBG	2.19 d	7.14 bc	0.55 e	0.34 e
	HBG	2.17 d	6.37 cd	0.89 cde	0.52 cde
	TF	2.31 cd	5.41 d	1.14 bcd	1.29 ab
Control	KBG	2.90 bc	9.01 a	0.92 cde	0.59 cde
	HBG	3.10 ab	9.07 a	1.30 abc	0.73 cde
	TF	3.73 a	9.49 a	1.60 a	1.78 a

† Means followed by the same letter within a column were not significantly different (P=0.05).

CHAPTER 2 - Rooting Characteristics and Responses of Photosynthesis to Irrigation Deficit of Two Hybrid Bluegrasses, Kentucky Bluegrass, and Tall Fescue

*This chapter has been submitted to Agronomy Journal

Abstract

Drought stress is common among cool-season turfgrasses during summer months in the U.S. transition zone. A two-year field study was conducted near Manhattan, Kansas, USA, to evaluate rooting characteristics and effects of drought on canopy physiology and appearance of 'Apollo' Kentucky bluegrass (*Poa pratensis* L.) (KBG), 'Dynasty' tall fescue (*Festuca arundincea* Schreb.) (TF), and two hybrid bluegrasses (HBG); HBG are genetic crosses between native Texas bluegrass (*Poa arachnifera* Torr.) and KBG. Rooting characteristics were measured in the field and greenhouse under well-watered conditions to evaluate root contributions to drought avoidance. Two irrigation treatments in the field included: 1) 60% (irrigation-deficit); and 2) 100% (well-watered) evapotranspiration (ET) replacement. Ninety to 96% of all root length (0-80 cm) in the field and 74 to 80% of all root length (0-120 cm) in the greenhouse were in the upper 30 cm among HBG1 'Thermal Blue', HBG2 'Reveille', and KBG while in TF, only 86% of all root length in the field and 70% of all root length in the greenhouse were in the top 30 cm. Tall fescue always had more roots deeper in the profile than HBG1, HBG2, and KBG. In the field, visual quality and gross photosynthesis (P_g) were greatest in TF among turfgrasses in both irrigation treatments. Reveille (HBG2) used more water from the 0-50 cm profile and performed better than HBG1 during irrigation-deficit, but both HBG recovered from irrigation-deficit slightly more quickly than KBG. Performances in visual quality and P_g generally ranked: TF > Reveille >= Thermal Blue = KBG.

Abbreviations: DOT, days of treatment; ET, evapotranspiration; HBG, hybrid between Texas bluegrass and Kentucky bluegrass; HBG1, Thermal blue- a HBG; HBG2, Reveille- a HBG; KBG, Kentucky bluegrass; MRE, maximum root extension; P_g, gross photosynthesis; RLD, root length density.

Introduction

Drought stress is common among cool-season turfgrasses during summer months in the U.S. transition zone, which spans 480 to 1120 km north to south between the northern regions where cool-season grasses are adapted and the southern regions where warm-season grasses are adapted (Dunn and Diesburg, 2004). As water for irrigation becomes increasingly limited, water restrictions are becoming more common for turfgrass managers and consequently, the problem of drought stress in cool-season turfgrasses will likely intensify (Reisner, 1993; Snow, 2001).

Kentucky bluegrass (KBG) is a cool-season turfgrass often used in home and commercial lawns, athletic fields, and golf courses fairways and roughs (Turgeon, 2002). During drought, however, KBG may go dormant and lose its green color, which is an undesirable trait to some turf managers. Tall fescue (TF), also a cool-season grass, is sometimes used in golf course roughs and is popular in lawns because of its good drought resistance (Carrow, 1996; Qian et al., 1997). Some turf managers, however, do not like TF because of its bunchgrass characteristics and coarser texture compared with KBG. Hybrid bluegrasses (HBG), which are genetic crosses between native Texas bluegrass and Kentucky bluegrass (KBG), may have similar visual quality as KBG but exhibit greater drought and heat resistance than other cool-season grasses (Read et al., 1999). Consequently, new cultivars of HBG are being investigated as potential drought-resistant alternatives compared with current cool-season turfgrasses.

Until recently, limited data have been available regarding the drought resistance of HBG. In a field study in Kansas, USA, differences were negligible in drought resistance among two HBG ('Thermal Blue' and 'Dura Blue') and a KBG ('Apollo') (Bremer et al., 2006). In a growth chamber study, no differences in drought resistance were observed among a HBG (Thermal Blue), a KBG (Apollo) and a TF ('Dynasty') although Thermal Blue was more resistant to heat than the other turfgrasses (Su et al., 2007). Considerable variation in drought resistance, however, was observed among thirty cultivars of HBG and their genetic parents in a growth chamber study (Abraham et al., 2004). Therefore, further research is needed to compare drought resistance among HBG in field trials, including new releases of HBG.

Even fewer data are available concerning the rooting characteristics of HBG. Suplick-Ploense and Qian (2005) investigated vertical rooting patterns of HBG ('Reveille') and KBG ('Bensun's A-34') and reported that HBG possessed a deeper more extensive root system, a

lower inherent ET rate, and exhibited greater drought avoidance than KBG. A deep, extensive root system is an important mechanism of drought avoidance in turfgrasses (White et al., 1993; Carrow, 1996; Qian et al., 1997, Jiang and Huang, 2001). Presumably, greater root density at deeper depths allows plants to extract soil water deeper in the profile (Fry and Huang, 2004).

Therefore, our objectives were to: 1) measure rooting characteristics of two HBG, a KBG, and a TF under well-watered conditions in the field and greenhouse; 2) measure soil water content in the profile, including at different depths and under water deficit, to evaluate patterns of soil water depletion by roots among species; and 3) measure the canopy characteristics of visual quality and photosynthesis in the same species under well-watered and irrigation-deficit conditions.

Materials and Methods

Study Site and Experimental design

This study was conducted from 3 August to 8 October, 2004 and from 27 June to 15 September, 2005 under an automated rainout shelter (12 m x 12 m) at the Rocky Ford Turfgrass Research Center near Manhattan, KS (39.14^oN, 96.35^oW); Manhattan lies in the U.S. transition zone. The rainout shelter rested north of the study area but automatically covered the research plots as precipitation began and retracted one hour after it ceased. The soil at the site was Chase silt loam (fine, montmorillonitic, mesic, Aquic, Argiudoll).

Plots were arranged in a randomized complete block design with four replications. Thirty two plots (1.36 m x 1.76 m) were bordered by metal edging (10 cm depth) to prevent lateral soil water movement between adjacent plots. Two irrigation treatments included well watered (replacement of 100% of the water lost from plants and soil via evapotranspiration [ET]) and water deficit (replacement of 60% of ET). Water was applied by hand on Monday and Friday twice a week through a fan spray nozzle attached to a hose; a meter (Model 03N31, GPI, Inc., Wichita, KS) was attached to ensure proper application rate. To determine irrigation requirements, evapotranspiration (ET) was calculated by using the Penman-Monteith equation (FAO, 1998) and climatological data obtained at an on-site weather station.

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Prior to seeding in 2003, the plot area was fumigated with dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) at 575 kg a.i.ha^{-1} to kill vegetation and weed, insect, and disease pests. Plots were seeded on 17 September 2003 with a hybrid bluegrass (HBG1 [Thermal Blue]) at 147 kg ha^{-1}, another hybrid bluegrass (HBG2 [Reveille]) at 293 kg ha^{-1} (rate recommended by manufacturer because of lower germination rates), Kentucky bluegrass (KBG [Apollo]) at 147 kg ha^{-1}, and tall fescue (TF [Dynasty]) at 293 kg ha^{-1}. All plots were fertilized with urea at 45 kg N ha^{-1} on 20 April, 73 kg N ha^{-1} on 17 September, and 45 kg N ha^{-1} on 19 November in 2004. In 2005, urea was applied at 54 kg N ha^{-1} on 27 April, 45 kg N ha^{-1} on 19 September, and 45 kg N ha^{-1} on 4 October. Plots were mowed twice a week with a walk-behind rotary mower at 7.62 cm mowing height.

Insecticide applications for controlling billbug grubs and white grubs in 2004 included imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) at 0.44 kg a.i. ha^{-1} on 19 April, bifenthrin (2-methyl [1,1'-biphenyl]3-y1)-methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate) at 0.12 kg a.i. ha^{-1} on 27 May, and halofenozide (Benzoic acid, 4-chloro-, 2-benzoyl-2-(1,1-dimethylethyl)hydrazide) at 1.69 kg a.i. ha^{-1} on 8 July. In 2005, the same insect pests were controlled with imidacloprid at 0.44 kg a.i. ha^{-1} on 18 June and bifenthrin at 0.06 kg a.i. ha^{-1} on 22 June.

Herbicides included dithiopyr (S,S'-dimethyl 2-(difluoro-methyl-4-(2-methylpropyl)-(trifluoromethyl)-3,5-pyridinedicarbothioate) applied at 0.58 kg a.i.ha^{-1} on 27 May in 2004 and 4 May in 2005 to control annual grassy weeds, and carfentrazone-ethyl (0.03 kg a.i. ha^{-1}) + 2,4-D, 2-ethyl hexyl ester (1.29 kg a.i. ha^{-1}) + mecoprop-p acid (0.27 kg a.i. ha^{-1}) + dicamba acid (0.08 kg a.i.ha^{-1}) were applied to control broadleaf weeds on 27 May and 22 October in 2004 and 4 May and 19 October in 2005.

Fungicide applications for controlling summer patch (*Magnoporthe poae*) included azoxystrobin (methyl(E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) at 0.61 kg a.i. ha^{-1} on 21 April, 24 May, and 22 June and triadimefon (1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4- trizol-1-yl)-2-butanone) at 1.53 kg a.i. ha^{-1} on 23 July in 2005.

Measurement of Rooting Characteristics

Field

Root cores (80 cm x 4.8 cm diam.) were collected on 13 October 2005 from well-watered plots using a direct-push coring machine (LWW; Concord Environmental Equipment, Hawley, MN) equipped with a 4.8-cm-i.d. sampling tube (D10006P; Concord Environmental Equipment) and single-use polyethylene terephthalate copolymer plastic liners (1024151; Concord Environmental Equipment). Two root cores were taken from each plot. After extraction, soil cores were cut into three segments representing the 0-30, 30-60, and 60-80 cm profiles and soaked in water for 3 h. After soil was washed from the roots, methyl blue (5 g L^{-1} water) was applied to enhance the image of finer roots. Root length, average root diameter, and root surface area at different segments were then measured using an image analysis system, WinRHIZO (Regent Instruments Inc. QC, CA). After root characteristics were measured, samples were dried in a forced-air oven for 48 h at 70°C and weighed separately. Root length density (RLD) was calculated as root length divided by the volume of soil inside each respective section of root core. Total RLD, average root diameter, and root surface area (0-80 cm profile) were also calculated by each depth. Root characteristics in the two root cores within each plot were averaged as a sample.

Greenhouse

This research was conducted from 13 December 2004 to 23 February 2005 in a greenhouse at Kansas State University. Sod plugs of HBG1 (Thermal blue), HBG2 (Reveille), KBG (Apollo), and TF (Dynasty) were collected from the field with a 3-cm-diam. corer. Six plugs of each species/cultivar were washed to remove the remaining soil, and existing roots were cut from the crown to stimulate new root formation. The plugs were then planted in 24 clear polyethylene tubes (3.5 cm in diam. by 120 cm long) that were filled with 100% calcined clay (Turface, Buffalo grove, IL). Calcined clay inside the tubes was saturated with water before planting grasses. Each tube was inserted into an opaque polyvinyl chloride (PVC) pipe. The method of Qian et al. (1997) was followed to prepare and position the tubes. Briefly, each PVC pipe was 120 cm long and 4 cm inside diam. The bottom was capped by a PVC plug in which

holes were drilled to allow for drainage. Twenty-four tubes were arranged on a tube rack at a 20° angle from vertical in a randomized complete block design with six replications.

Irrigation was applied with a mist system which was automatically turned on 6 times per day for 10 min each. Fertilizer was applied once weekly with irrigation water at 250 ppm N of Peters Peat-lite Special 20-10-20 water-soluble fertilizer (Scotts-Sierra Horticultural Product Co., Marysville, OH). Average day/night air temperature was $24/15^{o}C$ and supplemental light with incandescent lamps was included for 14-h/day. Grasses were clipped weekly at 6.5 cm.

Maximum root extension (MRE) was determined by measuring the length of the deepest root visible at the calcined clay- clear polyethylene tube interface every two weeks. When MRE reached the bottom of the first tube, the study was halted. Tubes were then cut into four 30-cm long sections. Roots in every section were also washed and measured as previously described.

Volumetric Soil Water Content

In all field plots, volumetric soil water content (θ_v) in the 0 to 50 cm profile was measured weekly in 2004 and 2005 using time domain reflectometry (TDR) (model 6050XI, Soilmoisture Equipment, Santa Barbara, CA); data from 2004 were discarded because of an instrument error. In drought plots, volumetric soil water content (θ_v) was also measured near the surface at 5 cm in 2004 and 2005, which presumably was a region of denser root mass compared to deeper depths (Ervin and Koski, 1998; Turgeon, 2002; Su et al., 2007). These θ_v measurements near the soil surface were used to investigate possible differences in root activity (i.e., water absorption) among HBG1, KBG, and TF during drought. Soil moisture was not measured at 5 cm in HBG2 because of practical limitations in sensor availability and datalogging capacity. The θ_v at 5 cm was measured using the dual-probe heat-pulse (DPHP) technique (Campbell et al., 1991; Tarara and Ham, 1997; Song et al., 1998); DPHP sensors were fabricated in the authors' laboratory as described by Basinger et al. (2003) and Bremer (2003). Measurements of θ_v were logged twice daily at 0600 and 1800 CST. All data acquisition and control were accomplished with a micrologger and accessories (CR10x, two AM16/32's, and one M25T, Campbell Scientific, Logan, UT).

Canopy Characteristics

Turf visual quality was rated on a scale of 1 to 9 (1=poorest quality, 6=minimally acceptable, and 9=highest quality) according to color, texture, density, and uniformity (Emmons, 2000). Quality ratings were recorded weekly in 2004 and 2005 by the same individual during the 2-year study.

Photosynthesis and respiration were measured biweekly on clear days in 2004 and 2005 between 1000 and 1400 CST with a LI-6400 portable gas exchange system (LI-COR Inc., Lincoln, NE) using a custom surface chamber described by Bremer and Ham (2005). Permanent PVC collars (10-cm diam.) were placed randomly at one location in each plot and were driven approximately 5 cm into the soil. The collars and foam gaskets provided an effective seal between the surface chamber and soil during photosynthesis and respiration measurements.

Calculations and Data Analysis

Gross photosynthesis in each plot was estimated from measurements of photosynthesis and respiration according to the method of Bremer and Ham (2005). Briefly, this method involves consecutive measurements with a sunlit and shaded chamber, respectively, at each collar; shaded measurements were obtained by covering the chamber with an opaque cloth that blocked solar radiation from the chamber. Using Equations 5 and 6 from Bremer and Ham (2005), sunlit measurements were used to determine $P_g - (R_c + R_s')$ and shaded chamber measurements to determine $R_c + R_s'$, where P_g is gross photosynthesis, R_c is canopy respiration, and R_s' is residual soil respiration in a pressurized chamber; all values are positive and units are $\mu mol\ CO_2\ m^{-2}s^{-1}$. Gross photosynthesis was calculated using their equation 8: P_g = sunlit chamber + shaded chamber.

All data were analyzed with the mixed procedure of SAS (SAS Institute Inc., Cary, NC). There were no significant interactions between turfgrass species and irrigation level on any given day after treatment initiation (Days of treatment) for θ_v in the 0-50 cm profile, visual quality, and P_g. Therefore, all differences between means were separated by the SAS PDIFF option (P=0.05). Average values of visual quality and Pg in each year were calculated by averaging each respective variable across all measurement days during the period of treatment.

Results and Discussion

Rooting characteristics and soil moisture in the field

In the 0-30 cm profile, RLD, dry root weight, and root surface area were the greatest in HBG1 and similar among HBG2, KBG, and TF (Table 2-1). Greater roots in the upper profile apparently resulted in a greater extraction of soil moisture near the surface by HBG1 than in KBG or TF (Fig. 2-1). At 5 cm in the drought treatment, the pattern of θ_v was consistently lower in HBG1 than in KBG and TF during both years although daily differences in θ_v were not significant among HBG1, KBG, and TF.

In the entire 80-cm profile in the field, HBG1, HBG2, KBG, and TF had 96%, 93%, 90%, and 86% of all root length in the top 30 cm of soil, respectively (Table 2-1). Therefore, most roots among species were distributed in shallow soil although the percentage was lowest in TF. Conversely, in the lower profiles (i.e., 30-60 cm and 60 to 80 cm) dry root weight and average root diameter were greatest in TF among the four turfgrasses. In the deepest part of the measured profile (60 to 80 cm), RLD and root surface area were also greatest in TF among turfgrasses. Thus, TF had more roots deeper in the profile than HBG1, HBG2, and KBG. Interestingly, the roots deep in the profile (60-80 cm) in TF were only 4% of all root length in the entire profile (0-80 cm). Greater quantities of roots deep in the soil, however, may play an important role in a plant's ability to avoid drought stress even if deep roots are a small percentage of the total root system (Garrot and Mancino, 1994; Carrow 1996).

Root characteristics were similar among HBG1, HBG2, and KBG at lower depths (Table 2-1). Our results contradicted those of Suplick-Ploense and Qian (2005), who found that HBG2 (Reveille) exhibited greater RLD at each soil depth (0-20, 20-40, and 40-60 cm) and total root mass than a KBG (Bensun's A-34). In our study the total RLD and root surface area in the 0 to 80 cm profile were lower in HBG2 than in KBG. These differing results may be attributable to the different cultivars of KBG used between studies, and perhaps to different soils and climates between sites.

In the 0 to 50 cm profile, θ_v was similar among HBG1, KBG and TF, but θ_v was consistently lower in HBG2 under well-watered and drought conditions (Fig. 2-2). Lower θ_v indicates that HBG2 (Reveille) extracted more water from the profile than HBG1, KBG, and TF given equal irrigation amounts. Presumably, greater water-extraction in soils is positively related to root characteristics (e.g., greater RLD and root surface area) (Carrow, 1996; Fry and Huang,

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2004; Fu et al., 2007). In our study, however, all measured root parameters in the 0-80 cm profile were numerically lowest in HBG2 among 4 turfgrasses (Table 2-1). This suggests that the rooting characteristics measured in our study were not necessarily an indication of root activity (i.e., capacity to absorb water).

Leaf characteristics (e.g., green leaf area index), which were not investigated in this study, may also directly impact ET and water use in turfgrass. For example, Fu and Huang (2003) reported that drought resistance in tall fescue is positively related to leaf thickness, epicuticular wax content, and tissue density but negatively related to stomatal density and leaf width. More information is needed on the relationship between physical root characteristics and root activity (e.g., root water potential, root electrolyte leakage, and root life span), and on leaf characteristics and their effects on water use in the particular cultivars used in our study.

Rooting characteristics in the greenhouse

In the first two weeks of the study, roots grew fastest in HBG1 among turfgrasses and at the end of the first month, MRE in HBG1 remained greater than in KBG (Fig. 2-3). After the first month, however roots grew faster in TF than in HBG1, HBG2, and KBG and differences in MRE diminished among HBG1, HBG2, and KBG (Fig. 2-3). By the end of the evaluation period (70 days after planting), MRE was 15 to 30% greater in TF than in the other three turfgrasses (Fig.2-3). Greater MRE in TF may partially explain faster establishment of TF than HBG (Thermal Blue and Dura Blue) and KBG in a field experiment in Kansas, USA (Bremer et al., 2006).

Tall fescue exhibited the greatest dry root weight and average root diameter at each depth (0-30, 3-60, 60-90, and 90-120) (Table 2-2). Seventy-four to 80% of all root length (0-120 cm deep) was in the upper 30 cm among KBG, HBG1, and HBG2, while only 70% of TF all root length was in the top 30 cm; this was similar to results from our field study. In the upper 30 cm, HBG1 had the greatest RLD and root surface area among turfgrasses, which is also consistent with results from our field study (Table 2-1).

Below 60 cm, TF also generally had greater RLD, dry root weight, average root diam., and root surface area than the other 3 turfgrasses, the lone exception being a similar RLD to KBG in the 60 – 90 cm layer (Table 2-2). In the deepest section (90-120 cm), TF was the only grass with measurable root surface area and dry root weight (Table 2-2). Overall, these results

were similar to the field study and indicated TF may be able to extract soil water from deeper depths than HBG1, HBG2, and KBG. A deep extensive root system, which is an important drought avoidance mechanism, has also been observed in TF in other studies (White et al., 1993; Carrow, 1996; Qian et al., 1997, Jiang and Huang, 2001).

Canopy characteristics in the field
Visual quality

In well-watered conditions, visual quality in TF was generally highest among the four turfgrasses during both years (Figs. 2-4A and 2-4B). Higher performance in TF than in HBG1 and KBG was also reported in another nearby field study (Bremer et al., 2006); HBG2 was not evaluated in that study. In 2004, visual quality of HBG2 was lower than the other grasses early in the treatment period, but differences diminished among bluegrasses and by 32 DOT, visual quality was similar among HBG1, HBG2, and KBG. In 2005, visual quality was lower in HBG1 than in HBG2 on 25, 31, 37, and 45 DOT (Fig. 2-4B). Lower visual quality in HBG1 in 2005 was likely caused by summer patch (*Magnaporthe poae*), which affected HBG1 more seriously than HBG2.

In the irrigation-deficit treatment, the visual quality of TF was consistently higher than the other turfgrasses during both years (Fig. 2-4C, D). The deep root system of TF (Table 2-1; White et al., 1993; Carrow, 1996; Qian et al., 1997, Jiang and Huang, 2001) likely contributed to its drought avoidance. In 2004, there were no differences in average visual quality among HBG1, HBG2, and KBG in the irrigation-deficit treatment. In 2005, however, visual quality of HBG2 was higher than HBG1 on 25, 31, and 37 DOT and KBG on 25 and 37 DOT (Fig. 2-4D). This indicated that HBG2 performed better than HBG1 under water deficit, heat stress, and during exposure to summer patch.

Gross Photosynthesis (P_g)

In well-watered plots, P_g was generally greatest in TF among turfgrasses during both years (Figs. 2-5A and 2-5B). The P_g in TF averaged 42 to 82% and 28 to 66% greater than Pg in the other 3 turfgrasses in 2004 and 2005, respectively. During both years, average P_g was similar among HBG1, HBG2, and KBG although Pg tended to be greater in KBG than HBG1 and HBG2; P_g in KBG averaged 28% higher than in HBG2 in 2004 and 29% higher than in HBG1 in

2005. Higher P_g in TF among turfgrasses may have been caused in part by higher green leaf area index in TF (Lee, 2008).

In the irrigation-deficit treatment, P_g was greatest in TF among turfgrasses in 2004. In HBG2, P_g was greater than HBG1 on 24 and 31 DOT and than KBG on 24, 31, and 38 DOT (Fig. 2-5C). In 2004, the average P_g of TF was 39 to 74% greater than that of the other three turfgrasses under the drought treatment. In 2005, P_g was greater in TF than in HBG1 and KBG during the first two weeks. Thereafter, P_g became similar among cultivars/species although the trend of higher P_g in TF apparently continued (Fig. 2-5D). Late in 2005, P_g decreased among turfgrasses, which was contrary to the upward trend late in 2004. The decline in 2005 was likely caused by a combination of drought stress and corresponding high temperature stress which did not occur in 2004; the drought treatment in 2004 ended in October, when temperature had cooled compared with 2005, when the drought treatment ended in early September. For example, weekly daytime air temperature (1000-1800 CST) after 28 DOT averaged 26.2 to 32.2 ^{o}C in 2005, but was only 19.4 to 28.5 ^{o}C in 2004. In 2005, P_g averaged greater in TF than in HBG1 and KBG, but was statistically similar to HBG2 under the drought treatment.

Recovery after drought

In 2005, after termination of the irrigation-deficit treatment and upon re-watering (on 70 DOT), HBG1 and HBG2 recovered more quickly than KBG from irrigation-deficit and summer stresses. Visual quality was statistically similar among HBG1 (4.0), HBG2 (4.8), and KBG (4.0) at the end of the irrigation-deficit treatment. At the end of the 4-wk recovery period, however, visual quality was higher in HBG1 (8.0) and HBG2 (8.0) than in KBG (7.3). Faster recovery in HBG after drought indicated a slight advantage in HBG1 and HBG2 over KBG as related to their drought resistances.

Conclusions

Under well-watered conditions, most roots were distributed in the shallowest soil layer (0-30 cm) although TF had fewer roots in the upper soil profile among turfgrasses. Tall fescue had a more developed root system at deeper depths, however, and its roots grew faster than the other three turfgrasses. In the upper layer (0-30 cm), HBG1 had the greatest RLD and root surface area among the four grasses although that didn't translate to greater performance in

HBG1 during irrigation-deficit, after water near the surface was depleted. In the irrigation-deficit treatment, HBG2 (Reveille) used more water from the 0-50 cm profile and performed better than HBG1 (Thermal Blue). Both HBG recovered from drought slightly faster than KBG.

In general, TF exhibited the highest visual quality and P_g among turfgrasses under irrigation-deficit and well-watered conditions. Our results were similar to those of Bremer et al. (2006), who reported that TF may be better suited than HBG in areas of the transition zone where soils are deep and if drought resistance is a priority. Further research may be needed to compare the drought resistance of newly released HBG with KBG and TF, including in areas of the transition zone with different soil types and depths. The performances of the turfgrasses in our field study, as evaluated by visual quality and Pg, generally ranked: TF > Reveille >= Thermal Blue = KBG.

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Figures and Tables
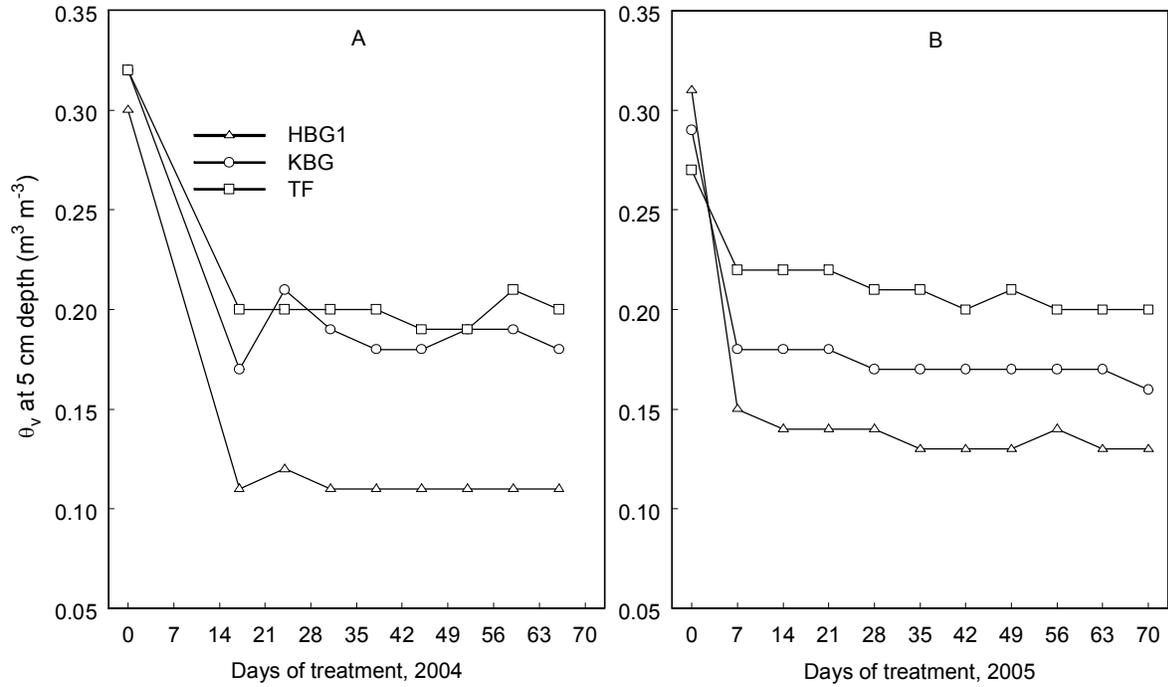
Figure 2-1 Volumetric soil water content (θ_v) at 5 cm in 2004 (A) and 2005 (B) in the irrigation-deficit treatment.

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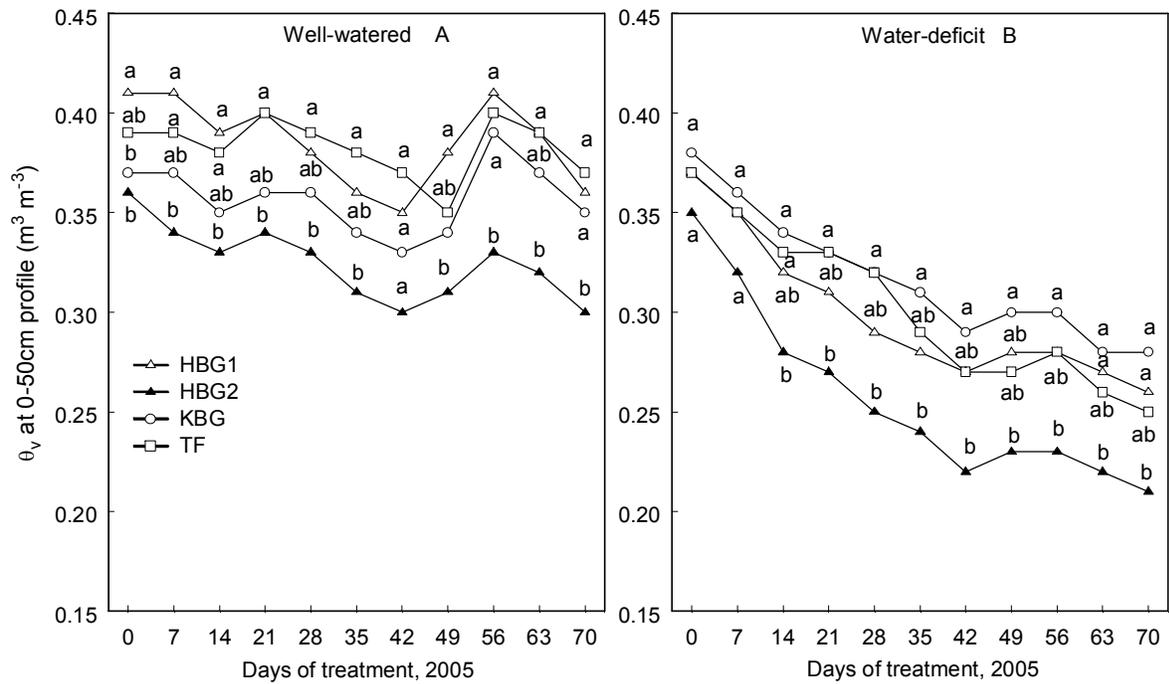
Figure 2-2 Volumetric soil water content (θ_v) in the 0-50 cm profile under well-watered (A) and irrigation-deficit (B) conditions in 2005. Means followed with the same letter on a given day after treatment initiation (Days of treatment) are not significantly different (P=0.05).
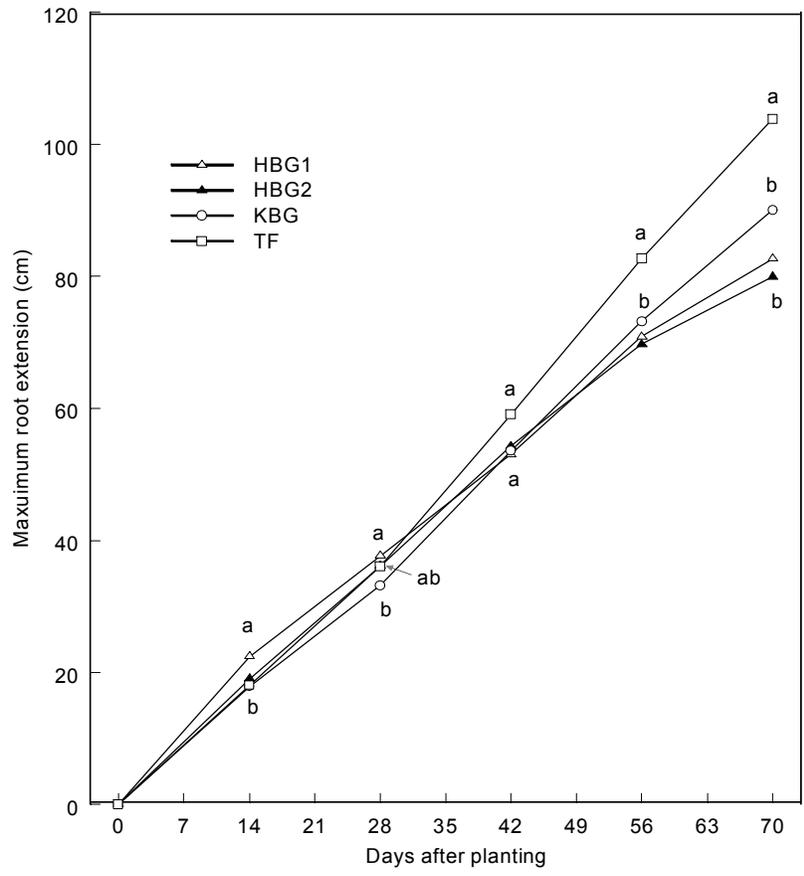
Figure 2-3 Maximum root extension (MSE) among turfgrasses under well-watered conditions in the greenhouse. Means followed with the same letter on a given day after planting are not significantly different (P=0.05).
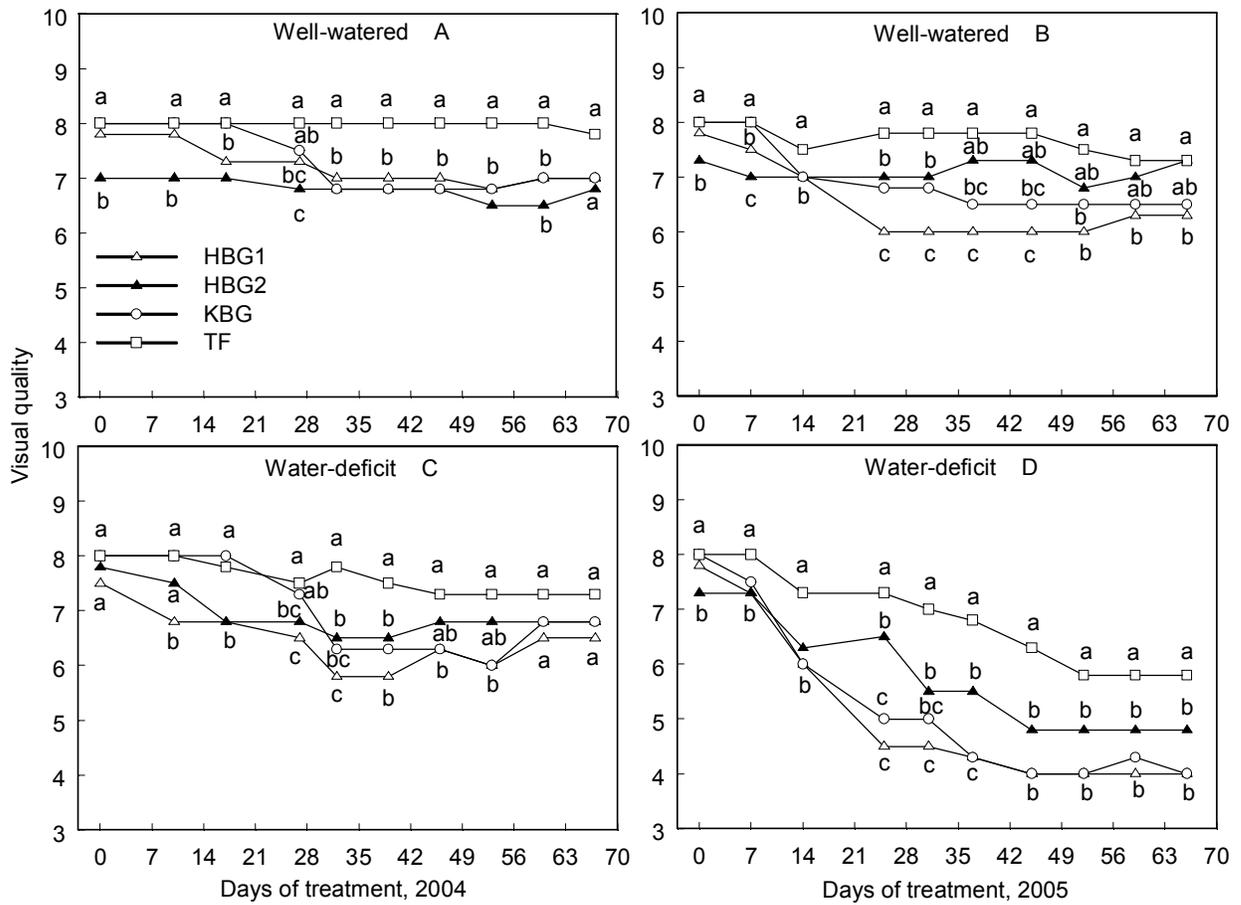
Figure 2-4 Visual quality among turfgrasses under well-watered (A, B) and irrigation-deficit (C, D) conditions in 2004 and 2005. Means followed with the same letter on a given day after treatment initiation (Days of treatment) are not significantly different (P=0.05).
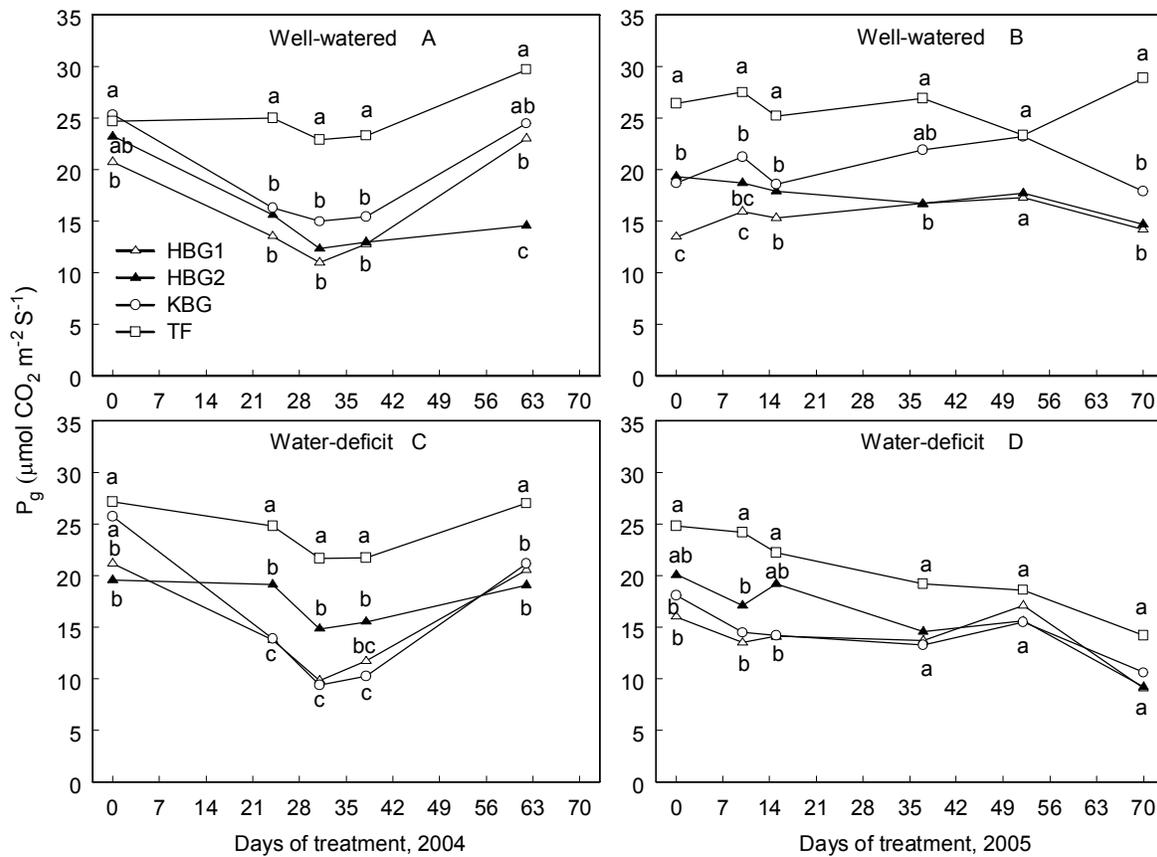
Figure 2-5 Gross photosynthesis (Pg) among turfgrasses under well-watered (A, B) and irrigation-deficit (C, D) conditions in 2004 and 2005. Means followed with the same letter on a given day after treatment initiation (Days of treatment) are not significantly different (P=0.05).

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Table 2-1 Root length density (RLD), dry root weight, average root diameter, and root surface area in different depths of HBG1 (Thermal Blue), HBG2 (Reveille), KBG (Apollo), and TF (Dynasty) under well-watered conditions in the rainout shelter at Manhattan, KS, in 2005.

Soil depth (cm)	Species	RLD (cm cm^{-3})	Dry root weight (g)	Average root diam (mm)	Root surface area (cm^2)
0-30	HBG1	14.10 a[†]	0.569 a	0.33 a	785 a
	HBG2	7.28 b	0.321 b	0.29 b	361 b
	KBG	10.35 b	0.401 b	0.31 a	547 b
	TF	8.17 b	0.408 b	0.32 a	454 b
30-60	HBG1	0.63 a	0.011 b	0.20 b	22 a
	HBG2	0.50 a	0.012 b	0.19 b	18 a
	KBG	1.08 a	0.019 b	0.20 b	39 a
	TF	0.97 a	0.036 a	0.29 a	48 a
60-80	HBG1	0.05 b	0.002 b	0.18 b	1 b
	HBG2	0.07 b	0.003 b	0.17 b	2 b
	KBG	0.19 b	0.004 b	0.17 b	4 b
	TF	0.61 a	0.013 a	0.29 a	20 a
0-80 (Total)	HBG1	5.54 a	0.581 a	0.32 a	808 a
	HBG2	2.93 c	0.336 b	0.28 b	381 c
	KBG	4.33 ab	0.423 ab	0.30 b	591 b
	TF	3.58 bc	0.457 ab	0.32 a	522 bc

[†] Means followed by the same letter within a specific soil depth and a column were not significantly different (P=0.05).

Table 2-2 Root length density (RLD), dry root weight, average root diameter, and root surface area in different depths of HBG1, HBG2, KBG, and TF under well-watered condition in the greenhouse in 2005. Dash (-) indicates amounts <0.001 g in dry root weight, <0.01 cm cm^{-3} in RLD, and < 1 cm^2 in root surface area, respectively.

Soil depth (cm)	Species	RLD (cm cm^{-3})	Dry root weight (g)	Average root diam (mm)	Root surface area (cm^2)
0-30	HBG1	41.75 a[†]	1.040 ab	0.33 b	1250 a
	HBG2	19.17 c	0.778 c	0.34 b	596 c
	KBG	34.46 b	0.892 bc	0.33 b	1014 b
	TF	18.90 c	1.199 a	0.42 a	728 c
30-60	HBG1	9.68 a	0.185 b	0.25 c	218 ab
	HBG2	5.91 b	0.169 b	0.29 b	154 b
	KBG	10.26 a	0.219 b	0.26 c	242 a
	TF	5.76 b	0.324 a	0.40 a	210 ab
60-90	HBG1	0.81 b	0.015 b	0.20 c	16 c
	HBG2	0.83 b	0.018 b	0.23 bc	19 c
	KBG	1.82 a	0.029 b	0.24 b	40 b
	TF	2.10 a	0.100 a	0.40 a	76 a
90-120	HBG1	-	-	0.06 b	-
	HBG2	-	-	0.04 b	-
	KBG	0.01 b	-	0.11 b	-
	TF	0.40 a	0.016 a	0.42 a	15 a
0-120 (Total)	HBG1	13.06 a	1.240 b	0.31 b	1484 a
	HBG2	6.48 b	0.965 c	0.33 b	769 c
	KBG	11.64 a	1.141 bc	0.31 b	1296 a
	TF	6.79 b	1.638 a	0.42 a	1028 b

[†] Means followed by the same letter within a specific soil depth and a column were not significantly different (P=0.05).

CHAPTER 3 - Mowing Height and Drought Effects on a Texas Bluegrass Hybrid Compared with Kentucky Bluegrass

Abstract

Texas bluegrass hybrids (HBG) resemble Kentucky bluegrass (*Poa pratensis* L.)(KBG) but HBG may have greater heat and drought tolerance. Little is known about the performance of HBG at low mowing and during drought. A two-year field study was conducted near Manhattan, Kansas, USA to investigate effects of mowing and drought on visual quality, gross canopy photosynthesis (P_g), and spectral reflectance in a KBG ('Apollo') and HBG ('Thermal Blue'). Treatments included three main factors at two levels each: 1) species (KBG and HBG); 2) mowing height (high =7.62 cm and low =3.81 cm); and 3) irrigation (100 and 60% ET replacement). Visual quality in HBG was equal to or lower than KBG during 2004 and 2005. At low mowing, visual quality in HBG was 9% to 13% lower than in KBG in 2005. During both years, normalized difference vegetation index (NDVI) was lower in HBG than in KBG except for late in 2004, indicating lower green leaf area and biomass and lower visual quality in HBG. Early in the study of both years, P_g was lower in HBG than in KBG but differences converged thereafter. Visual quality, NDVI, and P_g declined with low mowing in both species in 2004 but only in HBG in 2005. Drought (60% ET replacement) reduced visual quality and NDVI of both grasses as the study progressed during both years. Generally, this HBG performed similar to or worse than this KBG. Further research is needed to identify new cultivars of HBG that may perform better than KBG at low mowing and during drought.

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Abbreviations: ET, evapotranspiration; HBG, a hybrid between Texas bluegrass and Kentucky bluegrass; KBG, Kentucky bluegrass; NDVI, normalized difference vegetation index; P_g, gross photosynthesis.

Introduction

A growing challenge facing the turfgrass industry is limited availability of water for irrigation (Snow, 2001). Local water-use restrictions may be imposed during drought that limit growth and cause severe declines in the visual quality of many cool-season turfgrasses (Perdomo et al., 1996; Bonos and Murphy, 1999). On golf courses, lower mowing heights in fairways may result in additional stress to turfgrass during drought because lower mowing typically reduces root growth and development (Parr et al., 1984; Liu and Huang, 2002). Research is needed to identify species or cultivars of cool-season turfgrasses that may perform better under drought stress and at lower mowing heights (2 to 4 cm).

Hybrid bluegrasses (HBG), which are genetic crosses between native Texas bluegrass and Kentucky bluegrass (KBG), may have greater drought and heat resistance than other cool-season grasses (Read et al., 1999). Hybrid bluegrasses have similar visual qualities as KBG, which is a fine-textured cool-season turfgrass that is commonly used on athletic fields and golf course fairways and roughs (Turgeon, 2002). For performance of KBG in the transition zone of the U.S., however, there are some difficulties with heat and drought stress and disease; the transition zone covers 480 to 1120 km north to south between the northern regions where cool-season grasses are adapted and the southern regions where warm-season grasses are adapted (Dunn and Diesburg, 2004). Consequently, new cultivars of HBG are being investigated as potential drought-resistant alternatives.

Limited data are available regarding the drought resistance of HBG. In a growth chamber study, the drought resistance of thirty cultivars of HBG and their genetic parents varied significantly (Abraham et al., 2004). In the same experiment, highly drought resistant HBG were the result of selecting first generation hybrids with good drought resistance and backcrossing them with elite drought resistant genotypes of KBG. In field tests in Colorado, USA, 'Reveille' HBG used significantly less water while maintaining higher quality than 'Bensun's A-34' KBG (Suplick-Ploense and Qian, 2005). Bremer et al. (2006) reported little difference in the general performance or drought resistance among two HBG (Thermal Blue and 'Dura Blue') and a KBG (Apollo) in a field study in Kansas, USA.

Even fewer data are available concerning the performance of HBG at different mowing heights, although lower mowing heights (< 3 cm) are typical in golf course fairways compared

with lawns. Stier et al. (2005) reported that HBG (Thermal Blue and Dura Blue) and KBG (Apollo and 'Unique') had similar visual quality at mowing heights of 2.5, 5.0, and 7.5 cm, and that Thermal Blue and Dura Blue may be acceptable replacements for KBG in the Upper Midwest, USA; those authors also reported earlier spring greenup in HBG than in KBG. Results from a study conducted further south in the transition zone where summers are warmer, however, indicated that mowing height for Thermal Blue should be greater than 3.5 cm to avoid decreased turf visual quality in late summer and fall (Teuton, 2006).

The combined effects of mowing height and drought on HBG have not been investigated. Therefore, the objectives of this two year study were to evaluate the effects of mowing height and drought stress on visual quality, photosynthesis, canopy spectral reflectance, and soil water content in a KBG and a HBG and to compare their performance under each treatment.

Materials and Methods

Study site and Experimental design

This study was conducted from 3 August to 8 October, 2004 and from 27 June to 15 September, 2005 under an automated rainout shelter (12 m x 12 m) at the Rocky Ford Turfgrass Research Center near Manhattan, Kansas (39.14^oN, 96.35^oW); Manhattan lies in the U.S. transition zone. The rainout shelter rested north of the study area but automatically covered the research plots as precipitation began and retracted one hour after precipitation ceased. The soil at the site was a Chase silt loam (fine, montmorillonitic, mesic, Aquic, Argiudoll).

The experiment was arranged in a randomized complete bock design with whole plot treatments in a two (mowing height) by two (irrigation) factorial (Fig. 3-1). Species was a split-plot factor. The mowing height factor (high mowing = 7.62 cm and low mowing = 3.81 cm) was randomized in a whole-plot strip to one of the two rows in each block. The irrigation factor (100% and 60% ET replacement) was arranged in a balanced design to the two columns in each block (Cochran and Cox, 1992). Therefore, in a block, each of the four combinations of mowing height by irrigation treatments were applied to two plots and the two species (HBG and KBG) were randomly seeded in those two plots. In this experimental design, arrangement of irrigation treatments was balanced across the four blocks.

Thirty two plots (1.36 m x 1.76 m) were bordered by metal edging (10-cm depth) to prevent lateral soil water movement between adjacent plots. Plots were seeded on 17 September 2003 with Apollo KBG and Thermal Blue HBG at a rate of 147 kg/ha. Plots were mowed twice a week with a walk-behind rotary mower. Water was applied twice a week on Monday and Friday through a fan spray nozzle attached to a hose; a meter (Model 03N31, GPI, Inc., Wichita, KS) was attached to ensure proper application rate. To determine irrigation requirements, evapotranspiration (ET) was calculated by using the Penman-Monteith equation (FAO, 1998) from climatological data obtained at a weather station located at the research site.

Plot Maintenance

Prior to seeding in 2003, the plot area was treated with 575 kg a.i./ha of dazomet (tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione) to kill vegetation and weed, insect, and disease pests. All plots were fertilized with urea at 163 kg N/ha in April, September, and November, 2004 and at 144 kg N/ha in April, September, and October, 2005. Insecticide applications for controlling billbug grubs and white grubs in 2004 included imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) at 0.44 kg a.i./ha on 19 April , bifenthrin (2-methyl [1,1'-biphenyl]3-y1)-methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropanecarboxylate) at 0.12 kg a.i./ha on 27 May, and halofenozide (Benzoic acid, 4-chloro-, 2-benzoyl-2-(1,1-dimethylethyl)hydrazide) at 1.69 kg a.i./ha on 8 July. In 2005, the same insect pests were controlled with imidacloprid at 0.44 kg a.i./ha on 18 June and bifenthrin at 0.06 kg a.i./ha on 22 June. Dithiopyr (S,S'-dimethyl 2-(difluoro-methyl-4-(2-methylpropyl)-(trifluoromethyl)-3,5-pyridinedicarbothioate) was applied at 0.58 kg a.i./ha of on 27 May in 2004 and 4 May in 2005 to control annual grassy weeds. Broadleaf weeds were controlled with carfentrazone-ethyl (0.03 kg a.i./ha)+ 2, 4- D, 2-ethyl hexyl ester (1.29 kg a.i./ha) + mecoprop-p acid (0.27 kg a.i./ha) + dicamba acid (0.08 kg a.i./ha) on 27 May and 22 October in 2004 and 4 May and 19 October in 2005. Fungicide applications for controlling summer patch (*Magnoporthe poae*) included azoxystrobin (methyl(E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) at 0.61 kg a.i./ha on 21 April, 24 May, and 22 June and triadimefon (1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4- trizol-1-yl)-2-butanone) at 1.53 kg a.i./ha on 23 July in 2005.

Measurements

Turf quality was rated visually on a scale of 1 to 9 (1=poorest quality, 6=minimally acceptable, and 9=highest quality) according to color, texture, density, and uniformity (Emmons, 2000). Quality ratings were recorded weekly by the same individual during the 2-year study.

Photosynthesis was measured biweekly on clear days between 1000 and 1400 CST with a LI-6400 portable gas exchange system (LI-COR Inc., Lincoln, NE) using a custom surface chamber described by Bremer and Ham (2005). Permanent polyvinyl chloride collars (10-cm diam.) were placed randomly at one location in each plot and were driven approximately 5 cm into the soil. A closed-cell foam gasket was used to maintain an effective seal between collars and the chamber during photosynthesis measurements.

Canopy spectral reflectance was measured weekly on clear days at approximately 1200 CST with a Cropscan multispectral radiometer (MSR) (model MSR16, Cropscan Inc., Rochester, MN). Canopy reflectance was measured in eight wavelengths including 507, 559, 613, 661, 706, 760, 813, and 935 nm. On measurement days, each plot was consecutively measured twice and the data were later averaged as a sample.

In all plots, the volumetric soil water content (θ_v) in the 0 to 50 cm profile was measured weekly in 2004 and 2005 using time domain reflectometry (TDR) (model 6050XI, Soilmoisture Equipment, Santa Barbara, CA); data from 2004 were discarded because of an instrument error. In drought plots, additional θ_v measurements were collected at a shallower depth (5 cm) in 2004 and 2005, which presumably is a region of denser root mass compared to deeper in the profile (Ervin and Koski, 1998; Turgeon, 2002; Su et al., 2007). These θ_v measurements near the soil surface were used to investigate possible differences in root activity (i.e., water absorption) between HBG and KBG in plots mowed at low and high heights, including during drought. The θ_v at 5 cm was measured using the dual-probe heat-pulse (DPHP) technique (Campbell et al., 1991; Tarara and Ham, 1997; Song et al., 1998); DPHP sensors were fabricated in the authors' laboratory as described by Basinger et al. (2003) and Bremer (2003). Measurements of θ_v at 5 cm were automated and logged twice daily at 0600 and 1800 CST. All data acquisition and control were accomplished with a micrologger and accessories (CR10x, two AM16/32's, and one AM25T, Campbell Scientific, Logan, UT).

Calculations and Data analysis

Gross photosynthesis in each plot was estimated from measurements of photosynthesis and respiration according to the method of Bremer and Ham (2005). Briefly, this method involves consecutive measurements with a sunlit and shaded chamber, respectively, at each collar; shaded measurements were obtained by covering the chamber with an opaque cloth that blocked solar radiation from the chamber. Using Equations 5 and 6 from Bremer and Ham (2005), sunlit measurements were used to determine $P_g - (R_c + R_s')$ and shaded chamber measurements to determine $R_c + R_s'$, where P_g is gross photosynthesis, R_c is canopy respiration, and R_s' is residual soil respiration in a pressurized chamber; all values are positive and units are μmol CO_2 $m^{-2}s^{-1}$. Gross photosynthesis was calculated using their equation 8: P_g = sunlit chamber + shaded chamber.

Using spectral reflectance data, the normalized difference vegetation index (NDVI) was computed as ([R935-R661] / [R935 +R661]) (Trenholm et al., 2000).

All data except for θ_v at 5 cm were pooled into three periods per year to evaluate treatment effects as the study progressed: 1) early (0-7 days of treatment); 2) mid (8-28 days of treatment); and 3) late (29-70 days of treatment). In each period, values of visual quality, NDVI, P_g, and θ_v (0-50 cm) were averaged across all measurement days during the period and the values were then analyzed with the mixed procedure of SAS (P=0.05; SAS Institute Inc.,Cary, NC). The model contained three factors: species, mowing height, irrigation, and their interactions. No significant interactions were found among main factors with the exception of species by mowing height in 2005 for the variables visual quality (early and mid periods) and θ_v at 0-50 cm (mid and late periods). For θ_v at 5 cm, weekly averages were analyzed, also with the mixed procedure of SAS, to evaluate differences in θ_v among treatments as the study progressed. Because soil moisture sensors were placed at 5 cm only in drought plots, the model contained only two factors (species and mowing height); sensors were only placed in drought plots because of practical limitations in sensor availability and datalogging capacity.

Results and Discussion

Because there were few interactions among the main factors of species, drought, and mowing height, data are presented and discussed by main factor. Interactions for visual quality and θ_v at 0-50 cm for certain periods in 2005 are denoted in the text and figures.

Species

The visual quality of HBG was generally similar to or lower than KBG (Fig. 3-2). For example, visual quality was similar between species in the early and late periods of 2004 but was lower in HBG than in KBG in the mid period of 2004 and the late period of 2005 (Fig. 3-2A). Although there was a significant species by mowing height interaction in visual quality during the early and mid periods of 2005, the trends revealed consistently lower visual quality in HBG than in KBG at both mowing heights (Fig. 3-2B; Murray et al., 1999). During the early and mid periods of 2005, visual quality in HBG ranged from 3 to 13% lower than in KBG at both mowing heights, with greater differences between species at low mowing. Lower visual quality in HBG was caused, in part, by its lighter color and coarser texture than KBG.

During both years, NDVI was lower in HBG than KBG for all periods except for late in 2004 (Figs. 3-3A and 3-3B). Lower NDVI may indicate lower visual quality (Trenholm et al., 1999; Lee et. al., 2006), but also suggests less green leaf area index and biomass and hence, less photosynthetic activity in HBG than in KBG (Asrar et al., 1984; Daughtry et al., 1992; Gallo et al., 1985; Goward and Huemmrich, 1992).

In the early period of both years, P_g was lower in HBG than in KBG (Figs. 3-4A and 3-4B), but differences between species diminished as the study progressed and presumably as drought stresses became more severe. In the mid and late periods of both years, no statistical differences (P=0.05) were observed in P_g between KBG and HBG although P_g was always numerically lower in HBG. The lack of difference in P_g between HBG and KBG in the mid and late periods of both years reveals there is no advantage in the photosynthetic capacity during drought of this HBG compared with KBG.

In the early period of 2005, θ_v in the 0-50 cm profile was 6% higher in HBG than in KBG (data not shown). Although there was a significant species by mowing height interaction during the mid and late periods of 2005, θ_v at 0-50 cm was also 19 to 22% higher in HBG than in KBG in low-mown plots (Fig. 3-5A); θ_v at 0-50 cm was similar between species at the high mowing

height during these periods. These results indicate that HBG extracted less water from the 0-50 cm profile than KBG, particularly at the low mowing height.

In low-mown drought plots, θ_v was also consistently higher in HBG than KBG at shallower depths (i.e., 5 cm) during both years (Figs. 3-6C and 3-6D). This indicates that when mown low and soil moisture is limiting, HBG extracts less water than KBG in a region of the soil profile where root density and soil-surface evaporation rates are presumably highest . Interestingly, the trend in θ_v and hence, soil water extraction at 5 cm was reversed in high-mown drought plots, because θ_v was consistently lower in HBG than in KBG (Figs. 3-6A and 3-6B). This indicates that when mown high, HBG extracts more water than KBG at shallower depths when soil moisture is limited. Higher mowing, which may improve rooting capacity (Parr et al., 1984; Liu and Huang, 2002), may have benefited root development in the upper profile more in HBG than in KBG.

Mowing height

Lower mowing reduced the visual quality of both species by 7 to 10% in 2004 (Fig. 3-7). In 2005, visual quality was not affected by mowing in the late period. As indicated earlier, however, there was a significant interaction in species by mowing height in visual quality during the early and mid periods of 2005. Interestingly, low mowing reduced visual quality in HBG by 4 to 5% in HBG during those periods in 2005, but the visual quality of KBG was 2 to 6% greater at the low than at the high mowing height (Fig. 3-2B). Our results generally indicated a poorer tolerance to low mowing in HBG than in KBG, and are similar to results from another transition-zone study (Teuton, 2006) that reported lower visual quality in Thermal Blue when it was mowed at or less than 3.5 cm.

Lower mowing reduced NDVI in three periods of 2004 (Fig. 3-8A), which probably indicates a reduction in green LAI and aboveground biomass with mowing (Asrar et al., 1984; Goward and Huemmrich, 1992) and may reflect a general reduction in visual quality (Trenholm et al., 1999; Lee et. al., 2006) (Fig. 3-7). In the three periods of 2005, NDVI was similar between high and low mowing heights (Fig. 3-8B). Our data indicate significant reductions in NDVI with mowing only in 2004. This was probably caused by some scalping of the turfgrass after the first mowing in low mowing height in late June, which was before the study began; the scalping exposed the leaf sheaths and resulted in a lighter color than in high mowing plots. The mowing

height in low mowing was later raised from 2.54 cm to 3.81 cm (12 July, 2004) to allow for recovery from scalping, and mowing height remained 3.81 cm thereafter. Thus, the mowing height in low–mown plots was 3.81 cm during the entire study, but residual effects of scalping may have affected reflectance data well into the evaluation period.

Lower mowing reduced P_g by 29 to 38% in 2004 and by 14 to 26% during 2005 although the reduction was not statistically significant for the mid period of 2005 (Figs. 3-8C and 3-8D). Greater reductions in P_g with mowing in 2004 may have been caused in part by the scalping that occurred earlier in the summer, before the study began. Presumably, however, lower P_g in low-mown plots was primarily caused by a reduction in green leaf area index (LAI) with mowing, which probably decreased the overall photosynthetic capacity of the canopy (Pearce et al., 1965; Bremer et al., 1998; Bremer and Ham, 2005).

Lower mowing did not affect θ_v in the 0-50 cm profile in the early period of 2005 (data not shown). During the significant species-by-mowing height interaction in the mid and late periods of 2005, however, θ_v at 0-50 cm decreased with mowing by 13 to 16% in θ_v in KBG but increased slightly (~2%) in HBG (Fig. 3-5A). During both years in the drought treatment, θ_v at 5 cm in KBG also averaged 13-14% lower in low- than in high-mown plots, while in HBG, θ_v at 5 cm averaged 17% greater in low- than in high-mown plots in 2004, but was similar between mowing treatments in 2005 (Figs. 3-6A to 3-6D). Consistently greater reductions in θ_v with mowing in KBG indicate that KBG extracted more water from the soil than HBG at the low mowing height, including during drought. Greater water extraction ability in KBG, in combination with generally higher visual quality in KBG in low-mown plots, suggests that this KBG (Apollo) is better suited for conditions of low mowing height and drought than this HBG (Thermal Blue).

Irrigation level

The θ_v in the 0-50 cm profile was similar between irrigation treatments early in 2005, before soil moisture began to decline as a result of the drought treatment (Fig. 3-5B); data were not available in the 0-50 cm profile in 2004. In the mid and late treatment periods of 2005, however, drought had significantly reduced θ_v in the 0-50 cm profile compared with well-watered plots (i.e., 100% ET replacement), which had subsequent effects on visual quality, NDVI, and Pg in drought plots.

Visual quality was similar between irrigation treatments early in the study in both years, before drought effects were evident (Figs. 3-9A and 3-9B). As the study progressed, however, drought significantly reduced the visual quality in the mid and late periods of both years although the effects of drought on visual quality were less severe in 2004 than in 2005. Drought reduced mean visual quality by 7% and 12% in mid and late periods of 2004 and by 14% and 32% in mid and late periods of 2005. The greater decline in visual quality with drought in 2005 than in 2004 was likely caused by higher temperature during 2005 (Table 3-1). This result is similar to other reports where the combined stresses of heat and drought caused a more rapid decline in visual quality of turfgrass than individual treatments of high temperature or drought (Jiang and Huang, 2001; Su et al., 2007).

Drought effect on NDVI was similar to drought effects on visual quality (Fig. 3-9A to 3-9D). The NDVI was similar between irrigation treatments early in the study, before the effects of drought began. Thereafter, in the mid and late periods, drought significantly reduced NDVI in both years. The decline in NDVI that resulted from drought indicated a decline in green LAI and biomass (Asrar et al., 1984; Daughtry et al., 1992; Gallo et al., 1985; Goward and Huemmrich, 1992) and in turfgrass visual quality (Trenholm et al, 1999). Drought reduced mean NDVI by 7% and 20% in late periods of 2004 and of 2005, respectively. This is consistent with the greater decline in visual quality with drought in 2005 than in 2004.

In both years, P_g was similar between 60 and 100% irrigation treatments except for late in 2005 (Figs. 3-9E and 3-9F). Drought significantly reduced average P_g by 25% in the late period of 2005 and by 17% in the mid period of 2005, although the reduction in the mid period was not statistically significant.

In summary, drought (60% ET replacement) caused declines in θ_v and consequently, in visual quality, NDVI, and P_g as the study progressed. Our results indicate that the effects of drought were similar between species and thus, we found no advantage in this HBG over KBG. This is similar to the results of Bremer et al. (2006) and Su et al. (2007).

Conclusions

Compared with KBG, visual quality in HBG was lower than in KBG in the mid period of 2004 and the late period of 2005. Visual quality in HBG was 9% and 13% lower than in KBG in the early and mid periods of 2005 under low mowing height. During both years, NDVI was

lower in HBG than in KBG except the late period of 2004. In the early period of both years, P_g was lower in HBG than in KBG. The HBG extracted less water from the 0-50 cm profile than KBG under low mowing height.

Low mowing reduced visual quality, NDVI, and P_g in 2004. In drought plots (60% ET replacement), visual quality and NDVI declined with θ_v in the 0-50 cm profile of the two grasses in the mid and late periods of both years.

In summary, this HBG (Thermal Blue) performed worse and did not demonstrate drought resistance or tolerance to low mowing height compared with this KBG (Apollo). Further field research is needed to identify newly-released cultivars of HBG that may perform better than KBG during drought and at lower mowing heights.

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↓
East

Row	Column 1		Column 2	
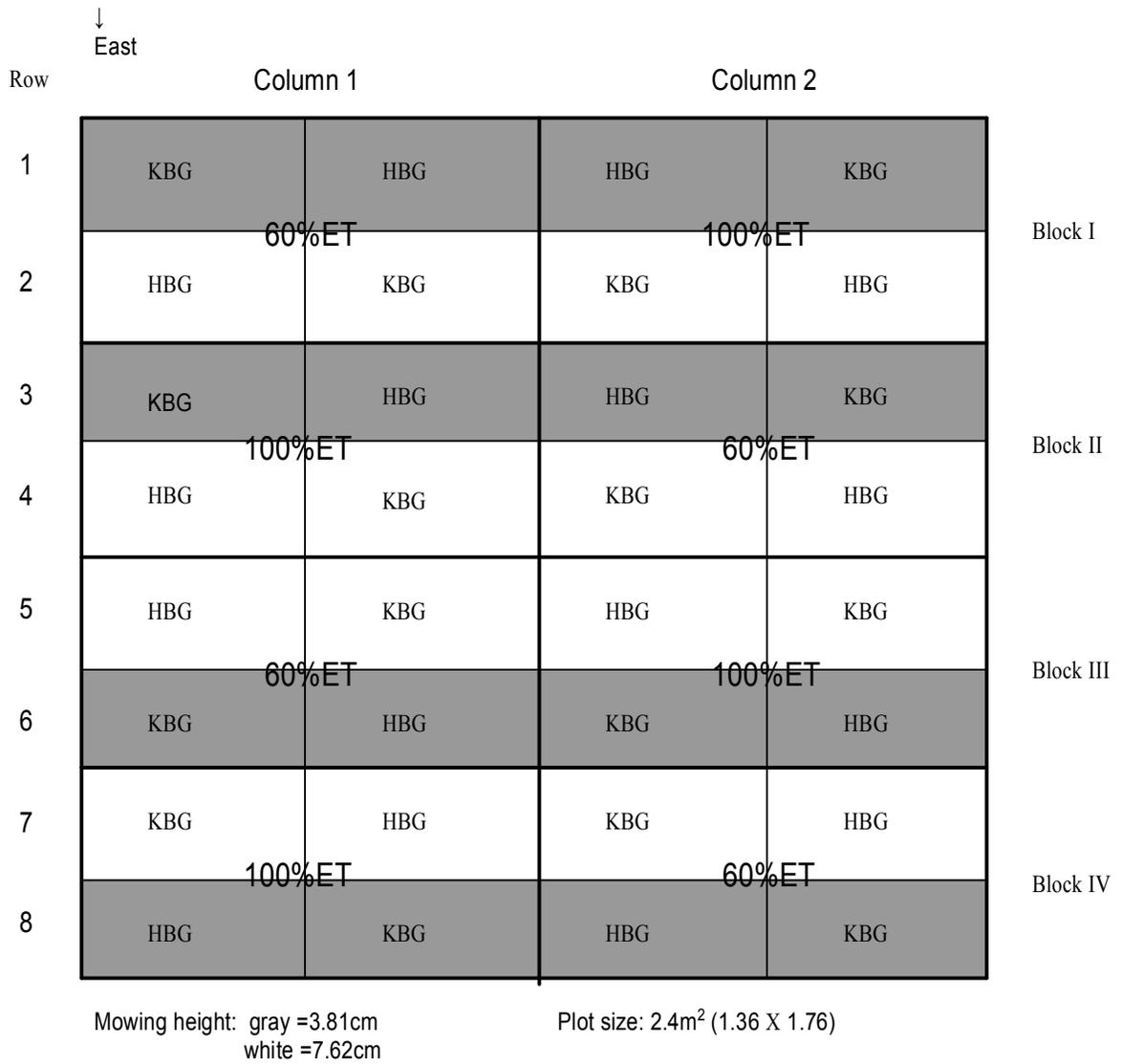
Figure 3-1 Plots layout diagram in the field.
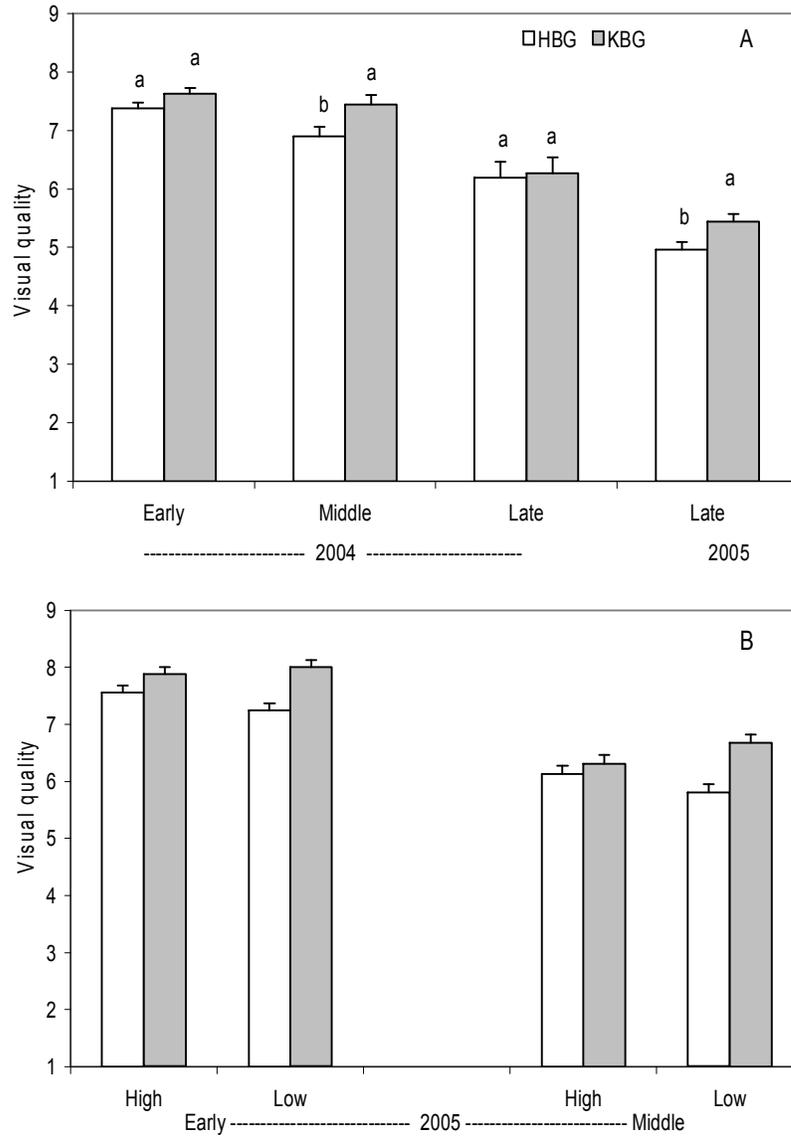
Figure 3-2 Visual quality in HBG and KBG in 2004 and in the late period of 2005 (3.2A) and during the interaction of species by mowing height in early and mid period of 2005 (3.2B). Means with the same letters during the same period were not significantly different (P=0.05).
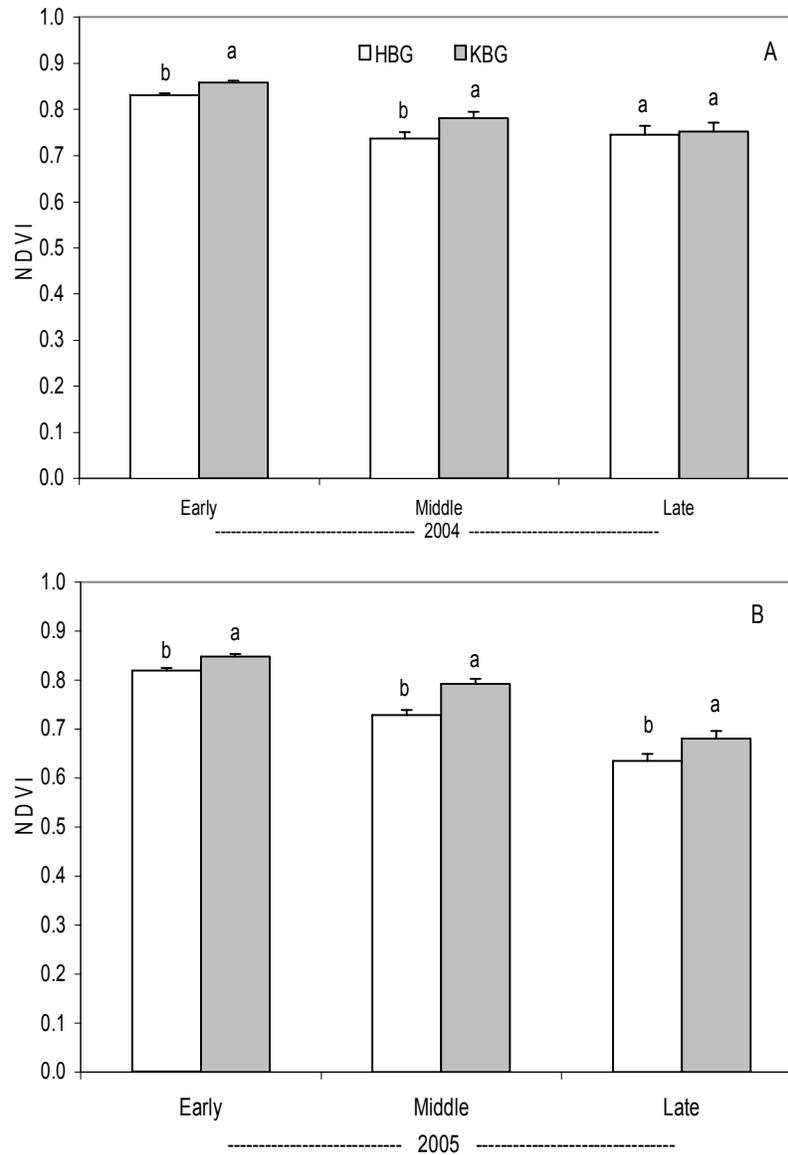
Figure 3-3 The normalized difference vegetation index (NDVI) in HBG and KBG in 2004 (3.3A) and 2005 (3.3B). Means with the same letters during the same period were not significantly different (P=0.05).
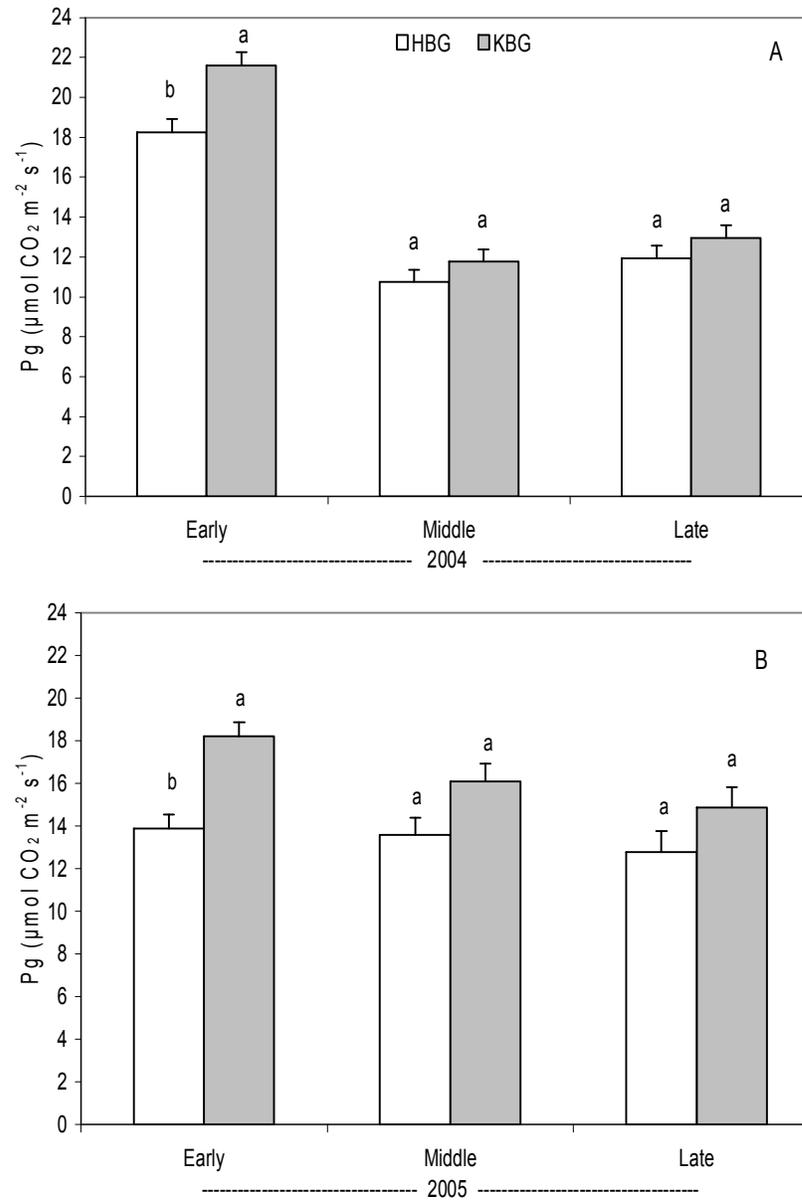
Figure 3-4 Gross photosynthesis (Pg) in HBG and KBG in 2004 (4A) and 2005 (4B). Means with the same letters during the same period were not significantly different (P=0.05).
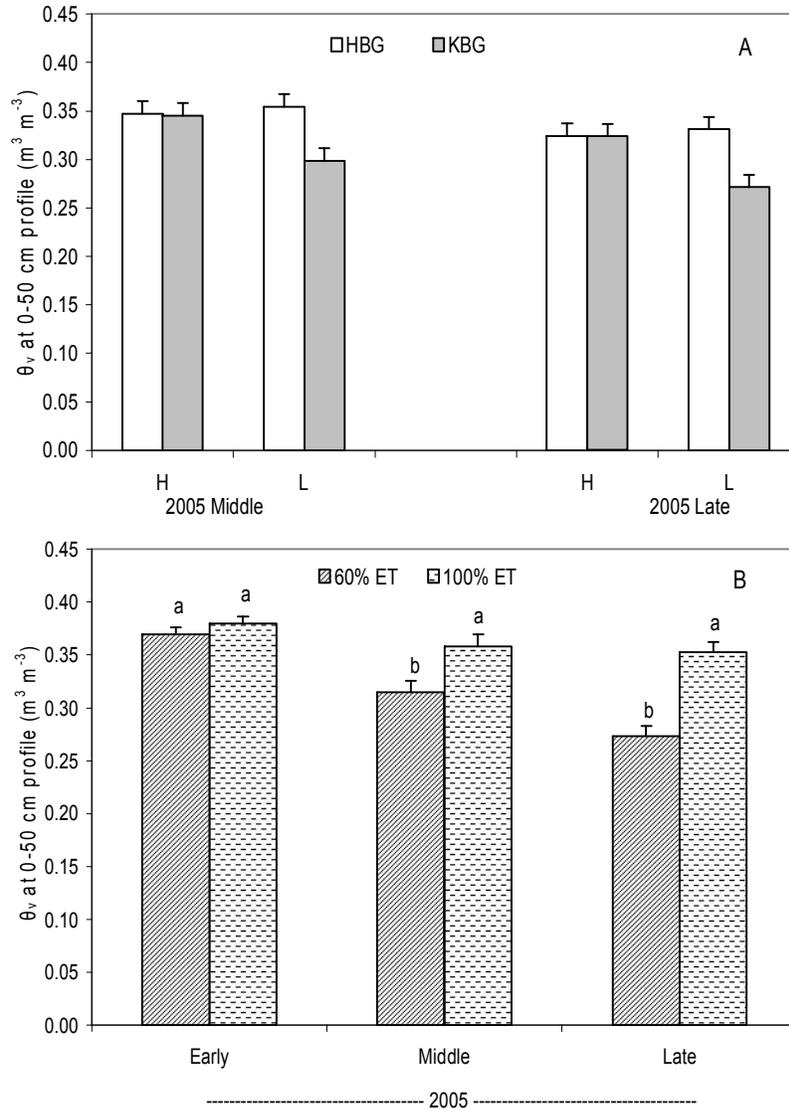
Figure 3-5 Volumetric soil water content (θ_V) at 0-50 cm during the species-by-mowing height interaction in the mid and late periods of 2005 (3.5A); and effects of irrigation on θ_V at 0-50 cm in 2005 (3.5B). Means with the same letters during the same period were not significantly different (P=0.05).
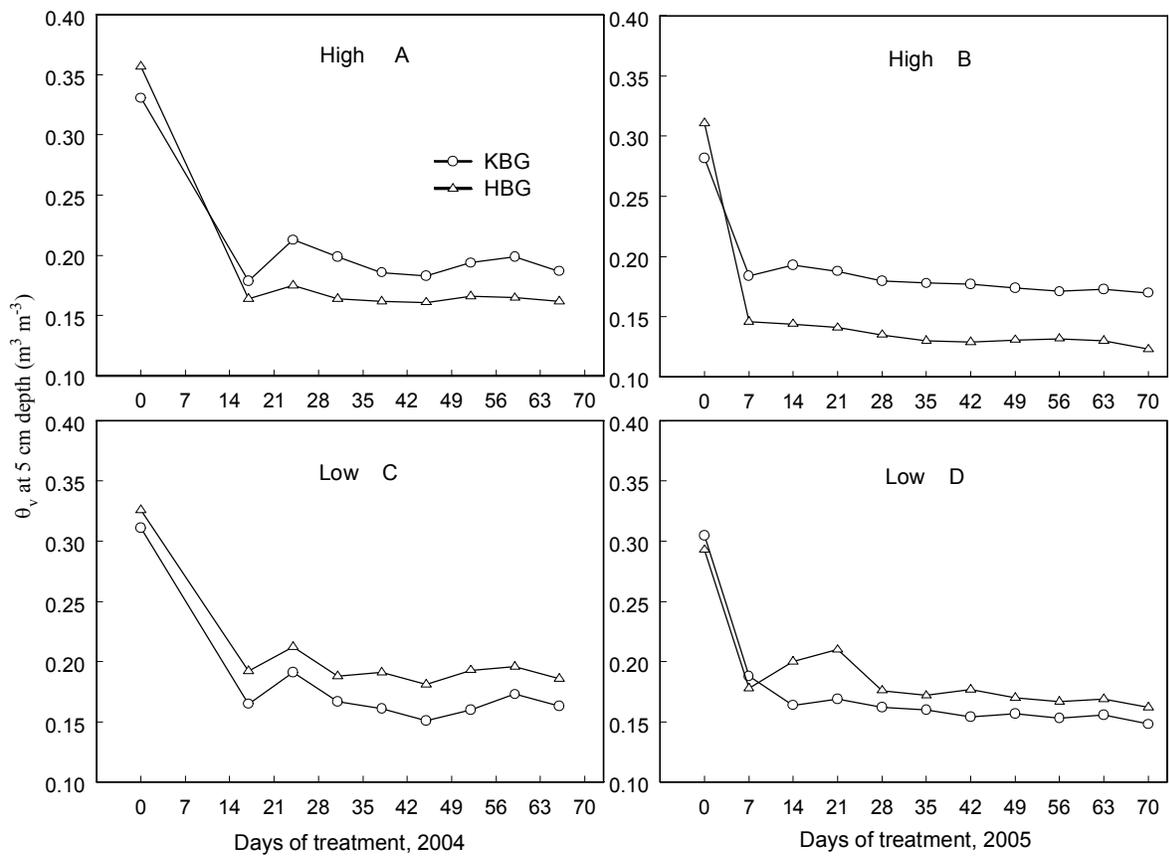
Figure 3-6 Volumetric soil water content (θ_v) at 5cm in KBG and HBG under 60% evapotranspiration (ET) replacement in high mown (3.6A and 3.6B) and low-mown plots (3.6C and 3.6D) in 2004 and 2005.
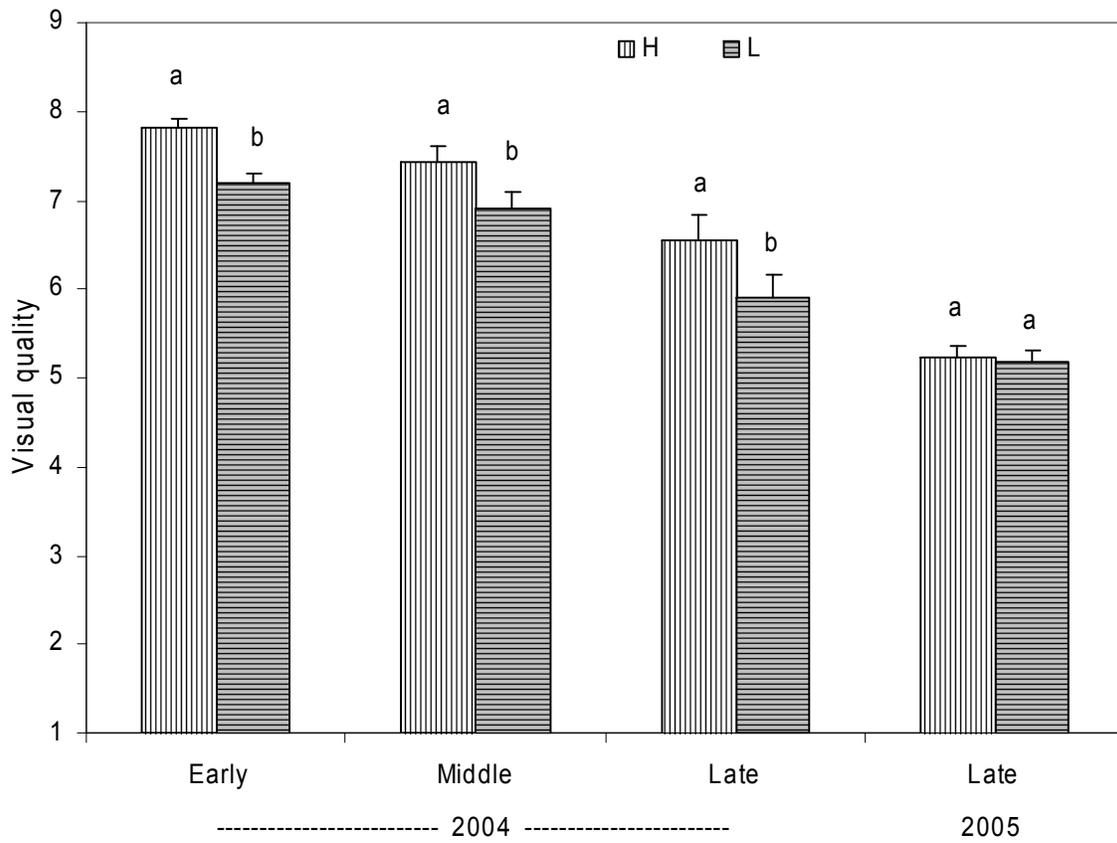
Figure 3-7 Effects of mowing height on visual quality in 2004 and in the late period of 2005. Means with the same letters during the same period were not significantly different (P=0.05).
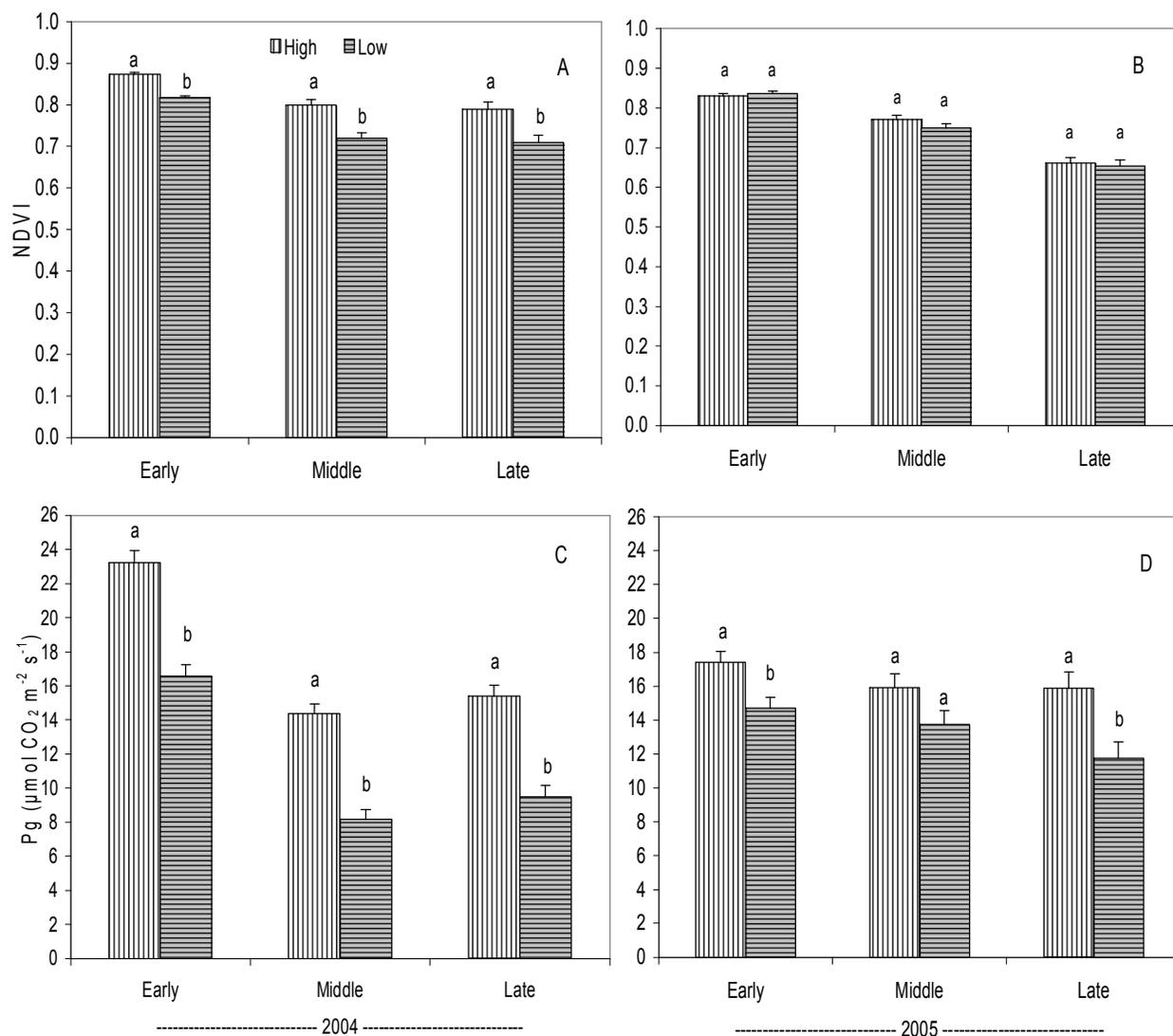
Figure 3-8 Effects of mowing height on the normalized difference vegetation index (NDVI) (3.8A and 3.8B) and gross photosynthesis (Pg) (3.8C and 3.8D) in 2004 and 2005. Means with the same letters during the same period were not significantly different (P=0.05).
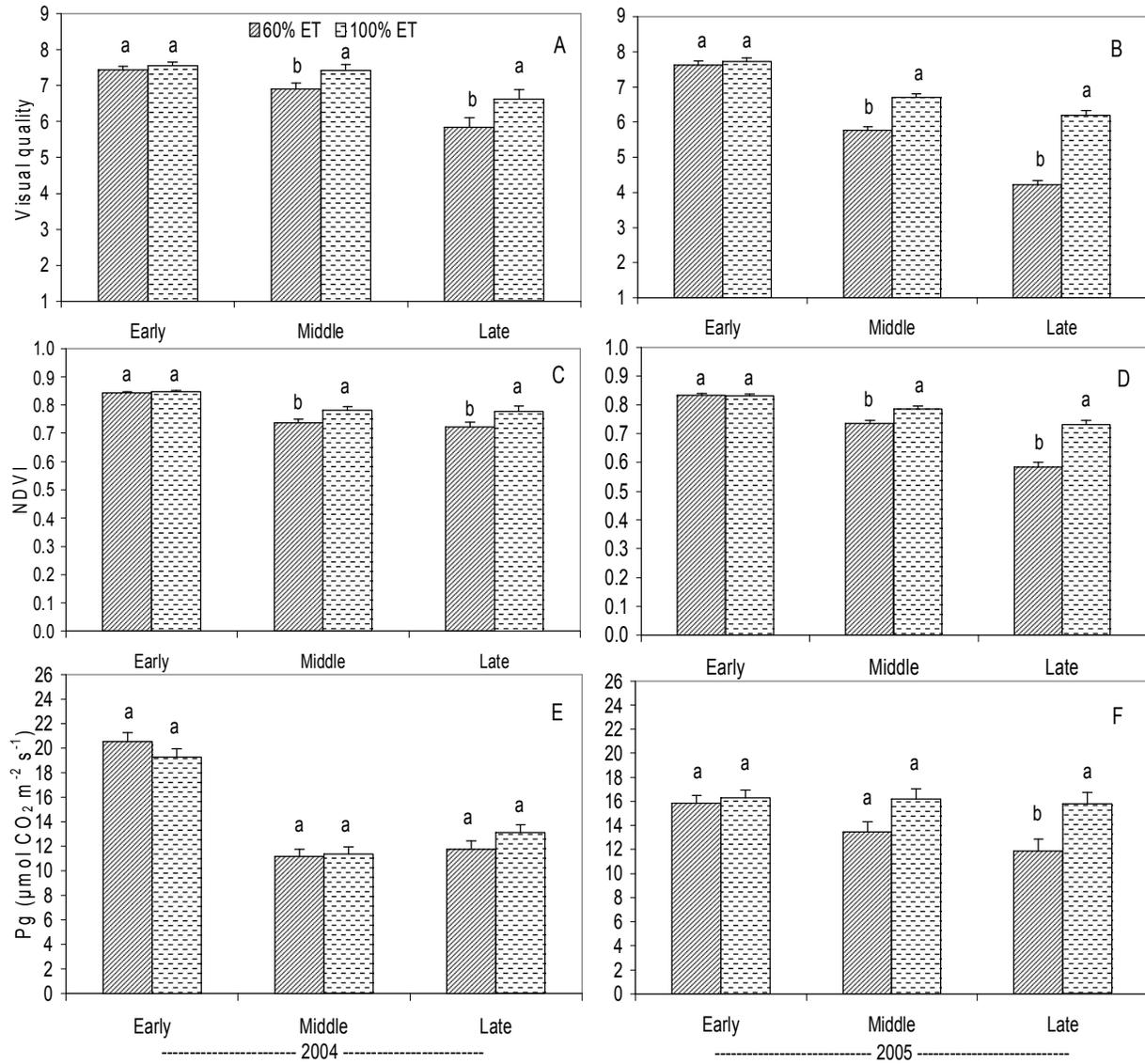
 Figure 3-9 Effects of irrigation level on visual quality (3.9A and 3.9B), normalized difference vegetation index (NDVI) (3.9C and 3.9D), and gross photosynthesis (Pg) (3.9E and 3.9F) in 2004 and 2005. Means with the same letters during the same period were not significantly different (P=0.05).

Table 3-1 Average daytime (1000-1800 CST) air temperature of each period during both years of the study and the difference between years.

Periods	2004 (°C)	2005 (°C)	Difference 2005-2004 (°C)
Early	26.9	29.2	2.4
Middle	25.5	30.9	5.4
Late	24.7	28.2	3.6

CHAPTER 4 - Membrane Lipid Composition and Heat Tolerance in a Hybrid Bluegrass Compared with Kentucky Bluegrass and Tall Fescue

Abstract

Heat stress is problematic for cool-season turfgrasses in some regions during summer. Hybrid bluegrasses have improved heat tolerance, but the underlying mechanisms of heat tolerance in turfgrasses are poorly understood. Our objectives were to quantitatively profile membrane lipid molecular species in three cool-season turfgrasses exposed to optimum ($22/15^\circ$C, 14-h light/10-h darkness) and supra-optimum temperature ($35/25^\circ$C and $40/30^\circ$C 14-h light/10-h darkness). The grasses included a heat tolerant hybrid bluegrass (HBG 'Thermal Blue'), a mid heat tolerant Kentucky bluegrass KBG (KBG 'Apollo') (*Poa pratensis* L.), and a low heat tolerant tall fescue (TF 'Dynasty')(*Festuca arundinacea* Schreb.). Under supra-optimum temperature, the ratio of the glycolipids, digalactosyldiacylglycerol (DGDG) to monogalactosyldiacylglycerol (MGDG), was highest in HBG and lowest in TF. In addition, heat tolerance was associated with a higher phosphatidylethanolamine (PE) content, a higher phosphatidylglycerol (PG) content, and reduced overall unsaturation, as compared to heat-sensitive TF. Overall, 20 lipid molecular species were higher and another 20 lipid molecular species were lower in the more heat tolerant HBG and KBG than in heat-sensitive TF. Results suggest that those 40 membrane lipid molecules are potential biomarkers for heat tolerance and that compositional changes in membrane lipids in response to heat may contribute to differences in heat tolerance among HBG, KBG, and TF cool-season grasses.

Abbreviations: DGDG, digalactosyldiacylglycerol; ESI-MS/MS, electrospray ionization tandem mass spectrometry; HBG, hybrid bluegrass; KBG, Kentucky bluegrass; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; TF, tall fescue.

Introduction

Heat stress is often a problem in cool-season turfgrasses during summer months in the transition zone of the U.S.A., which covers 480 to 1120 km north to south between the northern regions where cool-season grasses are adapted and the southern regions where warm-season grasses are adapted (Dunn and Diesburg, 2004). Predictions of higher temperature from global warming suggest that heat stress in cool-season turfgrasses may become more common in some regions, including in and north of the present-day transition zone (National Assessment Synthesis Team 2000). Thus, understanding the mechanisms of heat tolerance is increasingly important for turfgrass breeders and managers.

Cellular membranes, which are selectively permeable lipid bilayers with associated or embedded proteins, have long been proposed as one of the prime sites of vulnerability or tolerance to heat and cold stress in plants (Armond et al., 1980; Quinn, 1998; Vigh et al., 1993). The composition of lipid molecular species in cellular membranes may change in response to environmental stress (Hazel and Williams, 1990). The major categories of plant cellular membrane lipids are glycolipids and phospholipids (Lea and Leegood, 1993). Glycolipids are the most abundant lipids in plastids, and thus in photosynthetic tissues, and are believed to be highly vulnerable to damage by heat stress (Armond et al., 1980; Weis and Berry, 1988; Welti et al., 2002); the two most abundatnt glycolipids in leaves are monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). Phospholipids are the structural building blocks of extraplastidic membranes. They modulate membrane trafficking of select chemicals, are precursors of intracellular signaling molecules, and participate in the regulation and control of cellular function and response to stresses or injury through signal transduction processes (Exton, 1990).

 To identify the possible roles of membrane lipids in heat tolerance mechanisms in plants, we sought to identify and quantify lipid molecular species in response to heat stress and in cultivars with varying heat tolerance. Until recently, it has been difficult to study membrane lipid species and their changes during environmental stresses because of the complexity of lipid molecular species and limited analytical procedures. Recently, however, a highly sensitive, selective, and efficient approach based on electrospray ionization tandem mass spectrometry (ESI-MS/MS) has been developed to comprehensively analyze lipid composition in plants

(Brügger et al., 1997; Welti et al., 2002, 2007; Welti and Wang, 2004; Han and Gross, 2005). In this study, the ESI-MS/MS approach was used to profile membrane lipid changes in three cool-season grasses under optimum and supra-optimum growth temperature. Turfgrasses selected for this study were the same as those in a related study (Su et al., 2007). In that study, we concluded that, of three cool-season turfgrasses, a hybrid bluegrass (HBG, Thermal blue) was most heat tolerant, a TF was least heat tolerant, and a KBG was midway between the HBG and TF in heat tolerance; the HBG turfgrass is a genetic cross between native Texas bluegrass (*Poa arachnifera* Torr.) and Kentucky bluegrass (KBG) (Read et al., 1999).

Our objectives in the current study were to: 1) quantify membrane lipid molecular species under optimum temperature and subsequent compositional changes in lipid species after exposure to supra-optimum temperature; 2) identify relationships between specific lipid compositions and heat tolerance; and 3) identify specific membrane lipid molecules as potential biomarkers for heat tolerance in turfgrass.

Materials and Methods

Plant preparation, maintenance, and treatments

Sod plugs of each grass were collected on September 9, 2005 from established swards at the Rocky Ford Turfgrass Research Center near Manhattan, Kansas (39.14°N, 96.35°W) and planted in 5 lysimeters (10 cm diameter by 40 cm deep). Lysimeters were filled with a mixture of sand and topsoil (1:1, v:v) and were maintained in a greenhouse for two weeks. Average day/night air temperature was 24/15°C and supplemental light with incandescent lamps was included for 14-h/day. Grasses were clipped weekly at 6.5 cm. During the first week, irrigation was applied with a mist system which was automatically turned on 4 times per day for 5 min each time to keep soil wet. During the second week, turfgrasses were watered twice with fertilizer water at 250 ppm N (Peters Peat-lite Special 20-10-20 water-soluble fertilizer, Scotts-Sierra Horticultural Product Co., Marysville, OH).

After two weeks in the greenhouse, on September 23, 2005, lysimeters were transferred to a growth chamber and maintained for 105 days at optimum growth temperature (22/15°C, 14-h light/10-h darkness) with photosynthetically active radiation at 580 μmol m^{-2} s^{-1} during the daylight period. Turfgrasses were mowed once a week at 6.5 cm and irrigated every 3 d. To

maintain well-watered conditions, 100% of water lost via evapotranspiration (ET) during the previous 3 d was replaced; 100% ET was determined gravimetrically with the lysimeters (Bremer, 2003). Using this method, lysimeters were irrigated, allowed to drain until free drainage ceased, sealed, and weighed. Lysimeters were weighed again after 3 d and the water loss was attributed to ET. Turfgrasses were fertilized every 6 d with a solution supplying 20 kg N ha^{-1} (15N-30P-15K).

On January 10, 2006, the growth chamber temperature was increased to 35/25ºC (14-h light/10-h darkness) and then to 40/30ºC (14-h light/10-h darkness) on February 15 through March 13. Thus, turfgrasses were maintained at 35 /25ºC (14-h light/10-h darkness) for 36 d and at 40/30ºC (14-h light/10-h darkness) for 27 d, for a total of 63 d under supra-optimum growth temperature.

Lipid Extraction

Lipids were extracted according to the procedure of Devaiah et al. (2006) with minor modifications. Briefly, five leaves about the same age were collected from each lysimeter on January 9, 2006 (i.e., end of 105 d period under optimum growth temperature) and March 13, 2006 (i.e., end of 63 d under supra-optimum growth temperature). Leaves were quickly immersed in 3 ml isopropanol with 0.01% butylated hydroxytoluene at 75ºC to inactivate lipolytic activity. After 15 minutes, 1.5 ml chloroform and 0.6 ml water were added and the tubes were shaken for 1 h. Thereafter, the extract was removed and replaced with chloroform/methanol (2:1) with 0.01% butylated hydroxytoluene and the tubes were shaken for 30 min. The latter procedure was repeated five times until the leaves of every sample appeared white. The remaining leaf material was then dried overnight at 105ºC and weighed. The combined extracts were washed once with 1 ml 1 M KCl and once with 2 ml water, evaporated under nitrogen, and dissolved in 1 ml chloroform.

Analysis of lipid molecular species

Analysis and quantification with ESI-MS/MS followed the procedure of Devaiah et al. (2006). Briefly, lipid samples were analyzed on a 'triple'quadrupole tandem mass spectrometer equipped for electrospray ionization. The lipids in each class were quantified in comparison with the two internal standards of that class.

Double-bond indices (DBI), which indicate the saturation level of lipids (i.e., greater DBI mean less saturation of lipid species or more unsaturation), were calculated by the equation: DBI = [sum of (N x mol% lipid molecular species)]/100, where N is the number of double bonds in each lipid molecular species and mol% referred to mol% of a complex lipid class (Chen et al., 2006).

Electrolyte leakage

The thermostability of turfgrass cell membranes was estimated by measuring leaf electrolyte leakage (EL). Five living leaves about the same age were collected from each lysimeter on January 9, 2006 (i.e., end of 105 d under optimum temperature) and March 13, 2006 (i.e., end of 63 d under supra-optimum temperature). Each leaf was cut into two to three 2-cm segments and rinsed three times with distilled deionized water. All rinsed leaf segments from each lysimeter were placed in a test tube containing 20 ml of deionized water. Test tubes were shaken on a shaker table at 120 RPM (Lab-Line Instruments Inc., Melrose Park, Illinois) for 24 h to dissolve electrolytes that had leaked from cells (e.g., due to membranes damaged by heat stress treatment). After measuring conductivity (C_1) with a conductivity meter (Model 32, Yellow Springs Instruments Inc., Yellow Springs, Ohio), the test tubes with leaf samples were placed in an autoclave at 140ºC for 20 min to destroy all cell membranes, shaken for 24 h to extract all electrolytes from the cells, and then the conductivity (C_2) was measured again. The percentage of the total electrolytes that had leaked from cells during treatments was calculated as C_1/ C_2 x 100. Lower EL indicated greater resistance to heat stress (i.e., greater cell membrane thermostability).

Experimental design and statistical analysis

A completely randomized experimental design with five replications was used. The Q-test for identification and rejection of outliers was done on the replicates of all individual lipid species in each head group class. Multiple comparisons were performed among lipid molecular species using Proc MULTTEST of the Statistical Analysis System (SAS Institute Inc., Cary, NC). Means among the grasses under the same temperature treatment were separated using adjusted *p*-values and the false discovery rate (FDR) method at the 0.05 level of probability in order to control the number of false positives obtained when performing multiple hypothesis

tests. For electrolyte leakage data, the GLM procedure of SAS was used and differences between means were separated by the SAS PDIFF option (P=0.05). Principal component analysis (PCA) was used to compare multivariate data from membrane lipid profiles under high supra-optimum growth temperature, thus reducing the numbers of variables to consider. PCA was performed using Proc PRINCOMP of SAS.

Results and Discussion

After exposure to high temperature, EL was lower in HBG than in TF (Fig. 4-1), indicating less damage to cellular membranes in HBG and thus, a greater tolerance to heat in HBG than in TF (Marcum, 1998). These data are similar to those in our previous study (Su et al., 2007), in which we investigated the same turfgrass genotypes. In that study, the overall heat tolerance of KBG was midway between HBG and TF. EL was also lower in HBG and KBG than in TF at optimum temperature (Fig. 4-1), but differences in EL among the three grasses were markedly smaller (i.e., EL ranged from 0.6 to 1.4%) than after the grasses were exposed to heat (i.e., 7.0 to 16.8%). Furthermore, in our previous work we observed no differences in EL among these three grasses at the optimum growth temperature (Su et al., 2007).

Glycolipids

When exposed to supra-optimum temperature, the amount of monogalactosyldiacylglycerol (MGDG) lipids decreased by 28% in HBG, 24% in KBG, and 7% in TF. Under supra-optimum temperature, MGDG in HBG was 6% and 20% lower, respectively, than in KBG and TF (Table 4-1). Research by others has indicated that high MGDG levels can threaten bilayer integrity by inducing a transition from a bilayer to a non-bilayer lipid phase (Quinn, 1988; Seddon, 1990; Webb and Green, 1991). Therefore, a greater reduction in MGDG in HBG among grasses after exposure to high temperature may partially explain the greater heat tolerance of HBG relative to KBG and TF.

When exposed to supra-optimum growth temperature, the amount of digalactosyldiacylglycerol (DGDG) increased in all three turfgrasses and the greatest increase was in HBG; DGDG increased by 36% in HBG, 20% in KBG, and 12% in TF (Table 4-1). Our results of DGDG were consistent with published results that reported increases in DGDG with temperature (Suss and Yordanov, 1986; Di Baccio et al., 2002; Chen et al., 2006). In our study, DGDG in HBG was 12 and 16% greater than in KBG and TF, respectively, under supra-optimum temperature. In particular, the ratio of DGDG to MGDG when the three grasses were exposed to supra-optimum growth temperature, increased to 1.12 in HBG, 0.93 in KBG, and 0.77 in TF. In fact, when exposed to supra-optimum growth temperature, the ratios of DGDG to MGDG increased by 90% in HBG, 58% in KBG, and 20% in TF. Our results were consistent with findings by others reporting that ratios of DGDG to MGDG increased with temperature (Di Baccio et al., 2000; Chen et al., 2006; Wang and Lin, 2006), and the largest increase in DGDG to MGDG ratio was associated with the most heat tolerance.

The exact role of glycolipids in plant thermotolerance is unclear. Much of the evidence linking membrane lipid composition with altered function is based on correlations and thus, it is difficult to obtain conclusive proof of cause and effect (Chen et al., 2006). The increased ratios of DGDG to MGDG with temperature, however, may help maintain chloroplast membrane integrity and normal membrane protein function at high temperature. DGDG is a large polar head group that forms a more robust bilayer in an aqueous environment, whereas with MGDG is a smaller head group that promotes the formation of a hexagonal phase (Hex $_{II}$) structure that

may result in the loss of bilayer integrity (Quinn, 1988; Seddon, 1990; Webb and Green, 1991). Consequently, greater DGDG to MGDG ratios in HBG than in KBG and TF, as found in this study, may partially explain the higher heat tolerance reported in HBG among these three turfgrasses (Su et al., 2007; Fig. 4-1).

All three turfgrasses had similar double-bond indices (DBI) for glycolipids (DBI of MGDG and DGDG) under optimum growth temperature with the exception of TF, which had a slightly higher DBI of MGDG than HBG or KBG (Table 4-2). When exposed to supra-optimum temperature, however, the DBI of MGDG and DGDG were reduced in all three turfgrasses (Table 4-2). Under supra-optimum temperature, the DBI of MGDG and DGDG were lowest in HBG, highest in TF, and middle in KBG. Lower DBI indicates greater saturation of a lipid species (Chen et al., 2006; Stupnikova et al., 2006). These data suggest that greater saturation levels in glycolipids contribute to greater heat tolerance. This is consistent with the established role of fatty acid saturation as related to heat tolerance (Kleinschmidt and McMahon, 1970; Pearcy, 1978; Quinn 1988).

Phospholipids

The major classes of phospholipids detected by ESI-MS/MS in grass leaves included phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylinositol (PI), and minor lipids including phosphatidylserine (PS), phosphatidic acid (PA), lysoPC, lysoPE, and lysoPG (Table 4-1). Under optimum growth temperature, the three grasses had similar phospholipid content, which ranged 20.1 to 20.8% of total polar lipids content. Under supra-optimum growth temperature, however, the composition of phospholipids changed and the total content of phospholipids was greater in HBG and KBG than in TF; the total polar lipid content was 24.9% in HBG, 26.4% in KBG, and 20.6% in TF after exposure to heat.

At high temperature, the relative abundances for the phospholipid classes ranged from 10.4 to 12.2% for PC, 4.9 to 7.5% for PE, and 2.7 to 4.3% for PG, and was approximately 1.8% for PI among grasses; PS, PA, and lysophospholipids were less prevalent and accounted for 0.5

to 0.7%, 0.2 to 0.3%, and 0.03 to 0.05% of the total polar lipid content, respectively. The content of PC, PI, PS, and PA was similar among three grasses under supra-optimum growth temperature. The content of PE and PG, however, was higher in HBG and KBG than in TF under high temperature. The content of lysophospholipid (lysoPC + lysoPE + lysoPG) was highest in KBG, lowest in TF, and midway between KBG and TF in HBG. Our results are inconsistent with the notion that the membrane-destabilizing effect of lysophospholipids (Cullis and Dekruijff, 1979) plays a critical role in heat tolerance. The content of lysophospholipids, however, was very small; the total content of the three lysophopholipids (lysoPC, lysoPE, and lysoPG) was less than 0.05% of total polar lipid content.

Phosphatidylethanolamine (PE), a major component of extraplastidic membranes (Moore, 1982; Williams and Harwood, 1994), was the second most abundant class of phospholipid in the leaves of these turfgrasses (Table 4-1). Interestingly, PE content was similar among grasses under the optimum growth temperature, but PE increased in the more heat tolerant grasses (i.e., HBG and KBG) and not in TF after exposure to high temperature. This result is inconsistent with the general idea that the important function of PE in heat stability is its role as a membrane-destabilizing hexagonal phase-promoting phospholipid (Seddon, 1990; Grunner, 1992).

Profiling of PE revealed some molecular species that contained two polyunsaturated acyl species, such as 36:4 (di 18:2, based on molecular composition of Arabidopsis), increased and they were higher in content in HBG and KBG than in TF after exposure to high temperature (Fig. 4-2; Welti et al., 2002). Our data also revealed that the DBI of PE was lower in HBG (3.32) and in KBG (3.27) than in TF (3.57) under optimum growth. After exposure to high heat, DBI of PE decreased in all three grasses and DBI of PE was lowest in HBG (2.94) than in KBG (3.05) and TF (3.10). These results suggest that the saturation level of PE was greater in HBG than in KBG and TF under supra-optimum growth temperature. In addition, PC was an abundant phospholipid class in all three grasses (Table 4-1). Thus, our results are consistent with other research that indicates PE systems in combination with abundant PC have the effect of stabilizing the bilayer structure (Tilcock et al, 1982; Silvius, 1986; Epand and Bottega, 1987; Seddon, 1990). Therefore, after exposure to high temperature, greater saturated PE in HBG coupled with abundant PC may have contributed to their greater heat tolerance than TF.

Phophatidylglycerol (PG) is the major phospholipid in the thylakoid membranes of plant chloroplasts (Hagio et al., 2000; Welti et al., 2003). Phosphatidylglycerol is considered to be essential for growth and development of the chloroplast. Hagio et al. (2000) and Sakurai et al. (2003), through their studies of a mutant that could not synthesize PG, determined that decreased PG content in thylakoid membranes inhibited photosynthesis. In our study, the content of PG was higher in HBG and KBG than in TF under both optimum and supra-optimum temperature regimes (Table 4-1). The content of PG declined, however, in all three grasses after exposure to supra-optimum temperature; PG content declined by 16% in HBG, 24% in KBG, and 37% in TF. This decline of PG content among grasses after exposure to high temperature was likely caused by decreased 34:4-PG (18:3-16:1) and 34:3-PG (18:3-16:0 and 18:2-16:1) (Fig. 4-3; Welti et al., 2002). These results are consistent with the findings by others that increased growth temperature led to reduction in linolenic acid (18:3) (Raison et al., 1982; Liu et al., 2006).

Potential membrane lipid molecular biomarkers for heat tolerance

Although there were differences in membrane lipid content among grasses under optimum temperature, the differences became much greater after exposure to high heat for a number of lipids (e.g. MGDG, DGDG, PE, PG, PS, PA, and lyso(PC + PE + PG)) (Table 4-1). Thus, different genotypes of turfgrasses responded differently to heat stress in their compositional changes in membrane polar lipids.

Similar to heat tolerance, which was greatest in HBG and least in TF, the lipid profiles among the three grasses after exposure to high temperature indicated that the differences among three grasses were greatest between HBG and TF (Table 4-2; Fig. 4-2; Fig. 4-3; profilings of molecular species of other phospholipids not shown). Although HBG and KBG were more similar to each other than to TF, lipid content in KBG was numerically (albeit not statistically) midway between HBG and TF in a number of instances (e.g., MGDG, DGDG, and PG) (Table 4-1; Fig. 4-3). The composition of membrane lipid molecules under supra-optimum temperature may contribute to the differences in heat tolerances observed among these three grasses. Principal component analysis (PCA) simplified an otherwise complex process by reducing 152 lipid molecular species from supra-optimum temperature treatment into 14 principal components

(Davies, 2005). Two of the 14 PCs explained about 57.4% of the variance in the dataset (Fig. 4-4). The biggest differences were between TF and the bluegrasses (i.e., HBG and KBG), which were separated along the PC1 axis; PC1 explained over 39.4% of the variance of the dataset. The HBG and KBG were separated along the PC1 axis and PC2 axis (PC2 explained over 18.0% of the variance).

Inspection of the loadings of PC1 revealed 40 individual lipid molecular species which separated the more heat-tolerant bluegrasses (HBG and KBG) from less heat tolerant TF (Fig. 4-4 and Table 4-3). Twenty of the 40 significant lipid molecular species, which were higher in content in the grasses with greater heat tolerance (i.e., HBG and KBG), had greater saturation levels (e.g., 34:1, 34:2, 36:1, 36:2, and 36:3). The remaining 20 lipid molecular species, which were lower in content in the two grasses with greater heat tolerance (i.e., HBG and KBG), have high unsaturation levels (i.e., 36:6 (di 18:3) and 36:5 (18:3-18:2)). These results were consistent with the finding that there was correlation between the low content of 18:3 and tolerance to high-temperature stress in tomato (Liu et al., 2006).

In general, HBG and KBG have more saturated lipid species and exhibit greater heat tolerance than TF. These results suggest a high probability that 40 membrane lipid species are potential biomarkers for heat tolerance in cool-season turfgrasses (Table 4-3). Further experiments are needed to validate candidate lipid biomarkers by analyzing additional heat tolerant cool-season turfgrasses.

Conclusions

Our data indicated that supra-optimum growth temperature caused compositional changes of membrane polar lipids among three grasses including in a heat-tolerant HBG (Thermal Blue), a medium heat-tolerant KBG (Apollo), and a heat-sensitive TF (Dynasty). Heat tolerant HBG and KBG had higher contents of DGDG, greater ratios of DGDG to MGDG, and greater saturation of fatty acids than a more heat-sensitive TF under supra-optimum growth temperature. There were also greater contents of PE and greater content of PG in HBG and KBG than in TF under high temperature. Results suggest that 40 membrane lipid molecular species are potential biomarkers for heat tolerance in cool-season turfgrasses.

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Figures and Tables
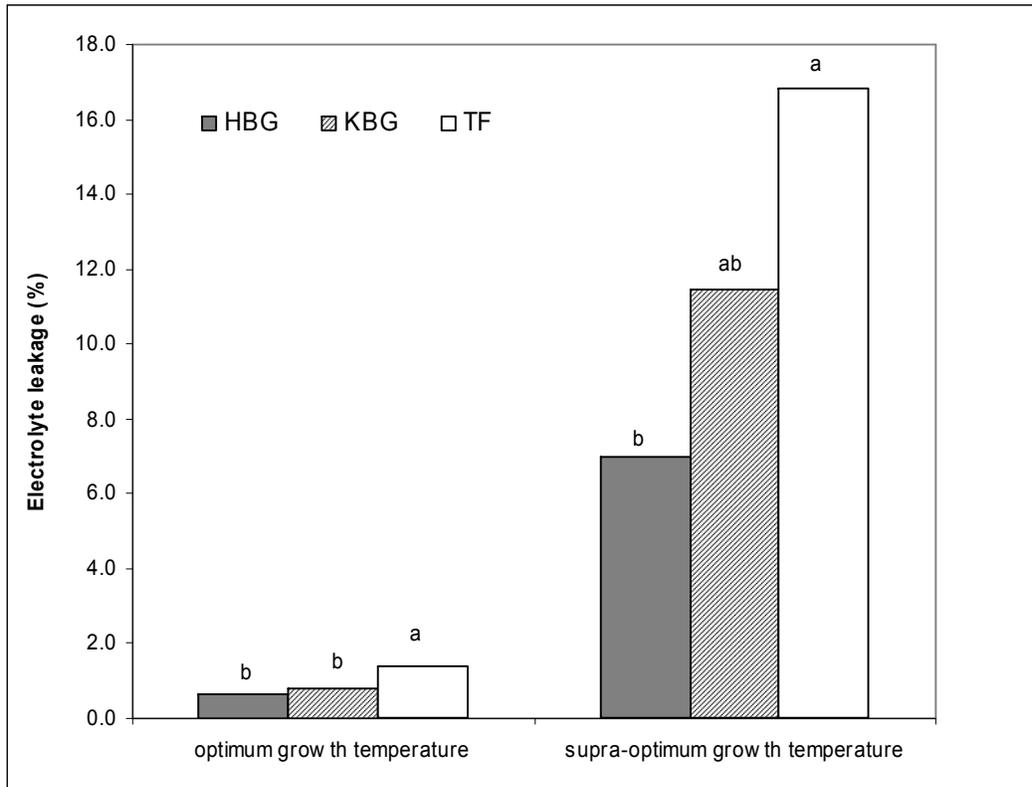
Figure 4-1 Electrolyte leakage (EL), indicating cell membrane stability, under optimum and supra-optimum growth temperatures. Means with the same letters under the same temperature treatment (i.e., optimum, supra-optimum) were not significantly different (P=0.05).
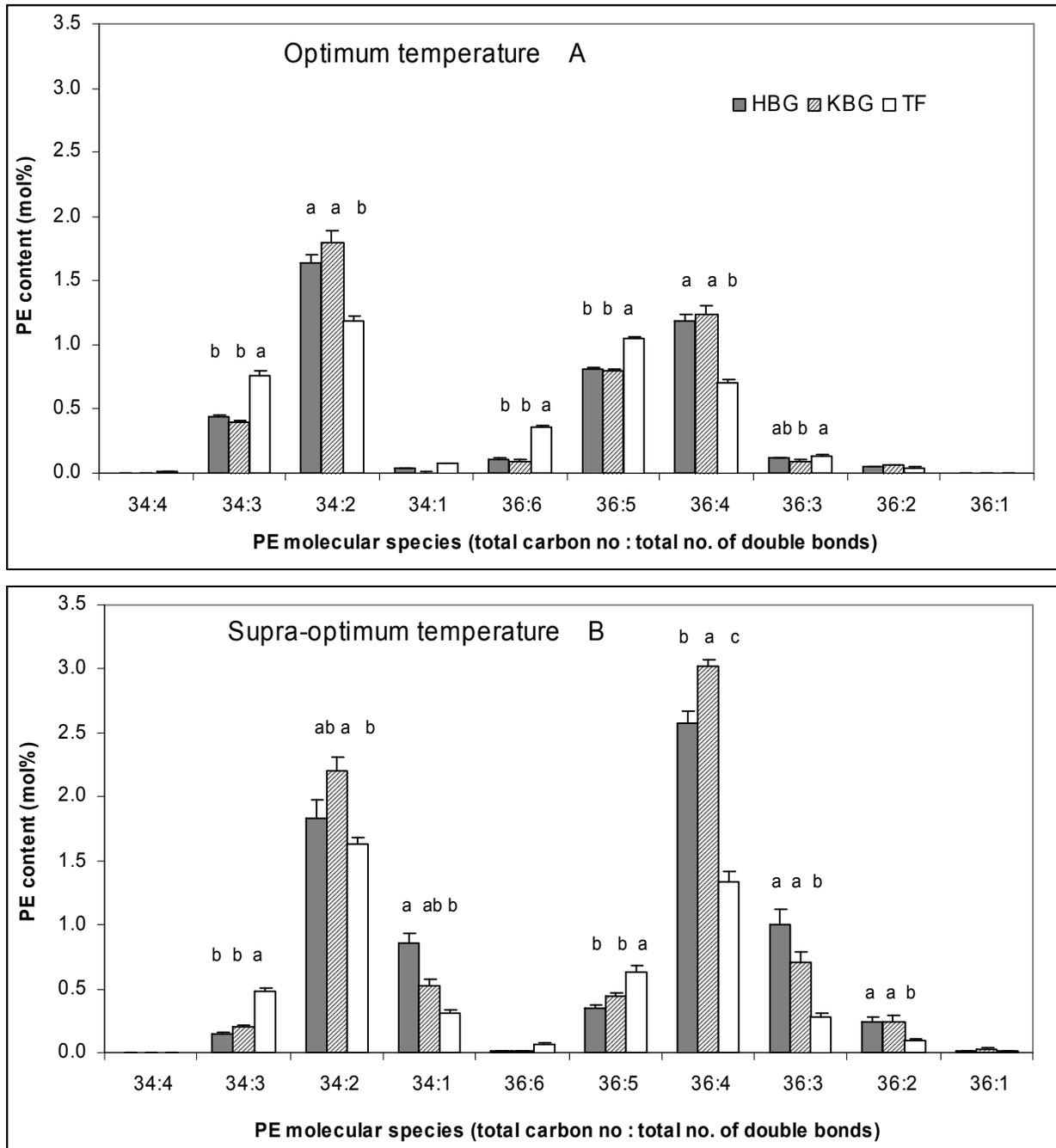
Figure 4-2 The PE molecular species composition (mol%) of HBG, KBG, and TF under optimal growth temperature (A) and supra-optimal growth temperature (B). Values were the means + S.E. (n = 4 or 5). Means with the same letters in each PE molecular species were not significantly different (adjusting P = 0.05 by the false discovery rate method). Means without letters indicated content <0.1%.
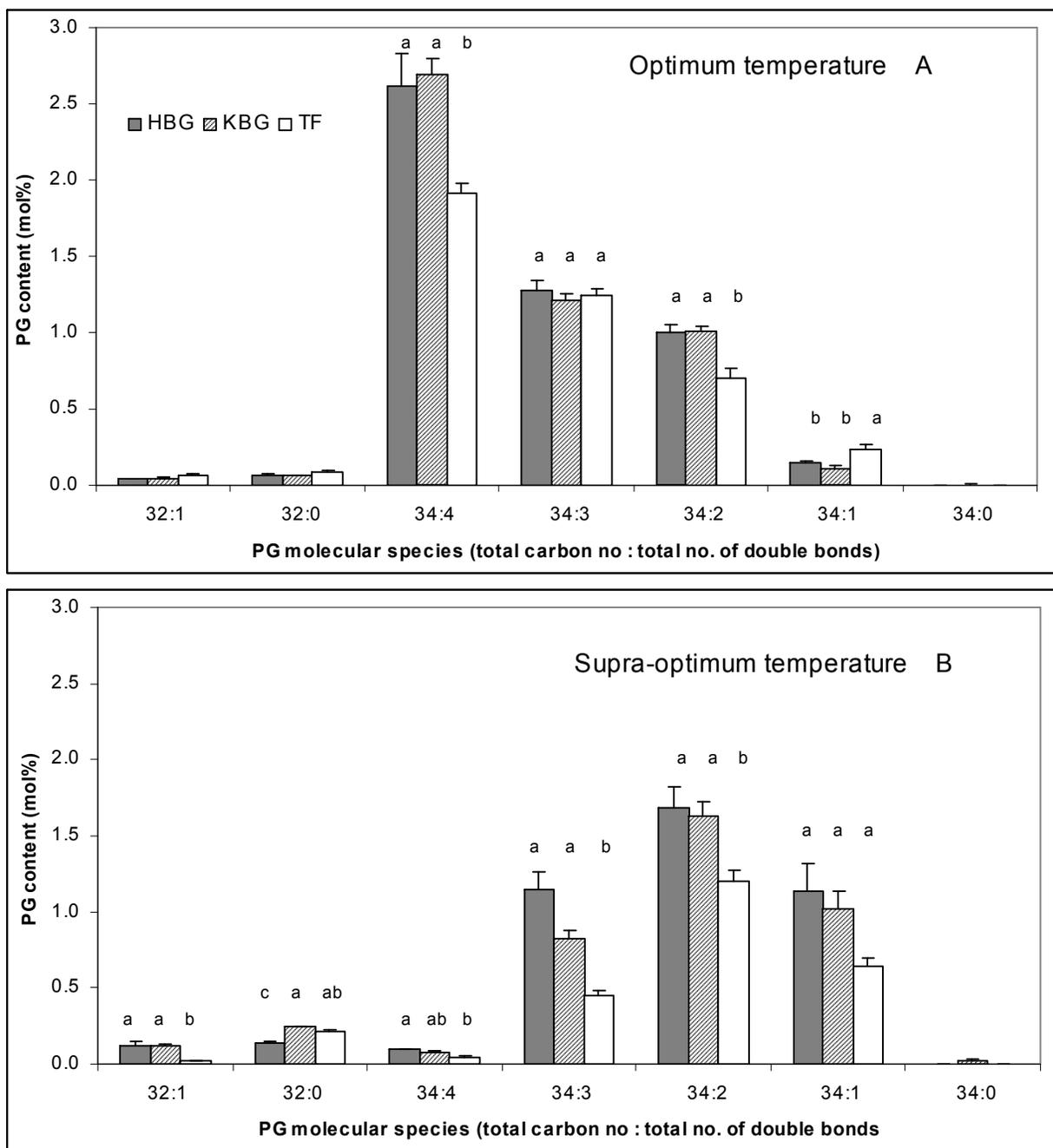
Figure 4-3 The PG composition (mol%) of HBG, KBG, and TF under optimal growth temperature (A) and supra-optimal growth temperature (B). Values were the means + S.E. (n = 4 or 5). Means with the same letters in each PG molecular species were not significantly different (adjusting P = 0.05 by the false discovery rate method). Means without letters indicated content <0.1 %.
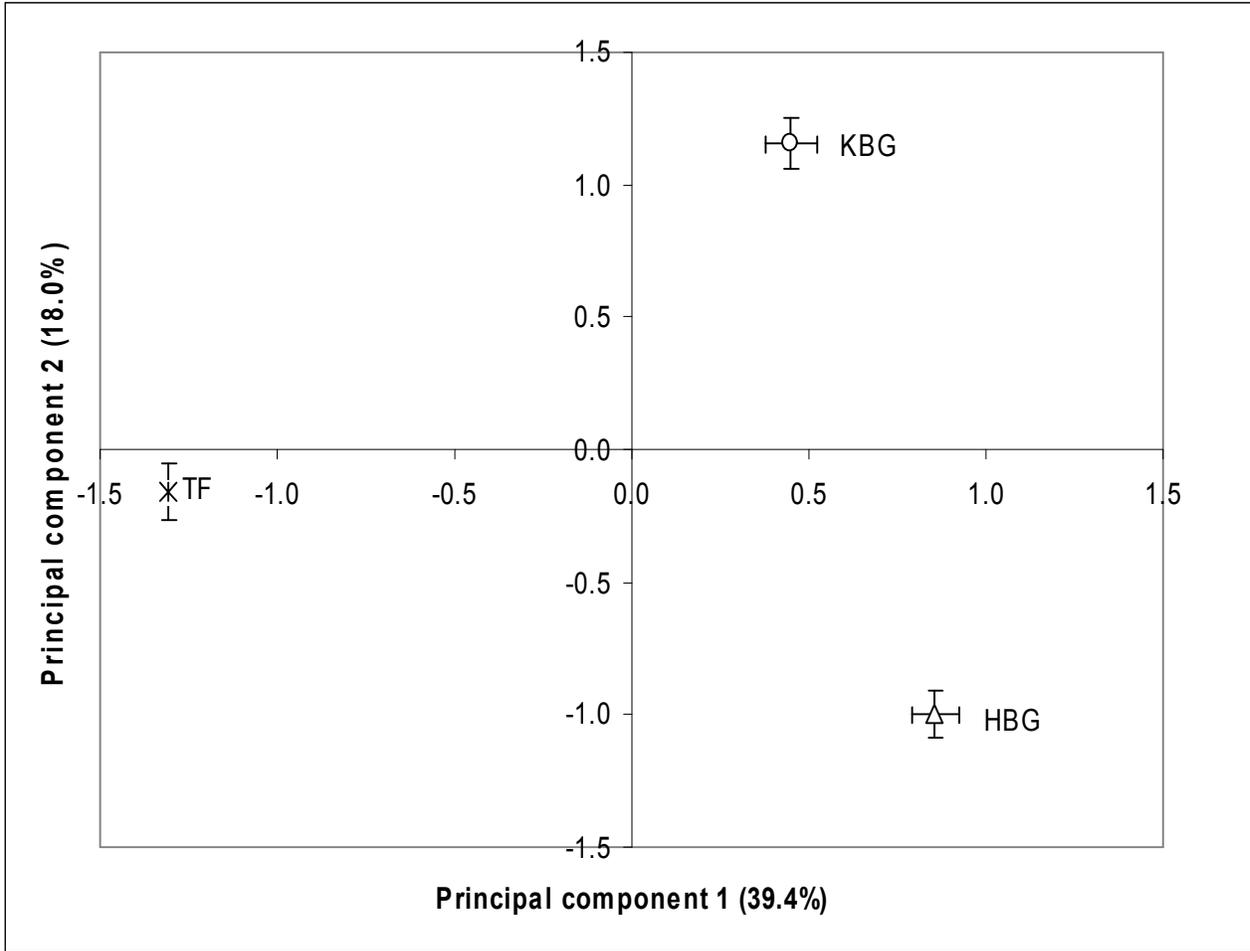
Figure 4-4 Principal component analysis (PCA) of 152 lipid molecular species in hybrid bluegrass (HBG), Kentucky bluegrass (KBG), and tall fescue (TF); Principal component (PC)1 and PC2 accounted for 39.4 and 18.0%, respectively, of the variance of the dataset, for a total of about 57.4%.

Table 4-1 Membrane lipid composition (mol%; percentages of individual species to total lipid content) of HBG, KBG, and TF under optimum and supra-optimum growth temperature.

Lipid species	Optimum growth temperature				Supra-optimum growth Temperature		
	HBG	KBG	TF		HBG	KBG	TF
			Glycolipids				
MGDG	50.12 a[†]	50.31a	48.41b		35.95 b	38.42 b	44.97 a
DGDG	29.39 b	29.59 b	30.99 a		39.97 a	35.61 b	34.58 b
Subtotal of Glycolipids	79.51 a	79.90 a	79.40 a		75.92 b	74.03 b	79.54 a
			Phospholipids				
PC	9.33 b	8.86 b	10.33 a		10.82 a	12.23 a	10.38 a
PE	4.50 a	4.58 a	4.39 a		7.10 a	7.49 a	4.87 b
PG	5.14 a	5.14 a	4.26 b		4.31 a	3.93 a	2.70 b
PI	1.13 b	1.15 b	1.35 a		1.80 a	1.81 a	1.76 a
PS	0.24 ab	0.20 b	0.29 a		0.61 a	0.67 a	0.48 a
PA	0.11 a	0.12 a	0.14 a		0.20 a	0.27 a	0.23 a
Sum of LysoPC, lysoPE, and lysoPG	0.04 a	0.03 ab	0.02 b		0.04 ab	0.05 a	0.03 b
Subtotal of Phospholipids	20.49 a	20.07 a	20.78 a		24.88 a	26.44 a	20.45 b

[†]Means followed by the same letter within a row and temperature subgroup (i.e., optimum, supra-optimum) were not significantly different (adjusting $P = 0.05$ by the false discovery rate method).

Table 4-2 Glycolipid species composition (mol%), DBI, and the ratios of DGDG to MGDG of HBG, KBG, and TF under optimum and supra-optimum growth temperature.

Major glycolipid species (total carbon no. : total No. of double bonds)	Optimum growth temperature			Supra-optimum growth temperature		
	HBG	KBG	TF	HBG	KBG	TF
MGDG 34:3 (18:3/16:0) [*]	1.85 b[†]	2.56 a	1.62 b	0.46 b	0.54 b	1.04 a
MGDG 34:2 (18:2/16:0)	0.17 b	0.19 a	0.08 c	0.51 a	0.58 a	0.38 a
MGDG 34:1 (18:1/16:0)	0.19 a	0.12 b	0.17 a	0.55 a	0.30 b	0.23 c
MGDG 36:6 (18:3/18:3)	35.86 c	37.93 b	39.92 a	1.02 b	2.18 b	8.74 a
MGDG 36:5 (18:3/18:2)	9.31 a	7.42 b	4.91 c	10.31 c	14.10 b	21.77 a
MGDG 36:4 (18:2/18:2)	2.48 a	1.84 b	1.36 c	17.12 a	17.20 a	11.25 b
MGDG 36:3 (18:2/18:1)	0.14 a	0.10 b	0.09 b	5.60 a	3.08 b	1.18 c
MGDG 36:2 (18:2/18:0)	-	-	-	0.67 a	0.34 b	-
DBI of MGDG	5.56 b	5.59 b	5.70 a	4.11 c	4.32 b	4.78 a
DGDG 34:3 (18:3/16:0)	5.84 b	6.95 a	7.05 a	1.51 b	1.55 b	3.94 a
DGDG 34:2 (18:2/16:0)	1.26 a	1.12 b	0.63 c	3.79 a	3.19 a	2.79 a
DGDG 34:1 (18:1/16:0)	0.37 b	0.29 c	0.54 a	3.27 a	1.80 b	1.31 b
DGDG 36:6 (18:3/18:3)	16.03 b	16.42 b	19.73 a	0.62 b	1.01 b	3.71 a
DGDG 36:5 (18:3/18:2)	4.69 a	3.37 b	1.40 c	8.50 b	9.61 b	12.81 a
DGDG 36:4 (18:2/18:2)	1.03 a	0.79 a	0.78 a	17.00 a	14.52 b	8.04 c
DGDG 36:3 (18:2/18:1)	0.45 b	0.52 b	0.69 a	4.10 a	2.98 b	1.29 c
DGDG 36:2 (18:2/18:0)	-	-	-	1.03 a	0.81 a	0.32 b
DBI of DGDG	4.97 a	4.86 a	4.98 a	3.61 c	3.82 b	4.11 a
Ratio of DGDG to MGDG	0.59 b	0.59 b	0.64 a	1.11 a	0.93 b	0.77 c

*Glycolipid molecular species (<0.1%) not shown in the table.

†Means followed by the same letter within a row and temperature subgroup (i.e., optimum, supra-optimum) were not significantly different (adjusting $P = 0.05$ by the false discovery rate method).

Table 4-3 Forty membrane lipid molecular species that were significantly different in the bluegrasses (HBG and KBG) compared to TF after exposure of grasses to high temperatures. These lipid molecular species may be potential biomarkers for heat tolerance in cool-season turfgrasses.

High content lipid molecular species in heat-tolerant turfgrasses†

PS 36:1	PS 34:1	PC 34:1	MGDG 36:4	PS 36:4
PG 34:2	PC 36:2	PS 36:3	PE 36:4	MGDG 36:2
DGDG 36:1	MGDG 36:3	PE 36:2	PE 34:1	PC 36:3
PC 36:1	DGDG 36:3	DGDG 36:4	PE 36:3	DGDG 36:2

Low content lipid molecular species in heat-tolerant turfgrasses

MGDG 34:3	MGDG 36:5	DGDG 34:3	DGDG 36:5	MGDG 36:6
DGDG 36:6	PC 34:3	PC 36:6	PI 34:3	PE 34:3
PC 36:5	PI 36:5	PS 42:3	PE 36:6	PS 34:3
PC 34:4	PE 34:4	PS 40:3	PE 36:5	PC 38:6

†Heat-tolerant turfgrasses referred to HBG and KBG in this study.