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The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 400 projects at a cost of \$31 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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# Selecting for Shade-Resistant Seeded Bermudagrass Cultivars

Greg Bell, Yanqi Wu, and Kyungjoon Koh

## SUMMARY

Researchers at Oklahoma State University are screening common bermudagrass selections to determine their capacity for shade resistance. Bermudagrass is aggressive, adapted to most soil conditions, has relatively good salt and drought tolerance, and is resistant or tolerant to most disease and insect pests. However, bermudagrass does not tolerate shade nearly as well as most grasses. Along tree-lined fairways and shaded golf course tees, bermudagrass is commonly replaced with less desirable species. The discovery or development of shade-resistant bermudagrasses would make an important contribution to the golf course and other turfgrass industries. The objectives of this study are to screen bermudagrass selections for their effectiveness in shaded environments and to determine turfgrass characteristics that may be useful for screening future selections for potential shade tolerance. Progress to date includes:

- A wide range of shade resistance and turf quality characteristics exists among the selections but three of the standards, 'TifGrand', 'Patriot', and 'Celebration' tend to perform better in terms of turf quality than all but a few of the selections.
- A single cross of two of the best performing selections was attempted in 2009 but was unsuccessful because of poorly matched physical characteristics (vigorous vs. non-vigorous growth).
- All but nine of the 45 selections maintained acceptable visual quality (greater than 6 on a 1-9 scale) throughout the 2010 growing season in sun, but none of the selections and only one standard ('Patriot') maintained acceptable visual quality throughout the season in shade.

**B**ermudagrass (*Cynodon* spp.) is the most widely used warm-season grass on golf courses and other turfgrass areas in the southern United States. Bermudagrass is aggressive, providing excellent recuperative ability and sod-forming characteristics. It is adapted to most soil conditions, has relatively good salt and drought tolerance, and is resistant or tolerant to most disease and insect pests. However, bermudagrass does not tolerate shade nearly as well as most grasses.

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Along tree-lined fairways and shaded golf course tees, bermudagrass is commonly replaced with less desirable species. The discovery or development of shade-resistant bermudagrasses would make an important contribution to the golf course and other turfgrass culture.

Bermudagrass is extremely variable (17). Harlan and de Wet (10) indicated that the morphological variation of bermudagrass is enormous, ranging from very small, fine turfgrass to large, leafy robust types. More recently, we reported that a large genetic variability existed in a Chinese bermudagrass collection of more than 120 original accessions for adaptive, morphological, and fertility traits (20, 21). Magnitudes of variances for environment and genotype by environment interactions in the collection are large, as well. Molecular markers and ploidy information further indicate substantial genotypic variation within the germplasm pool (20, 21, 22). A worldwide bermudagrass collection has been amassed, and is in place for use at the OSU turfgrass breeding program. We believe similar or substantial genetic variation for shade tolerance in bermudagrass exists in the collection.

A reduction in solar irradiance caused by shade is usually combined with other environmental stresses such as airflow restriction and tree root competition to reduce turfgrass quality in shade. Shade alters several physiological and morphological characteristics of plants. Low irradiance results in increased stem elongation, longer leaf sheaths, higher chlorophyll content, and higher leaf succulence (7). Plant growth is more vertical in shade because of the inactivation of phytochrome influenced by far-red irradiance resulting in increased gibberellic acid (16). Low radiant flux increased stem elongation, lengthened leaf sheaths, and reduced tillering in 'Diamond' zoysiagrass (*Zoysia matrella* (L.) Merr.) (14), but under certain conditions, moderate shade may increase tillering in some tropical grasses (11) and shoot growth in some forage grasses (8).





Research site at mid-morning (above) and in late afternoon (below) in 2007 prior to construction. The site meets the most important parameters for effective shade research. Vegetative shade is provided for several hours by the conifers on the west side of the plots. These conifers also provide root competition and reduce the predominately westerly airflow.





Light quality also affects grasses in shade. Bell et al. (3) determined that the quality of light in shade varies with the type of shade present, and vegetative shade is significantly different from neutral shade such as the shade found next to a building or under black shade cloth. The use of shade cloth and other neutral shade sources for studies of plant response to shade is a common practice and is a valuable source of information.

Radiance filtered by shade cloth, however, is not consistent with radiance filtered by plants. The ratio of red wavelengths to near infrared wavelengths differs in vegetative shade compared to shade produced by a neutral source. Therefore, the most effective shade studies are probably those conducted under vegetative shade. Bell and Danneberger (2) reported that the duration of shade was more detrimental to creeping bentgrass health than either the density or the temporal period of shade. Consequently, a means of varying the period that plants are exposed to shade to match the species or to select the most shade resistant germplasm would be beneficial to a shade research study.

Koh et al. (12) demonstrated that airflow restriction was equally detrimental to creeping bentgrass growth and development as light reduction. Trees, smaller plants, and structures provid-

ing shade also reduce air circulation and increase relative humidity causing leaf surfaces to remain wet for many hours. These wet leaf surfaces combined with reduced evapotranspiration create a microclimate conducive to disease development. Disease development, however, may be less of a detrimental factor in shade than gas exchange. As photosynthesis occurs, the air around the turf canopy becomes relatively high in oxygen and low in carbon dioxide. An oxygen-rich, carbon dioxide-deficient atmosphere discourages photosynthesis.

With sufficient airflow, the carbon dioxide-deficient air is replaced with fresh air and carbon dioxide deficiency does not limit photosynthesis. Airflow restriction represents another factor that helps to create a realistic shade environment for field research. A third component, tree root competition, may also be an important factor affecting the survival of turfgrasses in vegetatively shaded environments.

Physiological turfgrass features such as pigment concentrations (13, 19) and carbohydrate reserve (6, 18) may be affected by shade stress. A reduction in the ratio of chlorophyll a to chlorophyll b has been used to indicate shade stress in many plants (4). Another indicator is the conversion of violaxanthin to zeaxanthin through anther-



The shade research site (facing north) in 2010, three years after planting. The site includes red maple trees on the south, eastern redbud trees on the east, and two 10 ft-wide strips of shade cloth overhead providing at least 60% shade for 89% of the day.

axanthin that is a potential measure of shade stress in turf (2). However, these factors measure shade stress rather than shade adaptation. It may be possible to identify factors that confer shade adaptation and enable breeders to select potentially shade tolerant plants from grasses growing in full sun.

For instance, in some plants, dwarfism is an indication of poor shade tolerance. However, studies have demonstrated that artificially induced dwarfism of otherwise normal plants through the application of plant growth regulators improves turfgrass performance in shade (9). Therefore morphological features such as internode length and shoot growth rate may be helpful for selecting potential shade resistant germplasm.

High carbohydrate reserve is a phrase often used to describe healthy turfgrass plants yet many researchers have unsuccessfully attempted to measure plant health by determining total non-structural carbohydrate levels. This is presumably because healthy plants are actively growing during the appropriate seasons and do not store large amounts of carbohydrates but use most excess carbohydrates for continuous growth and development. Therefore, a cultivar that has high shoot density may also have high photosynthetic efficiency and may be capable of superior performance in shade. The light compensation point is an obvious measure of potential shade tolerance and the light saturation point and/or other measures of photosynthesis may also be appropriate selection criteria.

Golf course managers consistently meet resistance from players when suggesting that trees be removed or canopies be thinned to improve air circulation and allow light to reach playing surfaces. Trees and shrubbery in the landscape are important sources of aesthetic beauty, and in many cases, contribute to the playability of a golf course. Improving the shade tolerance of bermudagrass cultivars could provide adequate cover of a highly desired turfgrass species and limit the need for tree removal.

The objectives of this study are to (1) screen bermudagrass germplasm collections and selections for their effectiveness in shaded envi-

ronments, (2) determine turfgrass characteristics that may be useful for screening future selections for potential shade tolerance, and (3) create one or two genetic populations by physiological and molecular selections of shade-tolerant and susceptible parents for future research.

The long-term goals of this work include using molecular markers to map major genes or genomic regions for shade tolerance with the mapping populations made in earlier investigations to develop cultivars with shade tolerance superior to currently used commercial cultivars.

## **Materials and Methods**

A research site has been specifically constructed to host this and future shade selection projects. The site receives mid to late afternoon shade, depending on season, from a dense, mature evergreen canopy on the west side of the site. Shade during this afternoon period has been suggested as the most detrimental for growth and development of bermudagrass (5).

In addition to the evergreens, we have planted deciduous shade trees along the southern borders of the shade research site to provide a longer shade period. However, over the next several years, as these deciduous shade trees mature, we have a unique opportunity to design neutral shade canopies that limit photosynthetic efficiency for whatever period we desire. Consequently, we can vary the length of the shade period during the study, if necessary, to increase the shade stress or to limit the loss of potential selections.

We currently have more than 600 turf bermudagrass selections including unique accessions obtained from China, African countries, Australia, and other nations. We chose 45 of these selections primarily from China as a starting point for the development of shade-tolerant bermudagrasses. Our primary focus for this project is to select the most shade-tolerant bermudagrasses from this germplasm base and use this knowledge for concurrent and future breeding. We will be using both traditional and molecular breeding techniques to develop shade-tolerant bermuda-



Planting of shade plots on June 22, 2007 (left) and planting of sun plots (right). The shade plots are located 50 feet to the west of the full-sun plots.

grass varieties and will extend our efforts as additional funding sources are identified and funding obtained.

Both qualitative and quantitative measures are used to identify the best selections. These measures include traditional visual evaluation to determine visual density and overall turf quality. In addition, normalized difference vegetation indices will be used to determine quantitative measures of cover + color = reflectance quality (1). Data are collected monthly to screen the selections for appropriate characteristics for use in fine turf.

We also hope to identify plant characteristics that will help to screen selections with potential shade adaptation. To do that, full-sun research sites will be utilized in close proximity to the shade sites. Selections tested in shade will also be tested in the full-sun locations in a similar experimental design. Our intentions are to plant in small plots with at least five replications to help improve statistical precision.

Potential plant indicators of shade tolerance will be measured in full sun and compared with quantitative measures of shade adaptation from the same germplasm in shade. Rate of photosynthesis based on the rate of CO<sub>2</sub> uptake was measured for each selection three times in 2010 using a LI-6400 photosynthesis system (LiCor, Lincoln, NE) and is being measured three times in

2011. The length of the third internode along a stolon from the parent plant was measured monthly during the growing season in 2010 and is being measured again in 2011. These plant parameters will be compared with the quantitative measure of shade adaptation (reflectance quality) to determine if they are significantly related to shade adaptation. Those plant parameters that influence shade adaptation will be used to determine a regression model that measures the potential of a bermudagrass selection for shade tolerance.

Once shade-tolerant and susceptible genotypes of bermudagrass have been identified from field screening and physiological experiments, those plants will be examined by AFLP and SSR markers to determine their genetic relatedness. Genotypes will be selected as parents to make mapping populations on the basis of their interactions to shade stress and molecular genetic distance. Hand-emasculation and hybridization methods of Richardson (15) will be followed to make the mapping populations in a future study.

The study consists of 45 bermudagrass selections and four standards, 'Celebration', 'Patriot', 'TifGrand', and 'Tifton 10'. Plot size is 24 in. x 24 in. (61 x 61cm) with 9 in. (23 cm) bare soil borders between plots. Each bermudagrass was replicated five times on the shade site and on an adjacent full-sun site. Visual turf quality (TQ) and NDVI were assessed every two weeks in 2008, 2009, and 2010 (Table 1).



Status	Bermudagrass	Shade		Sun		Shade/Sun Decline*
		VisualTQ	Visual Rank*	VisualTQ	Visual Rank	
		1-9=best	--- LSD ---	1-9=best	--- LSD ---	---- % ----
<b>2008</b> (mean decline in shade = -7.5%)						
Standard	Patriot	7.1	A	7.7	A	-8.4
Best	C19	6.8	B	7.2	BC	-6.4
Best	C13	6.7	BC	7.0	DEFGH	-4.2
Best	C17	6.6	BCD	7.0	CDEFG	-5.7
Best	C28	6.6	BCD	7.0	CDEF	-6.6
Best	C4	6.6	BCD	6.9	EFGHIJ	-4.2
Standard	Tifton10	6.5	BCDE	7.0	CDEF	-7.3
Standard	Celebration	6.5	CDEF	6.7	GHIJKLM	-3.6
Best	C24	6.5	CDEFG	6.8	EFGHIJKL	-5.2
Best	C27	6.4	CDEFGH	6.8	EFGHIJKL	-5.5
Best	C20	6.4	CDEFGHI	7.0	CDEFG	-8.3
Best	C30	6.4	CDEFGHI	6.5	MNOP	-0.7
<b>2009</b> (mean decline in shade = -12.0%)						
Standard	Patriot	7.8	A	8.7	A	-11.0
Standard	Celebration	7.1	B	7.5	BC	-5.3
Standard	Tifton4	7.0	B	7.8	B	-9.7
Best	C116	6.9	BC	7.4	BCD	-6.5
Best	C28	6.9	BC	7.4	BCD	-6.5
Best	C118	6.7	BCD	7.0	DEFGHIJ	-4.0
Tifton10	Tifton10	6.6	BCDE	7.0	EFGHIJK	-4.6
Best	C24	6.6	CDE	7.3	CDE	-10.4
Best	C35	6.5	CDEF	7.2	CDEFGH	-8.9
Best	C72	6.5	CDEF	7.0	DEFGHIJ	-6.9
Best	C23	6.5	CDEF	7.2	CDEFG	-9.4
Best	C34	6.5	CDEF	7.4	BCD	-11.9
Best	C79	6.5	CDEF	7.2	CDEFGH	-9
<b>2010</b> (mean decline in shade = -23.9%)						
Patriot	Patriot	6.0	A	7.6	A	-21
Tifton4	Tifton4	5.6	AB	7.3	BC	-23
Celebration	Celebration	5.6	ABC	7.0	CDEF	-19
Best	C116	5.6	ABC	6.8	BC	-20
Best	C34	5.2	BCD	6.7	CDEFGH	-23
Best	C28	5.1	CD	6.9	BCD	-26
Best	C118	5.1	DE	6.8	CDEF	-25
Tifton10	Tifton10	5.1	DE	6.4	GHIJKLM	-20
Best	C125	5.0	DEF	6.5	EFGHIJK	-23
Best	C72	5.0	DEFG	6.5	FGHIJK	-24
Best	C73	4.8	DEFGH	6.7	CDEFG	-29

**Table 1.** The best bermudagrass selections including four standards determined by visual quality means in shade collected every two weeks in 2008, 2009, and 2010.



Season	2010 Photosynthesis					
	Shade F1	LSD	Sun F2	LSD	Sun F4	LSD
	----- $\mu\text{mol}/\text{m}^2/\text{sec}$ -----					
Summer	31.9	A	30.0	A	37.1	A
Fall	23.6	B	25.8	B	28.2	B
Spring	20.2	C	23.2	C	24.2	C

**Table 2.** Photosynthesis based on CO<sub>2</sub> exchange rate differed significantly (P = 0.05) by season in 2010, summer > fall > spring on the shade site (F1), the sun site (F2), and a second sun site (F4).

### Results

In 2008, shade stress occurred on the shade site for 12% longer each day than on the sun site. This short duration of shade stress caused an average 7.5% decline in turfgrass quality (TQ) and a 5.2% decline in NDVI in 2008 (Table 1). On May 7, 2009, a black woven shade cloth with 75% light reduction (10 ft x 160 ft) was installed on a hoop structure over the shade site to provide longer and more uniform shade. Consequently, the shade duration increased from 12% in 2008 to 52% in 2009. The additional shade caused an

increased decline in TQ from 7.5% in 2008 to 12% in 2009 and a decline in NDVI of 5.2% in 2008 to 7.4% in 2009. A second significant decline in TQ was observed by adding additional 75% black woven shade cloth in 2010. TQ decline increased from 7.5% in 2009 to 38.9% in 2010 and NDVI decline also deepened from 7.5% to 26.8% in 2010. The bermudagrass selections differed significantly (P=0.05) in TQ and in NDVI both in full sun and in shade in 2008, 2009, and 2010 (Table 1).

In 2010, photosynthesis rates were measured in spring, summer, and fall from the five best,

Status	Selection	Ps Shade	Ps Sun	Shade Performance
		----- ( $\mu\text{mol}/\text{m}^2/\text{sec}$ )-----		(%)
Standard	Celebration	29	37	-12
Standard	Tifton10	28	33	-17
Standard	Patriot	21	28	-14
Standard	TifGrand	20	19	-14
Best	C72	32	30	-21
Best	C28	27	25	-18
Best	C35	23	25	-20
Best	C116	23	24	-13
Best	C118	22	22	-10
Worst	C31	32	26	-22
Worst	C84	28	27	-21
Worst	C130	26	20	-27
Worst	C83	22	27	-29
Worst	C74	21	26	-29

\*Shade performance = (mean visual rating in 2010 in shade – mean visual rating in 2010 in sun) / mean visual rating in sun in 2010 x 100.

**Table 3.** Mean photosynthesis CO<sub>2</sub> gas exchange rate of the five best, five worst, and four standard bermudagrass selections measured in spring, summer, and fall 2010. Status group is based on the monthly visual quality rating among selections and standards in 2010.

Status	Selection	Internode Length	
		Shade	Sun
		----- cm -----	
Standard	TifGrand	2.0	1.4
Standard	Patriot	2.3	2.2
Standard	Celebration	2.7	2.3
Standard	Tifton10	3.0	2.4
Best	C118	2.2	1.3
Best	C116	2.1	1.4
Best	C35	2.6	2.2
Best	C72	3.0	2.2
Best	C28	1.9	2.3
Worst	C130	2.6	2.4
Worst	C74	2.3	2.5
Worst	C31	3.4	2.8
Worst	C84	2.3	3.3
Worst	C83	N/A*	3.4

\*Not enough stolons that extended to the third internode were present to allow measurement of three sub-samples per plot.

**Table 3.** Mean photosynthesis CO<sub>2</sub> gas exchange rate of the five best, five worst, and four standard bermudagrass selections measured in spring, summer, and fall 2010. Status group is based on the monthly visual quality rating among selections and standards in 2010.

five worst, and the four standard bermudagrass selections. CO<sub>2</sub> gas exchange rate was always highest in summer and lowest in spring for plants in both full sun and shade (Table 2). From photosynthesis data obtained in 2010 and what is being obtained in 2011, we may be able to estimate the amount of shade that each selection can tolerate. However, the 2010 data do not show much promise for use photosynthesis as a shade indicator (Table 3). These data do not indicate a relationship between photosynthesis in shade or in sun with bermudagrass performance.

As reported in previous studies (19), internode length was typically longer in the best performing (according to visual quality) selections and standards grown in shade than the same selections and standards grown in full sun (Table 4). However, longer internode in shade was not a consistent measure in the poorest performing selections. For that reason, internode length may have some promise for rapid selection of shade tolerant species. However, more than one season's research is needed to support that contention.

At the completion of this study in 2014, we expect to have made adequate progress toward the production of a shade-resistant common bermudagrass cultivar(s) propagated from seed. Once the appropriate germplasm has been identified, we expect to be able to make parental combinations that result in improved shade tolerance and to be able to identify physiological components that may confer shade tolerance. We hope to provide a reasonably accurate method for screening potentially shade-tolerant selections.

We intend to identify parental genotypes to make genetic populations that will be used in future research, molecular mapping the major or genomic regions responsible for shade tolerance and identification of molecular markers closely linked to the major genes at a more precise level. We will also be able to provide useful information for future research in this area for our colleagues and ourselves.

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