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Screening St. Augustinegrass For USDA Zone 7

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SCREENING ST. AUGUSTINEGRASS [STENOTAPHRUM SECUNDATUM (WALT.) KUNTZE] FOR USDA ZONE 7

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ABSTRACT

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] generally has poor cold tolerance yet excellent shade tolerance. As mostly hot summers follow cold winters in USDA Hardiness Zone 7, severely damaging tall fescue [*Festuca arundineacea* Schreb.] and centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], a St. Augustinegrass cultivar cold tolerant enough to be grown for shady lawns would greatly benefit both home owners and sod growers in USDA Hardiness Zone 7. Eight St. Augustingrass samples were selected, including industry standards 'Raleigh' and 'Palmetto', for further testing from an established germplasm collection of material collected from lawns grown in USDA Hardiness Zone 7. Morphological differences, establishment rates, shade tolerance, and most importantly cold tolerance were evaluated through field trials, greenhouse trials, and growth chamber trials. When applicable experimental samples were compared to industry standards to determine either similar or improved performance. The studies revealed several germplasm samples with differences compared to industry standards indicating possible increased performance capabilities. These findings warrant further investigation and possible DNA testing to determine genetic differences.

DEDICATION

I dedicate this work to my parents, Brian and Densie, for raising me with a desire to learn, work, and strive to follow my dreams. I love y'all!

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I am very grateful to my advisor, Dr. McCarty, for his guidance throughout my graduate studies. I am inspired by his passion and drive in the turf science industry and look up to him as a leader to follow as I continue to chase my goals. I would also like to thank my other committee members, Dr. Wells and Dr. Gerard for serving on my committee. Their guidance, and expertise was essentual in the completion of my thesis.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Table Page

List of Tables (Continued)

List of Tables (Continued)

Table Page

LIST OF FIGURES

List of Figures (Continued)

Figure Page

INTRODUCTION

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is one of the most popular turfgrass species used for home lawns throughout the southern United States (Busey, 2003). It is believed to be native to open-to-lightly shaded, high rainfall, and humid regions of coastal South and Central America, including the West Indies (McCarty, 2011a). This species is adaptable to many soil conditions but does best on moist, well-drained sandy soils. Irrigation is necessary during periods of dry weather because its drought tolerance is only fair (Emmons, 2000).

On occasion, USDA Hardiness Zone 7 experiences years of drought and above average heat during summer months, followed by below average cold temperatures during winter months. Often, these conditions create a turf void in shady locations throughout the landscape. Such areas suffer as temperatures become too hot for the survival of tall fescue [*Festuca arundineacea* Schreb.], a C_3 plant, survival, yet too cold for the survival of centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], a C_4 plant, survival.

This weather pattern, combined with a growing population in the upstate of South Carolina, opens a niche demand for a warm season (C4) grass that is cold tolerate enough to survive below average temperatures in shaded lawns.

Upstate South Carolina lies along the Interstate 85 corridor connecting Charlotte, North Carolina and Atlanta, Georgia. Atlanta and Raleigh (north of Charlotte) are among the top 10 fastest growing cities in America (Fisher, 2011). For example, Greenville

County, lying along I-85 between Atlanta and Charlotte, has experienced a 70% population growth since 1990. Such growth rates demand more housing, more lawns, thus an increased demand for quality turf. Improvement of cold tolerance in St. Augustinegrass would increase the area of adaptation and potential use of this important turfgrass species (Philley et al., 1998).

Germplasm collection is an effective approach for cultivar development (Li, 2010). For example, tall fescue (*Festuca arundineacea* Schreb. cv. 'Kentucky 31'), one of the most popular tall fescue cultivars, and 'Raleigh', currently the most cold tolerant St. Augustinegrass cultivar, were both selected from plants collected from the field (Maier et al., 1994a).

The purpose of this study was to evaluate a st. augustinegrass germplasm collection from upstate South Carolina for potential sod production. Comprehensive evaluations of these plant collections could open new opportunities for sod growers to provide homeowners with a highly shade tolerant warm season turfgrass capable of surviving unusually cold winters in USDA Hardiness Zone 7.

ST. AUGUSTINEGRASS [*Stenotaphrum secundatum* (Waltz.) Kuntze]

MORPHOLOGICAL DIFFERENCES

Introduction

It is difficult to prove morphological differences in st. augustinegrass without field trials to examine morphological stability. As homeowners demand greater energy efficiency, demands for better performing, more shade tolerant turfgrass also increase. Current trends toward energy conservation in home landscaping present problems in warm-season turfgrass selection since all species grow best in full sunlight. As the use of shade for cooling homes and buildings has increased, the need for a shade tolerant turfgrass by homeowners and landscapers has arrived (Beard, 1970; Boardman, 1977).

To determine if these st. augustinegrass germplasm samples from upstate, South Carolina are truly different cultivars than ‘Raleigh’ or ‘Palmetto’, morphological traits must first be evaluated. In addition, turf height and seedhead production differences would be valuable information for those turf managers interested in cultivars that require less frequent mowing.

The objective of this study was to determine if experimental germplasm samples possessed different morphological characteristics than industry standards ‘Raleigh’ and ‘Palmetto’.

Materials and Methods

A field trial was conducted for eight st. augustinegrass samples, two industry standard cultivars, 'Raleigh' and 'Palmetto', and six experimental samples. Plots were located at the Clemson University Cherry Farm in Clemson, South Carolina. Plots were sprayed with glyphosate twice, three weeks apart, at a rate of 4.48 kg ai ha^{-1} during June 2012, plowed and disked, then fumigated with methyl bromide at 73 kg ai ha^{-1} in July 2012. Plots were 3 x 4.5 m with 0.5 m alleys between plots. St. augustinegrass was established by evenly plugging 7 plugs totaling 0.24 m^2 (2.6 ft^2) per plot. Plots were fertilized with a 1-1-1 complete fertilizer after plugging and once a month thereafter during the growing season. Plots were irrigated as needed to prevent drought stress. Plots were mown twice per week during the growing season with a 1.52 m pto driven finishing mower behind a John Deere 955 tractor set at 5.1 cm height. Plots were sprayed with a postemergent herbicide, Celsius (iodosulfuron + dicamba + thiencarbozone), at a rate of 217 g ha^{-1} as needed to reduce weed competition. Plot edges were mechanically trimmed monthly to prevent encroachment and contamination from alleys. Plots were not treated with a fungicide or insecticide. Plots were covered with wheat straw from December 2012 through April 2013 to reduce winter damage.

Measurements were taken in July 2013 and July 2014 using five stolons from each plot to quantify morphological characteristics of leaf width, leaf length, and internode distance. Measurements were taken at the third internode of each stolon. Internode distance was measured between the third and fourth internode. Turf height measurements were taken in July 2013. After removing the wheat straw in April 2012, plots were left unmown for six weeks. Five height measurements were taken from randomly selected areas within each plot and measured with a ruler. Visual seedhead density counts were also taken in July 2013.

Experimental design was a randomized complete block with three replications. The study was repeated in time. Data were subjected to ANOVA for evaluation of main effects. Further mean comparisons between grasses were performed using Fisher's protected LSD. Where appropriate, mean comparisons to industry standards were performed using Dunnett's test. All comparisons were based on an $\alpha = 0.05$ significance level. All analyses were conducted using JMP version 10 (SAS Institute Inc., Cary, NC).

Results and Discussion

Significant differences leaf width, leaf length, and internode distance occurred among germplasm samples (Table 1). A grass-by-year interaction also occurred; therefore data will be presented separately by year for these morphological differences. Significant differences in turf height and seedhead density also occurred among samples in 2013. A grass-by-block interaction was detected for turf height, therefore date will be presented separately by block (Table 1).

In 2013, grasses ‘A’, ‘E’, and ‘H’ had significantly different leaf widths when compared to ‘Raleigh’ but only ‘F’ had a significantly different leaf width compared to ‘Raleigh’ in 2014. ‘A’, ‘E’, and ‘G’ had significantly different leaf lengths compared to ‘Raleigh’ in 2013 while ‘E’ and ‘F’ had significantly different leaf lengths compared to ‘Raleigh’ in 2014. ‘E’ was the only grass to show differences to ‘Raleigh’ both years. ‘C’, ‘Palmetto’, ‘E’, and ‘G’ had significantly different internode lengths compared to ‘Raleigh’ in 2013, while all grasses but ‘G’ had significantly different internode lengths than ‘Raleigh’ in 2014. ‘E’ was the only grass to have a significantly different seedhead density compared to ‘Raleigh’ in 2013 (Table 2). 53% of ‘Raleigh’ plots possessed seedheads compared to 78% of grass ‘E’ plots (Table 5).

In 2013, only grass ‘E’ had a significantly different leaf width compared to ‘Palmetto’ while ‘A’ and ‘C’ had significantly different leaf widths compared to ‘Palmetto’ in 2014. Grasses ‘E’ and ‘G’ had significantly different leaf lengths compared to ‘Palmetto’ in 2013 while ‘E’ and ‘F’ had significantly different leaf lengths in 2014. ‘E’ was the only grass to have significantly different leaf lengths than ‘Palmetto’ in both years. In 2013, only ‘E’ and ‘F’ had similar internode lengths compared to ‘Palmetto’ while ‘Raleigh’ and ‘G’ were the only grasses to have significantly different internode lengths than ‘Palmetto’ in 2014 (Table 3).

In 2013, grass ‘C’ was the only grass to have significantly different turf height in more than one block compared to ‘Raleigh’. ‘A’, ‘F’, and ‘G’ also had significantly different turf heights in one of the three blocks compared to ‘Raleigh’. Compared to ‘Palmetto’, only ‘C’ and ‘E’ had significantly different turf heights in just one of the three blocks. All other grasses had similar turf heights to ‘Raleigh’ and ‘Palmetto’ (Table 4).

Table 1.3. ANOVA for morphological differences of st. augustinegrass germplasm samples. 2013 and 2014 in Clemson, South Carolina.

Source	DF	Leaf Width	Leaf Length	Internode Distance	Turf Height	Seedhead Density
			2013 & 2014 Combined			
Grass	7	*	*	*	-	-
Block	2	ns	ns	ns	-	-
Year	1	ns	*	*	-	-
Grass-by-Year	7	*	*	*	-	-
			2013			
Grass	7	*	*	*	*	*
Block	2	ns	ns	ns	*	*
Grass-by-Block		ns	ns	ns	*	ns
			2014			
Grass	7	*	*	*	-	-
Block	2	ns	*	ns	-	-
Grass-by-Block	14	ns	ns			

Abbreviations: ns, not significant.
*Significant at $\alpha = 0.05$ level.
(-) Not applicable.

Table 1.2. DUNNETT for comparing leaf width, leaf length, internode distance, and seedhead densities of st. augustinegrass germplasm samples to industry standard ‘Raleigh’. 2013 and 2014 in Clemson, South Carolina.

Grass	Industry Standard	Leaf Width (mm)	Leaf Length (cm)	Internode Distance (cm)	Seedhead Density (%)
			2013		
A	Raleigh	*	*	ns	ns
C	Raleigh	ns	ns	*	ns
Palmetto	Raleigh	ns	ns	*	ns
E	Raleigh	*	*	*	*
F	Raleigh	ns	ns	ns	ns
G	Raleigh	ns	*	*	ns
H	Raleigh	*	ns	ns	ns
			2014		
A	Raleigh	ns	ns	*	-
C	Raleigh	ns	ns	*	-
Palmetto	Raleigh	ns	ns	*	-
E	Raleigh	ns	*	*	-
F	Raleigh	*	*	*	-
G	Raleigh	ns	ns	ns	-
H	Raleigh	ns	ns	*	-

*Significant at $\alpha = 0.05$ level.
Abbreviation: ns, not significant.
(-) Not applicable.

Table 1.3. DUNNETT for comparing leaf width, leaf length, internode distance, and seedhead densities of st. augustinegrass germplasm samples to industry standard ‘Palmetto’. 2013 and 2014 in Clemson, South Carolina.

Grass	Industry Standard	Leaf Width (mm)	Leaf Length (cm)	Internode Distance (cm)	Seedhead Density (%)
			2013		
A	Palmetto	ns	ns	*	ns
Raleigh	Palmetto	ns	ns	*	ns
C	Palmetto	ns	ns	*	ns
E	Palmetto	*	*	ns	ns
F	Palmetto	ns	ns	ns	ns
G	Palmetto	ns	*	*	ns
H	Palmetto	ns	ns	*	ns
			2014		
A	Palmetto	*	ns	ns	-
Raleigh	Palmetto	ns	ns	*	-
C	Palmetto	*	ns	ns	-
E	Palmetto	ns	*	ns	-
F	Palmetto	ns	*	ns	-
G	Palmetto	ns	ns	*	-
H	Palmetto	ns	ns	ns	-

*Significant at α = 0.05 level.
Abbreviation: ns, not significant.
(-) Not applicable.

Table 1.4. DUNNETT for turf height measurements of st. augustinegrass germplasm samples compared to industry standard 'Raleigh'. July 2013 in Clemson, South Carolina.

Grass	Industry Standard		Turf Height (in)	
			2013	
		Block 1	Block 2	Block 3
A	Raleigh	*	ns	ns
Raleigh	Raleigh	ns	ns	ns
C	Raleigh	*	*	ns
E	Raleigh	ns	ns	ns
F	Raleigh	ns	*	ns
G	Raleigh	ns	*	ns
H	Raleigh	ns	ns	ns
A	Palmetto	ns	ns	ns
Raleigh	Palmetto	ns	ns	ns
C	Palmetto	ns	ns	*
E	Palmetto	ns	*	ns
F	Palmetto	ns	ns	ns
G	Palmetto	ns	ns	ns
H	Palmetto	ns	ns	ns

*Significant at $\alpha = 0.05$ level

Abbreviation: ns, not significant

Table 1.5. Leaf width, leaf length, internode distance, and seedhead densities of st. augustinegrass germplasm samples. 2013 and 2014 in Clemson, South Carolina.

Grass	Leaf Width (mm)	Leaf Length (cm)	Internode Distance (cm)	Seedhead Density (%)
		2013		
A	6.20	2.30	5.5	80
Raleigh	7.00	2.87	5.18	53
C	6.60	2.50	4.25	53
Palmetto	6.80	2.75	6.39	61
E	5.90	1.99	6.20	78
F	6.36	3.23	5.86	76
G	6.43	4.31	2.88	50
H	6.26	2.58	5.13	71
$LSD_{0.05}$	0.51	0.39	0.51	17.8
		2014		
A	7.03	2.74	5.25	-
Raleigh	6.60	2.58	3.77	-
C	7.10	2.65	5.24	-
Palmetto	6.17	2.60	5.44	-
E	6.00	2.08	5.19	-
F	5.67	1.92	5.60	-
G	6.17	2.39	3.22	-
H	6.23	2.54	5.32	-
$LSD_{0.05}$	0.49	0.35	0.60	-

(-) Not applicable.

Table 1.6. Turf height measurements of st. augustinegrass germplasm samples. July 2013 in Clemson, South Carolina.

Grass	Turf Height (in)		
	2013		
	Block 1	Block 2	Block 3
A	5.60	7.80	9.80
Raleigh	9.00	7.80	7.80
C	4.80	5.00	5.40
Palmetto	6.80	5.40	8.80
E	9.60	8.00	9.00
F	7.20	4.80	10.60
G	6.60	5.00	6.60
H	8.40	7.80	7.20
$LSD_{0.05}$	2.35	1.85	2.24

Conclusions

‘A’ was different than ‘Raleigh’ in four morphological traits at least once throughout the study and different than ‘Palmetto’ in two morphological traits at least once throughout the study. ‘C’ was different than ‘Raleigh’ in two morphological traits at least once throughout the study and different than ‘Palmetto’ in three morphological traits at least once throughout the study. ‘E’ was different than ‘Raleigh’ in four morphological traits at least once throughout the study and different than ‘Palmetto’ in three morphological traits at least once throughout the study. ‘F’ was different than ‘Raleigh’ in one morphological trait at least once throughout the study and different than ‘Palmetto’ in one morphological trait at least once throughout the study. ‘G’ was different than ‘Raleigh’ in three morphological traits at least once throughout the study and different than ‘Palmetto’ in two morphological traits at least once throughout the study. ‘H’ was different than ‘Raleigh’ in two morphological traits at least once throughout the study and different than ‘Palmetto’ in one morphological traits at least once throughout the study.

These findings support preliminary work done on these germplasm samples that express the possibility they may be different cultivars. Differences in morphological characteristics is one tool used to determine differences between cultivars. Further research is needed to prove this claim, however, this study justifies further testing such as DNA assays to determine if these grasses are truly different.

ST. AUGUSTINEGRASS ESTABLISHMENT RATES, GRAY LEAF SPOT, AND CHINCH BUG SUSCEPTIBILITY

Introduction

St. augustinegrass is one of the most popular turfgrass species used for home lawns throughout the southern United States (Busey, 2003). Propagation is primarily vegetative by plugs or sod as few seedheads are formed. St. augustinegrass has strong, thick stolons, course leaf texture, and produces a turf of medium density. Recuperation is good because of the aggressive stolons, but wear tolerance is only fair. Salt tolerance is very good (Emmons 2000). The maintenance requirement is medium, though the grass has a vigorous growth rate while moderate fertilization is necessary. St. augustinegrass is maintained at a height of 3.8 cm – 7.6 cm (1.5 to 3 inches) with either a reel or rotary mower. St. augustinegrass decline (SAD), a disease caused by a virus, is a potential problem; however southern chinch bugs (*Blissus insularis* Barber) can cause extensive injury as can gray leaf spot disease (caused by *Pyricularia grisea* Cooke) (Emmons 2000).

The sod industry is an important sector of the South Carolina turfgrass industry. There are many commonly produced cultivars of St. Augustinegrass, which show different physiological and morphological responses. The sod industry demands new st. augustinegrass cultivars with pest resistance and quicker establishment rates.

The objective of this study was to determine if experimental samples from Clemson’s germplasm collection of st. augustinegrass possessed increased establishment rates or pest resistance over current industry standards ‘Raleigh’ and ‘Palmetto’. Comprehensive evaluations of these plant collections could open new opportunities for turf managers and homeowners.

Materials and Methods

Three field trials were conducted for eight st. augustinegrass samples, two industry standards, 'Raleigh' and 'Palmetto', and six experimental samples from a germplasm collection at Clemson University. Trial 1 was conducted between August 3 2012 and July 14 2014. Trials 2 and 3 were conducted between July1 2013 and July 14 2014. Plots were sprayed with glyphosate twice, three weeks apart, at a rate of 4.48 kg ai ha^{-1} during June 2012, plowed and disked, then fumigated with methyl bromide at 73 kg ai ha^{-1} in July 2012. Trial 1 plots were 3 x 4.5 m with 0.5 m alleys between plots. St. augustinegrass was established by evenly plugging 7 plugs totaling 0.24 m^2 (2.6 ft^2) per plot. Trial's 2 & 3 plots were 1 x1 m with 0.5 m alleys and established with one plug per plot measuring 10.8 cm in diameter. Plots were fertilized with a 1-1-1 complete fertilizer after plugging and once a month thereafter during the growing season. Plots were irrigated as needed to prevent drought stress. Plots were mown twice per week during the growing season with a 1.52 m pto driven finishing mower behind a John Deere 955 tractor set at 5.1 cm height. Plots were sprayed with a postemerge herbicide, Celsius (iodosulfuron + dicamba + thiencarbozone), at a rate of 217 g ha^{-1} as needed to reduce weed competition. Plot edges were trimmed monthly with a weed eater to prevent encroachment and contamination. Plots in all three trials were not treated with a fungicide or insecticide.

Density counts were taken weekly and quantified by placing a 3 x 4.5 m 150 square grid with 30 x 30 cm centers within each plot, for trial 1, and a 1 x1 m 36 square grid with 16 x 16 cm centers within each plot for trials 2 & 3. Grid squares containing st.

augustinegrass were denoted as a ‘hit’ and counted. Establishment percentage was calculated as number of hits divided by number of squares in the grid ((total hit/total squares) x 100). Binomial counts for gray leaf spot and chinch bugs were also taken in trial 1. If visual symptoms were present a hit was recorded.

After the final density count for trial 1 in 2012, all plots were covered with wheat straw. Six square bales weighing approximately 18 kg each were evenly spread over trial 1. This was to ensure survival of plots through initial winter to continue growth and density counts the following year. Trial 1 remained covered in wheat straw from December 3 2012 through April 22 2013. Density counts resumed following the straw removal.

Experimental design was a randomized complete block with three replications. Treatments were arranged in a single factor design (grass samples) with 8 levels. The study was replicated with 3 trials during the same timeframe. Data were subjected to ANOVA for evaluation of main effects and interaction between factor levels. Where appropriate, mean comparisons between factor levels were performed using Fisher’s protected LSD. Also where appropriate, further mean comparisons between industry standard grasses were performed using Dunnett’s test. All comparisons were based on an $\alpha = 0.05$ significance level. All analyses were conducted using JMP version 10 (SAS Institute Inc., Cary, NC).

Results and Discussion

St. augustinegrass samples had similar establishment rates throughout trial 1 (Table 1). All samples also had similar susceptibility to gray leaf spot. No chinch bug damage was observed during trial 1. An establishment-by-week interaction occurred most weeks as expected as densities of each sample increased over time.

The samples maintained steady growth through September each year. No winter damage was seen the following spring of 2013. Plots remained green in color throughout the winter and continued to grow. It should be noted that no significant growth occurred in 2013 until week 43 (table 2). Although days often reach into the 80's (ºF) during the late spring, cool night temperatures can prevent warm-season turf from growing aggressively.

By the end of August 2013 all samples were roughly 95% established. It took these st. augustinegrass samples approximately 56 weeks to reach 95% established. Ideally, one would like to begin the 'grow-in' process at the start of summer rather than the end (June 1 vs. August 3). However, based on this study, a sod grower could anticipate spending more than one growing season establishing a field of st. augustinegrass to be harvested as sod.

Although growth ceased towards the end of October 2012, all samples still held their green pigment through the first of December. As a homeowner, this would be an attractive advantage of st. augustinegrass over other warm-season grasses, such as bermudagrass which typically tends to lose its color much earlier in the fall.

Both industry standards and all experimental samples were susceptible to gray leaf spot at the same time. Once plots became infested, symptoms remained throughout remainder of the growing season (Table 3). GLS was first noticed during the beginning of September 2012 (week 6). In 2013, GLS was first recorded during the June (week 47). Extensive irrigation and fertilization during early stages of establishing turf could have led to increased disease pressure. Also, afternoon thunderstorms in the summer lead to excessive hours of leaf moisture. With night temperatures on the rise, this combination can also lead to an increase in disease pressure, as seen during June of 2013.

The winter of 2013-2014 plots were left uncovered, unlike the previous winter. A final establishment rating was taken on July 14, 2014 (week 102). This data was analyzed separately because of being uncovered over the winter. All grass samples were similar in establishment percentage upon conclusion of the trial (table 4).
All st. augustinegrass samples had a significantly lower establishment percentage in July 2014 compared to July 2013 (Figure 1). Based on these findings, it appears a difficult task for sod growers to maintain established st. augustinegrass fields in USDA Zone 7 during cold winters such as the winter of 2013-2014 when st. augustinegrass is unprotected.

Grass samples in trials 2 and 3 have statistically different establishment percentages (Table 6). An interaction between trials also occurs, therefore mean establishment percentages were analyised separately by trial (Table 6). The weekly ratings were pooled across the studies as no differences in establishment-by-week occurred.

Trial 2 had a mean establishment percentage of 27% while Trial 3 had a mean establishment percentage of 32.3% (Table 7). The increase in established turf in Trial 3 seems logical as it was located on the low end of Trial 1. Trial 3 probably retained greater soil moisture than Trial 2, which was located on the high side of Trial 1.

In Trial 2, 'E' and 'H' had greater establishment percentages than 'G', yet were still similar to all other samples. In Trial 3, 'H' had a greater establishment percentage than 'C' or 'Palmetto', and 'C' also had a lower establishment percentage than 'A'. All other samples had similar establishment percentages (Table 8).

When experimental samples were compared back to standards 'Raleigh' and 'Palmetto', using Dunnett's test, all samples had similar establishment rates (Table 9 & Table 10).

Trials 2 & 3 were established July 1, 2013 and left uncovered during the winter of 2013-2014, similar to Trial 1. Because plots in 2013 did not undergo a winter season, the final rating was analyzed separately. Data from both trails were pooled together as no trial interaction was observed. All samples had similar establishment percentages on the final rating date, July 14, 2014 (Table 11).

All samples had less than a 12% establishment percentage on the final rating, July 14, 2014. Unlike Trial 1, Trial's 2 & 3 had just part of one growing season to establish before going into winter uncovered. More winter damage was sustained by these immature, un-established plots. This supports the practice of growing well established turf before the onset of winter.

Similar to Trial 1, Trial's 2 & 3 suffered significant winter damage during 2013 – 2014 (Figure 2). Figure 2 provides similar results as Figure 1 and supports the conclusion that growing st. augustinegrass in open areas in USDA Zone 7 will be difficult if left unprotected from winter temperatures.

Table 2.4. ANOVA for establishment, gray leaf spot damage & chinch bug damage of st. augustinegrass germplasm samples in trial 1. 2012 - 2013 in Clemson, South Carolina.

Source	DF	Establishment (%)	Gray Leaf Spot	Chinch Bug
		August 2012 – September 2013		
Grass	7	ns	ns	ns
Week	27	*	*	ns
Block	2	*	ns	ns
Grass-by-Week	189	ns	ns	ns
Grasses-by-Block	14	ns	ns	ns

Abbreviations: ns, not significant
*Significant at $\alpha = 0.05$ level.

Table 2.2. Least square means for establishment by week of st. augustinegrass germplasm samples in trial 1.
2012 – 2013 in Clemson, South Carolina.

Date	Week	Establishment (%)
	August 2012 – September 2013	
8/3/12	0	0.5
9/10/12	6	32.8
9/17/12	7	37.7
9/24/12	8	40.6
9/30/12	9	42.5
10/5/12	10	43.9
10/11/12	11	45.5
10/18/12	12	46.8
10/29/12	14	48.5
11/5/12	15	49.0
11/12/12	16	49.2
11/19/12	17	49.9
11/26/12	18	49.6
12/3/12	19	49.3
4/22/13	38	50.5
4/30/13	39	51.3
5/6/13	40	51.9
5/15/13	42	52.6
5/21/13	43	55.8
5/28/13	44	59.5
6/11/13	46	70.4
6/19/13	47	75.9
6/27/13	48	80.1
7/16/13	50	85.5
8/2/13	52	88.6
8/29/13	56	93.3
9/19/13	59	93.7
9/27/13	60	94.5
$LSD_{0.05}$	4.1	

Table 2.3. Gray leaf spot counts by week of st. augustinegrass germplasm samples in trial 1.
2012 - 2013 in Clemson, South Carolina.

Date	Week	Count
	August 2012 – September 2013	
8/3/12	0	-
9/10/12	6	*
9/17/12	7	*
9/24/12	8	*
9/30/12	9	*
10/5/12	10	*
10/11/12	11	*
10/18/12	12	*
10/29/12	14	*
11/5/12	15	*
11/12/12	16	*
11/19/12	17	*
11/26/12	18	*
12/3/12	19	*
4/22/13	38	-
4/30/13	39	-
5/6/13	40	-
5/15/13	42	-
5/21/13	43	-
5/28/13	44	-
6/11/13	46	-
6/19/13	47	*
6/27/13	48	*
7/16/13	50	*
8/2/13	52	*
8/29/13	56	*
9/19/13	59	*
9/27/13	60	*

Binomial counts.
Weeks with (*) contained GLS damage, weeks with (-) contained no damage.

Table 2.4. ANOVA for establishment of st. augustinegrass germplasm samples in trial 1. 2014 in Clemson, South Carolina.

Source	DF	Establishment (%)
		July 14, 2014
Grass	7	ns
Block	2	ns
Grasses-by-Block	14	ns

Abbreviations: ns, not significant

*Significant at $\alpha = 0.05$ level.

Table 2.5. Least square means for establishment of st. augustinegrass germplasm samples in trial 1. 2014 in Clemson, South Carolina.

Grass	Establishment (%)
	July 14, 2014
A	52
Raleigh	62
C	67
Palmetto	61
E	71
F	69
G	55
H	70
$LSD_{0.05}$	22.5

Figure 2.1. Comparing mean establishment rates of July 2013 and July 2014 following winter of 2013-2014.

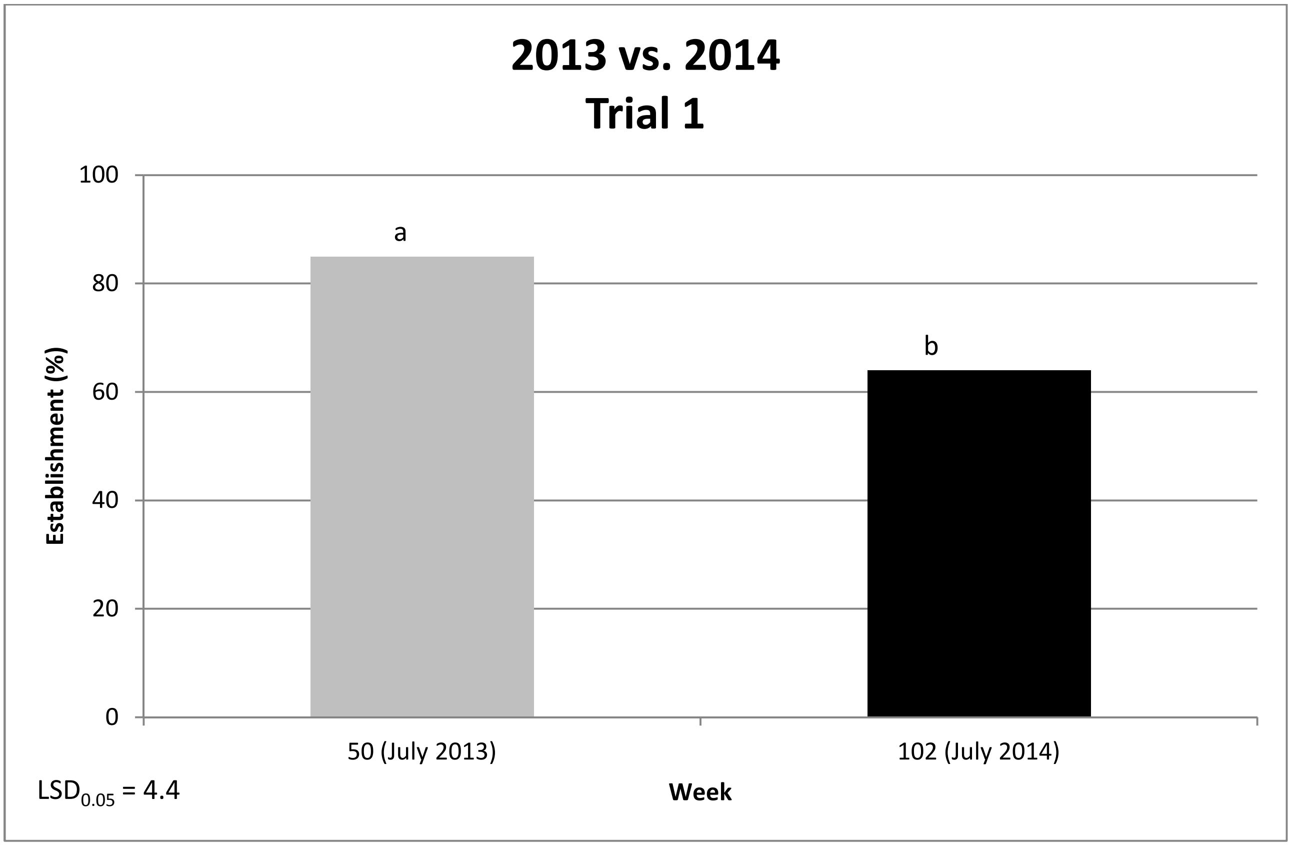


Table 2.6. ANOVA for establishment of st. augustinegrass germplasm samples in trial's 2 & 3. 2014 in Clemson, South Carolina.

Source	DF	Establishment (%)
		September 2013
Trial	1	ns
Grass	7	*
Week	1	ns
Block	2	ns
Trial	1	*
Grass-by-Week	7	ns
Grass-by-Block	14	ns
Grass-by-Trial	14	ns

Abbreviations: ns, not significant

*Significant at α = 0.05 level.

Table 2.8. Least square means for establishment of st. augustinegrass germplasm samples in trial's 2 & 3. 2014 in Clemson, South Carolina.

Grass	Establishment (%)	Establishment (%)
	September 2013	
	Trial 2	Trial 3
A	27.8 ab	38.8 ab
Raleigh	27.3 ab	29.6 abc
C	22.7 ab	25.5 c
Palmetto	25.7 ab	27.0 bc
E	34.3 a	35.3abc
F	29.8 ab	33.3 abc
G	16.7 b	29.5 bc
H	31.3 a	39.3 a
$LSD_{0.05}$	13.2	9.8

Table 2.9. DUNNETT's test for establishment of st. augustinegrass germplasm samples in trial's 2 & 3. 2014 in Clemson, South Carolina.

Grass	Industry Standard	Establishment (%)
	Trial 2	
A	Raleigh	ns
C	Raleigh	ns
Palmetto	Raleigh	ns
E	Raleigh	ns
F	Raleigh	ns
G	Raleigh	ns
H	Raleigh	ns
	Trial 3	
A	Raleigh	ns
C	Raleigh	ns
Palmetto	Raleigh	ns
E	Raleigh	ns
F	Raleigh	ns
G	Raleigh	ns
H	Raleigh	ns

Abbreviations: ns, not significant
*Significant at $\alpha = 0.05$ level.

Table 2.10. DUNNETT’s test for establishment of st. augustinegrass germplasm samples in trial’s 2 & 3. 2014 in Clemson, South Carolina.

Grass	Industry Standard	Establishment (%)
	Trial 2	
A	Palmetto	ns
Raleigh	Palmetto	ns
C	Palmetto	ns
E	Palmetto	ns
F	Palmetto	ns
G	Palmetto	ns
H	Palmetto	ns
	Trial 3	
A	Palmetto	ns
Raleigh	Palmetto	ns
C	Palmetto	ns
E	Palmetto	ns
F	Palmetto	ns
G	Palmetto	ns
H	Palmetto	ns

Abbreviations: ns, not significant
*Significant at $\alpha = 0.05$ level.

Table 2.11. ANOVA for establishment of st. augustinegrass germplasm samples in trial's 2 & 3. 2014 in Clemson, South Carolina.

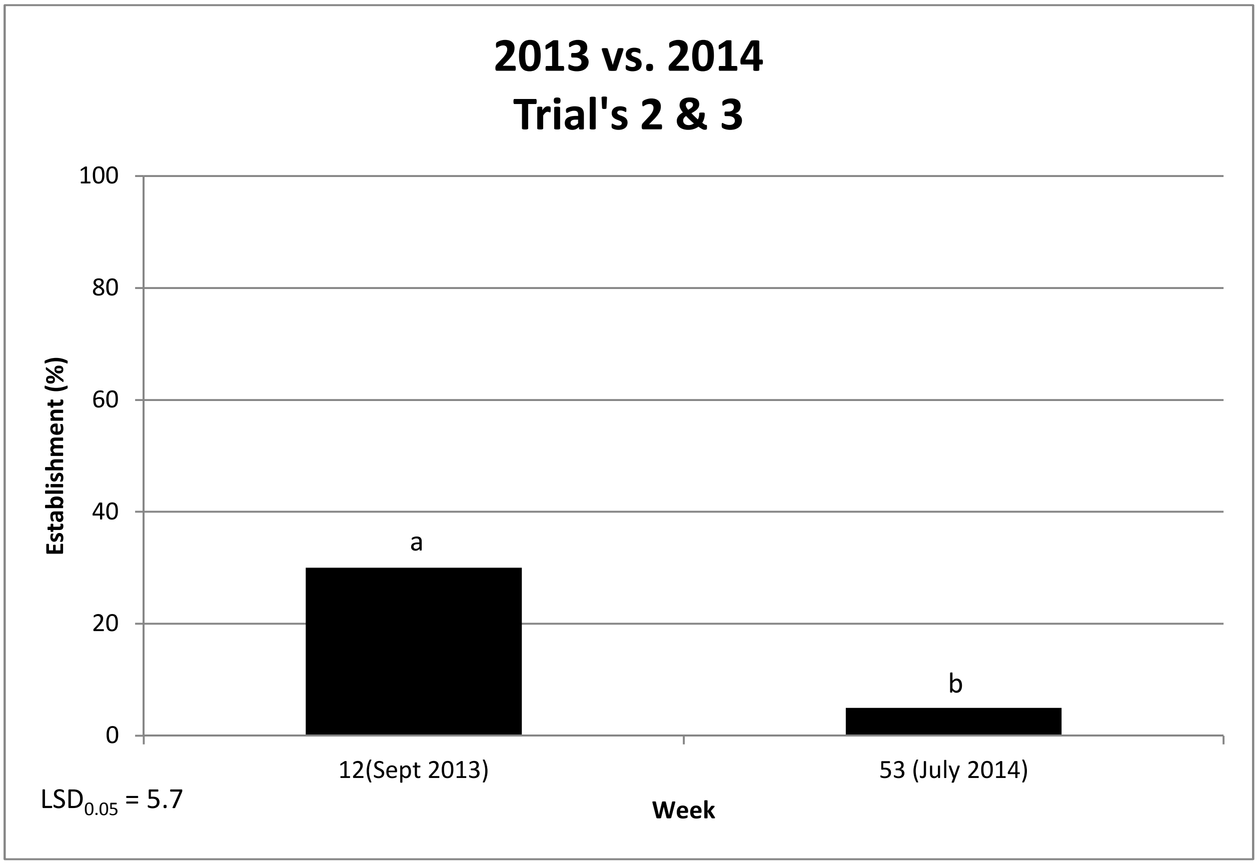
Source	DF	Establishment (%)
		July 14, 2014
Trial	1	ns
Grass	7	ns
Block	2	ns
Grass-by-Block	14	ns
Grass-by-Trial	7	ns

Abbreviations: ns, not significant
*Significant at α = 0.05 level.

Table 2.12. LEAST SQUARE MEANS TABLE for establishment of st. augustinegrass germplasm samples in trial's 2 & 3. 2014 in Clemson, South Carolina.

Grass	Establishment (%)
	July 14, 2014
A	4
Raleigh	5
C	2
Palmetto	3
E	6
F	6
G	6
H	12
LSD	16.5

Figure 2.2. Comparing mean establishment rates of September 2013 & July 2014 following winter of 2013-2014.



Conclusion

Sod growers are interested in establishment rates of turfs to help determine the length of time expected to harvest a crop. From this study, the experimental samples established in a similar amount of time compared to available industry standard cultivars. Therefore, no extra delays in time could be expected for a grower if they chose to establish a crop of sod from one of these experimental lines.

All samples held their color through the first of December. For homeowners, this would be an attractive advantage of a st. augustinegrass cultivar over another warm-season grass which typically loses color much earlier in the fall.

Figure 1 tells the take home message of this study, however. Even though these samples appear to grow at the same rates, left uncovered or unprotected over winter in an open field, even after a crop reaches maturity, severe damage over winter could delay harvests, create additional imput costs, thus resulting in an unprofitable field of sod.

ST. AUGUSTINEGRASS [*Stenotaphrum secundatum* (Waltz.) Kuntze] RESPONSE TO REDUCED LIGHT ENVIRONMENTS

Introduction

Shade is a major issue for turfgrass managers. A shade environment can be particularly challenging to warm-season turfgrasses, which have inherently higher light compensation points relative to cool-season grasses (Beard, 1973).

St. augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is considered one of the most shade-tolerant warm-season turfgrass species (Beard, 1973; Stier and Gardner, 2008), and one of the most popular turfgrass species used for home lawns throughout the southern United States (Busey, 2003). Propagation is primarily vegetative by plugs or sod as few seedheads are formed. St. augustinegrass has strong, thick stolons, course leaf texture, and produces a turf of medium density. Recuperation is good because of the aggressive stolons, but wear tolerance is only fair. Salt tolerance is very good (Emmons 2000). The maintenance requirement is medium, though the grass has a vigorous growth rate while moderate fertilization is necessary. St. augustinegrass is maintained at a height of 3.8 cm – 7.6 cm (1.5 to 3 inches) with either a reel or rotary mower. St. augustinegrass decline (SAD), a disease caused by a virus, is a potential problem; however southern chinch bugs (*Blissus insularis* Barber) can cause extensive injury as can gray leaf spot disease (caused by *Pyricularia grisea* Cooke) (Emmons 2000).

Previous research has identified typical turf responses to shaded environments (Trenholm and Nagata, 2005). Morphological characteristics include longer leaf length (Wilkinson and Beard, 1974), longer internode lengths (Peacock and Dudeck, 1981; Winstead and Ward, 1974), reduced clipping weights (Barrios et al., 1986; Wilkinson and Beard, 1974), and increased leaf area (Beard, 1973; Peacock and Dudeck, 1981). Reduced tillering and stand density are also typical responses to shaded conditions (Beard, 1973; Schmidt and Blaser, 1967; Winstead and Ward, 1974). Physiological changes generally include greater chlorophyll concentration (Beard, 1973; Winstead and Ward, 1974), although Peacock and Dudeck (1981) noted shade did not affect the chlorophyll content of various st. augustinegrass cultivars. They also saw differences in shoot growth between cultivars under shaded conditions, which may have contributed to lower chlorophyll content. Barrios et al. (1986) noted turfgrass quality generally declined as shade increased, particularly under severe. Knowledge of what cultivar might perform best under shade is an important issue for builders, landscape designers, and horticulturists (Trenholm, 2005).

There are many commonly produced cultivars of st. augustinegrass, which show different physiological and morphological responses to shade. Peacock and Dudeck (1981) reported that 'Bitter Blue' st. augustinegrass performed best in shade. Trenholm and Nagata (2005) and Cai (2011) reported best shade tolerance in dwarf cultivars of St. Augustinegrass and optimal turf performance at 30% shade compared with 0%, 50%, or 70% in all cultivars.

Dudeck and Peacock (1981) stated shade adaptation of a turfgrass ground cover is influenced by a complex of microclimatical, pathological, and physiological responses. Primary factors involve: reduced irradiance; tree root competition for nutrients and water; microclimate that favors disease activity; succulent grass tissue; and, reductions in shoot density, root growth, and carbohydrate reserves (Beard, 1965, 1973; Schmidt & Blaser, 1967; Wilkinson et al., 1975).

Plants which adapt to shade environments do so by a combination of physiological or morphological adaptations (Leopold & Kriedmann, 1975). Plants capable of shade adaptation develop a higher photochemical efficiency, which is expressed by a steeper slope in the early phases of their light response curves. Boardman (1977), however, concludes no one factor is the primary cause of altered photosynthetic capacity. Wilkinson et al. (1975) concluded the photosynthetic respiratory balance is a critical factor in shade adaptation. For a plant to survive, net photosynthesis must exceed respiration (Waddington, 1992).

Dudeck and Peacock (1981) discussed cultural practices for shaded areas which include: Raising mowing heights as high as possible to provide maximum leaf area for absorption of limited radiant energy and to increase turfgrass rooting depth which and helps maintain turf density. Irrigation should be applied only when turf shows signs of stress, including folded leaf blades, blue-gray color overall, and footprints or wheel marked impressions due to loss of turgor. Water deeply to promote deep rooting of turf as shallow, light, and frequent irrigations enhances disease activity and encourages development of shallow root systems. Excessive N fertilization should be avoided as this encourages shoot growth over root growth which places a further stress on carbohydrate reserves. Excessive N also increases tissue succulence which again increases disease susceptibility and decreases ability of turf to withstand environmental stress. Minimize traffic in shaded areas since wear tolerance is reduced. As disease pressure often increases on shade-grown turf, fungicide use may be needed if that occurs (Waddington, 1992).

Only limited published information exists pertaining to comparative shade tolerance of st. augustinegrass (Wherley et al. 2013). In recent years, newer cultivars with reportedly improved shade tolerance such as ‘Amerishade’ and ‘Captiva’ have been developed (Brosnan and Deputy, 2008; Trenholm and Kenworthy, 2009); however, data on their comparative shade tolerance in relation to other commercially available cultivars are lacking (Wherley, 2013).

Current trends toward energy conservation in home landscaping present problems in warm-season turfgrass selection (Beard 1970; Boardman 1977). It is not uncommon for builders of homeowners to attempt to establish turfgrasses in densely shaded environments (Wherley et al. 2013). Determining and comparing shade limits for currently available cultivars along with new experimental lines is necessary information for turf managers and homeowners.

The purpose of this study was to evaluate shade tolerance of a St. Augustinegrass germplasm collection from upstate South Carolina, including commercial cultivars

‘Raleigh’, ‘Palmetto’ and ‘Palisades’ zoysia (*Zoysia japonica* Steud.). Comprehensive evaluations of these plant collections could open new opportunities for turf managers and homeowners.

Materials and Methods

In 2006, a germplasm collection was established with samples from thirty St. Augustinegrass lawns grown in USDA Hardiness Zone 7. These samples, along with commercial standards, ‘Raleigh’ and ‘Palmetto’, were established by plugs under natural low light (~50% full sunlight) conditions in Clemson, South Carolina.

In June of 2012, plugs from the top six performing grasses, along with ‘Raleigh’ and ‘Palmetto’, were collected and transplanted into 24 plastic trays (53 x 38 x 8 cm), filled with river sand, using four, 5 cm, plugs per tray. Trays were transported to Clemson University’s Greenhouse Facility and grown for 12 months at 25 $\pm$ 10ºC. Once established, shade studies were conducted for further evaluations.

Two 8-week experiments were conducted at Clemson University’s Greenhouse Research Complex during 2013. Study 1 was conducted from 7 June 2013 to 2 August 2013. While study 2 was conducted from 18 November 2013 to 6 January 2014. Greenhouse temperatures averaged 31°C/22°C (day/night) for study 1. For study 2 greenhouse temperatures averaged 26°C/20°C (day/night).

Grass samples were established from 10 cm diameter x 3 cm deep, round cup cutter plugs from previously established trays into 15 cm diameter x 11 cm deep round pots filled with a potting soil medium (Faford 3B mx, Concord Faford Inc., Agawam, MA). Pots were fertilized at 25 kg N/ha on a three week interval with a 1-1-1 complete fertilizer, watered every other day to field capacity, and mowed at 5.5 cm twice weekly until all grasses attained ideal turf quality and density. This length of pre-treatment grow-in period required to reach ideal canopy density was 6 weeks for study one and 10 weeks for study 2. Natural sunlight and day length were used during the greenhouse establishment phase.

Shade treatment structures were constructed to evaluate the response of experimental and commercial lines of st. augustinegrass to four levels of a reduced light environment (RLE): 0, 30, 50, and 70%. Two shade structures of each RLE level were constructed.

Light reduction of each shade cloth was determined by comparing photosynthetic photon flux (PPF) (μmol m^{-2} s^{-1}) under the shade cloths at soil level to full-irradiance PPF measurements with a LI-28663 quantum light sensor (LiCor, Inc., Lincon, NE) $[(PPF_{full\ sun} - PPF_{under\ shade\ cloth})/PPF_{full\ sun}] \times 100$.

Reduced light environments were applied continuously using neutral density, poly-fiber black shade cloth (model SC-black30, SC-black50, SC-black70; International Greenhouse Company, Danville, IL) that removed equal amounts of light across the photosynthetically active light spectrum. Individual shade cloth tent frames were 1 × 1 m and constructed with 5.3 cm diameter polyvinyl chloride (PVC) pipes. Shade cloths were attached to PVC frames with zip-ties and pulled taut to maintain shade cloths at a consistent height above the soil surface and maintain consistent surface temperature and air movement among all treatments.

Underneath each shade structure, pots were arranged in a completely randomized design with four replications per treatment (shade) level with nine different grass samples totaling 36 pots per treatment (shade) level. Equal numbers of pots were placed under each shade structure with two shade structures per treatment (shade) level. A 20 cm buffer around the perimeter was used to reduce potential border effects during the study. Pots were re-randomized every two weeks when ratings were taken and mown to avoid localized environmental conditions.

Photoperiod in the greenhouse was extended to 12 h with 1000-W lamps (300 μmol m^{2} s^{1} light intensity) located approximately 2 meters above the turf canopy for study two to provide similar photoperiod length as study one. Irrigation was maintained as needed to meet evapotranspiration at the varying shade levels throughout the experiment. At study initiation, pots were trimmed vertically to a height of 5.5 cm as well as laterally back to the original 15 cm diameter pot size.

Morphological ratings of leaf width, leaf length, and internode distance were evaluated every two weeks. The average distance of the two longest leaves from the soil surface to the tip were measured from each pot for length and measured mid-way up the length of the leaf for width using a ruler. Internode distance was measured using the average of two longest stolons growing to the third internode past the pot edge. The distance between the second and third internode was measured. Daily leaf elongation rate ($mm \cdot d^{-1}$) was calculated by subtracting height of cut from leaf length and divided by number of days since previous mowing before determining daily leaf elongation rate ($mm \cdot d^{-1}$). After measurements, pots were trimmed back to original 5.5 cm height.

Visual turf quality and turf density was evaluated every two weeks using a 1 to 9 scale, where 1 = dead turf, 6 = minimally acceptable quality, and 9 = perfect green turf. Clipping weights were calculated monthly (weeks 4 & 8). Pots were trimmed to original 5.5 cm height, clippings were collected, dried for 48h at 60°C, then weighed (g). Root weights were collected upon conclusion of studies. Roots were removed from pots, clipped below thatch, washed and sieved to remove any attached soil, dried for 48h at 60°C, then weighed.

For study 2, chlorophyll content was measured monthly (week 4 & 8) using a chlorophyll meter (Field Scout CM 1000; Spectrum Technologies, Inc., Plainfield, IL), which measured ratios of reflected red and far-red light to calculate relative chlorophyll content, or greenness. The output is a unitless index of chlorophyll content on a scale of 0 to 999 (Bunderson et al. 2009). Two measurements were taken per pot and averaged. All measurements were taken between 1200 and 1400h with pots removed from shade structures. Measurements were taken with the meter 1m from turf surface.

Data were analyzed using PROC MIXED repeated measures analysis. Means separation procedures were performed by Fisher's protected LSD. All comparisons were based on an $\alpha = 0.05$ significance level and conducted using SAS version 9.3 (SAS

Institute Inc., Cary, NC). Final (week 8) data was presented to provide comparison of experimental lines and commercially available cultivars.

Results and Discussion

Visual Ratings. At all RLE's (0, 30, 50, & 70%), differences were not seen among commercial cultivars and experimental lines for final turf quality (Tables 1-4) as all entries maintained acceptable quality levels (greater than 6). Final turf density scores had a similar trend as no differences were found between entries at any RLE. At 70% RLE (heavy shade), experimental line 'E' and experimental line 'F' numerically has turf density values below acceptable levels but statistically weren't significant. Visually, experimental lines collected from USDA Hardiness Zone 7 performed comparable to current commercially available cultivars 'Palmetto' and 'Raleigh' as well as 'Palisades' zoysia.

Morphological Ratings. 0% RLE All st. augustinegrass entries measured similar leaf widths, between 7.0 – 8.4 mm (Table 1). As expected, all st. augustinegrass entries were statistically different from 'Palisades' leaf width of 3.6 mm. A similar trend was seen for leaf elongation rate. All st. augustinegrass entries provided a similar elongation rate between 1.3 – 2.7 $mm \cdot d^{-1}$ compared to 4.8 $mm \cdot d^{-1}$ elongation rate of 'Palisades'. Internode distance for experimental 'A' (4.5 mm) was significantly longer than experimental 'G' (2.1 mm) but similar to all other entries.

30% RLE All st. augustinegrass entries had similar leaf widths between 7.6 – 8.6 mm and again significantly wider than 'Palisades' zoysia (3.5 mm) (Table 2). All st. augustinegrass entries provide a similar elongation rate between 4.0 – 2.3 $mm \cdot d^{-1}$. 'Palisades' had a leaf elongation rate of 6.0 $mm \cdot d^{-1}$, which was different from four experimental (‘A’, ‘C’, ‘F’, & ‘G’) and one commercial (‘Palmetto’) st. augustinegrass entries. The lack of stolons with a 3rd internode past the edge of the pot failed to generate internode distance ratings in the 30% RLE.

50% RLE All st. augustinegrass entries had similar leaf widths between 6.8 – 8.2 mm, again, significantly wider than ‘Palisades’ zoysia (3.4 mm) (Table 3). All entries had similar leaf elongation rates between 2.9 – 7.1 mm. Experimental line ‘F’ had a longer internode distance (6.3 cm) than experimental lines ‘C’ (3.2 cm) and ‘G’ (2.6 cm) as well as commercial cultivar ‘Raleigh’ (3.5 cm). These four entries were the only entries to produce measurable stolons with a 3rd internode past the pot edge at 50% RLE.

70% RLE All st. augustinegrass entries had similar leaf widths between 7.0 – 8.4 mm, again significantly wider than ‘Palisades’ zoysia (3.4 mm) (Table 4). All entries had similar leaf elongation rates between 4.4 – 7.1 $mm \cdot d^{-1}$. Similar to 30% RLE, insufficient stolon lengths prevented internode distance ratings at 70% RLE.

Overall increased leaf elongation rates from 0% RLE to 70% RLE supports previous research stating shaded environments result in longer leaf lengths (Wilkinson and Beard, 1974). Experimental entry ‘F’’s internode distance increase from 0% RLE to 50% RLE also supports previous research that shaded environments increase internode distances (Peacock and Dudeck 1981). In the field, st. augustegrass would generally be mowed with greater frequency and perhaps at a higher height, however, this study was designed to maximize differences to shade responses among entries. A 14-d clipping interval was also used by Trenholm and Nagata (2005) in screening st. augustinegrass cultivars for shade tolerance. It is plausible that a greater clipping frequency could have potentially resulted in somewhat improved quality of plants in this study, as greater frequency of mowing can promote increased tillering (Beard, 1973; Bell, 2011).

Shade tolerance indicators. Clipping weights, root weights, and chlorophyll content were all measured for indicators of shade tolerance. At 0% RLE (Table 1), clipping weights and root weights were similar for all entries ranging from 2.3 - 3.1 g and 8.6 – 10.9 g, respectively. Five experimental entries (‘C’, ‘E’, ‘F’, ‘G’, & ’H’) and both commercial cultivars (‘Raleigh’ & ‘Palmetto’) had similar chlorophyll content readings, measuring from 276.8 – 384.3 chlorophyll content index (CCI). Experimental entry ‘A’ (192.8) and ‘Palisades’ zoysia (188.0) had a significantly lower CCI than experimental ‘F’ (384.3) and experimental ‘C’ (337.8). At 30%, 50%, & 70% RLE (Table 2, 3, & 4), clipping weights, root weights, and chlorophyll content were similar for all entries. At 30% RLE, clipping weights ranged from 1.8 – 2.3 g while root weights ranged from 7.3 – 8.8 g. Chlorophyll content ranged from 262.5 – 336.5 CCI. At 50% RLE, clipping weights ranged from 1.4 – 2.2 g, root weights ranged from 7.4 – 8.8 g. Chlorophyll content ranged from 141.3 – 332.0 CCI. At 70% RLE, clipping weights ranged from 0.9 – 1.5 g and root weights ranged from 6.0 – 11.0 g. Chlorophyll content ranged from 167.8 – 225.8 CCI. Among these parameters evaluated, considerable variability existed but experimental lines still performed comparable to commercially available cultivars.

Table 3.1. Final st. augustinegrass parameter measurements under no shade [0% reduced light environment (RLE)][1]

	No Shade (0% RLE)							
Entry	Turf quality (1-9)	Turf density (1-9)	Clipping weight (g)	Root weight (g)	Chlorophyll content (0-999)	Leaf width (mm)	Leaf elongation rate ($mm \cdot d^{-1}$)	Internode distance (cm)
A	6.6	7.4	3.1	9.9	192.8	7.1	1.3	4.5
'Raleigh'	7.1	7.5	2.5	8.6	293.5	7.5	2.1	2.8
C	8.1	8.4	2.4	10.6	337.8	7.4	1.0	2.9
'Palmetto'	7.6	7.4	2.9	10.9	313.8	7.1	2.1	2.7
E	7.3	7.3	2.6	10.1	323.5	7.6	2.7	3.5
F	8.0	7.6	2.5	10.2	384.3	8.4	1.4	2.9
G	8.3	8.6	2.8	10.7	276.8	7.5	1.4	2.1
H	7.3	7.8	2.3	9.1	295.8	7.0	1.4	3.1
'Palisades'	8.0	8.3	2.4	10.9	188.0	3.6	4.8	2.0
LSD (0.05)	NS	NS	NS	NS	136.27	2.01	1.834	2.375
P-value	0.3457	0.3992	0.1806	0.0666	0.0008	0.0167	0.0372	0.0359

[1]Except for chlorophyll content, which was measured only for study 2, means have been pooled across experiments.

Abbreviations: LSD = least significant difference; NS = not significant (α=0.05)

Table 3.2. Final st augustinegrass parameter measurements under light shade [30% reduced light environment (RLE)][1]

	Mild Shade (30% RLE)							
	Turf quality	Turf density	Clipping weight	Root weight	Chlorophyll content	Leaf width	Leaf elongation rate	Internode distance
Entry	(1-9)	(1-9)	(g)	(g)	(0-999)	(mm)	($mm \cdot d^{-1}$)	(cm)
A	7.0	7.0	1.8	7.3	289.8	8.1	2.7	.
'Raleigh'	7.0	7.8	2.2	8.5	335.3	8.0	3.8	.
C	7.8	7.5	2.0	8.8	332.5	7.8	3.2	.
'Palmetto'	6.8	6.9	1.8	7.4	262.5	8.6	3.5	.
E	7.1	7.4	2.3	8.6	280.5	7.6	3.8	.
F	7.3	7.3	1.9	8.0	283.0	7.9	2.3	.
G	7.6	7.6	1.9	7.8	336.5	8.6	2.9	.
H	7.1	7.3	1.9	8.1	296.5	8.3	4.0	.
'Palisades' zoysia	7.5	7.3	2.1	8.6	273.3	3.5	6.0	.
LSD (0.05)	NS	NS	NS	NS	NS	1.6234	2.5952	.
P-value	0.155	0.3531	0.8214	0.3214	0.2927	0.0053	0.0204	.

[1]Except for chlorophyll content, which was measured only for study 2, means have been pooled across experiments.

. Internode distance not measureable due to stolons lacking third internode.

Abbreviations: LSD = least significant difference; NS = not significant (α=0.05)

Table 3.3. Final st augustinegrass parameter measurements under moderate shade [50% reduced light environment (RLE)][1]

	Moderate Shade (50% RLE)							
	Turf quality	Turf density	Clipping weight	Root weight	Chlorophyll content	Leaf width	Leaf elongation rate	Internode distance
Entry	(1-9)	(1-9)	(g)	(g)	(0-999)	(mm)	($mm \cdot d^{-1}$)	(cm)
A	7.3	7.0	1.6	8.3	281.0	6.8	4.4	.
'Raleigh'	6.8	6.8	1.5	7.4	202.3	8.2	2.9	3.5
C	7.1	7.5	2.0	8.7	240.5	7.4	3.1	3.2
'Palmetto'	6.9	6.9	1.6	7.3	290.3	7.1	3.8	.
E	6.9	7.0	2.1	8.3	313.8	7.9	4.1	.
F	7.5	7.3	1.69	8.8	299.0	7.6	3.3	6.3
G	7.3	7.5	1.4	7.9	230.8	7.8	4.3	2.6
H	7.1	6.9	1.5	7.5	141.3	8.0	4.2	.
'Palisades' zoysia	7.4	7.3	2.2	8.7	332.0	3.4	7.1	.
LSD (0.05)	NS	NS	NS	NS	NS	3.48	NS	2.697
P-value	0.178	0.4465	0.2251	0.2763	0.2403	0.0002	0.0639	0.049

[1]Except for chlorophyll content, which was measured only for study 2, means have been pooled across experiments.

. Internode distance not measureable due to stolons lacking third internode.

Abbreviations: LSD = least significant difference; NS = not significant (α=0.05)

0

Table 3.4. Final st augustinegrass parameter measurements under heavy shade [70% reduced light environment (RLE)][1]

	Heavy Shade (70% RLE)							
	Turf quality	Turf density	Clipping weight	Root weight	Chlorophyll content	Leaf width	Leaf elongation rate	Intern distan
Entry	(1-9)	(1-9)	(g)	(g)	(0-999)	(mm)	$(mm \cdot d^{-1})$	(cm
A	6.4	6.5	1.0	7.6	167.8	7.9	5.4	.
'Raleigh'	7.0	6.8	1.2	6.0	225.8	7.6	4.6	.
C	6.6	6.9	1.0	6.6	183.3	8.4	4.6	.
'Palmetto'	6.9	6.8	1.1	6.7	186.3	7.8	5.3	.
E	6.3	5.5	0.9	6.7	195.3	7.6	4.4	.
F	6.8	5.9	1.0	7.0	175.8	7.6	4.7	.
G	7.6	7.8	1.2	5.6	225.5	7.9	5.0	.
H	6.5	6.6	1.2	11.0	199.0	7.0	6.4	.
'Palisades' zoysia	6.9	6.4	1.5	7.3	202.5	3.4	7.1	.
LSD (0.05)	NS	NS	NS	NS	NS	1.8832	NS	.
P-value	0.335	0.0913	0.1541	0.4034	0.9676	0.0011	0.1924	.

[1]Except for chlorophyll content, which was measured only for study 2, means have been pooled across experiments.

· Internode distance not measureable due to stolons lacking third internode.

Abbreviations: LSD = least significant difference; NS = not significant (α=0.05)

Conclusions

In conclusion, based on 8-week greenhouse studies under ideal temperature and moisture conditions with no disease or insect pressure indicated, the experimental lines had similar shade tolerance compared to commercial standards ‘Raleigh’ ‘Palmetto’ and ‘Palisades’ zoysia. Field studies are needed to validate greenhouse studies to help further evaluate shade tolerance of experimental and commercial lines.

ST. AUGUSTINEGRASS [*Stenotaphrum secundatum* (Waltz.) Kuntze] LOW TEMPERATURE TOLERANCE

Introduction

St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is one of the most popular turfgrass species used for home lawns throughout the southern United States (Busey, 2003). It is believed to be native to open-to-lightly shaded, high rainfall, and humid regions of coastal South and Central America including the West Indies (McCarty, 2011a). This species is adaptable to many soil conditions, but does best on moist, well-drained sandy soils. Irrigation is necessary during periods of dry weather because its drought tolerance is only fair (Emmons, 2000).

On occasion, USDA Hardiness Zone 7 experiences years of drought and above average heat during summer months, followed by below average cold temperatures during winter months. Often, these conditions create a turf void in shady locations throughout the landscape. Such areas suffer as temperatures become too hot for tall fescue [*Festuca arundineacea* Schreb.], a C_3 plant, survival, yet too cold for centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], a C_4 plant, survival. For Clemson, SC, average summer high and low temperatures are 32°C and 20°C, with a record high of 41°C. Average winter high and low temperatures are 11°C and -1°C, with a record low of -20°C. Warm-season grasses exhibit optimum growth between 27°C and 38°C as cool-season grasses exhibit optimum growth when temperatures range between 15°C and 25°C (McCarty, 2011b). As summer nights fail to cool below 18°C, tall fescue struggles to recover from daytime heat. When ambient temperatures rise above a specific level, known as the temperature compensation point, C_3 plants cannot produce enough carbohydrates to fulfill the demand for respiration. At this temperature, the amount of CO_2 fixed by the dark reactions of photosynthesis is equal to the amount of CO_2 released by mitochondrial respiration (McCarty, 2011b). Prolonged exposure of temperatures in excess of the temperature compensation point leads to continuous depletion of carbohydrate reserves and eventual weakening of the turf. Ultimately, this leads to exhaustion of carbohydrate reserves via respiration. Low nighttime temperatures (<18°C) provide cool-season turfgrass with a recovery time to mobilize stored carbohydrate reserves for energy production (McCarty, 2011b).

Permanent turfgrass injury to warm-season turfgrass, such as centipedegrass, often occurs if ambient temperatures drop rapidly below -5°C and gradually below -12.2°C (McCarty, 2011b). This weather pattern, combined with a growing population in the upstate, opens a niche demand for a warm season (C4) grass cold tolerate enough to survive below average temperatures in shaded lawns.

Upstate South Carolina lies along the Interstate 85 corridor connected Charlotte, North Carolina and Atlanta, Georgia. According to Forbes.com, Atlanta and Raleigh (north of Charlotte) are among the top 10 fastest growing cities in America (Fisher, 2011). For example, Greenville County, lying along I-85 between Atlanta and Charlotte, has experienced a 70% population growth since 1990. Such growth rates demand more housing, more lawns, thus an increase demand for quality turf.

As homeowners demand greater energy efficiency, demands for better performing, greater shade tolerant turfgrass also increase. Current trends toward energy conservation in home landscaping present problems in warm-season turfgrass selection since all species grow best in full sunlight. As the use of shade for cooling homes and buildings has increased, the need for a shade tolerant turfgrass by homeowners and landscapers has arrived (Beard, 1970; Boardman, 1977).

Shade and cold tolerance are a must for new turfgrass species to fill demands of this niche market. Winterkill is often a problem in St. Augustinegrass sod production (Philley et al., 1998), as St. Augustinegrass is the least freezing-tolerant of the warm-season turfgrasses (Beard et al., 1980). St. Augustinegrass is adapted to U.S. Dept. of Agriculture hardiness zones 8, 9, and 10. However, severe freezing injury may occur during periodic winters in zones 8 and 9. While residential sites may offer some protection from cold, sod is usually produced in large open fields (Philley et al., 1998). Improvement of cold tolerance in St. Augustinegrass would increase the area of adaptation and potential use of this important turfgrass species (Philley et al., 1998).

Freezing temperatures that result in ice formation within plant cells can cause multiple types of tissue damage and death of the entire plant under severe conditions (Livingston et al., 2006). During a period of low but non-freezing temperatures in a process called cold-acclimation (Thomashow, 1999; Xin and Browse, 2000; Livingston et al., 2006), plants can increase their ability to withstand freezing temperatures. In nature, cold-acclimation is initiated by decreasing temperatures in late autumn or early winter.

Selecting plants with increased tolerance to winter freezing is an important aspect of plant improvement. However, fluctuating winter temperatures make it necessary for experiments to be conducted in multiple locations and years (Tcacenco et al., 1989). Such tests are costly and time-consuming. Therefore, cold tolerance evaluations have been conducted in field trials, cold simulation chambers (Beard et al., 1980) and by excised stolon regrowth tests (Maier et al., 1994a).

Electrolyte leakage and differential thermal analysis (DTA) have been used to predict lethal low temperatures for St. Augustinegrass genotypes. Lethal temperatures determined by electrolyte leakage ranged from -4.0°C for ‘Floratam’ to -6.8°C for ‘Raleigh’ (Fry et al., 1991; Maier et al., 1994b). Lethal temperatures predicted by DTA ranged from -4.7°C to -7.7°C for 14 genotypes that displayed a wide range of winter survival (Philley et al., 1995).

Cold tolerance in most plants is controlled by multiple genes and additive gene action (Marshall, 1982; Fowler et al., 1993; Philley et al., 1998). Maier et al. (1994a) acclimated plants in the field then froze them in a chamber at various temperatures. They found freezing survival of ‘Raleigh’ (>60%), was much better than ‘Floratam’ and ‘FX-332’ (< 20%). Maier, Lang, and Fry (1994a) reported St. Augustinegrass cultivars have also been exposed to freezing temperatures in a controlled environment to determine freezing tolerance. Common St. Augustinegrass was killed after a 16-h exposure to -4.4°C (Reeves and McBee, 1972). Fry et al. (1991) reported lethal temperatures for ‘Floratam’ to vary monthly from -6.1°C to -5.3°C between December and March in

Louisiana. In contrast, Murdoch et al. (1990) reported 'Floratam' nodes from actively growing turf were killed following exposure to -4°C.

Germplasm collection is an effective approach for cultivar development (Li, 2010). For example, tall fescue (*Festuca arundineacea* Schreb. cv. 'Kentucky 31'), one of the most popular tall fescue cultivars, and 'Raleigh', currently the most cold tolerant St. Augustinegrass cultivar, were both selected from plants collected from the field (Maier et al., 1994a).

The purpose of this study was to evaluate cold tolerance of a St. Augustinegrass germplasm collection from upstate South Carolina, for potential sod production. Comprehensive evaluations of these plant collections could open new opportunities for sod growers to provide homeowners with a highly shade tolerant warm season turfgrass capable of surviving unusually cold winters in USDA Hardiness Zone 7.

Materials and Methods

In 2006, a germplasm collection was established with samples from thirty St. Augustinegrass lawns grown in USDA Hardiness Zone 7. These samples, along with commercial standards, 'Raleigh' and 'Palmetto', were established by plugs under natural low light (~50% full sunlight) conditions in Clemson, South Carolina.

In June of 2012, plugs from the top eight performing grasses were collected and transplanted into 24 plastic trays (53 x 38 x 8 cm), filled with river sand, using four, 5 cm, plugs per tray. Trays were transported to Clemson University's Greenhouse Facility and grown for 12 months at 25 ± 10ºC. Sprigs of each grass sample were transplanted into 10 cm diameter pots filled with a potting soil medium (Faford 3B mix, Concord Faford Inc., Agawam, MA) using six sprigs with 3 internodes and 8-12 cm in length, per pot. Pots were placed in a growth chamber and established for 6 weeks at 32ºC with a 16 h photoperiod (500 µmol m^2 s^1). Pots were fertilized at 25 kg N/ha on three week intervals using a 1-1-1 complete fertilizer, watered every other day to field capacity, and mowed at 5.5 cm, twice weekly.

Pots were selected for preliminary testing to identify the target freezing temperature by exposing plants to -2°C, -4°C, -6°C, and -8°C for 3 h following the 'two-step acclimation' protocol by Li et al. (2010) to simulate the natural acclimation in late fall or winter. Greater than 95% of plants provided regrowth at -2°C, while < 10% of plants provided regrowth at -8°C. Therefore, both -4°C and -6°C were selected as optimum temperatures for testing as 60% of plants provided regrowth at -4°C and 20% of plants provided regrowth at -6°C.

Remaining pots were relocated into three separate growth chambers and simultaneously subjected to a cold acclimation period. Growing conditions were reduced to 13°C with a photoperiod of 12 h for one week. This period was followed by a temperature reduction to 3°C and photoperiod reduction of 10 h for another week. The control growth chamber was maintained at 3°C. Growth chambers were then lowered at 1°C h^{-1} to target temperature (-4°C and -6°C) and maintained for 3 h. Temperatures were then raised back to 3°C at 2°C h^{-1}. After three hours at 3°C, pots were moved into the greenhouse. Plants recovered for four weeks during a re-growth period under natural light, at 25°C ± 5°. Plants were then mown at 5.5 cm, clippings collected, dried for 48 hours at 60°C, and weighed (g). Regrowth weight was calculated as a percentage to control (cold acclimation only) plant's growth weights.

Pots were labeled 'A' through 'H' with 'Raleigh' designated as 'B', and 'Palmetto' designated as 'D'. All samples were grown in 10 cm diameter pots, allowed to establish under optimum growing conditions in the growth chamber, then underwent the two-step acclimation process before freezing. Pots were rotated within the three growth chambers every week to avoid localized environmental effects. Mean clipping weights were calculated at each temperature (0°C, -4°C, -6°C), then divided by mean clipping weights at 0°C to calculate a percent regrowth. Percent regrowth's were then compared to 'Raleigh's percent regrowth to determine improved cold tolerance versus the industry standard in this region.

Experimental design was a completely randomized design (CRD) including three temperature regime treatments: cold acclimation, cold acclimation followed by a freezing period of -4°C, and cold acclimation followed by a freezing period of -6°C and six replications of each grass sample were used. Calculations were performed using the NLMIXED Procedure in SAS version 9.3 to compare regrowth percentages (SAS Institute, Inc., Cary, NC). Regrowth percentages of experimental grasses were compared to industry standard 'Raleigh' to determine statistically similar or significant differences

(P<0.05). Study one was conducted from 2 December 2013 thru 17 January 2014, with study two from 31 January 2014 thru 17 March 2014.

Results and Discussion

Significant interactions occurred between the two studies; therefore, data are presented separately. In study one (Fig. 1), at -4°C, 'Raleigh' regrew 90% compared to its clipping weight at 0°C, while six grass samples had statistically similar regrowth compared to 'Raleigh' including: 'C', 'Palmetto', 'E', 'F', 'G' and 'H'. Sample 'A' had a significant (67%) increase in regrowth compared to 'Raleigh', at -4°C. 'A' regrew 156% compared to its clipping weight at 0°C.

In study one (Fig. 2), at -6°C, 'Raleigh' regrew 38% compared to its clipping weight at 0°C and all grass samples showed statistically similar regrowth compared to 'Raleigh'. Although statistically similar, sample 'A' regrew 43% compared to its clipping weight at 0°C, which was the only sample with numerically greater regrowth (5%), compared to 'Raleigh'.

In study two (Fig. 3), at -4°C, 'Raleigh' regrew 70% compared to its clipping weight at 0°C and all grass samples showed statistically similar regrowth compared to 'Raleigh'. Although statistically non-significant (P<0.05), grass 'H' did have numerically greater regrowth compared to 'Raleigh' with a p-value of 0.069. This is worth noting due to its significant regrowth compared to 'Raleigh' at -6°C (Fig. 4), during study two. In contrast to study one, at -4°C, Sample ‘A’ had similar regrowth compared to ‘Raleigh’.

In study two (Fig. 4), at -6°C, ‘Raleigh’ again regrew 38% compared to its clipping weight at 0°C. Five grass samples had statistically similar regrowth compared to ‘Raleigh’ including: ‘A’, ‘C’, ‘Palmetto’, ‘E’, and ‘F’. Two samples, ‘G’ and ‘H’, had significant increased regrowth compared to ‘Raleigh’ of 56% and 87%, respectively. Although ‘Palmetto’ regrew more than ‘G’ numerically (59%), its large standard error reveals statistically similar regrowth compared to ‘Raleigh’ unlike sample ‘G’, with a smaller standard error.

North Carolina is the northern edge of St. Augustinegrass distribution range (Li et al., 2010). ‘Raleigh’, a release from North Carolina State University, is considered the most cold-tolerant cultivar currently available (Busey et al., 1982). However, the use of ‘Raleigh’ is limited to areas that rarely experience temperatures lower than -5°C (Li et al., 2010).

Our effort was to determine if plant material collected from USDA Hardiness Zone 7 provided improved cold tolerance. In one of two studies, three samples, ‘A’, ‘G’, ‘H’ provided statistically greater regrowth rates compared to current industry standard ‘Raleigh’ with similar regrowth rates in another study.

Various methods have been developed to predict, or correlate to, freezing tolerance in St. Augustinegrass, which include electrolyte leakage technique (Maier et al. 1994b) and differential thermal analysis (Philley et al. 1995). In our experiments, we measured clipping weights and divided by clipping weights at 0°C to calculate a percent regrowth at 4 weeks after freezing. Cold-acclimation has been shown to be a crucial prerequisite for plants to survive freezing temperatures in nature as well as in laboratory tests (Li et al., 2010). However natural acclimation is impossible to duplicate because acclimating conditions vary from year to year. Li et al., (2010) suggested the two-step acclimation protocol closely assimilates the natural acclimation period. Incorporating this cold acclimation protocol with plugs grow in 10 cm pots, opposed to individual internodes, provided the most comparable methods to natural freezing by using a controlled environment.

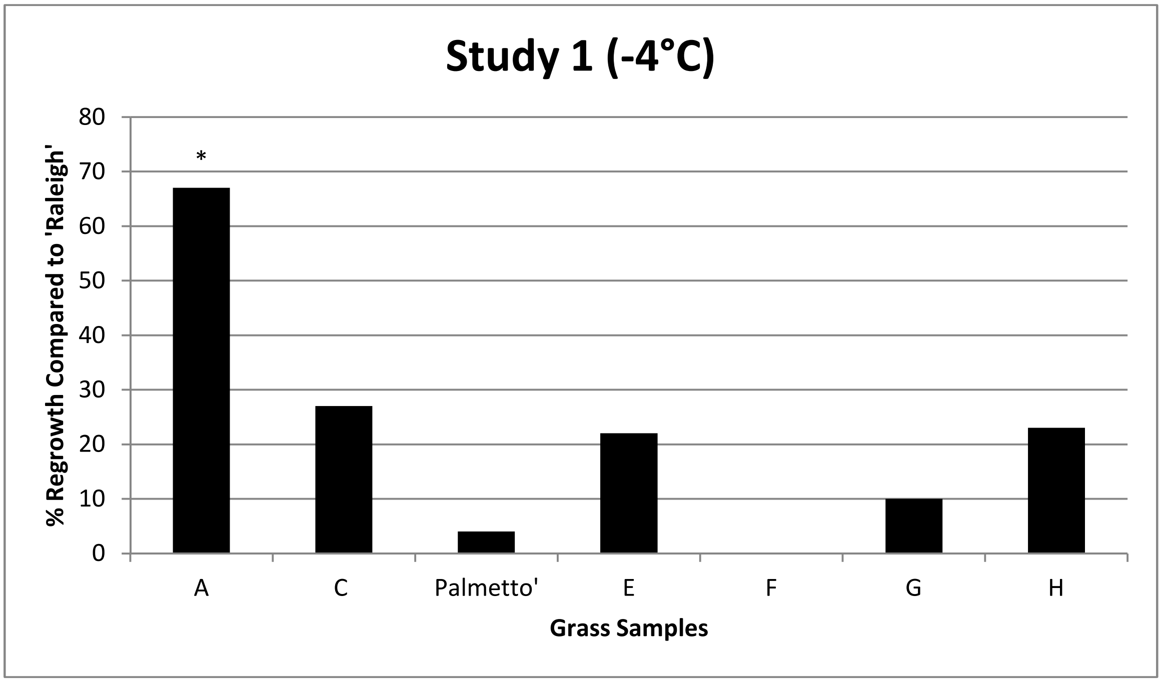


Fig. 4.1. Study 1 regrowth percentage of seven germplasm samples frozen at -4°C for 3 h after two-step cold acclimation under controlled growth chamber conditions. Columns represent mean percent regrowth subtracted from mean percent regrowth of 'Raleigh' of six replicates. Grass samples containing (*) were significantly different from 'Raleigh' according to the NLMIXED Procedure in SAS (P=0.05).

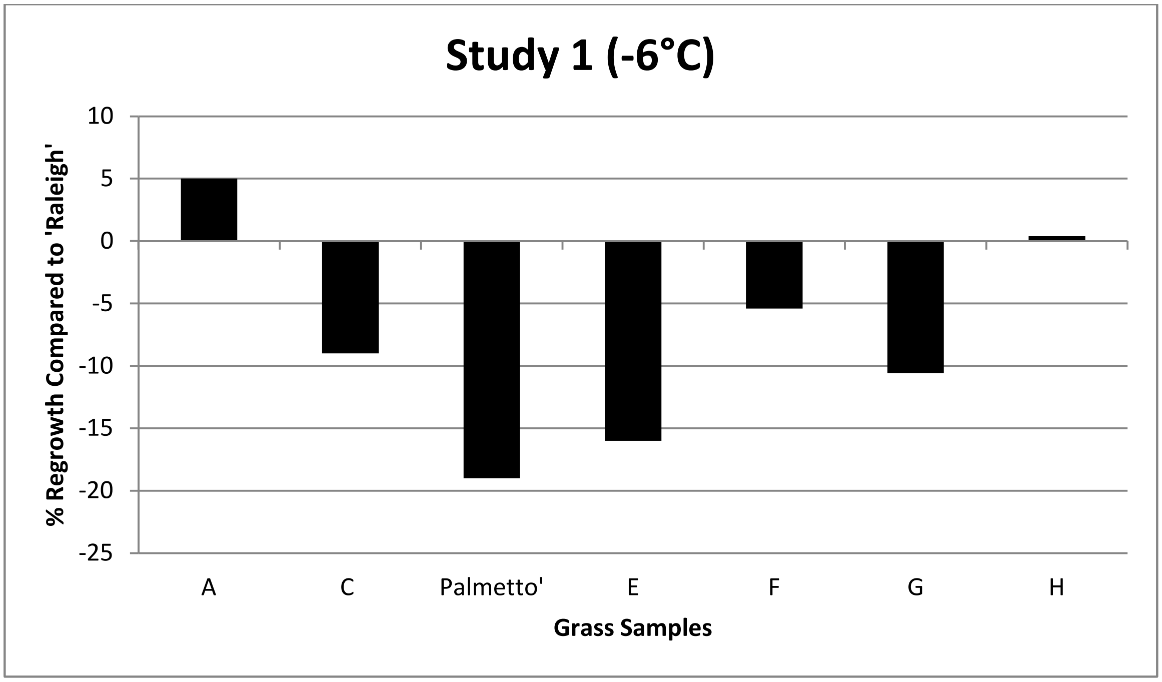


Fig. 4.2. Study 1 regrowth percentage of seven germplasm samples frozen at -6°C for 3 h after two-step cold acclimation under controlled growth chamber conditions. Columns represent mean percent regrowth subtracted from mean percent regrowth of ‘Raleigh’ of six replicates. Grass samples containing (*) were significantly different from ‘Raleigh’ according to the NLMIXED Procedure in SAS (P=0.05).

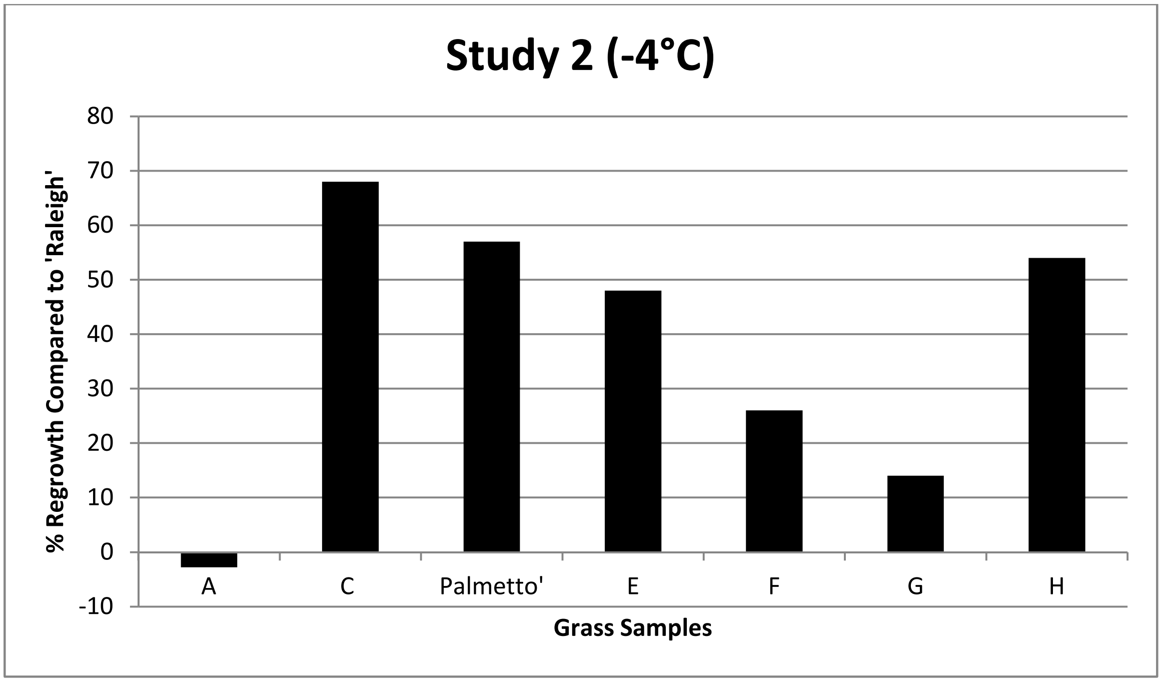


Fig. 4.3. Study 2 regrowth percentage of seven germplasm samples frozen at -4°C for 3 h after two-step cold acclimation under controlled growth chamber conditions. Columns represent mean percent regrowth subtracted from mean percent regrowth of ‘Raleigh’ of six replicates. Grass samples containing (*) were significantly different from ‘Raleigh’ according to the NLMIXED Procedure in SAS (P=0.05).

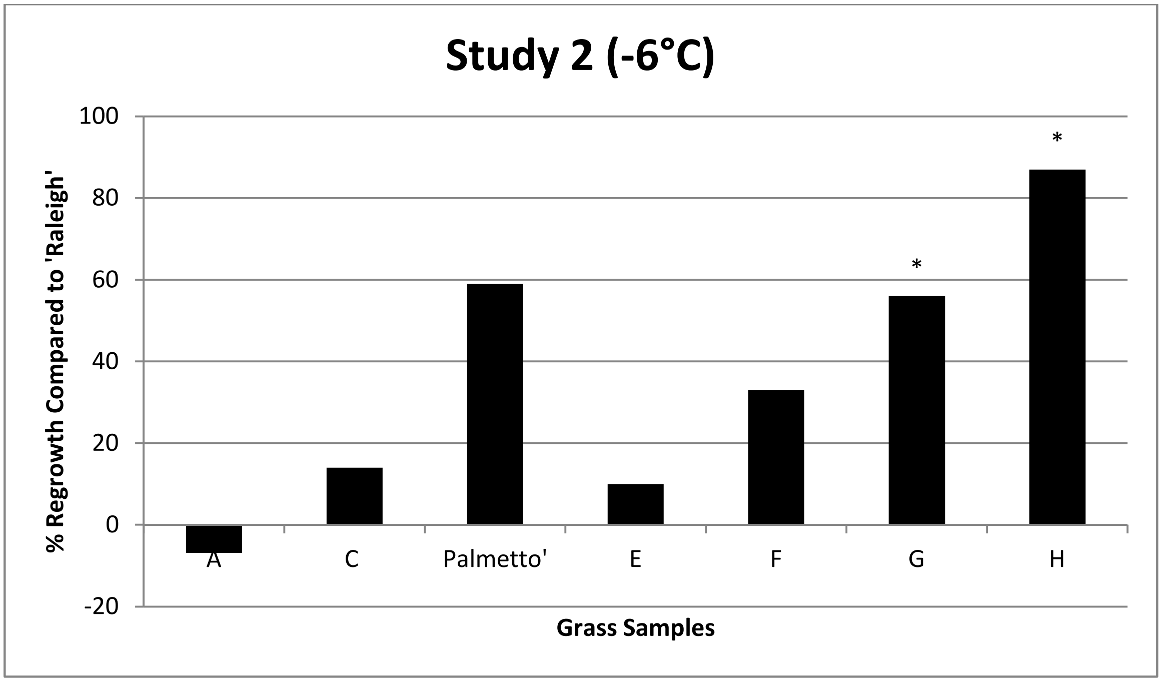


Fig. 4.4. Study 1 regrowth percentage of seven germplasm samples frozen at -6°C for 3 h after two-step cold acclimation under controlled growth chamber conditions. Columns represent mean percent regrowth subtracted from mean percent regrowth of ‘Raleigh’ of six replicates. Grass samples containing (*) were significantly different from ‘Raleigh’ according to the NLMIXED Procedure in SAS (P=0.05).

Conclusions

These experimental grass samples appear to have similar or improved cold tolerance, especially grasses ‘A’, ‘G’, and ‘H’, compared to the industry standard ‘Raleigh’. Field studies are needed to validate greenhouse growth chamber studies to help further evaluate cold tolerance of experimental and commercial lines.

REFERENCES

Beard, J.B. 1970. Turfgrass shade adaption. p. 273-283. In: R.R. Davis (ed.) Proc. First Int. Turfgrass Res. Conf., Harrogatte, England. 1969. Sports Turf Res. Inst., Bingley, England.

Beard, J.B. S.M. Batten, and G.M. Pittman. 1980. St. Augustinegrass cultivar characterization. Texas Turf Res. p. 44-47. Turf Research, Texas.

Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. 28:355-377.

Busey, P. 2003. St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. p. 309-390. In: Casler. M.D. and Duncan. RR (eds) Biology, Breeding, and Genetics of Turfgrasses, John Wiley & Sons, Inc, Hoboken, NJ.

Emmons, Robert D. 2000.Turfgrass science and management. p. 45-46. 3rd ed. Albany, N.Y. Delmar, Thomson Learning.

Fisher, Daniel, and Forbes Staff. 2011. America's Fastest-Growing Cities. 25 June 2014. <http://www.forbes.com/2011/05/20/fastest-growing-cities.html>.

Fowler, D.B., A.E. Limin, A.J. Robertson, and L.V. Gusta. 1993. Breeding for low temperature tolerance in field crops. p. 357-362. In: D.R. Buxon et al. (ed.) International Crop Science I. CSSA, Madison, WI.

Fry, J.D., N.S. Lang, and R.G.P. Clifton. 1991. Freezing resistance and carbohydrate composition of 'Floratam' St. Augustinegrass. Hort. Sci. 26:1537-1539.

Li, R., R. Qu, A.H. Bruneau, and D.P. Livingston. 2010. Selection for freezing tolerance in St. Augustinegrass through somaclonal variation and germplasm evaluation. Plant Breed. 129(4):417-421.

Livingston, D.P., R. Premakumar, and S.P. Tallury. 2006. Carbohydrate partitioning between upper and lower regions of the crown in oat and rye during cold acclimation and freezing. Cryobiology. 52:200-208.

Maier, F.P., N.S. Lang, and J.D. Fry. 1994a. Freezing tolerance of three St. Augustinegrass cultivars as affected by stolon carbohydrate and water content. J. Am. Soc. Hort. Sci. 119(3):473-476.

Maier, F.P., N.S. Lang, and J.D. Fry. 1994b. Evaluation of an electrolyte leakage technique to predict St. Augustinegrass freezing tolerance. Hort. Sci. 29:316-318.

Marshall, H.G. 1982. Breeding for tolerance to heat and cold. p. 47-70. In: M.N. Christiansen and C.F. Lewis (ed.) Breeding plants for less favorable environments. John Wiley & Sons, New York.

McCarty, L.B. 2011a. Cultivars and Their Characteristics. p. 18-32. In: L.B., McCarty, ed. Best Golf Course Management Practices. 3rd ed. Upper Saddle River, NJ: Prentice Hall.

McCarty, L.B. 2011b. Turfgrass Physiology and Environmental Stresses. p. 51-81. In: L.B., McCarty, ed. Best Golf Course Management Practices. 3rd ed. Upper Saddle River, NJ: Prentice Hall.

Murdoch, C.L., A.E. Dudeck, and C.L. Guy. 1990. Development of a technique for screening St. Augustinegrass for cold tolerance. Turfgrass Res. Fla. p. 53-62.

Philley, H.W., C.E. Jr. Watson, J.V. Krans, J.M. Jr. Goatley, V.L. Maddox, and M. Tomaso-Peterson. 1998. Inheritance of cold tolerance in St. Augustinegrass. Crop Sci. 38(2):451-454.

Philley, H.W., C.E. Jr Watson, J.V. Krans, J.M. Jr Goatley, and F.B. Matta, 1995: Differential thermal analysis of St. Augustinegrass. Hort. Sci. 30:1388-1389.

Reeves, S.A., Jr. and G.G. McBee, 1972. Nutritional influences on the cold hardiness of St. Augustinegrass. Agron. J. 64:447-450.

Tcacenco, F.A., C. F. Eagles, and B. F. Tyler, 1989: Evaluation of winter hardiness in Romanian introductions of *Lolium perenne*. J. Agric. Sci. (Camb.) 112:249-255.

Thomashow, M. F., 1999: Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:571-599.

Xin, Z., and J. Browse, 2000: Cold comfort farm: the acclimation of plants to freezing temperatures. Plant Cell Environ. 23:893-902.