

DISSERTATION

PHYSIOLOGICAL, MORPHOLOGICAL, AND SPECTRAL REFLECTANCE
CHARACTERISTICS OF KENTUCKY BLUEGRASS, TEXAS BLUEGRASS,
AND THEIR HYBRIDS IN RESPONSE TO WATER STRESS

Submitted by

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In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

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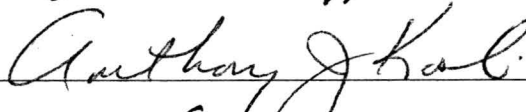
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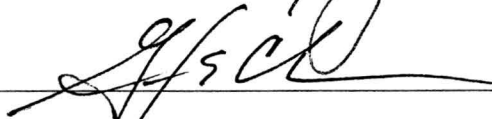
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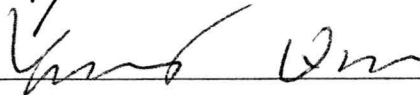
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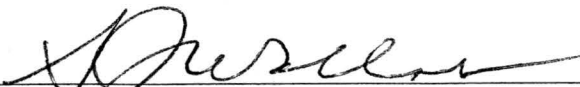








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ABSTRACT OF DISSERTATION

PHYSIOLOGICAL, MORPHOLOGICAL, AND SPECTRAL REFLECTANCE CHARACTERISTICS OF KENTUCKY BLUEGRASS, TEXAS BLUEGRASS, AND THEIR HYBRIDS IN RESPONSE TO WATER STRESS

Demands on fresh water resources, especially throughout the arid and semi-arid western USA, have resulted in escalation of government regulations to force water conservation. Such measures have included limitations on the permissible area devoted to turfgrass, restrictions on the quantity of potable water relegated to turfgrass irrigation, and mandates on the types of turfgrass permitted. As a result, identification and development of turfgrasses exhibiting improved drought resistance as well as those able to withstand secondary irrigation waters high in soluble salts has become paramount. Towards this end, three studies were conducted. In Study 1 Kentucky bluegrass (*Poa pratensis* L.) (KBG), Texas bluegrass (*Poa arachnifera* Torr.) (TBG), and their hybrids (HBG) were evaluated for salt tolerance. A broad range of variability in leaf firing and shoot and root growth reduction in response to salinity was found to exist within and among these *Poa* spp. and their hybrids, indicating that improvement in the salt tolerance of bluegrasses may be possible. In study 2 HBG and KBG water use characteristics and response to drought were compared. Hybrid bluegrass exhibited a lower inherent ET rate, as well as the ability to moderate its ET as evaporative demand increased, relative to KBG, thereby reducing water loss. Hybrid bluegrass also exhibited lower leaf water content and

slower shoot growth than KBG under non-limiting soil moisture conditions, characteristics advantageous to reduce water use. Additionally, HBG possesses a significantly deeper, more extensive root system than KBG enabling water extraction from a greater volume of depth when surface soil water is depleted. These factors combined contributed significantly to the drought avoidance ability observed in HBG. In study 3 we found spectral reflectance within the far-red region of the visible portion of the spectrum to be most sensitive to, and highly correlated with, progressive dehydration. These wavelengths might effectively be used as an irrigation management tool in non-destructively monitoring leaf water status. Computation of reflectance difference between non-stressed and dehydrated leaves of HBG, KBG, and perennial ryegrass (*Lolium perenne* L.) revealed consistent differences in their reflectance sensitivity to dehydration. This sensitivity ranking was comparable with previous reports of drought resistance among these grasses, suggesting that the magnitude of reflectance change may be used as an indicator of drought resistance.

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“Grass is the forgiveness of nature ... her constant benediction ...
it softens the rude outline of the world.”

John James Ingalls 1872

CHAPTER 1

RELATIVE NaCl TOLERANCE OF KENTUCKY BLUEGRASS,
TEXAS BLUEGRASS, AND THEIR HYBRIDS

ABSTRACT

Government regulations to force water conservation have escalated the use of secondary water high in soluble salts for turfgrass irrigation in the arid and semiarid western USA, thus increasing the need for more salt tolerant turfgrasses. This study was initiated to determine the variability in salt tolerance within and among two *Poa* spp. and their hybrids. Two experiments were conducted during 2000 in the greenhouse at Fort Collins, CO in solution culture to examine the effects of NaCl on leaf firing, and shoot and root growth reduction of 9 Kentucky bluegrass (*Poa pratensis* L.) (KBG) cultivars representing 3 ecotypes, 3 Texas bluegrass (*Poa arachnifera* Torr.) (TBG) accessions, and 5 of their hybrids (*P. pratensis* x *P. arachnifera*) (HBG). In Experiment I, conducted during late winter through spring 2000, overall salt tolerance based on leaf firing and electrical conductivity of 50% shoot growth reduction ($EC_{shoot\ 50}$) placed 7 KBG in the most tolerant group. In Experiment II, conducted during summer through early fall 2000, overall salt tolerance rankings placed 4 KBG and 3 TBG in the most tolerant group. Based on % leaf firing and the salinity levels that caused 25 and 50% shoot growth

reduction, compact (low, compact growth habit) and aggressive (aggressive, lateral growth habit) KBG ecotypes showed more salt tolerance than common ecotypes in both Experiments I and II. A broad range of variability in leaf firing and shoot and root growth reduction in response to salinity was found to exist within and among these *Poa* spp. and their hybrids, indicating that improvement in the salt tolerance of bluegrasses may be possible. Additionally, differences in salt tolerance of KBG and TBG between Experiment I and II suggested that environmental conditions could affect bluegrass salt tolerance expression.

INTRODUCTION

Demands on fresh water resources in the western USA have resulted in codified limitations on the permissible area devoted to turfgrass (City of Aurora, CO, 1998; Clark Co., NV 1992) as well as the quantity of potable water relegated to its irrigation (City of Albuquerque, NM, 2000; AZ Dept. of Water Res., 1999). As a result, secondary or effluent water, often containing high concentrations of NaCl, the only municipal water supply expected to increase in this region in the future (CO Water Resources Res. Inst., 1996), is increasingly being used as an irrigation source for turfgrass whenever available (Gill and Rainville, 1994; Marcum et al., 1998; San Diego Co., CA, 2000). Fresh water conservation mandates in rapidly growing metropolitan areas of the arid and semi-arid western USA, where saline soils are common, has increased the need for more salt tolerant turfgrasses.

Kentucky bluegrass (KBG) (*Poa pratensis* L.), considered to be salt-sensitive with an average threshold electrical conductivity (EC) of 3 dS m⁻¹ (Carrow and Duncan, 1998), is the most widely used cool-season turfgrass species in the more temperate regions of the western USA (Christians, 1998). Texas bluegrass (TBG) (*Poa arachnifera* Torr.) is native to the southern Great Plains, and persists under extended periods of high temperature (Gould, 1975). 'Reveille' hybrid bluegrass (HBG), a hybrid between KBG and TBG (*P. pratensis* x *arachnifera*) and recently released as a heat resistant hybrid

bluegrass for the southwestern USA (Read et al., 1999), may increase the area of bluegrass planted under saline conditions.

Several studies have been conducted to assess the effect of salinity on KBG (Grueb et al., 1983; Horst and Taylor, 1983; Torello and Spokas, 1983; Torello and Symington, 1984; Butler et al., 1985; Qian et al., 2001). However, screening of the considerable amount of KBG plant material released during the past 15 yr for salt tolerance has received little attention. Although KBG is generally ranked as a salt-sensitive turfgrass, variability in salt tolerance has been shown to exist among cultivars. Horst and Taylor (1983), examining germination and initial growth in saline solution culture, reported significant differences in salt tolerance during germination and initial growth between 44 KBG cultivars. Variability in salt tolerance at the species level has likewise been demonstrated in bluegrass. Grueb et al. (1983) found rough bluegrass (*Poa trivialis* L.) to be more salt tolerant than a group of 6 KBG cultivars, within which group there was significant variability in visual appearance under salt stress. Demonstrated inter- and intra- specific variability in salt tolerance among the *Poa* spp. suggests that genetic improvement in the salt tolerance of bluegrasses is possible. More current information regarding the salt tolerance of KBG is needed. Nothing is known of the salt tolerance of TBG or HBG.

Therefore, the objective of this study was to examine the growth and turf quality responses to salinity within and among KBG cultivars, TBG accessions, and their hybrids.

MATERIALS AND METHODS

Plant Material

Five KBG were chosen for this study: 'Huntsville', 'Dellwood Fine', and 'H-86-386' because they served as the pollen source for the HBG examined in this study, and 'Kenblue' and 'Bensun A-34' because previous work indicated that they exhibited contrasting salinity tolerance, respectively (Qian, unpublished data). Dellwood Fine, Huntsville, and Kenblue represented 3 common KBG ecotypes (erect growth habit, narrow leaf blade) and Bensun A-34 and H86-386 represented 2 aggressive ecotypes (aggressive lateral growth habit, high shoot density) as described by Murphy et al. (1997). Additionally, 4 unidentified Kentucky bluegrass plants discovered persisting on a highly sodic site at College Station, TX which exhibit the appearance of compact ecotypes (low, compact growth habit) were collected and included in this study. Three TBG accessions, 20-11, 10-30, and 10-24 which served as the female parents of the released hybrid, 'Reveille', and four HBG experimental lines (TXKY 96-260-6, TXKY 96-260-7, TXKY 96-260-7, and TXKY 94-8-8) were examined.

Plant Culture and Treatment Procedures

From 30 December 1999 to 30 May 2000 (Experiment I) and from 1 June to 30 October 2000 (Experiment II) plant material was screened for salinity tolerance in a greenhouse at the W.D. Holly Plant Environmental Research Center at Colorado State

University, Fort Collins, CO in a solution culture system (Qian et al., 2000). New sets of plants not previously subjected to salinity treatment were used at the initiation of each experiment. Greenhouse air temperatures ranged from 20.9 to 28.2 °C in Experiment I and from 20.8 to 36.1 °C in Experiment II. Because no supplemental light source was used during either experiment, mean photosynthetically active radiation (PAR) in the greenhouse increased from 33.8 to 78.1 W m⁻² in Experiment I (Dec to May) and decreased from 35.7 to 80.6 W m⁻² in Experiment II (Jun to Oct) (Table 1.1).

Table 1.1. Greenhouse mean minimum and maximum temperatures and irradiance during Experiment II and I in 2000.

	Mean min	Mean max	Mean PAR†
	°C		W m ⁻²
Experiment I			
Jan	20.9	27.2	33.8
Feb	21.5	27.3	45.5
March	21.7	26.8	58.2
April	21.5	27.4	71.8
May	21.9	28.2	78.1
Experiment II			
June	23.3	31.3	80.6
July	24.9	36.1	70.9
Aug	22.8	33.6	63.7
Sep	20.8	32.7	52.9
Oct	20.9	27.4	35.7

† PAR = photosynthetically active radiation.

Sod pieces of each grass were planted into 7-cm-diam. by 4-cm-deep plastic cups filled with a 1-cm layer of Isolite (Sundine Enterprises Inc., Arvada, CO). The cup bottom was removed and covered with nylon screen to hold the Isolite and allow roots to grow

through. Seventeen cups consisting of 1 entry per cup were placed into holes of a 1.5-cm-thick wooden lid, with the lid suspended over a 38-L-tank. A total of 15 tanks were used with each accommodating 17 cups. The tanks contained 36 L of constantly aerified full strength Hoagland solution, which was replaced weekly. This volume allowed the bottom of each cup to be submersed approximately 2 cm into the solution.

When plants had fully established, approximately 10 wk after planting, shoots and roots were clipped and discarded prior to initiation of salt treatments. Roots were clipped at the base of the cups and shoots were clipped to a 2.5-cm height. For salinity treatment, NaCl was gradually added daily, for a period of 5 days, to bring the tank solutions to their randomly assigned treatment level of 3, 5, 7, or 9 dS m⁻¹, which was measured using an EC meter (Hach Co., Model 50150). Sodium chloride was chosen because its presence in irrigation water produces the most detrimental effects on plant growth and soil structure. Electrical conductivity in the control tanks was maintained at 1.8 dS m⁻¹ throughout the study. Twelve tanks were subjected to salinity treatments while three tanks were maintained as controls for 10 wk. Experimental design was a split plot with three replications, salt treatment (tank) as the main effect, and entries within each tank being the subplot effect.

Data Collection and Analyses

Data were collected on shoot and root growth and leaf firing percentage. After reaching the designated treatment level, shoots were clipped weekly to a 6.4-cm-height and discarded. Beginning 4 wk after salinity treatment, when grasses had fully exhibited response to the salt treatments, clippings were collected weekly for about 6 wk. Clippings were immediately dried for 48 h at 70°C and weighed. Six of these harvests were

combined to determine shoot growth. At the conclusion of each experiment, roots were harvested and dried for 48 h at 70°, then weighed for dry mass. Leaf firing percentage was determined by visually estimating the total percentage of chlorotic leaf area at the conclusion of each experiment.

Analysis of variance indicated significant difference in all measured parameters between Experiments I and Experiment II (Table 1.2). Therefore, data on shoot and root growth and leaf firing percentage are presented separately. Linear and quadratic regression analysis was conducted to determine relationships between shoot and root growth vs. salinity level. By regression analysis, threshold EC (salinity level at which growth reduction began relative to control) and the slope of growth reduction were determined for each entry. The regression slopes were then used to derive salinity levels that caused 25 and 50% shoot and root growth reductions ($EC_{shoot\ 25}$, $EC_{shoot\ 50}$, $EC_{root\ 25}$, and $EC_{root\ 50}$) (Maas and Hoffman, 1977). Resulting data were then subjected to ANOVA tests, and cultivar means were separated by Fisher's LSD (SAS Institute, 1990). Pearson's correlation analysis between % leaf firing and threshold EC, $EC_{shoot\ 25}$, and $EC_{shoot\ 50}$ was conducted using the CORR procedure of SAS (SAS Institute, 1990). Group response comparisons were made using the TTEST procedure assuming both equal and unequal variance (SAS Institute, 1990). Using two factors, leaf firing percentage at 5 dS m⁻¹ and $EC_{shoot\ 50}$ as source data, cluster analysis was performed on all 17 entries using the non-hierarchical FASTCLUS procedure to place entries into groups not defined apriori (SAS Institute, 1990). Electrical conductivity of 50% shoot growth reduction values was transformed ($\max EC_{shoot\ 50} - xEC_{shoot\ 50}$) so that a lower

Table 1.2. Analysis of variances with mean square, experiment, and treatment significance.

Parameters	Experiment	Salinity	Grass	Experiment x salinity	Experiment x grass	Salinity x grass
Leaf firing	1151.9**	6900.1****	7718.6****	648.4 ns	4573.9****	229.5ns
Shoot wt.	27.9***	1.4****	0.266****	0.018ns	0.533**	0.046ns
Root wt.	132.9****	12.4****	3.2****	0.218ns	4.4****	0.238ns

ns, **, ***, **** = not significant or significant at the 0.01, 0.001, and 0.0001 levels, respectively.

score indicated greater tolerance by both factors. Thus both axis values increased with increasing distance from the point of origin.

RESULTS

Leaf Firing

Experiment I

All entries exhibited increased leaf firing with increasing salinity (data not shown). Analysis of variance indicated that greater variability among entries in leaf firing was exhibited at 5 dS m⁻¹ than other salinity levels; therefore leaf firing at this salinity level is presented in Table 1.3. Leaf firing response at 5 dS m⁻¹ ranged from 1.7 - 5.0 % in the most tolerant group (CS-ST 1 KBG, CS ST 3 KBG, CS ST 4 KBG, H-86-386 KBG and Bensun A-34 KBG) to 97.7% in the least tolerant group (TBG 10-24). Inter-specific comparisons revealed differences in leaf firing response at 5 dS m⁻¹ among KBG, TBG, and their hybrids, with KBG as a group exhibiting less leaf firing than TBG and their hybrids (Table 1.4). No significant difference in leaf firing was found between TBG and HBG. Among ecotypes, compact types showed less leaf firing than common types.

Experiment II

In agreement with Experiment I, all entries showed increased leaf firing with increasing salinity. Greatest differences within and among *Poa* spp. and their hybrids were exhibited at 5 dS m⁻¹ (Table 1.3). Leaf firing at 5 dS m⁻¹ ranged from 5 - 10% in the more tolerant group (CS ST 3 KBG, CS ST 4 KBG, and TBG 10-30) to 93.3 - 95% in the more susceptible group (Huntsville KBG and TXKY 96-270-7 HBG). Hybrid bluegrass as a group exhibited greater leaf firing than TBG. Kentucky

Table 1.3. Leaf firing, threshold salinity levels, and salinity levels of 25 and 50% shoot and root growth reduction for 3 Texas bluegrasses, 9 Kentucky bluegrasses, and 5 hybrid bluegrasses.

Entry	Type	Leaf firing (EC _w =5 dS m ⁻¹)	Growth reduction					
			Threshold	Shoot	50%		Root	50%
				25%	Threshold	25%		
Experiment I								
		—— % ——	EC (dS m ⁻¹)					
Texas bluegrass								
TXBG 10-30	unknown	91.7 ef	1.6 bc	3.3 cd	6.5 cdef	3.0 cdef	7.4 bcd	8.6 bcd
TXBG 10-24	unknown	97.7 f	1.0 c	2.2 cde	4.4 efg	1.0 f	2.5 e	5.1 cd
TXBG 20-11	unknown	46.7 bcd	3.0 a	5.6 b	8.1 bc	3.0 cdef	7.3 bcd	11.6 ab
Kentucky bluegrass								
Dellwood Fine KBG	common	30.0 abc	1.0 c	3.1 cde	6.2 cdefg	1.06 ef	2.4 e	4.8 cd
Huntsville KBG	common	18.3 ab	2.3 ab	2.8 cde	5.5 defg	5.0 abc	9.3 b	11.0 ab
Kenblue KBG	common	58.3 cd	2.3 ab	5.5 b	7.9 bcd	1.0 f	5.1 de	7.1 bcd
Bensun A-34 KBG	aggressive	1.7 a	1.6 bc	3.2 cde	6.3 cdefg	5.0 abc	6.7 bcd	8.1 bcd
H86-386 KBG	aggressive	3.3 a	1.0 c	6.3 b	9.5 b	1.6 ef	7.5 bcd	9.0 bcd
CS ST 1 KBG	compact	1.7 a	1.0 c	3.4 c	6.7 cde	7.0 a	13.3 a	15.8 a
CS ST 2 KBG	compact	20.0 ab	1.0 c	2.3 cde	4.7 efg	1.0 f	5.8 cd	11.7 ab
CS ST 3 KBG	compact	5.0 a	1.0 c	3.3 cd	6.6 cdef	3.7 cde	8.4 bc	10.8 ab
CS ST 4 KBG	compact	5.0 a	2.3 ab	7.8 a	12.6 a	4.3 bcd	8.4 bc	9.9 bc
Hybrid bluegrass								
TXXY 96-260-7	H-86-386 KBG x TXBG 10-30	89.3 e	1.6 bc	2.1 de	4.2 fg	1.0 f	2.4 e	4.7 cd
TXXY 96-260-6	H-86-386 KBG x TXBG 10-30	41.7 bcd	1.6 bc	2.2 cde	4.4 efg	1.6 ef	2.3 e	4.7 cd
TXXY 94-8-8	Dell. Fine KBG x TXBG 10-24	40.0 bcd	3.0 a	5.4 b	7.9 bcd	6.3 ab	5.9 cd	7.2 bcd
TXXY 96-260-22	H-86-386 KBG x TXBG 10-30	68.3 de	1.6 bc	2.4 cde	4.9 efg	2.3 def	8.3 bcd	10.5 ab
Reveille HBG	Huntsville KBG x TXBG 20-11	88.3 ef	1.0 c	2.0 e	4.0 g	1.6 ef	2.2 e	4.4 d
LSD (P < 0.05)		29.0	1.3	1.2	2.5	2.1	3.3	5.4
F test		12.0****	2.4**	16.4****	6.9****	7.3****	8.2****	3.2***
CV		42.0	46.5	20.1	22.7	42.3	29.4	34.7

†Means followed by the same letter within a column are not significantly different at the 0.05 probability level using Fisher's LSD test.

ns, **, ***, **** indicate non-significant or significant at the 0.01, 0.001, and 0.0001 levels, respectively.

Table 1.3. Continued.

Entry	Type	Leaf firing (EC _w =5 dS m ⁻¹)	Growth reduction					
			Threshold	Shoot		Root		50%
				25%	50%	Threshold	25%	
Experiment II								
		— % —	EC (dS m ⁻¹)					
Texas bluegrass								
TXBG 10-30	unknown	10.0 a	1.6 bc	5.2 cdefg	10.5 abcd	3.0 a	7.2 b	8.6 b
TXBG 10-24	unknown	18.3 abc	1.6 bc	5.7 cdefg	11.4 abcd	1.6 a	8.5 a	12.1 a
TXBG 20-11	unknown	16.7 ab	1.6 bc	4.4 efg	8.8 bcd	1.6 a	3.1 cdef	6.2 cdef
Kentucky bluegrass								
Dellwood Fine KBG	common	35.0 abcd	1.6 bc	6.3 bcdef	12.5 abc	1.0 a	2.6 cdef	5.2 defg
Huntsville KBG	common	93.3 e	2.3 bc	6.3 bcdef	9.5 abcd	1.0 a	2.4 ef	4.8 defg
Kenblue KBG	common	38.3 abcd	1.6 bc	4.8 defg	6.7 cd	1.0 a	2.9 cdef	6.3 bcdef
Bensun A-34 KBG	aggressive	15.0 ab	3.0 ab	10.0 a	15.0 a	1.6 a	2.5 def	5.0 defg
H86-386 KBG	aggressive	35.0 abcd	4.3 a	8.9 ab	14.8 ab	1.6 a	3.4 cde	6.9 bcd
CS ST 1 KBG	compact	20.0 abc	2.3 bc	8.2 abc	13.4 ab	3.0 a	3.8 cd	7.6 bc
CS ST 2 KBG	compact	53.3 cd	1.0 c	3.5 fg	7.0 cd	1.6 a	2.4 ef	4.8 efg
CS ST 3 KBG	compact	5.0 a	4.3 a	5.6 cdefg	11.2 abcd	1.0 a	8.6 a	11.2 a
CS ST 4 KBG	compact	10.0 a	1.6 bc	7.1 abcde	14.2 ab	1.6 a	2.3 ef	4.6 fg
Hybrid bluegrass								
TXXY 96-260-7	H-86-386 KBG x TXBG 10-30	95.0 e	1.0 c	3.1 g	6.2 d	1.0 a	1.9 f	3.9 g
TXXY 96-260-6	H-86-386 KBG x TXBG 10-30	46.7 bcd	2.3 bc	6.4 bcdef	9.8 abcd	1.0 a	2.3 ef	4.7 fg
TXXY 94-8-8	Dell. Fine KBG x TXBG 10-24	65.0 de	3.0 ab	7.5 abcd	10.0 abcd	3.0 a	6.9 b	8.9 b
TXXY 96-260-22	H-86-386 KBG x TXBG 10-30	63.3 de	3.0 ab	3.6 fg	7.3 cd	1.6 a	3.1 cdef	5.3 defg
Reveille HBG	Huntsville KBG x TXBG 20-11	33.3 abcd	2.3 bc	5.9 bcdefg	8.8 bcd	2.3 a	3.9 c	6.8 bcde
LSD (P < 0.05)		35.7	1.9	3.0	6.1	2.1	1.3	3.3
F test		4.9****	2.1**	3.7***	2.0**	1.4***	22.7****	9.7****
CV		55.9	50.7	28.1	32.9	38.6	19.6	18.8

†Means followed by the same letter within a column are not significantly different at the 0.05 probability level using Fisher's LSD test.

ns, **, ***, **** indicate non-significant or significant at the 0.01, 0.001, and 0.0001 levels, respectively.

bluegrass had intermediate leaf firing percentage but was not statistically different from TBG or HBG. Unlike in Experiment I, there was no difference in leaf firing among KBG ecotypes at 5 dS m⁻¹. As a group, TBG exhibited a significantly lesser degree of leaf firing in Experiment II than Experiment I (78.7 vs. 15.0%) (Table 1.4).

Shoot Growth Reduction

Experiment I

Differences were found for threshold EC, EC_{shoot} 25 and EC_{shoot} 50 (Table 1.3). Threshold EC ranged from 1.0 - 3.0 dS m⁻¹. The highest threshold EC of 3.0 was observed in TBG 20-11, Kenblue and H-86-386 KBG, and TXKY 94-8-8 HBG. Salinity levels that caused 25% shoot growth reduction ranged from 2.0 dS m⁻¹ in Reveille HBG to 7.8 dS m⁻¹ in CS ST 4 KBG. Salinity levels that caused 50% shoot growth reduction ranged from 4.0 dS m⁻¹ in Reveille HBG to 12.6 dS m⁻¹ in CS ST 4 KBG. Variability in shoot growth reduction both within and among *Poa* spp. and their hybrids increased from EC_{shoot} 25 to EC_{shoot} 50 indicating the influence of slope on shoot growth reduction in response to increasing salinity. No differences were found in threshold EC, EC_{shoot} 25, and EC_{shoot} 50 between *Poa* spp., their hybrids, or among KBG ecotypes as groups (Table 1.4).

Experiment II

Again, significant variability in shoot growth reduction parameters was found within and among *Poa* spp. and their hybrids (Table 1.3). Threshold EC ranged from 1.0 to 5.0 dS m⁻¹, with the highest threshold EC of 5.0 observed in Bensun A-34 KBG, H-86-386 KBG, and TXKY 94-8-8 HBG. Values of EC_s 25 ranged from 3.1 dS m⁻¹ in

Table 1.4. Mean performance of bluegrass entries in 3 groups for leaf firing at 5 dS/m, threshold salinity level and salinity level causing 25 and 50% shoot and root growth reductions.

Group	Entries (No.)	Leaf firing (EC _w =5 dS m ⁻¹)	Growth reduction												
			Shoot								Root				
			Threshold	25%		50%		Threshold	25%		50%				
				EC (dS m ⁻¹)											
		— % —													
		Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II	Exp. I	Exp. II
Texas bluegrass	9	78.7a	15.0 b*	1.9 a	1.6 a	3.7 a	5.1 a	6.3 a	10.2 a*	2.3 a	2.1 a	5.7 a	6.3 a	8.4 a	9.0 a
Kentucky bluegrass	3	15.9 b	33.9 ab	1.5 a	2.4 a	4.2 a	6.7 a*	7.3 a	11.6 a*	3.3 a	1.5 a*	7.4 a	3.4 a*	9.8 a	6.3 a*
Hybrid bluegrass	5	65.5 a	60.7 a	1.8 a	2.3 a	2.8 a	5.3 a*	5.1 a	8.4 a*	2.6 a	1.8 a	4.2 a	3.6 a	6.3 a	5.9 a

*, indicates significant difference from Exp. I at the 0.05 probability level

TXKY 96-260-7 HBG to 10.0 dS m^{-1} in Bensun A-34 KBG. Both HBG and KBG significantly increased their mean $EC_{\text{shoot } 25}$ from Experiment I to II. Among KBG ecotypes, aggressive types exhibited higher $EC_{\text{shoot } 25}$ than both common and compact types (Table 1.4).

Salinity levels that caused 50% shoot growth reduction values ranged from 6.2 dS m^{-1} in TXKY 96-260-7 HBG to 15.0 in Bensun A-34 KBG. Although the most and least tolerant entries were the same from $EC_{\text{shoot } 25}$ to $EC_{\text{shoot } 50}$, greater difference in $EC_{\text{shoot } 50}$ was found between KBG and HBG than $EC_{\text{shoot } 25}$, indicating influence of slope of reduction on decreased shoot growth. Both species and their hybrids increased their mean $EC_{\text{shoot } 50}$ in Experiment II compared to Experiment I (Table 1.4). Among KBG bluegrass ecotypes, aggressive types exhibited higher $EC_{\text{shoot } 50}$ than common types.

Root Growth Reduction

Experiment I

Relationships between root growth and level of salinity were determined for each entry. Variability existed within and among *Poa* spp. and their hybrids for each measured or derived root growth reduction parameter (threshold EC, EC that caused 25 and 50% root growth reduction) (Table 1.3). Threshold EC ranged from $1.0 - 7.3 \text{ dS m}^{-1}$. The highest threshold EC of 7.3 was observed in CS ST 1 KBG. Salinity levels that caused 25% root growth reduction ranged from as low as $2.2 - 2.5 \text{ dS m}^{-1}$ in Reveille HBG, TXKY 96-260-6 HBG, TXKY 96-260-7 HBG, Dellwood Fine KBG, and TBG to as high as 13.3 dS m^{-1} in CS ST 1 KBG. Electrical conductivity of 50% root growth reduction ranged from 4.4 dS m^{-1} in Reveille HBG to 15.8 in CS ST 1 KBG. No difference in EC_{root}

25 was found between *Poa* spp. and their hybrids. No difference in any root growth reduction parameter was exhibited among KBG ecotypes.

Experiment II

Significant variability in all measured and derived root growth reduction parameters were found within and among *Poa* spp. and their hybrids (Table 1.3). Threshold EC ranged from 1.0 - 5.0 dS NaCl m⁻¹, with the highest threshold EC of 5.0 observed in TBG 10-24 and TXKY 94-8-8 HBG. Values of EC_{root} 25 ranged from a low of 1.9 dS m⁻¹ in TXKY 96-260-7 HBG to 8.5 dS m⁻¹ in TBG 10-24. No difference was found among KBG ecotypes for EC_{root} 25.

Although salinity levels that caused EC_{root} 50 ranged from 3.9 dS m⁻¹ in TXKY 96-260-7 HBG to 12.1 dS m⁻¹ in TBG 10-24, no difference in EC_{root} 50 was found between *Poa* spp., their hybrids, or among KBG ecotypes. Even though the most and least tolerant entries ranked the same from EC_{root} 25 to 50, variability within and among *Poa* spp. and their hybrids was greater at EC_{root} 50 than EC_{root} 25.

Kentucky bluegrasses exhibited a significantly lower (7.3 to 3.4 dS m⁻¹) EC_{root} 25 in Experiment II than I, because of poorer performance of the compact and aggressive ecotypes in Experiment II. The same occurred for EC_{root} 50, however at this level it was primarily attributable to the poorer performance of only compact ecotypes.

Salt Tolerance Ranking

To assess the overall salt tolerance of all entries, cluster analysis was performed based on relative leaf firing and EC_{shoot} 50 (Fig. 1.1). These factors were chosen because turfgrass appearance and growth are two important factors influencing overall turf

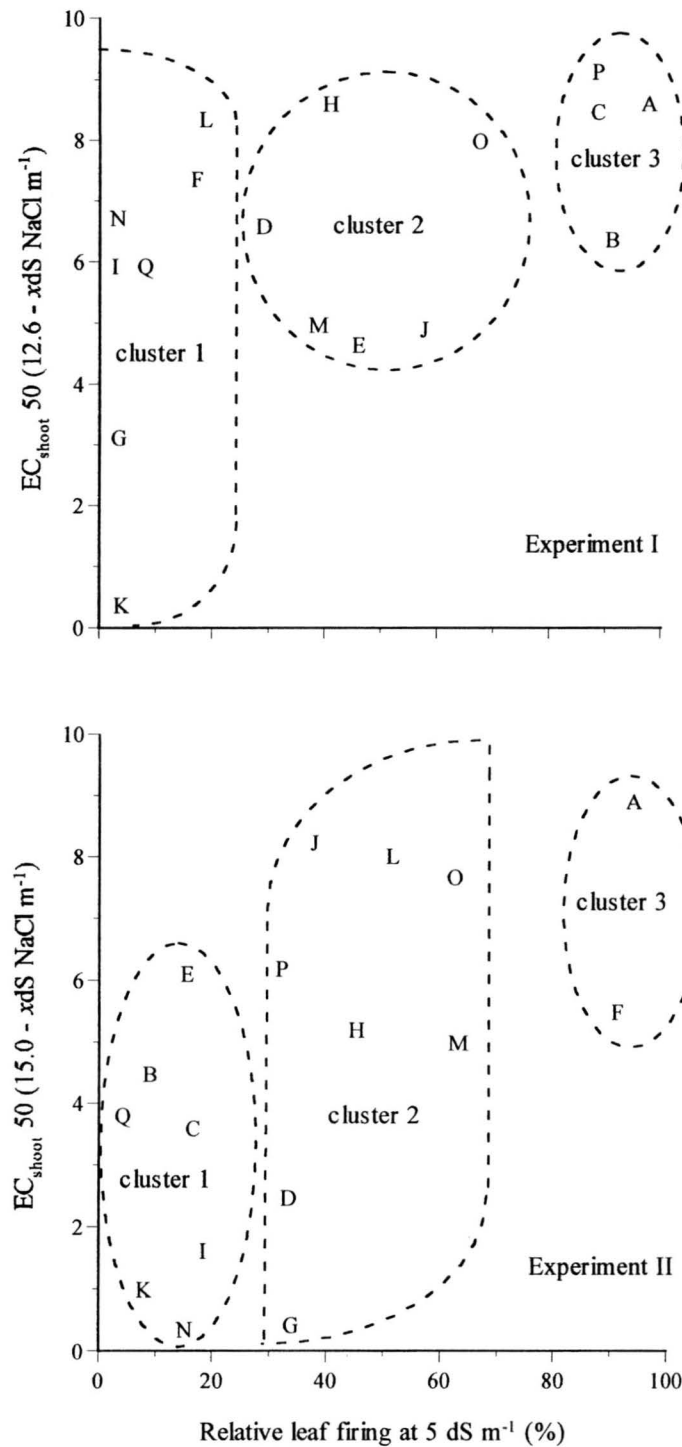


Figure 1.1 Cluster analysis of Experiment I and Experiment II based on relative leaf firing at 5 dS m⁻¹ and salinity level resulting in 50% shoot growth reduction (EC_{shoot} 50) for 3 Texas bluegrasses (TXBG), 9 Kentucky bluegrasses (KBG), and 5 of their hybrids (HBG). A=TXKY 96-260-7 HBG, B=TXBG 10-30, C=TXBG 10-24, D=Dellwood Fine KBG, E=TXBG 20-11, F=Huntsville KBG, G=H-86-386 KBG, H=96-260-6 HBG, I=CS ST 1 KBG, J=Kenblue KBG, K=CS ST 4 KBG, L=CS ST 2 KBG, M=TXKY 94-8-8 HBG, N=Bensun A-34 KBG, O=TXKY 96-260-22 HBG, P=Reveille HBG, Q=CS ST 3 KBG.

performance, and the highest correlation between relative leaf firing and shoot growth as found between EC_{shoot} 50 (Table 1.5).

Table 1.5. Correlation coefficients for turfgrass appearance (% leaf firing) and growth (threshold EC, EC of 25 and 50% shoot growth reduction) measurements of salt treated and control plants for 17 bluegrasses grown in the greenhouse in solution culture.

	Threshold EC	EC _{shoot} 25	EC _{shoot} 50
Experiment I			
% Leaf firing (5 dS m ⁻¹)	-0.208	-0.412	-0.506*
Experiment II			
% Leaf firing (5 dS m ⁻¹)	0.111	-0.353	-0.578*

* Significant correlation at the 0.05 probability level.

Experiment I

The group with the highest salt tolerance ranking (cluster 1) was composed only of KBG (CS ST 4 KBG, H-86-386 KBG, CS ST 1 KBG, CS ST 3 KBG, Bensun A-34 KBG, Huntsville KBG, and CS ST 2 KBG) including 4 compact, 2 aggressive, and 1 common ecotypes (Fig. 1.1). The intermediate and least tolerant groups (clusters 2 and 3, respectively) included KBG, TBG, and HBG.

Experiment II

In Experiment II the most salt tolerant group (cluster 1) was composed of 4 KBG (Bensun A-34, CS ST 1, CS ST 3, and CS ST 4), including 3 compact and 1 aggressive ecotype, and all 3 TBG examined (10-24, 10-30, and 20-11) (Fig. 1.1). Again, the

intermediate and least tolerant groups (clusters 2 and 3, respectively) included KBG, TBG, and HBG.

DISCUSSION

Results indicate that significant variability in leaf firing, and shoot and root growth responses to salinity exist within and among *Poa* spp., and their hybrids. The range of salinity tolerance among the 9 KBG in relative leaf firing response at 5 dS m⁻¹ in both Experiment I (1.7 - 58.3%) and Experiment II (5.0 - 93.3%) was considerably broad. Among the KBG examined in this study, greatest salt tolerance was seen in the aggressive and compact ecotypes. Qian et al. (2001) also reported that 'Limousine' KBG, an aggressive ecotype, was more salt tolerant than Kenblue, a common ecotype KBG. Nevertheless, KBG is still a salt sensitive turfgrass species when compared with other species, such as tall fescue (*Festuca arundinacea* Schreb.) and creeping bentgrass (*Agrostis palustris* Huds.) (Carrow and Duncan, 1998). Selecting relatively salt tolerant KBG cultivars is beneficial for sites where salinity level marginally high.

Differences between Experiment I and II suggested that environmental conditions could substantially affect bluegrass salt tolerance (Table 1.4). In a previous study (Qian and Suplick, 2001), involving the interactive effect of temperature and salinity on KBG seed germination, the effect of salinity became more pronounced as temperature deviated from optimum growth range. Optimum temperature range for KBG shoot growth is 16 to 24 °C, and 10 to 18°C for root growth (Beard, 1973). Optimum shoot and root growth temperature ranges have not been determined for TBG or HBG.

Texas bluegrass is distributed throughout several vegetative regions of Texas, where mean maximum summer temperatures range from 34 to 37 °C (Gould, 1975; NOAA-CIRES Climate Diagnostics Center, 2001). This region of origin suggests that TBG may have a warmer optimum temperature range than KBG. Current and ongoing research (Suplick and Qian, 2000) suggests that HBG may have a broader temperature adaptation than those of KBG and TBG.

This speculation may be supported by the differences in performance from Experiment I to Experiment II of the 3 *Poa* groups examined in this study in many measured and derived parameters (Table 1.4). Experiment I was conducted during winter and spring. Experiment II was conducted throughout summer into fall when daily warm temperatures were higher, and their duration prolonged possibly creating an environment more favorable to TBG but less favorable to KBG growth. This change in environment may explain several observations:

- (1) There was an 81% decrease in TBG mean leaf firing at 5 dS m⁻¹ from Experiment I to Experiment II.
- (2) KBG root threshold EC, EC_{root} 25, and EC_{root} 50 were significantly decreased from Experiment I to II, whereas no change was seen in TBG or HBG.
- (3) There was an improvement in overall salt tolerance for all three TBG, reduction in tolerance for several KBG, and relatively steady ranking of HBG from Experiment I to II.

This research supports that of Horst and Taylor (1983) and Grueb et al. (1983), in that significant variability in salt tolerance exists within and among *Poa* spp., indicating that improvement in the salt tolerance of bluegrasses may be possible. Additionally, our

findings, with respect to the effect of sub- and supra-optimum temperature regimes on the expression of that tolerance, are in agreement with previous research (Qian and Suplick, 2001), confirming the importance of temperature in evaluating the salt tolerance of *Poa* spp.

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CHAPTER 2

EVAPOTRANSPIRATION, ROOTING CHARACTERISTICS, AND DROUGHT RESPONSE: A COMPARISON BETWEEN HYBRID BLUEGRASS AND KENTUCKY BLUEGRASS

ABSTRACT

Kentucky bluegrass (*Poa pratensis* L.) (KBG) is the predominant cool-season turfgrass used throughout temperate regions and the transition zone of the USA. In the arid and semi-arid west, some government agencies have imposed restrictions on its use due to its sensitivity to drought. Hybrid bluegrass (HBG), a cross between native Texas bluegrass (*Poa arachnifera* Torr.) and KBG, has been released as a heat resistant bluegrass for the southern USA where KBG is unable to withstand high summer temperatures. It is unknown if HBG possesses the ability to avoid drought, unlike KBG. Hence, our objectives were to compare water use rate and drought avoidance of KBG and HBG. Studies were conducted in both the field and greenhouse during 2000 and 2001 at Fort Collins, CO to examine ET rates under non-limiting soil moisture conditions, vertical rooting patterns, and relationships among surface soil water depletion (SWD), shoot water content (SWC), and turfgrass response to drought. In the field, mean HBG ET (5.0 mm d^{-1}) was significantly less than that of KBG (6.1 mm d^{-1}) during the period of July through Sep during 2000 and 2001. As evaporative demand increased, the magnitude of difference in ET rate between HBG and KBG increased. Mean HBG clipping yield in the

greenhouse was 12% that of KBG. In both the field (clay) and greenhouse (sand), at each soil depth examined (0-20, 20-40, and 40-60 cm) HBG exhibited significantly greater root length density (RLD), total root length (TRL), and total root mass (TRM) than KBG. In the field HBG exhibited greater drought avoidance, maintaining turf quality longer during prolonged dry-down than KBG. These factors combined contribute significantly to the reduced water use and greater drought avoidance observed in HBG relative to KBG.

INTRODUCTION

Kentucky bluegrass (*Poa pratensis* L.) (KBG) is the predominant cool-season turfgrass used on home lawns, golf courses, and athletic fields throughout temperate regions and transition zone of the USA due to its high quality appearance and desirable growth characteristics (Christians, 1998). However, as demand on limited fresh water resources continues to grow, especially throughout the arid and semi-arid west, some government agencies have begun imposing restrictions or limitations on the use of KBG due to its poor drought resistance (City of Santa Fe, NM, 2001). Drought resistance is a general term encompassing a range of mechanisms employed by plants to withstand periods of drought; drought-tolerating plants possess mechanisms to endure tissue water deficits, whereas drought-avoiding plants possess mechanisms to maintain positive tissue water balances. Kentucky bluegrass does in fact possess drought resistance, however, it is manifested in the form of drought tolerance. Under periods of prolonged drought KBG enters dormancy, appearing brown, and thus, visually unappealing. This has created demand for a comparable alternative; a turfgrass possessing the valued appearance and growth characteristics of KBG but maintaining green color and not entering dormancy quickly. 'Reveille' hybrid bluegrass (HBG), a cross between native Texas bluegrass (*Poa arachnifera* Torr.) and KBG, has recently been developed and released as a heat resistant bluegrass for the southern USA, where high summer temperatures are detrimental to

KBG (Read et al., 1999). At present, the mechanism by which HBG resists heat stress, and whether it is an indication of drought resistance, is not known.

In the transition zone, where high summer temperatures are typically the most limiting factor to maintaining KBG, tall fescue (*Festuca arundinacea* Schreb.) is often recommended based on its better heat resistance (Duble, 1996; Christians, 1998). However, Wallner et al. (1982) reported no difference in the killing times and temperatures between these two grasses in vitro. This finding would suggest sustained transpirational cooling, via a deeper, more extensive root system, during periods of high temperature stress as the primary mechanism of heat avoidance in tall fescue. Strong positive correlations between possession of a deeper, more extensively developed root system with both heat and drought resistance have repeatedly been demonstrated in several turfgrass species (Lehman and Engelke, 1993; Qian et al., 1997; Ervin and Koski, 1998; Bonos and Murphy, 1999; Jiang and Huang, 2000). It is unknown if the improved heat resistance of HBG relative to Kentucky bluegrass is also associated with a deeper and more extensive root system.

In addition to deeper and greater root production and distribution throughout the soil profile, an inherently low rate of evapotranspiration (ET), as well the ability to maintain ET rate as the soil dries, have also been identified as mechanisms of drought avoidance (Beard, 1989; Carrow, 1996a). When soil is covered by a dense turfgrass stand, water loss is due mainly to transpiration. Low rates of transpiration may be achieved by inherent shoot characteristics such as leaf cuticle thickness, fewer exposed stomata, and slowed shoot growth rate (Beard, 1973). Research has indicated that KBG ET rate generally ranges from 3.6-6.3 mm d⁻¹ under varying climatic, edaphic, and

management conditions, yet no clear relationship between ET rate and drought resistance in KBG has emerged (Feldhake et al., 1983; Shearman, 1986; Aronson et al., 1987; Fernandez and Love, 1993; Keeley, 1996). No information is available regarding neither the water use characteristics of HBG nor its response to drought conditions.

Hence, our objectives were to evaluate HBG, relative to KBG, for (i) ET rates under non-limiting soil moisture conditions in the field, (ii) vertical rooting patterns in the field and greenhouse, and (iii) relationships among surface soil water depletion (SWD), shoot water content, and turfgrass leaf wilting in the field.

MATERIALS AND METHODS

Field Study

Experimental Design and Cultural Conditions

This study was conducted during the summers of 2000 and 2001. Reveille HBG and Bensun's A-34 KBG were established by sod at the Colorado State University Horticulture Field Research Center, Fort Collins, CO in Oct. 1999 on a Nunn clay loam soil (fine montmorillonitic mesic aridic argiustoll). The climate is semi-arid with an average annual precipitation of 365 mm. Plots measuring 2.5 by 2.5 m were arranged in a randomized complete block design with four replications and were separated from each other by a 0.3 m perennial ryegrass (*Lolium perenne* L.) strip. The experimental area contained four semi-circle, popup spray heads at the corners providing uniform irrigation. Plots were mowed 2 to 3 times weekly to maintain a height of 6.4 cm and were fertilized with 149 Kg N ha⁻¹ yr⁻¹ (as urea 46-0-0) between April and October.

Environmental parameters were monitored and recorded throughout the 2000 and 2001 by a weather station over a field of actively growing alfalfa maintained at approx. 30 cm. located ~ 200 m east of the experimental site. With the climate data reference evapotranspiration (ET_r) was estimated based on the 1982 Kimberly-Penman equation (ASCE, 1990). These estimates were summed every 2 to 3 d and multiplied by a crop coefficient (K_c) of 0.91 determined by the Northern Colorado Water Conservancy District for KBG mowed at 6.4 cm in this area. The resulting number minus any

precipitation that had occurred represented the estimated amount of irrigation needed to replace 100% ET.

Evapotranspiration Measurements

Weighing bucket lysimeters to determine ET were installed in the middle of each of the eight plots in April 2000. The polyvinyl chloride (PVC) lysimeters measured 30.5 cm in diameter and 80 cm deep with soil occupying the top 61 cm, separated by a 1.3 cm thick PVC plate covered by a filter fabric. Below the drainage plate was a 6 cm deep air space area connected to a 1 cm diameter PVC air inlet tube open to the air at the top of the lysimeter. Native soil was removed for each lysimeter in 15.2 cm increments and placed aside. Sheet metal sleeves were inserted to stabilize the area within which lysimeters were to be placed. Lysimeters were then filled with each appropriate 15.2 cm layer of soil, packed to an approximate bulk density of 1.30 g cm^{-3} , and placed in the ground at a height uniform with the surrounding turf. Lysimeters were mowed concurrently with the experimental site the day before lysimeters were weighed. During each irrigation application lysimeters were covered with plastic lids. Evapotranspiration measurements were collected every 2 to 3 days between 5 June and 1 Sep. 2000 and 2 July and 5 Oct. 2001. An electronic load cell (Revere Transducers, Tustin, CA) connected to a calibrated strain gauge meter (Model: MDS41, Omega Engineering, Stamford, CT) was used to weigh lysimeters. The load cell had a resolution equivalent to 0.025 mm of water. Five readings per lysimeter were made and the average value was used for ET calculation. Evapotranspiration was calculated by mass difference and water lost was returned to individual lysimeters by hand.

Root Measurements

Vertical rooting patterns to a 60 cm depth plots were determined by obtaining soil cores (5.5 cm in diam.) using a truck-mounted Gidding's hydraulic soil probe (Gidding's Manufacturing, Fort Collins, CO) on 28 Sep. 2000 and 5 Oct 2001. Four sub-samples were collected from each plot, cut into 20 cm long segments representing depths of 0-20, 20-40, and 40-60 cm., and washed of soil with a hydropneumatic elutriation system (Smucker et al., 1982). After washing, a methyl-based violet staining solution was applied to enhance the image of finer roots. Root length density at 0-20, 20-40, and 40-60 cm were determined with a digital image analyzing system (Delta-T Devices Ltd., Cambridge, UK). Total root length was defined as the sum of root lengths from all depths. Subsequent to length measurements, samples were dried and root mass determined.

Shoot Measurements and Surface SWD

Leaf water content, leaf wilting, and SWD from the top 20 cm of the soil profile were measured concurrently every other day during the summer of 2001. Within each plot 1 by 1 m areas were selected and 20 cm time domain reflectometry (TDR) waveguides installed to evaluate drought responses. During the first year of the study 40 and 60 cm TDR waveguides were installed, however, data collection proved problematic due to strong signal attenuation. Thus, SWD data at these depths is not provided. Prior to each drought cycle, plot soils were slowly brought to field capacity over a one-week period. Subsequently, irrigation was withheld from the sample areas by placement of 1.2 by 1.5 m clear polycarbonate shelters. To determine leaf water content on each sampling date, four sub-samples of leaf clippings from each experimental unit were collected.

After measuring fresh weight, clippings were dried and leaf water content was then calculated. Volumetric soil water content was recorded by means of TDR (Trace, Model: 6050X1, Soil Moisture Equipment Corp., Santa Barbara, CA). Turfgrass was visually evaluated for leaf wilting on a scale of 0 to 9, where 0 = 100% leaf firm and 9 = no wilting symptoms.

Greenhouse Study

Shoot and Root Measurements

This study was conducted from 6 July to 5 Nov. 2001 in the greenhouse at Colorado State University. Clear PVC root tubes measuring 10.2 cm in diam. and 122 cm in length contained a 7.6 cm gravel base with drainage holes at the bottom. These tubes were filled with washed sand and vibrated by hand until a uniform column formed. Each tube was placed in a black PVC sleeve with a bottom cap drilled with holes for drainage. Sod pieces, approximately 10 cm in diam., of Reveille HBG and Bensun's A-34 KBG were collected from the field plots with a cup cutter. They were hand-washed to remove soil and planted in the clear PVC tubes after roots were trimmed to the crown. Tubes, arranged in a randomized complete block design, were then placed in a rack and supported at 15° angles from vertical so that root growth could be observed along the side of the tubes. Average day/night air temperature was 22.5/17 °C. A 20-10-20 N-P-K soluble fertilizer containing all the micronutrients was applied weekly to each tube through the study period. Irrigation was applied by an injection system that provided 5.5 mm d⁻¹. Both grasses were clipped weekly at 6.4 cm. Clippings were collected, dried, and weighed to determine rates of shoot growth. Roots in all tubes were harvested when no further advance of root was observed in any of the tubes. When the study ended, roots

were washed free of sand, stained, and RLD, TRL, and TRM at 0-20, 20- 40, and 40-60 cm were determined as described previously. Maximum root extension was determined by measuring the length of the deepest root visible at the sand-root tube interface.

Statistical Analysis

Data from the field were analyzed using the general linear model procedure to determine differences between grasses (SAS, 1990). Due to the effect of climatic conditions, dry-down duration was shorter in the first study than in the second. Therefore data on SWD, LWC, and turfgrass wilting over time are presented separately. However, ET and vertical rooting patterns in the field were not statistically different between the first and second study, therefore data for both years were combined for analyses. Simple linear regression was used to compare lysimeter measured ET to KBG reference ET. Means were separated using protected LSD at $P < 0.05$ for both the field and greenhouse studies.

RESULTS AND DISCUSSION

Evapotranspiration

Mean HBG ET of 5.0 mm d^{-1} was significantly less than that of 6.1 mm d^{-1} found in KBG during the months of July through Sep during 2000 and 2001. While measured KBG mean ET was at the upper end of the range of those previously reported ($3.6\text{--}6.3 \text{ mm d}^{-1}$), throughout the duration of this study measured KBG ET was well correlated ($R^2 = 0.954$) to that predicted by the Northern Colorado Water Conservancy District for KBG mowed at the same height (6.4 cm) (Fig. 2.1). As evaporative demand increased, the magnitude of difference between HBG and KBG ET increased (Fig. 2.1). For example, regression analysis showed that HBG ET was 85, 82, 81, and 80% that of KBG as predicted demand increased to 4, 6, 8, and 10 mm d^{-1} , respectively.

Our data is similar to that of Aronson et al. (1987) and Sheffer (1979) who found changes in the relative ranking of cool-season turfgrass ET as evaporative demand increased. Under non-limiting soil moisture conditions turfgrass water use is understood to be a function of climatic conditions and stand density. However, shoot characteristics which decrease shoot surface area and increase canopy resistance have been shown to moderate transpiration and increase drought resistance in turfgrasses (Burt and Christians, 1990; Kim and Beard, 1988). Relative to KBG, HBG exhibited coarser leaf texture and slower shoot growth rate (as we will discuss below) that may have served to moderate transpiration.

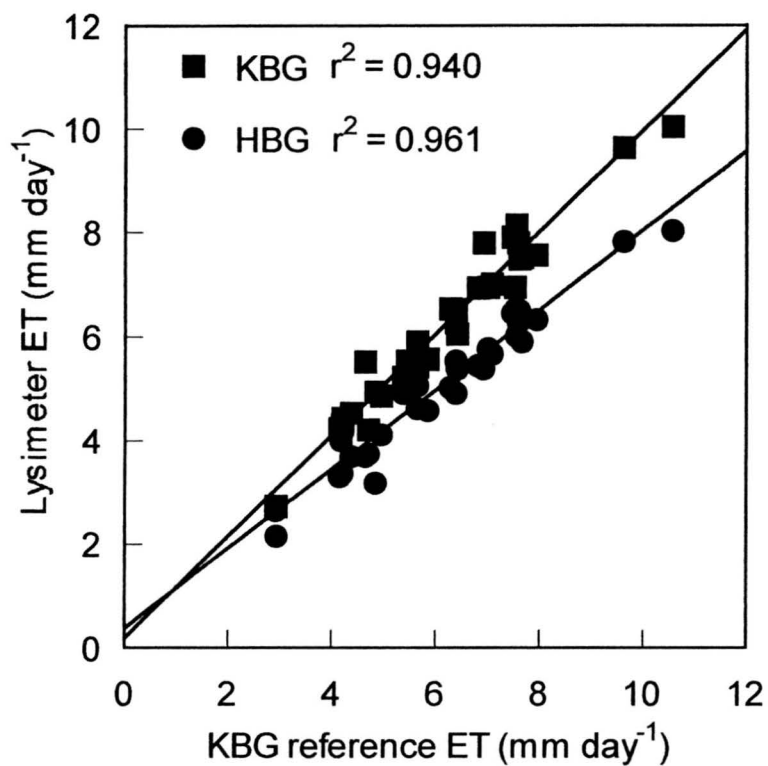


Fig. 2.1. Evapotranspiration of A-34 Kentucky bluegrass (KBG) and Reveille hybrid bluegrass (HBG) during summer 2000 and 2001 at Fort Collins, CO. Each data point represents the mean of four replications.

Shoot Growth

Greenhouse Study

Mean HBG clipping yield over the five-month period was 12% that of KBG, indicating an inherently slower rate of shoot growth. Interspecific differences in inherent leaf growth rates have been reported in cool season turfgrasses previously (Hull, 1992). As discussed previously, an inherently slower rate of shoot growth is one characteristic by which turfgrasses may reduce transpiration.

Rooting Characteristics

Field Study

At each soil depth HBG exhibited significantly greater RLD TRL, and TRM than KBG (Table 2.1). Greatest RLD was observed within the 0-20 cm in both grasses, with HBG distributing 82.9% and KBG 90.3% of their TRL at this depth. At the 20-40 cm depth HBG produced 9.8% and KBG 7.3% of their roots. The remaining 7.3% of HBG and 2.4% of KBG TRL were distributed within the 40-60 cm soil level.

Kentucky bluegrass roots were observed to be finer and more pliable than HBG roots, which appeared thicker and coarser. Calculation of the mass to length ratio (TRM: TRL) for each grass supported this observation, with HBG exhibiting a TRM: TRL of $0.0978 \text{ mg cm}^{-1}$ and KBG $0.0523 \text{ mg cm}^{-1}$.

Greenhouse Study

As in the field, HBG exhibited greater RLD, TRL, and TRM than KBG (Table 2.1). Differences were seen, however, in both grasses, in RLD distribution throughout the

Table 2.1. Root length density (RLD) at three depths, total root length (TRL), total root mass (TRM), and maximum root extension (MRE) of A-34 Kentucky bluegrass and Reville hybrid bluegrass grown in the field and the greenhouse at Fort Collins, CO.

Grass	Field					Greenhouse					
	RLD (cm cm ⁻³)					RLD (cm cm ⁻³)					
	0-20	20-40	40-60	TRL	TRM	0-20	20-40	40-60	TRL	MRE	TRM
	(cm)				(mg)	(cm)					(mg)
A-34 Kentucky bluegrass	3.7	0.3	0.1	935.8	48.9	5.0	1.2	0.1	1264.7	43.2	72.2
Reville hybrid bluegrass	6.8	0.8	0.6	1739.2	170.1	6.7	2.4	1.2	2668.1	57.8	229.2

† Within each column, all means are significantly different at the 0.05 probability level.

sand profile when compared to the field. In the greenhouse, HBG root distribution from 0-20 cm was reduced to 65% and KBG to 79.4%, indicating greater distribution of roots deeper in the profile under sandy conditions when compared to the clayey field soil. At 20-40 cm, HBG RLD was increased to 23.3% and KBG to 19%. Compared to field study, greater difference in RLD between two grasses was in the sand based soil profile in the greenhouse study where HBG distributed 11.7 % and KBG 1.6% of their RLD. Hybrid bluegrass MRE was 14.6 cm longer that of KBG.

These results support those of Carrow (1996b) who noted that under field conditions genetic rooting potential is rarely achieved due to physical and chemical stresses. Hybrid bluegrass and KBG TRM: TRL were 0.0859 and 0.0571 mg cm⁻¹, respectively, which were not significantly different from those under field conditions. Fine root systems are generally believed to be more efficient investments than coarser root systems. However, as Eissenstat (1992) points out, this ignores other functions of roots that may permit coarse root to be more adaptive under adverse edaphic conditions such as greater longevity.

Drought Response

Leaf Water Content

Depletion of water from the top 20 cm of the soil profile progressed from field capacity to approximately 23% volumetric soil water content over a 25-day period in Study I and a 30-day period in Study II (Fig. 2.2). During Study I KBG maintained leaf water content at approximately 72% for 12 days after irrigation ceased, after which time it began to decline rapidly (Fig. 2.3). Hybrid bluegrass maintained an inherently lower initial leaf water content of approximately 67% for 10 days after irrigation ceased, which

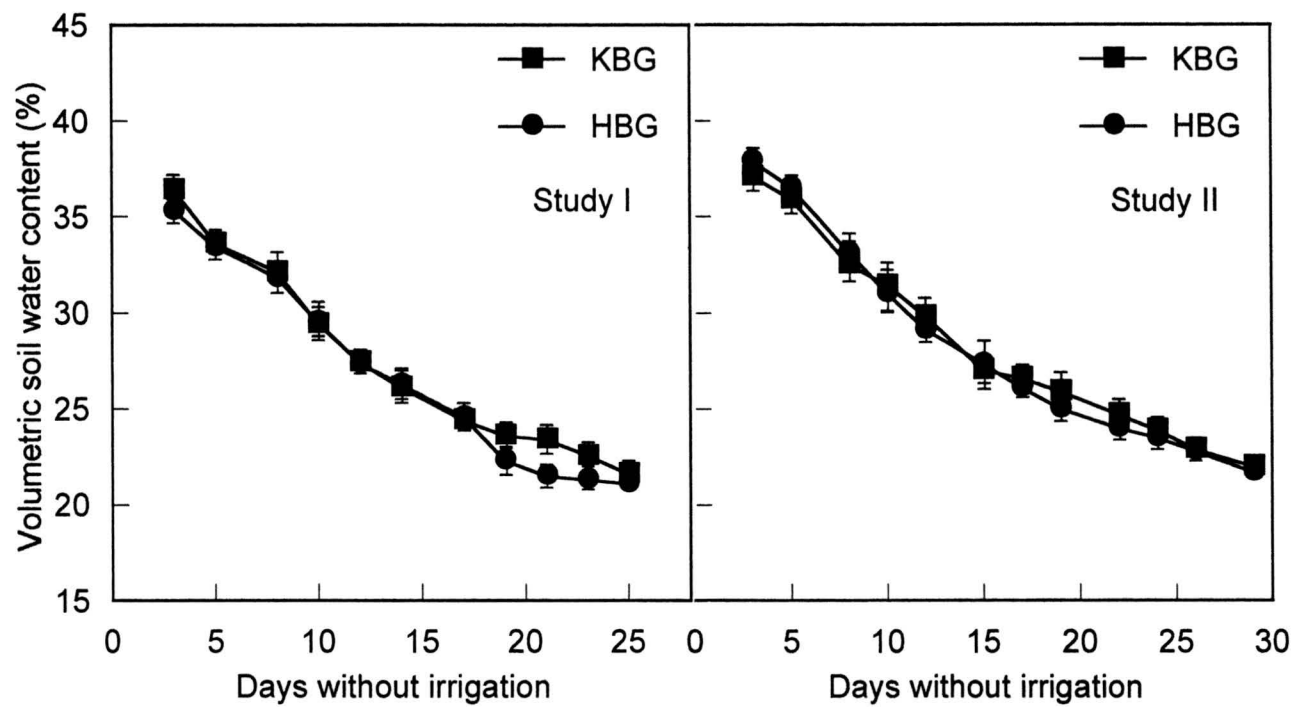


Fig. 2.2. Decline of volumetric soil water content in the 0-20 cm soil layer for Bensuns A-34 Kentucky bluegrass (KBG) and Reville hybrid bluegrass (HBG) after irrigation ceased. Each point represents the mean of four replications. Bars indicate SE.

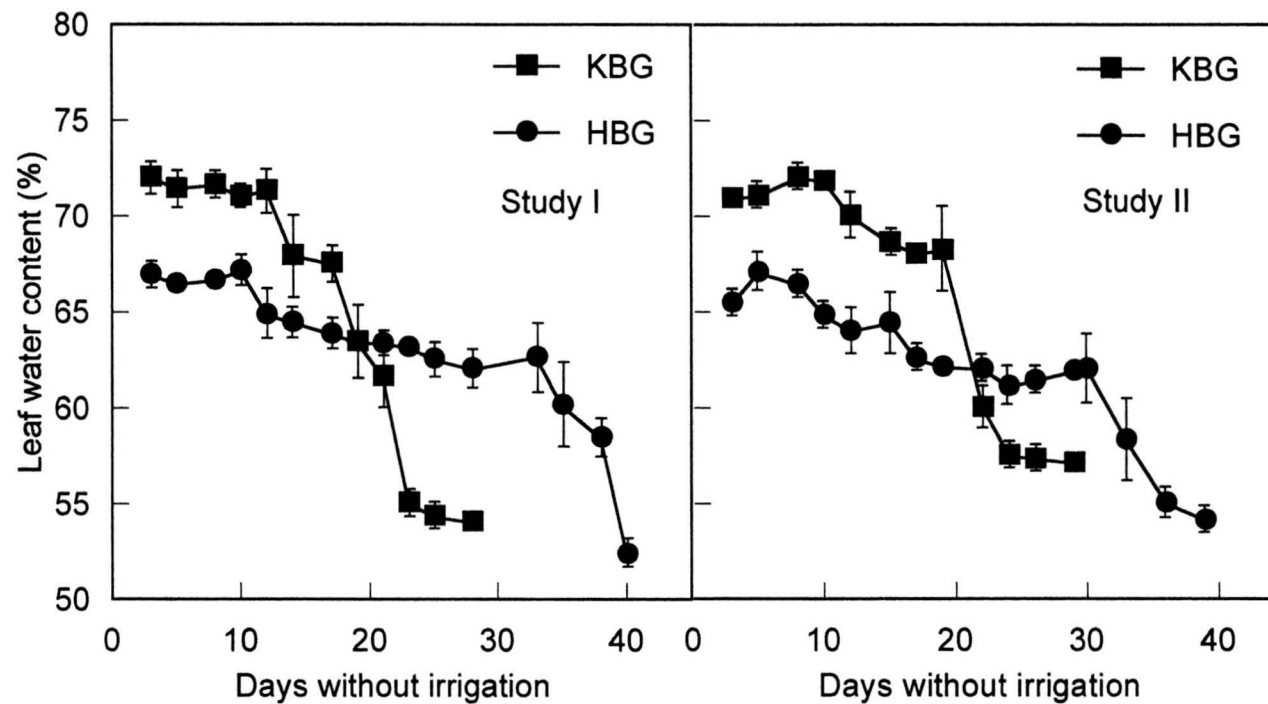


Fig. 2.3. Shoot water content of Bensuns A-34 Kentucky bluegrass (KBG) and Reveille hybrid bluegrass (HBG) after irrigation ceased. Each point represents the mean of four replications. Bars indicate SE.

slowly declined to approximately 62% over the next 23 days, after which time it began to decline dramatically.

Similar trends in leaf water content were observed in Study II (Fig. 2.3), however, because SWD of the top 0-20 cm of soil occurred more slowly, KBG was able maintain an approximate leaf water content of 70% for approximately 19 days, until it began declining. Hybrid bluegrass leaf water content began to decline 30 days after irrigation ceased. In both grasses and in both studies, initial decline of leaf water content coincided, approximately, with depletion of available water from the 0-20 cm soil level (28%) as previously determined for this field (Dahlin, 1992).

Turfgrass Wilting

In Study I KBG began exhibiting leaf wilt on day 12 of dry down and by day 25 showed > 45% leaf firing (Fig 2.4). In contrast, HBG did not begin to exhibit leaf wilt until day 22 and by day 28 showed only 15% leaf firing. In Study II KBG again exhibited leaf wilting earlier than HBG (at 22 vs. 33 days for KBG and HBG, respectively). After 40 days of dry down KBG exhibited > 90% leaf firing while HBG only exhibited 35% leaf firing. During both dry down cycles, HBG gradually exhibited an increasingly grayish appearance as SWD progressed, whereas KBG progressed more quickly to leaf firing.

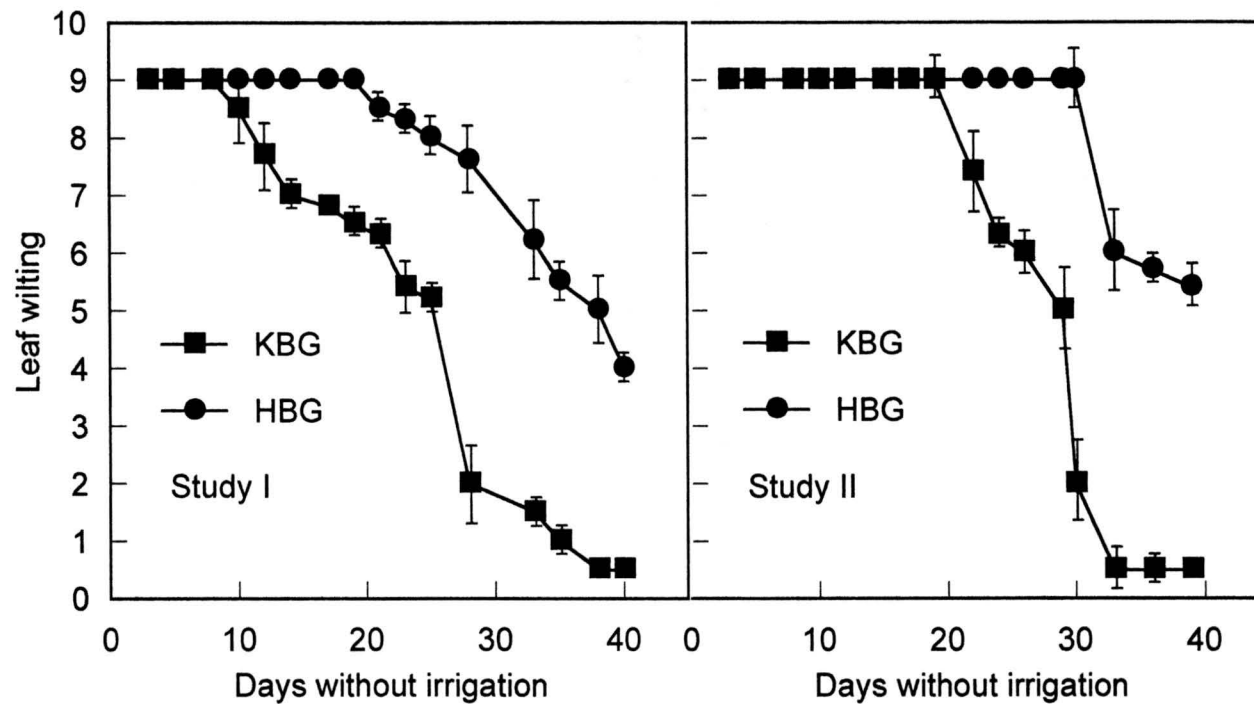


Fig. 2.4. Turfgrass wilting ratings (where 0 = 100% leaf firing and 9 = no wilting symptoms) of Bensun's A-34 Kentucky bluegrass (KBG) and Reville hybrid bluegrass (HBG) after irrigation ceased. Each data point represents the mean of four replications. Bars indicate SE.

SUMMARY AND CONCLUSIONS

Our results indicate that HBG exhibits a lower inherent ET rate than KBG, as well as the ability to moderate its ET as evaporative demand increases relative to KBG, thereby reducing water loss. Hybrid bluegrass exhibited lower leaf water content and slower shoot growth than KBG under non-limiting soil moisture conditions, characteristics advantageous to reduce water use. Additionally, HBG possesses a significantly deeper, more extensive root system than KBG, enabling water extraction from a greater volume of depth when surface soil water is depleted. We conclude that these factors combined contribute significantly to the reduced water use and greater drought avoidance observed in HBG relative to KBG in these studies.

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CHAPTER 3

SPECTRAL REFLECTANCE RESPONSE OF THREE TURFGRASSES TO LEAF DEHYDRATION

ABSTRACT

Spectral reflectance assessment of turfgrass canopies is likely to enhance our ability to non-destructively detect turfgrass water stress and refine irrigation management technology. Reliable spectral detection of impending water stress is dependent upon knowledge of wavelengths most sensitive to leaf water content. The purposes of this study was to determine wavelengths at which turfgrass canopy reflectance is most sensitive to progressive dehydration in the visible (400-700 nm) and NIR (700-1100 nm) portions of the spectrum, and investigate whether different turfgrass species exhibit spectrally unique canopy reflectance responses to progressive dehydration. Two consecutive studies were conducted using established field plots of hybrid bluegrass (*Poa pratensis* L. x *Poa arachnifera* Torr.) (HBG), Kentucky bluegrass (*Poa pratensis* L.) (KBG) and perennial ryegrass (*Lolium perenne* L.) (PRG) during summer 2001 at Fort Collins, CO. During each study field plots were brought to field capacity, then irrigation was withheld and spectral reflectance and leaf water content measured as dehydration progressed. Changes in turfgrass canopy spectral reflectance within the red region of the visible range (400-700 nm) were well correlated to decreasing leaf water content. In

Study 1 significant coefficients of determination (r^2) were found within 616-696 nm in HBG, 658-688 nm in KBG, and 630-688 in PRG, with maximum r^2 occurring at 664, 672, and 664 nm for HBG, KBG, and PRG, respectively. In Study 2 significant coefficients of determination ranged from 642-694, 594-698, and 638-678 nm, with maximum r^2 occurring at 668, 672, and 660 for HBG, KBG, and PRG, respectively. Within the near infrared range only KBG exhibited correlation between canopy reflectance and decreasing leaf water content. Within 734-878 nm range, three species exhibited different degrees of reflectance difference between fully turgid and wilted turf; indicating the rank of canopy reflectance sensitivity to dehydration was KBG > PRG > HBG, the reverse order of their drought resistance ranking.

INTRODUCTION

Use of multispectral radiometry as a tool in detecting initial onset and progression of biotic and abiotic turfgrass stresses prior to appearance of visible symptoms is receiving increased attention. Early detection of stress could reduce the application of costly or environmentally sensitive corrective inputs and maintain visual turfgrass quality. Strong relationships between many plant health indicators with spectral reflectance have been well proven in several agronomic crops and forest canopies (Carter and Miller, 1994; Cibula and Carter, 1992; Hoque and Hutzler, 1992; Penuelas et al., 1997; Tarpley et al., 2000). At present, limited information is available on the use of spectral imaging as a tool in the evaluation of turfgrass stand health. Initial research has demonstrated strong correlations between changes in spectral reflectance at specific and narrow wavelengths and visual observations of disease, drought, and soil compaction in several turfgrass species (Richardson and Everitt, 1987; Fenstermaker-Shaulis et al., 1997; Green et al., 1998, Trenholm et al., 1999a; Trenholm et al., 1999b). However, high-resolution continuous multispectral reflectance response curves for turfgrass species in response to directly quantified stress agents are not presently available due to the instrumentation limitations of these previous studies.

Perhaps most promising is the potential use of spectral reflectance data as a turfgrass water conservation management tool. However, reliable detection of impending water stress is dependent upon knowledge of wavelengths most sensitive to leaf water

content. A variety of plant stressors, including dehydration, are known to increase reflectance of green vegetation in the visible portion of the spectrum (400-700 nm) due to decreased chlorophyll content and in the near infrared region (700-1100 nm) to changes in cell structure (Datt, 1998; Gausman and Allen, 1973; Lichtenthaler et al., 1996; Schepers et al., 1996; Sinclair et al., 1973). Carter (1993) reported increased reflectance in the 535-640 nm and 685-700 nm wavelength ranges to be most consistent and particularly sensitive to leaf dehydration in switchcane (*Arundinaria tecta* [Walt.] Muhl.). Reflectance response to leaf dehydration in the near infrared region was found to be consistent only when water stress had developed sufficiently to cause severe leaf dehydration resulting in alterations in cell structure. The wavelengths at which turfgrass canopy reflectance is most sensitive to water stress, and whether variability in wavelength sensitivity exists between species, generally remains uninvestigated and undefined.

Therefore, the purposes of this study were to (i) determine the wavelengths at which turfgrass canopy reflectance is most sensitive to progressive dehydration across the visible and NIR portions of the spectrum, and (ii) investigate whether different turfgrass species exhibit spectrally unique canopy reflectance responses to progressive dehydration.

MATERIALS AND METHODS

This investigation consisted of two studies conducted consecutively using fully established field plots of hybrid bluegrass (*Poa pratensis* L. x *Poa arachnifera* Torr.) (HBG), Kentucky bluegrass (*Poa pratensis* L.) (KBG) and perennial ryegrass (*Lolium perenne* L.) (PRG) at the Colorado State University Horticultural Research Center at Fort Collins, CO. Study 1 was conducted from 7 July to 12 Aug. and Study 2 from 2 Sep. to 5 Oct. 2001. Plots measuring 6 by 6 m were arranged in a randomized complete block design with three replications. Plots were mowed 2 to 3 times weekly to maintain a height of 6.4 cm and were fertilized with 149 hg N ha⁻¹ yr⁻¹ (as urea 46-0-0) in three split applications over the growing season. The experimental area contained semi-circle pop-up spray heads at the corners providing uniform irrigation. Plots were irrigated to replace 100% ET prior to initiation of each study after which irrigation was withheld to induce dehydration.

During each evaluation, leaf water content was measured by collecting leaf clippings from the experimental areas. After fresh weight determination, clippings were dried in a forced air oven at 70 °C to determine clipping dry weight. Leaf water content was then calculated. Concurrent with leaf water content determination, turf canopy spectral reflectance from 400 to 1100 nm at 2 nm resolutions was measured with a LI-COR spectral radiometer model LI1800 (LI-COR, Lincoln, NE) equipped with a 1800-06 Telescope Receptor. The telescope receptor was mounted on a tripod at a height of

approximately 0.9 m from the turfgrass canopy measuring canopy reflectance from an approximately 0.1-m circular area. Reflectance readings were taken during each evaluation between 1100 to 1300 h MST under conditions of no or minimal cloud cover and three scans averaged during each measurement.

At each 2 nm wavelength from 400 – 700 nm, in both Studies II and I, leaf water content was regressed against canopy reflectance and tested for significant linear relationships. Reflectance differences at each 2 nm wavelength from 400-1100 nm between non-stressed and dehydrated turf were calculated as:

$$\text{Reflectance difference (\%)} = [\text{canopy reflectance of dehydrated turf} - \text{canopy reflectance of non stressed turf} / \text{canopy reflectance of dehydrated turf}] \times 100.$$

The calculated reflectance differences were then subjected to ANOVA test to determine species effects. Species means were separated by protected LSD.

RESULTS AND DISCUSSION

Changes in turfgrass canopy spectral reflectance within the red region of the visible range (400-700 nm) were well correlated to decreasing leaf water content in a manner typical of reflectance response to plant stress; as canopy dehydration progressed reflectance was increased (Fig. 3.1). In Study 1 significant coefficients of determination (r^2) for linear regressions of leaf water content with canopy reflectance ranged from 616-696 nm in HBG, 658-688 nm in KBG, and 630-688 in perennial ryegrass (Fig. 3.2). Maximum r^2 in Study 1 occurred at 664, 672, and 664 nm for HBG, KBG, and perennial ryegrass, respectively. In Study 2 significant coefficients of determination ranged from 642-694, 594-698, and 638-678 nm, with maximum r^2 occurring at 668, 672, and 660 for HBG, KBG, and perennial ryegrass, respectively (Fig. 3.2).

Kentucky bluegrass was the only turfgrass that exhibited the same reflectance maxima from Study 1 to Study 2. Spectral reflectance in the visible portion of the spectrum is generally characterized to be a sole function of chlorophyll *a* and *b*, with strict absorption maxima having been identified at 420, 435, 663, and 668 *in vitro* (Hopkins, 1995). However, *in situ*, attachment of chlorophylls to a variety of proteins, as well as the presence of other plant pigments, can alter and expand the peak and range of wavelengths at which green leaves harvest light energy as these results indicate. We observed no statistically significant association between decreasing leaf water content

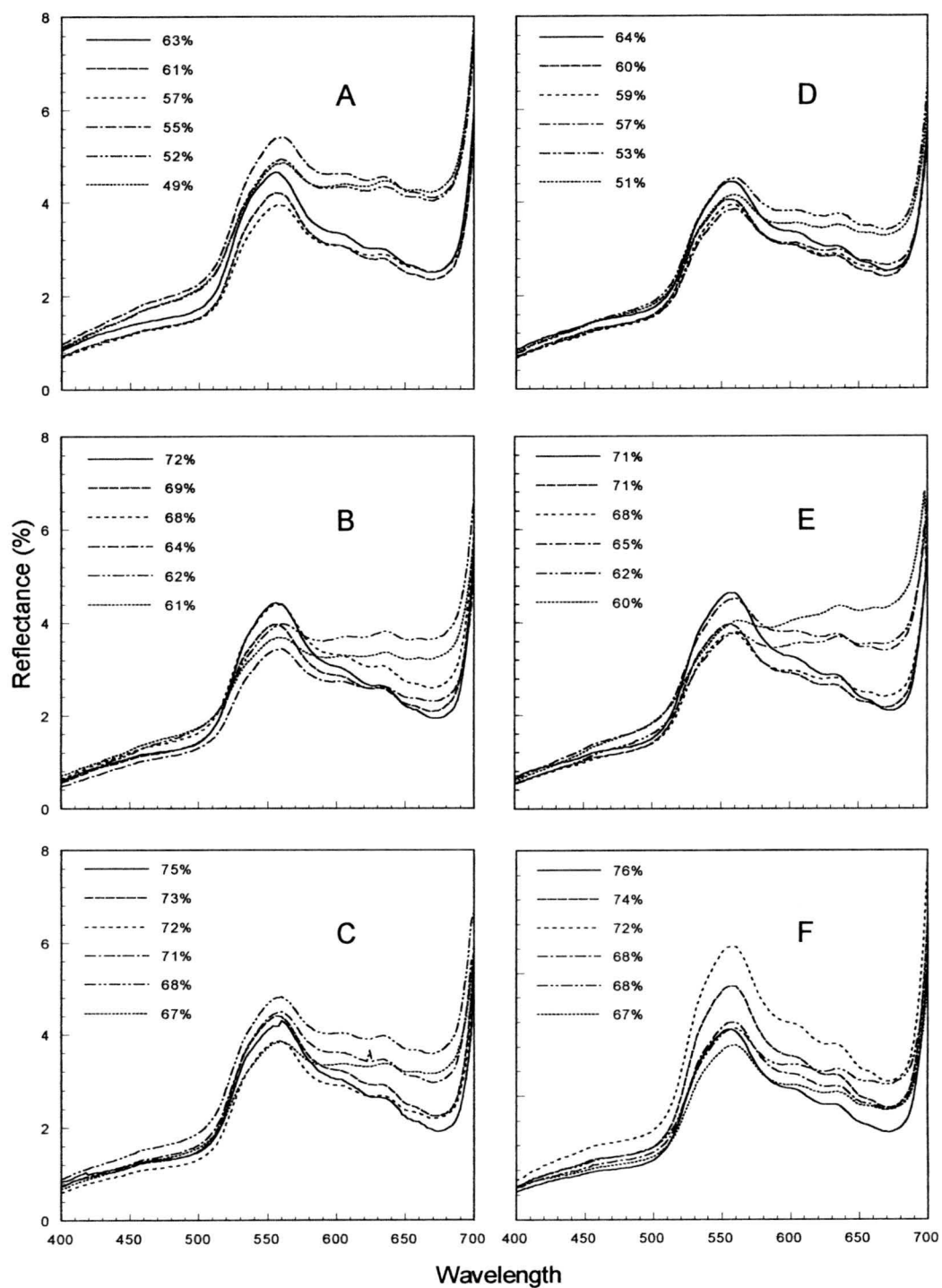


Fig. 3.1. Responses of turfgrass canopy spectral reflectance within the visible wavelength range (400-700 nm) to decreasing leaf water content in Study 1 (A, B, C) and Study 2 (D, E, F) for hybrid bluegrass (A, D), Kentucky bluegrass (B, E), and perennial ryegrass (C, F).

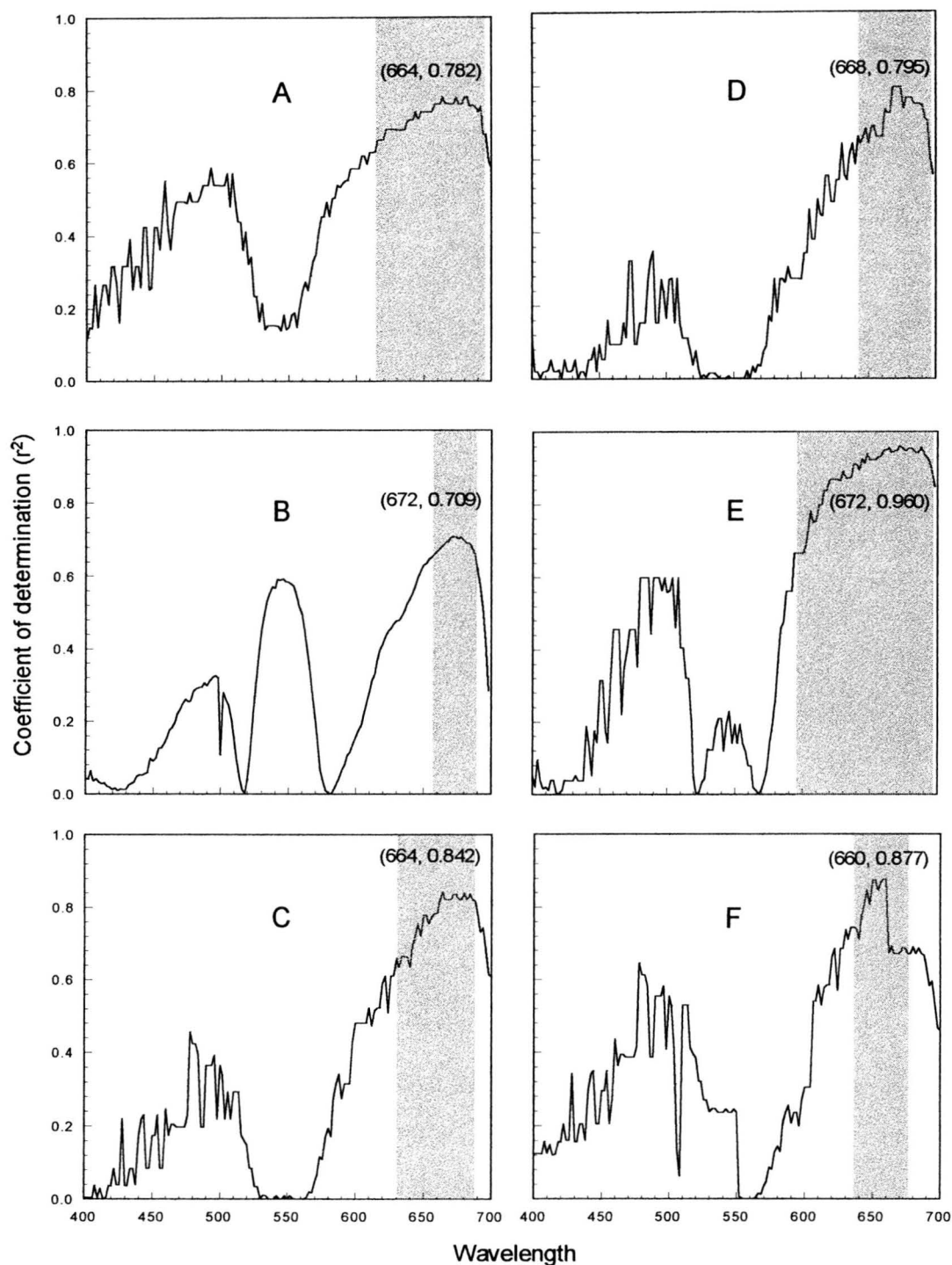


Fig. 3.2. Coefficient of determination (r^2) vs. wavelength for simple linear relationships of turfgrass canopy reflectance with decreasing leaf water content within the visible wavelength range (400-700 nm) in Study 1 (A, B, C) and Study 2 (D, E, F) for hybrid bluegrass (A, D), Kentucky bluegrass (B, E) and perennial ryegrass (C, F). Wavelengths at which best-fit relationships were identified and corresponding r^2 are indicated in parentheses. Shaded regions under the curve denote significant correlations at the 0.05 probability level.

and chlorophyll *a* and *b* near their absorption maxima at 420 and 435 nm. These results are consistent with those of Carter (1993) and Carter and Knapp (2001) in that leaf reflectance is altered by stress most reliably and consistently, and therefore detected earliest, within the far-red region of the spectrum due to the high sensitivity of chlorophyll concentrations to physiological disturbances at these wavelengths.

Within the near infrared range (700-1100 nm) only KBG exhibited correlation between canopy reflectance and decreasing leaf water content (Fig 3.3). Significant coefficients of determination (r^2) for KBG ranged from 722-948 nm and 734-952 nm in Studies 1 and 2, respectively (Fig. 3.4). Hybrid bluegrass and PRG exhibited no such correlation.

The effect of slow and progressive dehydration in the near infrared region of the spectrum can be subtler, and therefore more difficult to evaluate, than in the visible region (Carter, 1991). However, broad bands of great differences in species reflectance difference (between fully turgid and wilted leaves) were observed in the near infrared region, at 736-878 nm in Study 1 and 734-874 nm in Study 2 (Fig. 3.5). Our results indicated the ranking for canopy reflectance sensitivity to dehydration was KBG > PRG > HBG, the reverse order of their drought resistance ranking (Beard, 2002; Sheffer et al. 1987; Suplick and Qian, unpublished data). Reflectance of leaves within the near infrared region is governed by the multiplicity of reflections inside the leaf, which can be altered by cell size, shape, and distribution. Dehydration can alter these characteristics resulting in a change in spectral reflectance (Gausman et al., 1969). These results indicate there exists sufficient difference in the leaf histology of these three grasses to influence their

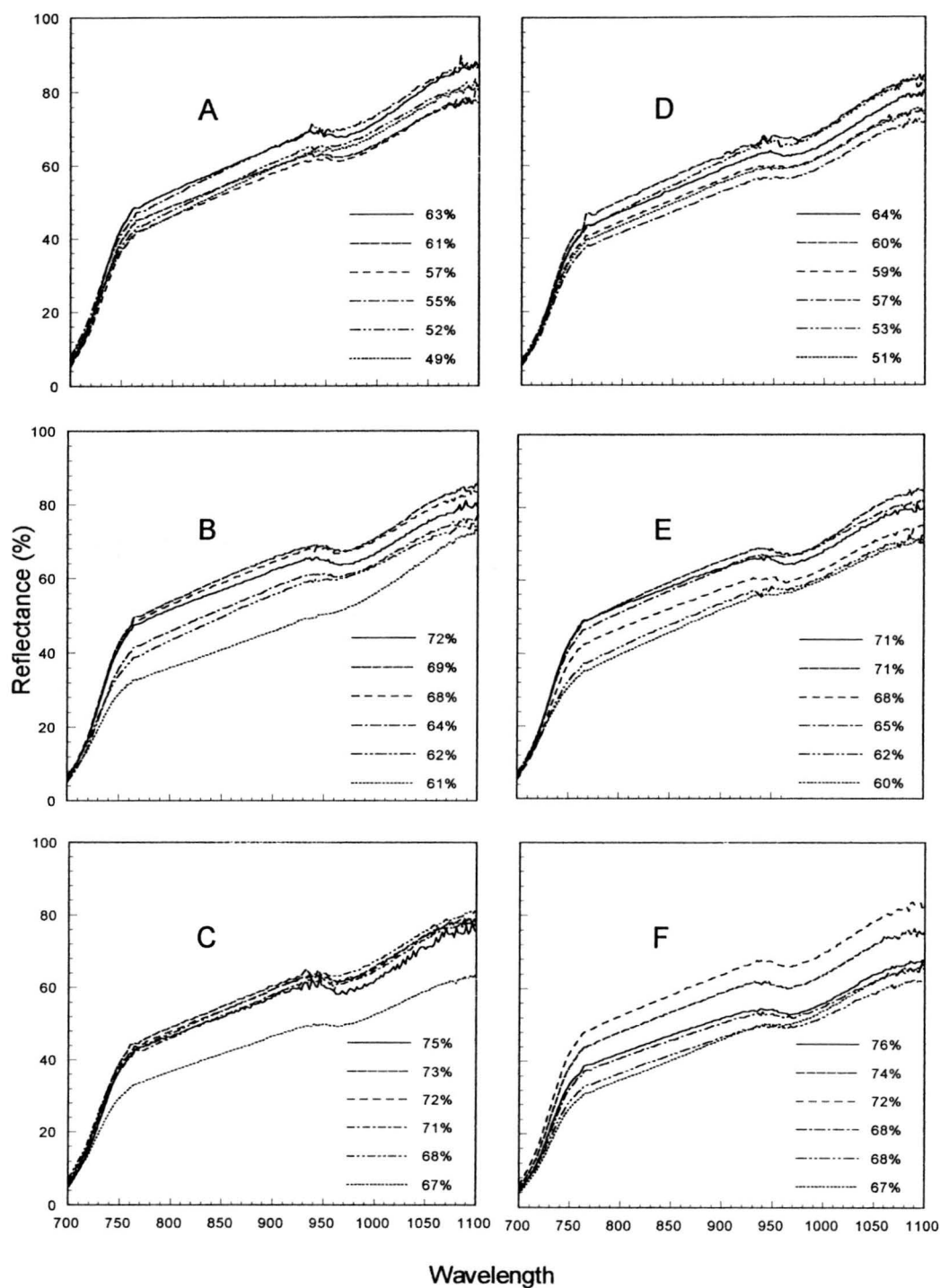
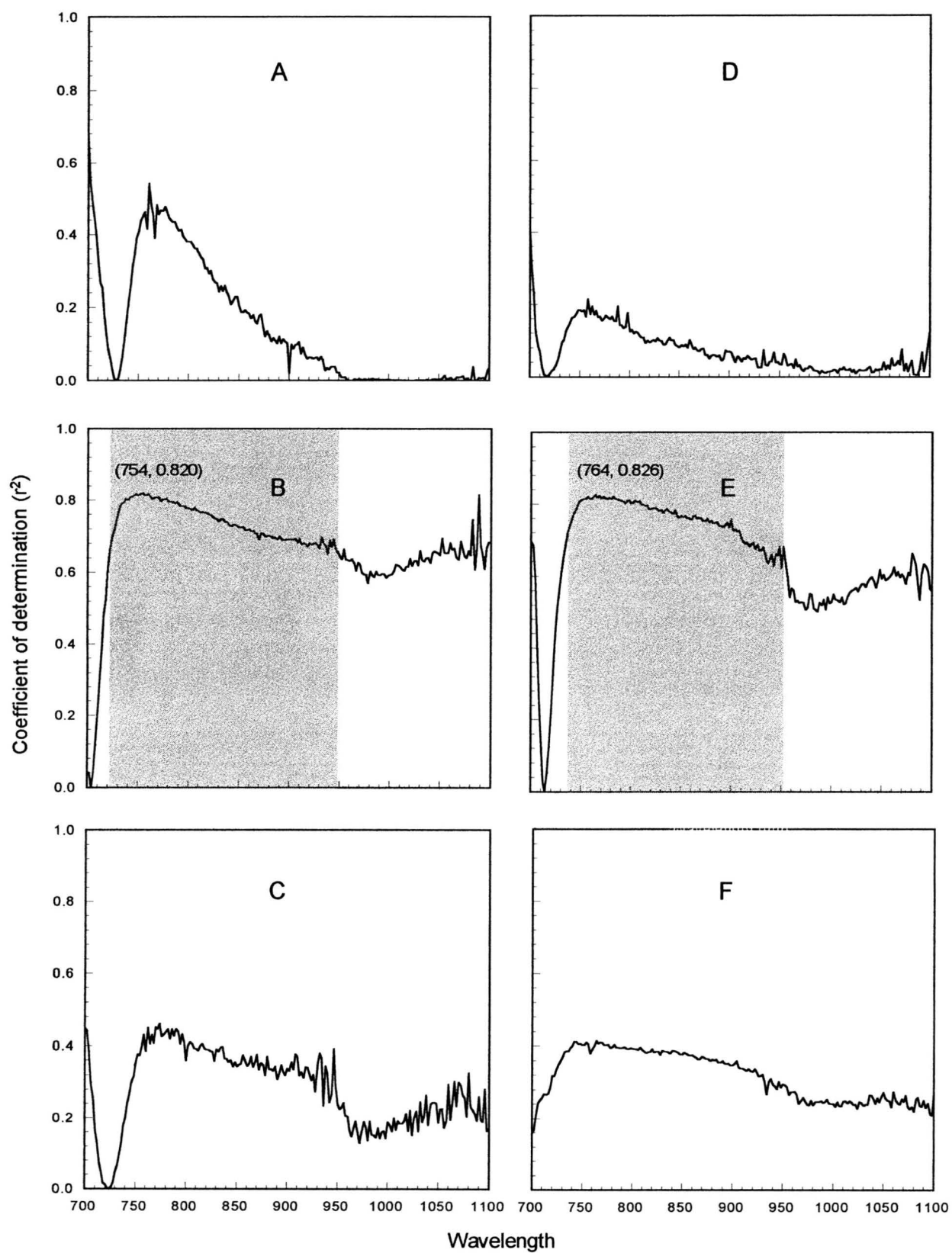


Fig. 3.3. Response of turfgrass canopy spectral reflectance within the near infrared wavelength range (700-1100) to decreasing leaf water content in Study 1 (A, B, C) and Study 2 (D, E, F) for hybrid bluegrass, (A, D), Kentucky bluegrass (B, E), and perennial ryegrass (C, F).



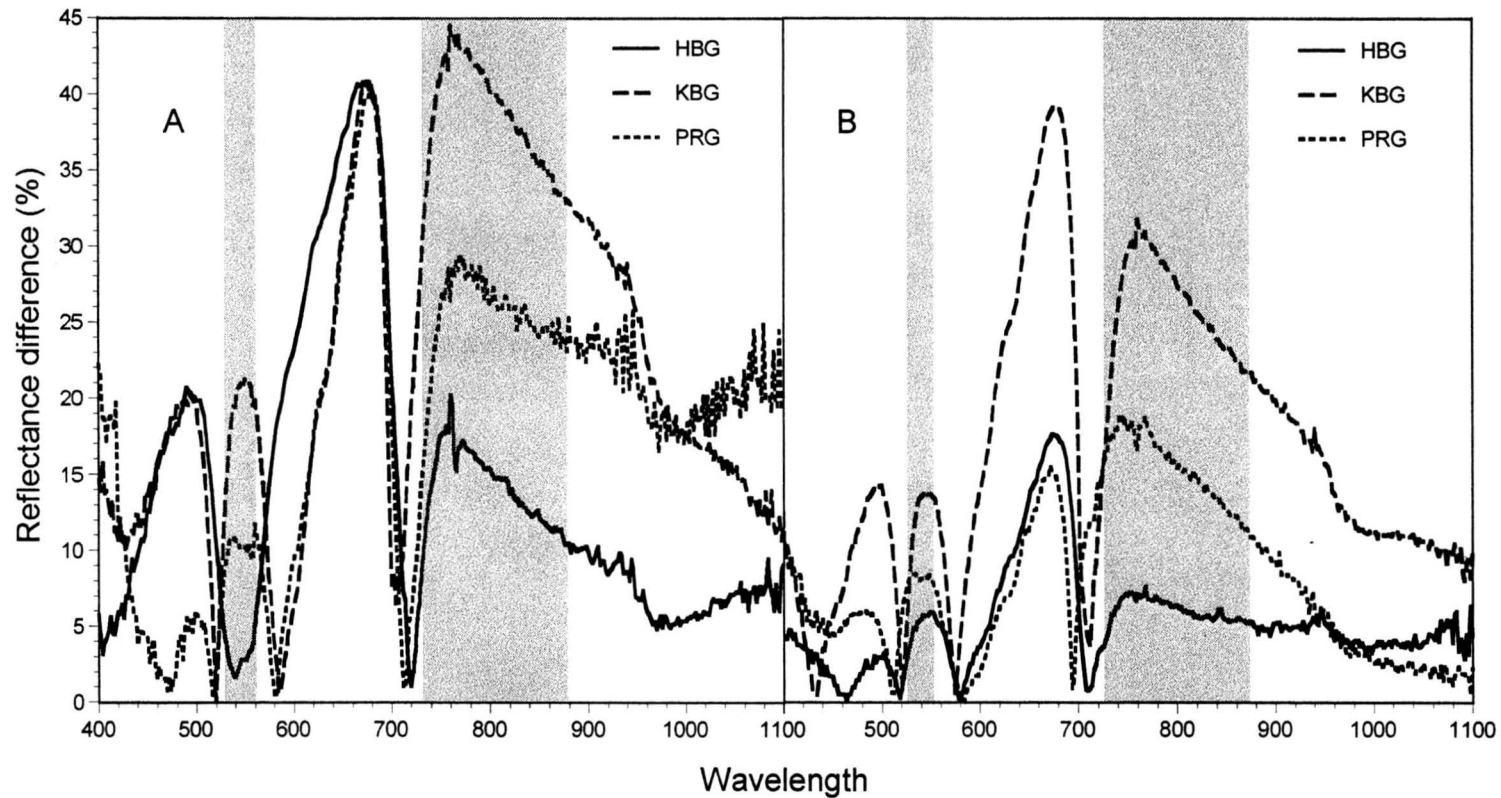


Fig. 3.5. Reflectance difference between fully turgid and wilted leaves vs. wavelength in Studies 1 (A) and 2 (B) for hybrid bluegrass, Kentucky bluegrass, and perennial ryegrass. Shaded regions under the curve denote statistical separation of the three grasses from one another. Significant species separation ($P \leq 0.001-0.0001$) occurred between 528-560 and 736-878 nm in Study 1 and 524-556 and 734-874 nm in Study 2.

reflectance response to dehydration in the near infrared region and influence their drought resistance.

Small wavebands of species separation were also observed to occur within the green portion of the visible range (528-560 and 524-556 nm in Studies 1 and 2, respectively) a pattern indicative of chlorophyll degradation and thus loss of green reflectance (Fig. 3.5). Although the species separation pattern was consistent with that observed in the near infrared range, the trend of the HBG curve was not between Studies 1 and 2.

SUMMARY AND CONCLUSIONS

In this study we found relatively narrow bands of spectral reflectance within the far-red region of the visible portion of the spectrum (660-672 nm) to be most sensitive to, and highly correlated with, progressive dehydration in three turfgrass species. These wavelengths might effectively be used as an irrigation management tool in non-destructively monitoring leaf water status. Computation of reflectance difference between non-stressed and dehydrated leaves of HBG, KBG, and PRG revealed consistent differences in their reflectance sensitivity to dehydration. This sensitivity ranking was comparable with previous reports of drought resistance among these grasses, suggesting that the magnitude of reflectance change may be used as an indicator of drought resistance. Our results also suggest that leaf histology has a strong influence on drought resistance.

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