

# Comparing Water Requirements of Diploid and Interploid St. Augustinegrass

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## Abstract

Compared to diploid St. Augustinegrasses (*Stenotaphrum secundatum* Walt. Kuntze) polyploids are more resistant to biotic stresses and drought (Busey, 1996). The development of interploid hybrids between diploid and polyploid St. Augustinegrass using embryo rescue offers to improve drought tolerance while maintaining visual quality. This study is aimed to compare the water requirements of seven diploid and nineteen interploid hybrids to four commercial checks, namely, Palmetto and Raleigh (diploids), TamStar (interploid), and Floratam (aneuploid) under short-term drought conditions. The study was planted as a randomized complete block with three replications on 22 Aug. 2014. Dry-down was initiated on 30 June 2015 under a rainout shelter for 86 days in year 1 and on 6 July 2016 for 91 days in year 2. Digital image analysis was used to determine average green cover (%) weekly beginning 16 July 2015 in year 1 and 13 July 2016 in year 2. Entries with green cover  $\leq 50 \pm 1\%$  were supplied with 2.54 cm of water. Quality was rated from 1 to 9 (1 = poor; 9 = excellent; 5 = minimum acceptable). The mean green cover and quality in year 1 were highest for interploids (77.4%, 5.2), followed by Floratam (65.7%, 4.4), and then diploids (64.3%, 4.1). Two diploids required water after 51 d of drydown. Two interploids and Floratam were watered at 72 d, and the remaining eighteen interploids, including TamStar, did not require water. In total, six diploids, including Palmetto and Raleigh, received between 5.08 cm and 12.7 cm of water, whereas Floratam and two interploids received 5.08 cm and 2.54 cm, respectively. Green cover (50%) was correlated to an average quality rating of 3.6 ( $R^2 = 0.81$ ), but to retain acceptable quality (5.0) the suggested percent green cover was 76.2%. Preliminary results suggest interploid St. Augustinegrasses have the potential to outperform Floratam and diploids for drought tolerance. Additionally, a higher threshold of at least 75% green cover may need to be considered for future drought studies in St. Augustinegrass. Results from year 2 are presented here.

## Experimental Design

- Twenty-six experimental DALSA St. Augustinegrass hybrids (7 diploids and 19 interploids) and four commercial checks, Floratam (aneuploid), Palmetto (diploid), Raleigh (diploid), and TamStar (interploid, Chandra et al., 2015) were arranged in a randomized complete block design with three replications.
- Plots were established on 1.5 x 1.5 m centers using six 10.2 x 10.2 cm plugs planted on 30.48 cm centers around the middle of each plot. Plots were planted on 22 Aug 2014 in Dallas, TX.
- The soil type was Austin silty clay composed of 45 % clay, 7% sand, and 48% silt with an available water holding capacity of 0.17 cm/cm and a 1 to 3 % slope (websoilsurvey.nrcs.usda.gov). The estimated available water supply was 4.5 cm to a soil depth of 25 cm.
- A rainout shelter (10.7 X 30.5 m) was erected in Jun 2015 and was covered with clear polypropylene (6mm) on 24 Jun 2015 and again on 28 Jun 2016. Photosynthetic active radiation (PAR) was reduced by 14% on clear days. All four sides were protected with manual roll-down curtains that were closed in anticipation of rainfall.
- Plots were irrigated 3x/wk for 1 h at each event before initiating dry-down on 30 Jun 2015 and 6 Jul 2016; the following day was recorded as the first day of each trial period.
- Plots were mowed at a height of 8.9 cm once every 2 wks during the trial period. Fertilizer (urea 46-0-0) was supplied at a rate of 0.8 lb N/1,000 ft<sup>2</sup> prior to starting and upon the conclusion of each trial year. Irrigation resumed upon each trial end date to be delivered 3x/wk for 1 h until the end of the growing season.
- Environmental conditions (PAR and RH%) were recorded hourly with loggers placed in the center of the field at a height of 8.9 cm. An on-site weather station was referenced for ambient temperature.

## Data Collection & Analysis

- Data was collected weekly between 1145 and 1300 h from 16 Jul through 24 Sept 2015 (86 d) and from 13 Jul through 5 Oct 2016 (91 d).
- Turfgrass quality was visually rated on a 1-9 scale (1 = brown/dead, 9 = excellent, 5 = minimum acceptable).
- Green Cover (%) was determined from digital image analysis (Richardson et al., 2001). Images were captured at a height of 56 cm using a light box (Turfgrass Water Conservation Alliance, www.twca.org) and batch analyzed (Karcher and Richardson, 2005) using SigmaScan Pro (v. 5.0, SPSS, Inc., Chicago, IL 60611). Hue settings were 45 to 120 with saturation settings at 0 to 100.
- Genotypes that had a average green cover  $\leq 50.0 \pm 1.0\%$  were hand watered using a construction grade watering hose fitted with a digital water meter (model 03N31GM, Great Plains Industries, Inc., Wichita, KS) and nozzle that was calibrated daily to deliver 2.54 cm (37.85 L) of water. Water was uniformly delivered within a 1.2 m x 1.2 m x 61 cm frame built from PVC, and an impermeable 6mm plastic barrier on four sides.
- Canopy temperature was collected from three random spots of each plot where green cover was present using an infrared thermometer (model 42510, Spectrum technologies) at a height of 20.3cm.
- Mean weekly performance by genotype and ploidy group (diploid, interploids, and Floratam) was compared by analysis of variance (ANOVA) in JMP (v.10, SAS Inc., Cary, NC) using the factors genotype (or ploidy), year, and genotype (or ploidy) x year; if the interaction was not significant ( $P \geq 0.05$ ), means were pooled across years and separated using Fisher's protected LSD ( $P \leq 0.05$ ). Interploids and Floratam are polyploids, but because Floratam is the commercial standard for drought tolerance, it was compared separately in the analyses.
- Linear and nonlinear (sigmoid) analyses were performed by ploidy to identify the best fit model (lowest error sum of squares, lowest AIC, and the closest parameter estimates to actual data). Predicted values from the chosen model were reanalyzed by ANOVA as a full model (ploidy, year, and ploidy x year).
- Weekly means for each ploidy group were calculated for green cover and quality for linear regression.

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## Acknowledgments

- Turfgrass Water Conservation Alliance – Light box donation
- Texas A&M AgriLife Research and Turfgrass Producers of Texas– Project funding
- Data collection support from Dr. Xiuli Shen and Justin Eads

## Genotype Comparison

Small differences in environmental conditions between years were captured by on-site weather stations (Table 1). Canopy temperature was collected starting 7/20/15 and 7/13/16 until the end of each trial year. On average, relative humidity was 3.0% higher and noon ambient temperature was 2.9°F warmer in 2016 than 2015. Noon-time differences in mean canopy and ambient temperatures were 14°F (2015) and 16.1°F (2016) indicating that canopy temperatures are generally much warmer than ambient temperatures, possibly due to stomata closure. Decline in overall green cover was evident throughout drydown (Fig. 1). Genotypic differences for green cover, total applied water, and turfgrass quality are presented in Table 2. Genotype x year interaction was not significant ( $P \leq 0.05$ ) for cover or quality. Days to decline to 50% cover ranged from 50.0 to 88.5 while mean cover ranged from 59.0% to 81%. Mean total water applied ranged from 0.0 cm to 11.5 cm. Days to decline to  $\leq 5.0$  ranged from 15 to 71 d while mean quality ranged from 3.4 to 5.7. Eight interploidy genotypes including TamStar had higher than average green cover ( $> 72.1\%$ ) and quality ( $\geq 4.6$ ), and did not require water in either year. These genotypes were high performers and would be found in the upper left quadrant of Fig. 2. Digital images for five high performing interploids and commercial checks at 51 d are shown in Fig. 3.

Table 2. The mean number of days to reach the initial  $\leq 50\%$  green cover, overall mean green cover from DIA, the mean total applied water, the mean number of days to reach an initial quality rating  $\leq 5.0$ , and the overall mean visual quality are presented for each experimental hybrid and commercial checks.

No.	Genotype	Green Cover		Mean Total Water Applied (cm)	Turfgrass Quality	
		Day $\leq 50\%$	Mean (%)		Day $\leq 5.0$	Mean (1-9)
<b>Diploids</b>						
1	TAES 5485-62	64.0 cde	63.7 ghi	7.6 abc	22.0 fgh	4.2 ghijkl
2	DALSA 1315	71.0 bcd	65.5 fghi	5.1 bcd	18.5 gh	3.8 klmn
3	DALSA 1318	81.5 ab	73.1 abcdef	1.3 de	39.5 cdef	4.5 fghijk
4	DALSA 1319	78.0 abc	68.8 defgh	2.6 cde	25.5 efgh	3.9 ijklmn
5	DALSA 1320	57.0 de	61.2 hi	6.4 bc	18.5 gh	3.4 n
6	DALSA 1333	78.0 abc	72.0 bcdef	2.6 cde	36.0 cdefg	4.5 fghij
7	DALSA 1401	50.0 e	59.0 i	11.5 a	15.0 h	3.8 ijklmn
8	Palmetto	64.0 cde	62.7 hi	7.7 ab	18.5 gh	3.5 mn
9	Raleigh	64.0 cde	65.7 fghi	5.1 bcd	18.5 gh	3.7 lmn
<b>Polyloids</b>						
10	DALSA 1316	88.5 a	79.1 abc	0.0 e	32.5 defgh	4.6 defghij
11	DALSA 1317	88.5 a	73.9 abcde	0.0 e	29.0 defgh	4.2 ghijklm
12	DALSA 1321	88.5 a	74.1 abcde	0.0 e	46.5 bcd	4.8 cdefg
13	DALSA 1322	78.0 abc	73.2 abcdef	2.5 cde	39.5 cdef	4.7 cdefgh
14	DALSA 1323	88.5 a	74.3 abcde	0.0 e	71.0 a	5.7 ab
15	DALSA 1324	88.5 a	79.3 abcde	0.0 e	43.0 abc	5.2 abcd
16	DALSA 1325	88.5 a	81.0 a	0.0 e	53.5 abc	5.4 abc
17	DALSA 1326	74.5 abc	73.5 abcdef	2.6 cde	32.5 defgh	4.5 efghi
18	DALSA 1327	88.5 a	74.8 abcde	1.3 de	64.0 ab	5.4 abc
19	DALSA 1328	88.5 a	76.1 abcde	1.3 de	29.0 defgh	4.1 hijklmn
20	DALSA 1329	81.5 ab	72.8 bcdef	1.3 de	39.5 cdef	4.7 cdefgh
21	DALSA 1330	88.5 a	77.3 abc	0.0 e	39.5 cdef	4.8 cdefg
22	DALSA 1331	88.5 a	79.3 ab	0.0 e	46.5 bcd	5.2 abcdef
23	DALSA 1332	85.0 ab	75.6 abcde	1.3 de	64.0 ab	5.7 a
24	DALSA 1402	71.0 bcd	71.9 bcdef	3.8 bcde	32.5 defgh	4.6 defgh
25	DALSA 1403	81.5 ab	68.7 defgh	2.5 cde	39.5 cdef	5.0 bcdef
26	DALSA 1404	88.5 a	71.1 cdefg	1.3 de	46.5 bcd	5.2 abcdef
27	DALSA 1405	81.5 ab	76.0 abcde	1.3 de	46.5 bcd	5.0 abcdef
28	DALSA 1406	81.5 ab	75.3 abcde	1.3 de	46.5 bcd	5.2 abcde
29	TamStar	88.5 a	79.2 ab	0.0 e	36.0 cdefg	4.6 defgh
30	Floratam	81.5 ab	66.9 efghi	3.8 bcde	32.5 defgh	4.5 fghij

† No. corresponds to Fig. 2 scatterplot.  
‡ Polyloids including interploid hybrids and Floratam (aneuploid).

## Ploidy Comparison

Logistic four-parameter sigmoid curves best fit the actual data to determine ploidy differences for green cover and turfgrass quality. Predicted green cover for the ploidy x year interaction was significantly different ( $P \leq 0.01$ ), so ploidy groups are presented separately by year (Fig. 4). Within each year, mean green cover x ploidy was significantly different ( $P \leq 0.0001$ ). Initial green cover was lower in 2015 following a harsh winter. Mean green cover for interploids (76.2%) was significantly higher than Floratam (64.3%) and diploids (63%). In 2016, initial green cover was higher than in 2015, and interploids and diploids showed a sigmoidal rate of loss whereas Floratam was almost linear. Green cover for interploids (74.6%) was not significantly different from Floratam (69.0%), but was significantly different from diploids (68.1%). Floratam and diploids were not significantly different. In both years, it was predicted that interploids would take longer to reach the 50% threshold, suggesting lesser requirements for irrigation than either diploids or Floratam. This can be partially explained by the fact that interploids have higher initial green cover. The ploidy x year interaction for predicted quality was not significantly different ( $P \leq 0.05$ ). Mean quality for each ploidy group were significantly different ( $P \leq 0.0001$ ). Interploids had the highest mean quality of 4.9 followed by Floratam (4.5) and diploids (3.9) (Table 2). Diploids were predicted to meet 5.0 earliest after 21 d, followed by Floratam at 30 d, and interploids at 42 d (Fig. 5). Regression of weekly green cover and quality means for each ploidy revealed that green cover was significant ( $P \leq 0.0001$ ), and highly and positively correlated to visual quality ratings (Fig. 6). The overall correlation ( $R^2 = 0.80$ ) (not shown) was also significant ( $P \leq 0.0001$ ). Mean green cover for diploids (71.7%) and interploids (70.3%) were significantly different ( $P \leq 0.001$ ) from Floratam (66.3%). It seems that diploids require a higher green cover of 77.9% to meet acceptable quality compared to Floratam (73.2%) and interploids (75.9%). Using the overall regression slope equation ( $y = 10.867x + 20.941$ ), it was calculated that a higher threshold of 75% green cover would be needed to maintain acceptable quality. We have interpreted these results to suggest that 50% green cover does not adequately represent the minimum acceptable quality when plants are under water and heat stress and that 75% should be used for future St. Augustinegrass drought studies when screening for tolerant germplasm. This study further suggests that interploids may provide greater green cover and quality with reduced inputs during water stressed periods as compared to diploids and Floratam.

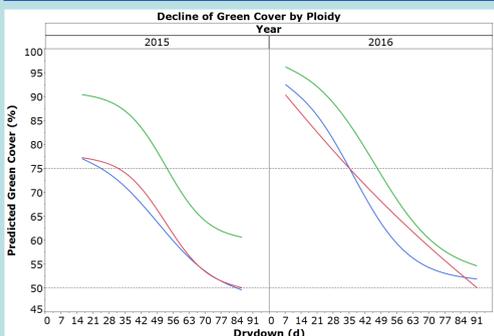


Fig. 4. Decline of green cover (%) by ploidy during each drydown period in 2015 (86 d) and 2016 (91 d).

Ploidy legend: Diploid (blue), Interploid (green), Floratam (red)

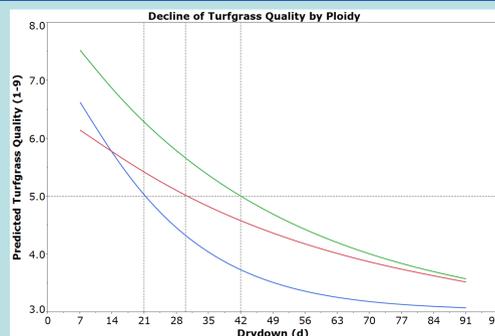


Fig. 5. The dotted line along the y-axis represents the minimum threshold for quality (5.0), and those along the x-axis correspond to 21 d, 30 d, and 42 d where each ploidy cross 5.0.

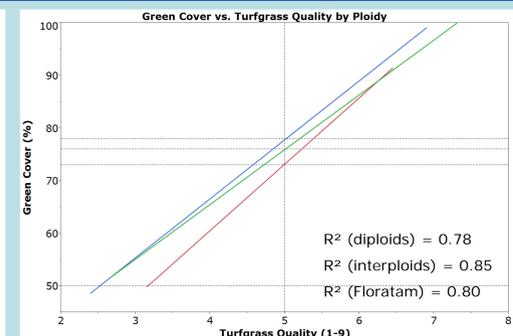


Fig. 6. Regression of weekly mean green cover (%) and quality (1-9) values for each ploidy group. Dotted lines were drawn at 50% green cover and where each ploidy crossed 5.0 at 73.2% (Floratam), 75.9% (interploids), and 77.9% (diploids).

Table 1. Environmental conditions (relative humidity (%) and ambient temperature (°F)) for each trial year at noon compared to mean canopy temperatures collected weekly at noon during drydown.

Date	2015										Mean	Stdev	
	7/20	7/23	7/30	8/6	8/13	8/20	8/27	9/3	9/10	9/17			9/24
Relative Humidity (%)	39.3	41.0	31.1	36.5	32.7	55.2	37.0	40.0	44.8	49.7	28.1	39.6	8.0
Ambient Temperature (°F)	88.8	89.1	95.2	88.6	86.8	69.6	83.0	83.6	81.4	82.2	86.1	84.9	6.5
Mean Canopy Temperature	91.1	104.2	110.7	95.4	95.3	76.1	97.2	102.2	106.2	101.4	109.0	99.0	9.7
Canopy - Ambient	2.3	15.1	15.5	6.8	8.5	6.5	14.2	18.6	24.8	19.2	22.9	14.0	7.2

Date	2016										Mean	Stdev		
	7/13	7/20	7/27	8/3	8/11	8/17	8/24	8/31	9/7	9/14			9/21	9/28
Relative Humidity (%)	39.3	35.9	48.7	36.6	37.8	46.4	40.0	44.0	48.6	43.2	38.9	43.8	50.5	4.9
Ambient Temperature (°F)	87.5	88.5	89.6	91.9	92.9	78.0	83.8	88.3	86.1	86.0	85.2	76.8	80.9	85.8
Mean Canopy Temperature	98.6	110.4	101.5	112.2	116.7	87.0	99.1	105.5	88.4	93.9	110.8	104.9	95.5	101.9
Canopy - Ambient	11.1	21.9	11.9	20.3	23.8	9.0	15.3	17.2	2.3	7.9	25.6	28.0	14.6	16.1



Fig. 1. Trial pictures taken in 2016 at 17 d, 28 d, and 84 d of drydown.

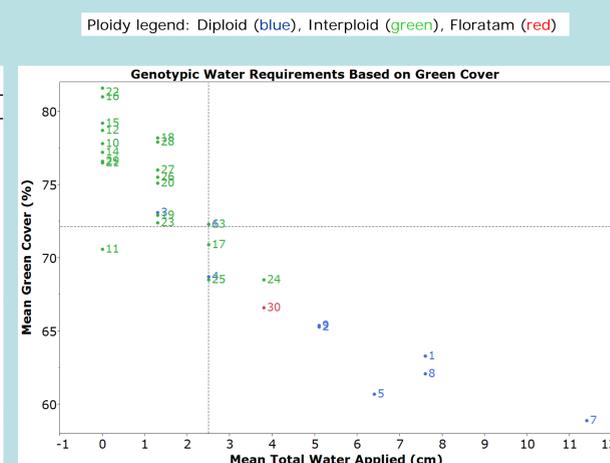


Fig. 2. Mean genotypic water requirements for each genotype based on green cover. Genotypes correspond to their respective number in Table 1 and by ploidy. Dotted lines represent an overall mean green cover of 72.1% and mean total water applied of 2.5 cm. Genotypes should be selected that appear in the upper left quadrant with above average green cover and below average irrigation needed.

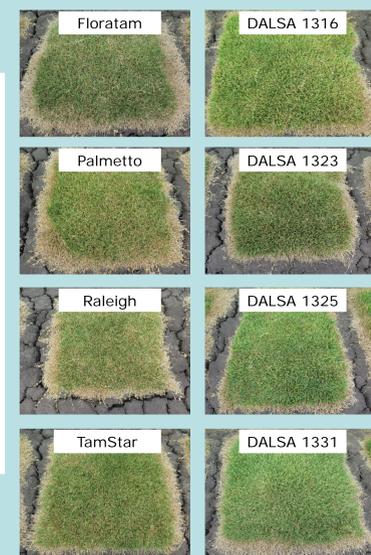


Fig. 3. Commercial checks and five high performing interploidy hybrids at 51 d of drydown.