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IMPROVING TALL FESCUE SHADE TOLERANCE: IDENTIFYING CANDIDATE GENOTYPES

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Abstract: Tall fescue (*Schedonorus arundinaceus*) is genetically variable for many agronomic traits, so it might be possible to increase its persistence and productivity in shaded agroforestry applications. The objective of this research was to identify high yielding, shade-tolerant genotypes. Seed was obtained from eight families: seven plant introductions of European origin: 234718, 234720, 234882, 234884, 235018, 235019, 235036, and one cultivar (Kentucky 31). Two sequential experiments were conducted to select genotypes for dry mass yield during April to September. Experiment (Exp) 1 included 30 genotypes of each of the eight families randomly assigned to each of two microenvironments: artificially shaded with fabric and unshaded. Maximum and minimum yields were 93.9 and 47.1 g family^{-1} for Kentucky 31 and 235036, respectively. After 1 yr, the proportion of vigorous survivors in Exp 1 was greater in the unshaded than shaded environment (0.40 and 0.09, respectively), and ranged from 0 to 0.56 (235036 and Kentucky 31, respectively). Forty robust genotypes (one later died) from four families (234718, 234720, 235019, and Kentucky 31) were selected from shaded and unshaded microenvironments of Exp 1, clonally propagated, and evaluated in pots for 2 yr in Exp 2. Shade-selected Kentucky 31 yielded more (31.0 g plant^{-1}) in shade than other shade-selected families (25.2 to 25.8 g plant^{-1}). Eleven genotypes in the top quartile (yield $\geq$ 33.0 g plant^{-1}) were selected for further testing. All genotypes were endophyte (*Neotyphodium coenophialum*)-infected. Future research will include seed increases and measuring yield in shaded, water-deficit conditions of a tree understory.

Key Words: Endophyte, *Genetic variability, Lolium arundinaceum, Schedonorus arundinaceus*

INTRODUCTION

Tall fescue is a cool-season, open pollinated grass widely used for hay, pasture, soil conservation, and turf because of its adaptability, yield, persistence, and nutritive value. It has suboptimal productivity in tree shade. Herbage should be managed to maintain at least a 10 cm residue height in silvopastoral practices (Belesky et al. 2008). In alley cropping, orchardgrass (*Dactylus glomerata*) persisted better under loblolly pine (*Pinus taeda*) shade than tall fescue (Burner 2003), and usually had greater yield.

When grown in the shade, grass species tend to have lower photosynthetic efficiency; larger specific leaf area; greater leaf light transmission coefficient; more photosynthate

partitioned to aboveground biomass; more air space in leaf lamina; thinner, wider leaf blades; decreased leaf area ratio; shifts in proportions of root cell types; and reduced foliar starch concentration compared with unshaded microenvironments (Ciavarella et al. 2006; Wahl et al. 2001; Zhai et al. 2006). Cumulative herbage N recovery and cumulative N acquisition efficiency decreased, and herbage NO_3^--N increased, when tall fescue was grown in shaded loblolly pine alleys relative to the unshaded control (Burner and MacKown 2005; 2006). In loblolly pine alleys (Burner and Belesky 2008), irradiance was a greater constraint than soil water to herbage specific leaf weight, leaf elongation rate, tillers plant^{-1}, mass tiller^{-1}, mass plant^{-1}, and total nonstructural carbohydrate (TNC) concentration. Irrigation generally failed to improve herbage productivity under intense shade (Burner and Belesky 2008).

Because of its wide adaptability and generally good performance, 'Kentucky 31' has historically been used as a check in tall fescue forage performance trials. Plant introductions (PI) and improved selections are an important source of germplasm for tall fescue cultivar development. In Kentucky, 'S-170', the predominant endophyte-free cultivar in Europe, and Kentucky 31 did not differ in total sugars, but S-170 matured earlier than Kentucky 31, and had lower seed yield, herbage digestibility, and fall vigor (R. Buckner and P. Burrus unpublished data). In Europe, distinct differences in morphology and agronomic characteristics of ecogeographic races have been reported for tall fescue (Robson and Jewiss 1968a, b). Twenty-six exotic PI from Europe, Central Asia, and Mediterranean area, and three U.S. cultivars, including Kentucky 31, differed in water soluble carbohydrate (WSC) concentration of summer herbage, yield, disease, relative maturity, and winter injury (Burner et al. 1988). Since U.S. cultivars and European PI did not differ in herbage regrowth yield (51 g plant^{-1}), and both these groups yielded more than those from Central Asia and Mediterranean area, breeding strategies for temperate U.S. should focus on domestic cultivars and European introductions (Burner et al. 1988).

Shade tolerance is an important trait for tall fescue turf. Germplasm screened in the National Turfgrass Evaluation Program is rated for turf quality, a subjective visual score of aesthetics (i.e. density, uniformity, texture, smoothness, growth habit and color), and functional use. The program annually screens entries under 'densely shaded conditions,' but experimental details on level of shading were not reported (Morris 2008). In August 2007, 113 entries ranged from a mean of 8.0 ('Essential' and 'ATM') to 3.3 ('GWTF'), where 1 and 9 were worst and best scores, respectively. Kentucky 31 scored 5.0, significantly different from 'best' and 'worst' entries. Our objective was to identify high yielding, shade-tolerant genotypes of tall fescue.

MATERIALS & METHODS

Seed of Kentucky 31 was purchased locally and seed of seven PI was obtained from USDA-ARS-GRIN (2006). The seven PI were bunch types of European origin: 234718 (France), 234720 (France), 234882 (Switzerland), 234884 (Switzerland), 235018 (Germany), 235019 (Germany), and 235036 (Sweden). Two sequential experiments (Exp) were conducted to measure dry mass yield during April to September. Exp 1 was

conducted to evaluate families, and Exp 2 was conducted to evaluate selections from Exp 1.

Exp 1

Sixty seeds (genotypes) per family were planted into greenhouse peat pots containing a peat moss medium in December 2005. Thirty genotypes from each of the eight families were randomly assigned to one of two field treatments: artificially shaded with fabric and unshaded, and transplanted in March 2006. The shaded microenvironment was created using black woven shade fabric of unknown manufacturer and shade intensity. The fabric was mounted horizontally about 2.5 m above ground level, and a fabric panel also was mounted vertically along the south edge of the structure. The unshaded microenvironment was immediately adjacent to the south side of the shade structure and outside the shaded microenvironment. Soil in each microenvironment was an Enders silt loam (fine, mixed, active, thermic Typic Hapludult).

There were six different genotypes per family in each of five replications. The six genotypes per family were transplanted in a row, with 15 cm between plants within rows, and 50 cm between rows. Extra plants were used as buffers between replications and at row ends. Plants were clipped to uniform 8 cm height after transplanting, and received fertilizer topdressing of 100 kg N ha^{-1}, 44 kg P ha^{-1}, and 83 kg K ha^{-1} in April 2006. Plants in both microenvironments were irrigated as needed with an oscillating sprinkler to minimize water-deficit, but timing and flow rate were not recorded.

Plants were harvested on 15 June, 1 August, and 5 September 2006. The six genotypes from each family row were clipped by hand to 8 cm stubble height, composited, oven dried at 60^{o}C, weighed for dry mass yield, and yield expressed as g $family^{-1}$. After the 15 June harvest, plants received fertilizer topdressing of 100 kg N ha^{-1}, 44 kg P ha^{-1}, and 83 kg K ha^{-1}. After the 1 August harvest, plants received fertilizer topdressing of 200 kg N ha^{-1}. Surviving genotypes were counted and rated for vigor in March 2007.

Exp 2

Based on Exp 1yields and observations, 40 of the most robust genotypes (visual basis) were selected from the following four families: 234718 (n = 9), 234720 (n = 11), 235019 (n = 6), and Kentucky 31 (n = 14). For each family, about one-half of the selections were from the shaded microenvironment and about one-half were from the unshaded microenvironment. The genotypes were clonally propagated by placing one tiller into each of four 14.5 cm-diameter pots containing about 1.8 L of a 1:1:1 mixture of silt loam soil : sand : peat moss. Pots were placed on benches about 0.7 m above ground level in the shade structure described in Exp 1. Plants were fertilized every two weeks with a water soluble fertilizer supplying 0.4 g N, 0.2 g P, and 0.3 g K per pot. Pots were kept continually moist by drip emitters connected to a watering timer.

Plants were harvested five times in 2007 at about 6 wk intervals (4 June, 2 July, 3 August, 11 September, and 1 November) to 5 cm stubble height, and weighed for dry

mass yield (g plant^{-1}). One genotype of 235019 subsequently died in late 2007. Genotypes were clonally propagated into fresh potting medium in fall 2007, one tiller per pot. The experiment was repeated as described above, except that clones were harvested four times at about 4 wk intervals in 2008 (19 May, 24 June, 26 July, and 16 September).

Environmental monitoring

Photosynthetically active radiation (PAR), measured 1.4 m above the soil surface, was continuously recorded at 0.5 h intervals from 1 July through 31 August 2006 (Exp 1), and 1 May through 30 September 2007 and 2008 (Exp 2) to characterize the microenvironments. The PAR was measured using Model 3668 quantum light sensors (Spectrum Technologies, Inc., Plainfield, IL). In 2006, two PAR sensors were placed in each of the shaded and unshaded microenvironments, and measurements were averaged at each recording interval. One (2007) or two PAR sensors (2008) were placed only in the shaded microenvironment (400 to 700 nm). Measurements were averaged at each recording interval in 2008. Shaded PAR was compared to that of an unshaded sensor located 470 m from the experimental area. The PAR data were expressed on a daily basis. In October 2008, genotypes were tested for endophyte using the tissue-print immunoblot method (Gwinn et al. 1991).

Statistical analysis

Daily PAR and soil temperature were presented as monthly means. Yield of each family (Exp 1) and genotype (Exp 2) was averaged across harvests within years. Analyses of variance of dry mass yield was conducted using a mixed linear model, Proc Mixed (SAS Inst. 2002). For Exp 1, fixed effects were microenvironment, family, and the family x microenvironment interaction. For Exp 2, fixed effects were year, family, selection microenvironment, and their interactions. Due to heterogeneity, Exp 2 yield was log-transformed for analysis of variance. For both experiments, random effects were replication and its interactions with fixed effects. Means were separated using the Tukey HSD test at $P \leq 0.05$ using (SAS Inst. 2002). The univariate procedure (SAS Inst. 2002) was used to test normality (Shapiro-Wilk test), and to identify genotypes in the top quartile (Exp 2). Untransformed means were presented for discussion purposes.

RESULTS & DISCUSSION

Environmental monitoring

Mean daily PAR of the shaded microenvironment varied relatively little from May to September (Fig. 1). The PAR of the shaded microenvironment was 0.41 (2006), 0.40 (2007), and 0.36 (2008) of the unshaded microenvironment (calculated from data of Fig. 1), indicating that the shade fabric provided about 60% shade. Mean daily PAR indicated that the microenvironment under shade fabric was relatively uniform compared to that provided by loblolly pine tree shade (Burner and Belesky 2008). Tall fescue can be grown under intense (83 to 89%) shade of loblolly pine (Burner and Belesky 2008), so this selection environment was rather mild.

All genotypes contained the endophyte, *Neotyphodium coenophialum* (Gwinn et al. 1991). This was important because the endophyte usually confers survival and productivity advantages to tall fescue (Malinowski et al. 2005).

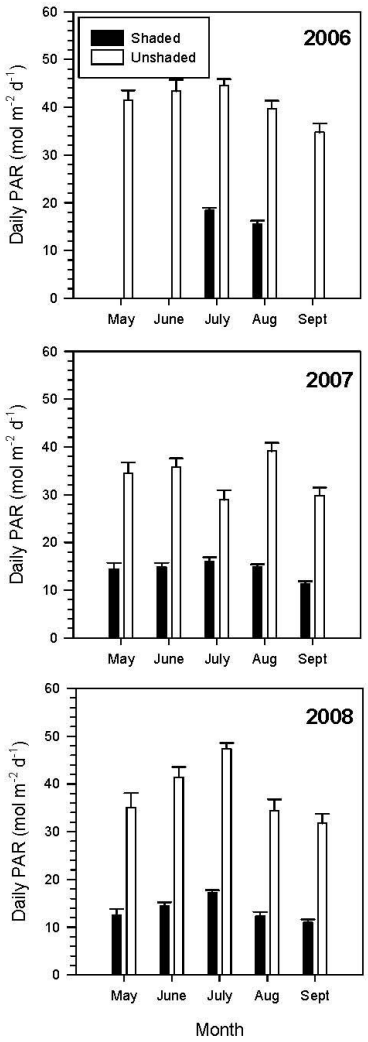

Figure 1. Mean daily photosynthetically active radiation (PAR) under the shade fabric and without shade for the May through September growth interval in 2006 to 2008. Vertical bars indicate standard error (n = number of days month^{-1} ≈ 30).

Exp 1

Mean dry mass yield was 78.2 and 68.4 g family^{-1} in unshaded and shaded microenvironments, respectively (P = 0.01). Compared to that of other studies with tall fescue (Burner and Belesky 2008; Burner and MacKown 2005), yield depression was relatively small perhaps because of the rather mild shade intensity (Fig. 1). There was a significant difference among families (P < 0.001), but the family x microenvironment

interaction was not significant (P = 0.13). Maximum and minimum yields were 93.9 and 47.1 g family^{-1} for Kentucky 31 and 235036, respectively (Table 1).

Table 1. Mean dry mass yield of tall fescue plant introductions (PI) harvested on 15 June, 1 August, and 5 September 2006 in shaded and unshaded microenvironments (Exp 1).

PI	Dry mass yield
	(g family^{-1})
Kentucky 31	93.9 a [a]
234720	92.7 a
234718	88.3 ab
235019	73.7 a-c
235018	71.8 a-c
234882	65.4 b-d
234884	53.5 cd
235036	47.1 d

[a] Means followed by a common letter do not differ by Tukey's HSD (P>0.05).

Survival had a significant (P < 0.001) family x microenvironment interaction. Kentucky 31 had the greatest survival across microenvironments, while virtually all plants of PI 235036 died regardless of microenvironment (Table 2). Two PI (234718 and 234720) did not differ from Kentucky 31 in either microenvironment. Mean survival was about four times greater

Table 2. Mean survival of tall fescue plant introductions (PI) in shaded and unshaded microenvironments (Exp 1).

	Unshaded	Shaded
PI	Survival [a]	
Kentucky 31	0.80 a [b]	0.32 a [b]
234718	0.68 a	0.30 a
234720	0.65 a	0.08 ab
235018	0.35 b	0.00 b
234882	0.32 b	0.00 b
235019	0.32b	0.02 b
234884	0.05 c	0.02 b
235036	0.00 c	0.00 b

[a] Survival = Number of vigorous survivors (2007) / number planted (2006). [b] Means within a microenvironment followed by a common letter do not differ by Tukey's HSD (P>0.05).

(P < 0.001) in the unshaded than shaded microenvironment (0.40 and 0.09, respectively), and ranged from 0 to 0.56 for 235036 and Kentucky 31, respectively (data not shown). Tall fescue exposed to combined stresses of intense shade and water stress in loblolly pine alleys had 67 to 75% survival (Burner and Belesky 2008).

Exp 2

All fixed effects except Exp 1 selection microenvironment and the year x family x selection microenvironment interaction were significant ($P \leq 0.04$) for dry mass yield. Shade yield of Kentucky 31 (26.1 g plant^{-1}) was greater than that of 235019 (17.7 g plant^{-1}) in 2007, but families did not differ in 2008 ($P \geq 0.89$). The family x selection microenvironment interaction was caused by a change in family ranking between selection microenvironments. Shade-selected Kentucky 31 yielded more (31.0 g plant^{-1}) in shade than other shade-selected families (25.2 to 25.8 g plant^{-1}). However, unselected families did not differ ($P \geq 0.26$) in shaded yield (range 27.0 to 29.8 g plant^{-1}). Yield was nearly twice as great in 2008 (40.1 g plant^{-1}) than in 2007 (15.2 g plant^{-1}). It was unclear why the effect of Exp 1 selection environment was not significant. This might have been caused by an insufficient level of shade (Fig. 1).

Yield was not normally distributed ($P = 0.22$), but had peak frequencies at about 33.0 ($n = 7$) and 25.0 g plant^{-1} ($n = 8$). Mean yield (Table 3) was 28.8 g plant^{-1} with a range of 18.4 g plant^{-1} (genotype 9, 234720) to 39.2 g plant^{-1} (genotype 8, 234720). Standard deviation (SD) was 5.7 g plant^{-1}. Eleven genotypes in the top quartile (yield $\geq$ 33.0 g plant^{-1}), at or about 1 SD of the mean, were selected for further testing (two to four genotypes per family). Mean yield of all selections of 234720 and Kentucky 31 exceeded 1 SD of the mean. Selection was biased about 2:1 toward those from the unshaded microenvironment ($n = 7$) compared to those from the shaded microenvironment ($n = 4$) of Exp 1. This suggested that one year of shade evaluation in Exp 1 was ineffective for identifying shade tolerant genotypes. Mean yield was about one-half that of regrowth yield (51 g plant^{-1}) in an unshaded study (Burner et al. 1988). More testing is needed to verify yield of the selections.

In unshaded conditions, genetic variance is nearly twice that of environmental variance for tall fescue yield, indicating that yield is highly heritable (Burner et al., 1983). However, improving shade yield could be a challenge because shade responses could have a non-genetic component (Cookson and Granier 2006; Wahl et al. 2001). Despite their reproductive divergence (Beuselinck et al. 1983), European and Mediterranean tall fescue varieties can exhibit different temperature-induced growth responses in winter which are absent in other seasons (Robson and Jewiss 1968b). In a preliminary study, five well established tall fescue plants chosen at random from a meadow and highly-shaded loblolly pine alley did not differ in CO_2 exchange rate (CER) when grown in a greenhouse (DM Burner 2003 unpublished data). If low CER was reversible in this small sample, shade-induced yield differences might also be.

This study was a first step in identifying sources of shade tolerance in tall fescue populations. Further testing of these selections will be needed to measure heritability of yield in shaded, water-deficit conditions of a tree understory. We plan to collect half- or full-sib seed of these genotypes and assess progeny performance in tree alleys as part of a recurrent selection program. Vigorous, shade tolerant germplasm developed from this study could improve forage productivity in agroforestry practices.

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Table 3. Tall fescue genotypes selected from four plant introductions (PI) in Exp 1, and two-year mean dry mass yield under shade (Exp 2). Genotypes in the top quartile (mean yield $\geq$ 33.0 g) are highlighted. Horizontal solid line indicates mean (28.8 g plant^{-1}), and dashed lines indicate $\pm$ one standard deviation (5.7 g plant^{-1}).

Genotype	PI [a]	Exp.1 envt. [b]	Yield [c] (g plant^{-1})
8	234720	U	39.2
7	234720	S	36.7
24	234720	U	36.6
36	Kentucky 31	S	36.5
1	Kentucky 31	U	36.4
31	Kentucky 31	U	35.7
17	Kentucky 31	S	35.5
22	234718	U	33.9
30	235019	U	33.7
10	234718	U	33.3
28	235019	S	33.2
37	Kentucky 31	S	32.8
2	234718	U	32.7
29	234718	U	32.3
5	234720	U	31.7
19	Kentucky 31	S	31.1
32	Kentucky 31	S	31.0
3	234718	S	30.8
26	234718	S	29.9
16	234720	S	28.1
39	234720	S	27.7
20	Kentucky 31	U	27.4
18	Kentucky 31	U	27.3
12	Kentucky 31	U	26.6
4	234720	S	25.9
34	Kentucky 31	U	25.9
35	Kentucky 31	S	25.9
38	235019	U	25.5
23	234720	U	24.7
33	234720	S	24.7
21	Kentucky 31	S	24.5
11	Kentucky 31	U	24.4
6	234718	S	23.4
15	235019	S	22.6
14	234718	S	20.4
27	234720	U	20.0
25	234718	U	19.7
13	235019	S	18.9
9	234720	S	18.4

[a] USDA-ARS-GRIN (2006). [b] S and U are shaded and unshaded microenvironment, respectively. [c] Mean of harvests in 2007 (n=5) and 2008 (n=4).

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