Composition and Characteristics of Blended Kentucky Bluegrass Stands

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Abstract. Kentucky bluegrass (*Poa pratensis* L.) cultivars are often blended to incorporate diverse characteristics. Factors that may contribute to the actual cultivar composition have not been evaluated. Through the use of DNA markers, individual plants in blended stands can be identified. This study evaluated changes in cultivar composition of 'Blacksburg', 'Midnight', and 'Unique' Kentucky bluegrass blends. Characteristics such as seed size, seed moisture content, percent germination, and seedling development did not affect the initial composition of blends at time of seeding. DNA markers were used to demonstrate how the composition of a blended 'Blacksburg', 'Midnight', and 'Unique' turf changed during the first growing season following establishment. The composition of blends did not significantly change from time of seeding in Sept. 1999 to Apr. 2000 or from Apr. 2000 to Oct. 2000. Two of the three blends were significantly different by Oct. 2000 relative to the percentages seeded in Sept. 1999.

Blending three or more turfgrass cultivars to include several desirable characteristics such as stress tolerance and disease resistance has become a common practice. The theory of blending indicates that combining cultivars is useful for capitalizing on characteristics from diverse genetic backgrounds (Golembiewski, 1999). Because Kentucky bluegrass is apomictic, lack of genetic variability within a cultivar may warrant blending several cultivars to gain genetic variability within a stand. Unfortunately, factors that may contribute to future composition of a blended turf have not been evaluated. With characteristics such as the number of seeds per unit weight differing between cultivars and even between seedlots of the same cultivar (Christians et al., 1979), achieving a stable composition is suspect. Furthermore, how composition of a blended stand changes over time is not understood.

Separating DNA fragments by molecular weight and visualizing by gel electrophoresis for the purpose of identifying an individual is referred to as DNA fingerprinting (Weising et al., 1995). Two individuals not having the same corresponding band on an electrophoretic gel is referred to as a polymorphism. Polymorphisms allow for distinguishing between

genetically dissimilar individuals. Random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland, 1990; Williams et al., 1990) have been used to distinguish between individual turfgrass plants of *Agrostis palustris* L. (Golembiewski et al., 1997), *Lolium perenne* L. (Forbes, 2000; Sweeney and Danneberger, 1994) and *Poa annua* L. (Sweeney and Danneberger, 1995). The fingerprinting of Kentucky bluegrass cultivars with DNA markers has also been evaluated (Barcaccia et al., 1997; Huff, 2001; Huff and Bara, 1993), but studies have also been conducted using isoenzymes (Freeman and Yoder, 1991; Wehner et al., 1976).

Contradictory to the theory of blending, Smiley and Fowler (1986) and Fowler and Hummel (1987) did not observe an improvement in disease resistance by blending Kentucky bluegrass cultivars. Vargas and Turgeon (1978) reported that blends of Kentucky bluegrass, which included a cultivar resistant to melting-out [*Drechslera poae* (Baudys) Shoemaker], were not resistant to the disease. They hypothesized that planting a susceptible cultivar with a resistant cultivar allowed disease inoculum to build-up beyond a threshold level at which point the resistant cultivar was no longer resistant.

In support of blending, Gregos et al. (1998) observed an improvement in *Drechslera* spp. resistance when Kentucky bluegrass cultivars were established as a blend. Furthermore, Watkins et al. (1981) reported stem rust (*Puccinia graminis* Pers.) severity was similar to the components grown as monostands. This indicated a relationship might exist between the quantity of resistant cultivars in a blend and disease severity. Only by identifying the composition of blended stands infected by pathogens will such relationships be quantified.

The first objective of this study was to determine how characteristics such as seed size, seed moisture content, percent germination and seedling development affect the initial composition of Kentucky bluegrass blends. The second objective was to determine how the composition of Kentucky bluegrass blends changes during their first growing season.

Materials and Methods

Three random samples of two seed lots of 'Blacksburg', 'Midnight', and 'Unique' Kentucky bluegrasses (Pure-Seed Testing, Hubbard, Ore.) were collected and the exact weight was recorded to the nearest 0.1 mg. The number of seeds in each sample was counted before drying for 48 h at 65 °C to determine seeds/g. Four additional 1-g samples were randomly selected from each of the six seedlots, weighed, dried at 65 °C for 48 h, then weighed again to determine percent moisture. Samples were again randomly selected, and 100 seeds of each seedlot were separated into four replicates of 25 seeds each. The seeds were planted as a randomized complete-block design in 133 cm² cells in greenhouse flats filled with a 1 soil : 1 peat : 1 perlite mixture. Blocking was by replication to minimize variation attributable to nonuniform light and mist irrigation conditions in the greenhouse. All flats received mist irrigation for 16 s every 20 min. The greenhouse was maintained without supplemental lighting at 20 ± 2 °C. Percent germination (seedlings per flat) was recorded at 7, 10, 14, 18, 21, and 25 d after seeding. The average number of leaves per tiller for each cultivar was calculated at 21 and 28 d after seeding.

Correlation analyses were used to test the relationship between seeds/g, percent seed moisture, and percent germination. Data were analyzed using the general linear model procedure of the Statistical Analysis System (SAS Institute, 1990). For the analysis of variance (ANOVA), the model [$y = m + \text{block} + \text{cultivar} + \text{seedlot}(\text{cultivar}) + e$] had seedlot nested within cultivar. The interaction terms that included blocks were nonsignificant ($P > 0.25$) so they were combined into a pooled error estimate for the ANOVA. Blocks were random effects while cultivar and seedlot were analyzed as fixed effects. Contrast statements were used in SAS to compare mean values for cultivars and seedlots within each cultivar.

A field trial was developed as a randomized complete-block design with four replications of three monostands ('Blacksburg', 'Midnight', 'Unique') and three blends [50 'Blacksburg':50 'Midnight', 50 'Blacksburg':50 'Unique', and 33 'Blacksburg':33 'Midnight':33 'Unique' (blended by weight)]. Plots (2.32 m²) were prepared at the Landscape Horticulture Field Research Laboratory in Urbana, Ill., on a Flanagan silt loam soil (fine, smectic, mesic Aquertic, Argiudoll, 52 g·kg⁻¹ organic matter, 1.38 g·cm⁻³ bulk density, pH 6.5). Soil preparation prior to seeding consisted of rotary tilling 15 to 20 cm deep, raking the surface by dragging a length of chain-link

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fence, then hand raking to create a uniform surface and remove large debris.

Blends were created by proportioning seed by weight rather than considering seeds per unit weight (volume) or percent germination. The percent germination and number of seeds per unit weight were used to calculate the degree of uncertainty in the intended blending percentages. All plots were seeded at 100 kg-ha⁻¹ (seedlots Blacksburg 21-9-150; Midnight GBS-6-3-02; and Unique 1994) on 13 Sept. 1999 by hand. Seed for each plot was premixed with 100 g of Milorganite® fertilizer [7-3-0 (Milorganite, Milwaukee)]. Plots were fertilized with urea (46-0-0) with N at 50 kg-ha⁻¹ at time of seeding and again on 10 Nov. 1999 and received 0.25 cm of daily irrigation until 10 Nov. 1999. Once established, plots were mowed once per week at a 5.1-cm height and given N at 100 kg-ha⁻¹ per year (46-0-0) split into two annual applications of N at 50 kg-ha⁻¹ (1 June and 1 Sept. 2000). No irrigation, fungicides, or insecticides were applied to the plots during the 2000 growing season. Trimec Classic® (PBI/Gordon Corp., Kansas City, Mo.) [2,4-dichlorophenoxyacetic acid, 2-(2-methyl-4-chlorophenoxy) propionic acid], and 3,6-dichloro-2-methoxybenzoic acid at 4.7 L-ha⁻¹ was applied on 31 Mar. and 31 Oct. 2000 for postemergent broadleaf weed control.

Each plot was sampled using a grid template that allowed 1.9-cm cores collected 5.1 cm apart in a pattern of 3 columns by 5 rows (15 plants per plot). The 15 samples were collected from the top center of each plot on 5 Apr. 2000 and the bottom center of each plot on 24 Oct. 2000. No samples were collected from within 15 cm of a plot's border to avoid the collection of contaminants from adjacent plots. Each core had tillers pruned so that only the most center tiller remained on each of the 15 cores. All single-plant cores were transplanted into 133 cm³ cells in greenhouse flats (1 soil : 1 peat : 1 perlite) then transferred to a greenhouse (no supplemental light, 20 ± 2 °C, mist irrigation applied for 16 s every 20 min). After 45 d, fresh leaf and stem tissue was collected for DNA analysis.

Total genomic DNA was extracted and purified using PlantDNAzol® reagent (GIBCO BRL, Grand Island, N.Y.—formerly Life Technologies) (Lin and Kuo, 1998; and Lickfeldt, 2001). This isolation method uses a buffer called PlantDNAzol® reagent that is a guanidine-detergent lysing reagent. The reagent allows for the selective precipitation of genomic DNA while hydrolyzing RNA. The procedures for determining DNA concentration, DNA amplification and PCR product separation were summarized by Lickfeldt (2001). Primers OPA-10 (5'-GTGATCGCAG-3') and OPB-10 (5'-CTGCTGGGAC-3') (Operon Technology, Alameda, Calif.) were used to distinguish among the three Kentucky bluegrass cultivars. To ensure accurate identification of individuals, two positive polymorphic bands for each of two different primers were required to declare the identity of an individual. This indicates that the three cultivars are not closely related because others have

required 15 RAPD markers to distinguish between Kentucky bluegrass cultivars (Huff, 2001).

The mean cultivar composition was calculated for each of the blends from the four replications. Chi-square values were calculated to determine the probability of differences between the intended blend composition and actual seeded composition (Sept. 1999). The actual seeded composition in Sept. 1999 (expected composition) was compared with the composition determined by DNA fingerprinting in Apr. and Oct. 2000 (observed composition) using chi-square analyses.

Results and Discussion

Characteristics such as seed size, percent seed moisture, percent germination, and number of leaves per tiller differed significantly between 'Blacksburg', 'Midnight', and 'Unique' Kentucky bluegrass and between seedlots of each cultivar (Table 1). More specifically, the number of seeds per unit weight (seed size) and percent germination were significantly different ($\alpha = 0.05$) between the three cultivar while seed size also varied significantly between seedlots. The smallest seed size was recorded for both seedlots of 'Unique' while the GBS-6-30-2 seedlot of 'Midnight' had the largest seeds. Correlation analysis of seeds/g with percent germination revealed a negative relationship ($r = -0.87$, $P < 0.05$), while percent moisture and percent germination were positively correlated ($r = 0.39$, $P < 0.05$).

Chi-square analyses did not reveal a significant difference between the intended (based on seed weight) and actual (based on seeds/g and percent germination in Sept. 1999) compositions (Table 2). Likewise, DNA fingerprinting of plots in Apr. 2000 determined that the composition of any blend did not change significantly from that seeded in Sept. 1999 (Table 3). Furthermore, the composition of all blends did not change from Apr. to Oct. 2000. The 3-way blend did change significantly from 37 'Blacksburg' : 37 'Midnight' : 26 'Unique' seeded in Sept. 1999 to 23:43:33 in Oct. 2000. Likewise, the 50 'Blacksburg' : 50 'Midnight' blend changed

significantly to 37:63, while the 50 'Blacksburg' : 50 'Unique' blend changed significantly to 62:38 after 13 months of establishment.

Variable growing conditions from season to season in seed production fields may be the contributing factor to seed size differences reported here and by Christians et al. (1979). With such variability being present in seed sources, the accurate creation of blends based on seed weight seems suspect. In this study, significant differences between the intended (designed) and actual (seeded) composition were not noticeable. With such inconsistencies in seed size and percent germination, obtaining actual blending percentages similar to the intended composition following the blending of seed seems doubtful. Since the smallest seeded cultivar (Unique) had more seeds per unit weight, while its rate of germination and leaf development were significantly less than that of the larger seeded cultivars, these two factors may counteract one another so that an intended composition resembles the actual composition as occurred here. Seedlots with higher germination rates should be tested in future studies.

After 13 months, the 3-way blend had a dramatic decrease in the prevalence of 'Blacksburg', while the percentage of 'Midnight' and 'Unique' increased slightly. Remarkably, this composition is similar to that observed following the sampling of two golf courses seeded to similar blends (Lickfeldt, 2001). Two sites, which were 14:46:40 and 14:47:39 based on percentage of 'Blacksburg' : 'Midnight' : 'Unique', had similar compositions as the 3-way blend in this study (23:43:33). This might lead to the conclusion that 'Blacksburg' is a weak competitor in blended stands, but the fact that 'Blacksburg' became the most prevalent cultivar in the 2-way blend with 'Midnight' suggests otherwise. With more time, 'Blacksburg' content of the blends may decrease even more, but factors contributing to its decline are unknown. The decrease in percentage of 'Blacksburg' in blends with 'Midnight' and 'Unique' could not be linked to disease resistance but disease-free controls were not included in this study to make such a determination. Future research tracking

Table 1. Seeds/g, percent seed moisture, percent germination after 21 d, and number of leaves per tiller at 21 d after seeding for two seedlots each of three Kentucky bluegrass cultivars.

Cultivar	Seedlot #	Seeds/g	Moisture	Germination	Leaves
		- # -	- - - - -	% - - - - -	- - - - -
Blacksburg	21-2-852	3522	5.1	35.5	2.0
Blacksburg	21-9-1500	3638	4.5	49.5	2.3
Midnight	GBS-6-30-2	2729	5.4	66.5	2.3
Midnight	GBS-8-180-F	3445	4.9	52.5	2.4
Unique	1994	4167	5.2	30.0	1.6
Unique	21-9-9016	5052	4.4	27.5	2.1
Significance level from contrast statements					
Cultivar contrasts		- - - - -	P > F	- - - - -	- - - - -
Blacksburg vs. Midnight		<0.001	0.006	0.003	NS
Blacksburg vs. Unique		<0.001	NS	0.015	0.050
Midnight vs. Unique		<0.001	0.006	<0.001	0.004
Seedlot contrasts					
21-2-852 vs. 21-9-1500		NS	0.003	NS	NS
GBS-6-3—2 vs. GBS-8-180-F		<0.001	0.005	NS	NS
1994 vs. 21-9-9016		<0.001	0.001	NS	0.014

Table 2. Cultivar composition of Kentucky bluegrass blends at the time of seeding based on seed size and percent germination. Chi-square analysis ($\alpha = 0.05$) determined there was not a significant difference between the intended and the actual composition at time of seeding.

Factor	Blacksburg	Midnight	Unique
	----- % -----		
Intended 3-way blend	33.3	33.3	33.3
Intended 2-way blend	50	50	
Intended 2-way blend	50	50	
Percent germination	49.5 (11.5) ^z	66.5 (11.1)	30 (18.9)
Seeds/g	3638 (79)	2729 (5.8)	4167 (112)
Live seeds/g ^y	1801 (457)	1815 (307)	1250 (820)
Actual 3-way blend ^x	37 (9.4)	37 (6.3)	26 (16.9)
Actual 2-way blend	50 (12.6)	50 (8.5)	
Actual 2-way blend	59 (15.0)	41 (26.9)	

^zStandard deviation in parenthesis.

^yLive seeds/g was calculated by seeds/g multiplied by percent germination.

^xThe actual composition of blends was calculated using Live seeds/g and weight values used to proportion seed for intended blend compositions.

Table 3. Cultivar composition of Kentucky bluegrass blends before and after the 2000 season with standard error in parentheses. Changes in composition were tested with chi-square analysis with critical values of 3.8 for 2-way blends and 6.0 for the 3-way blend ($\alpha = 0.05$).

Dates	Blacksburg	Midnight	Unique
	----- % -----		
	<i>3-Way Blend</i>		
Sept. 1999 - Seeding	37 (4.7)	37 (3.2)	26 (8.5)
Apr. 2000 - Sampling ^z	26 (9.7)	43 (6.4)	31 (5.6)
Oct. 2000 - Sampling ^y	23 (6.5)	43 (6.5)	33 (8.6)
	<i>2-Way Blend</i>		
Sept. 1999 - Seeding	59 (7.5)	NA	41 (13.5)
Apr. 2000 - Sampling ^z	58 (5.7)	NA	42 (5.7)
Oct. 2000 - Sampling ^x	62 (8.4)	NA	38 (8.4)
	<i>2-Way Blend</i>		
Sept. 1999 - Seeding	50 (6.3)	50 (4.3)	NA
Apr. 2000 - Sampling ^z	44 (3.8)	56 (3.8)	NA
Oct. 2000 - Sampling ^y	37 (6.2)	63 (6.2)	NA

^zBlends in Apr. 2000 were not significantly different than the blends seeded in Sept. 1999.

^yThese blends had a significant change in composition ($\alpha = 0.05$) from the intended composition and the actual composition seeded in Sept. 1999. The change from Apr. to Oct. 2000 was nonsignificant.

^xThis 2-way blend was significantly different from the intended 50%:50% composition ($\alpha = 0.05$), but the composition in Oct. 2000 was not significantly different from that observed in Sept. 1999 or Apr. 2000.

changes in cultivar composition with proper control treatments may lead to a much greater understanding of the dynamics involved when a turfgrass cultivar persists following biotic or abiotic stress.

More research is needed to determine if 60 samples (15 per plot in 4 replications) are sufficient for estimating the composition of blended turfgrass plots. Lickfeldt (2001) reported that 60 samples would be sufficient to estimate percentages within ± 10 percentage points ($P = 0.05$). Therefore, declaring actual composition based on means with large standard deviation values (Table 3) requires sensible limitations. Still, chi-square analysis was successful for detecting differences between actual percentages based on DNA analysis and percentages based on what was seeded by weight.

The number of seeds per unit weight and percent germination differed significantly from cultivar to cultivar and from seedlot to

seedlot within a cultivar, but blending Kentucky bluegrass seed using seed weight resulted in an actual composition similar to the intended composition. Therefore, additional testing of seed sources for seed size and percent germination may not be necessary to accurately create blends of the intended composition. The actual composition obtained following full establishment is still unpredictable because many factors can contribute to a particular cultivar's success, but DNA markers are valuable tools for identifying individual Kentucky bluegrasses in ecology studies.

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