

Morphological Identification of St. Augustinegrass Cultivars¹

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ABSTRACT

A vegetatively propagated crop such as St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] should consist of distinctive, easily identifiable cultivars. The purpose of this study was to improve identification criteria for this species and measure the frequencies of various St. Augustinegrass cultivars in Florida. Morphological traits were recorded in 242 unknown Florida St. Augustinegrass lawn and sod samples, which had been transplanted and grown outdoors in containers with 16 known clonal cultivars and taxonomic representatives. Lawn and sod samples conformed mostly to the expected morphological distributions of 'Floritam', 'Bitterblue', and a heterogeneous 'Roselawn'-like complex. The latter clones were distinctive because of their intermediate-sized (4.6 to 4.8 mm) spikelets, relatively long floral regions (measured from the base of the lowermost spikelet to the tip of the inflorescence), and long, narrow internodes. Therefore, they were named the Longicaudatus ("long-stemmed") Race. Clones with short (<4.5 mm) spikelets were also distinctive and were named the Breviflorus Race, however, this race was virtually absent in Florida. Spikelet length and length of the floral region separated known cultivars in 84 of 91 pairwise comparisons ($P < 0.05$), and these two traits showed no detectable environmental variance between containers and field plots. Inclusion of floral traits in discriminant analysis increased the proportion of unknown clones that could be identified from 49% (with vegetative traits only) to 95%. Keys using vegetative field traits and replicated container measurements were 93% accurate in identification of knowns, and 86 to 96% consistent with discriminant analysis in identification of unknowns.

Additional index words: *Stenotaphrum secundatum* (Walt.) Kuntze, Turf, Discriminant analysis.

TURFGRASSES present a special problem in cultivar identity. Unlike annual crops requiring recurrent seeding, turfgrasses frequently persist in the landscape and are used for many years without replacement. As a result, their identity may be lost. Yet sound management recommendations may depend upon the correct identification of turfgrass cultivars, or such things as preventive pesticide treatments might be applied needlessly. In Florida, the chinch bug resistant cultivar 'Floritam' St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] is sometimes treated with preventive chinch bug spray. Such a practice is expensive and unnecessary, and may have nontarget effects which are harmful. In this and other possible instances, accurate identification of turfgrass cultivars could facilitate their sound management.

The taxonomy of commonly grown cultivars has tended to be ignored, both by botanists (Anderson, 1967) and by agriculturists. Yet, multivariate taxonomic methods have been long used in anthropology (Pearson, 1926; Rao, 1948), botany (Fisher, 1936), and zoology (Jolicoeur, 1959). The specific problem of identifying individuals in closely related populations was solved by Fisher (1936) by using the linear discriminant function involving several variables. At the same time, Mahalanobis (1936) summarized a taxonomic similarity coefficient, the "Generalized Distance" statistic, D^2 , later called "Mahalanobis Distance", which eliminates the bias caused by correlation among characters that was present in Pearson's (1926)

method. Multivariate approaches have been used in maize classification (Goodman, 1968) and in measuring statistical distance between maize parents (Martinez et al., 1983), but cultivar identification of unknowns has not been commonly examined quantitatively (see Porter and Smith, 1982, for a qualitative approach).

Discriminant analysis is appropriate for identification where, "there are a few close groups in which identification must be as certain as possible" (Sneath and Sokal, 1973). Such an approach utilizes multiple variables simultaneously and is amenable to a probabilistic model. In contrast, the more traditional monothetic keys are sequential, require less data, and are appropriate to large studies involving well-separated taxa (Sneath and Sokal, 1973). As will be shown here, the identification of St. Augustinegrass cultivars presents both situations. Because the objectives of an identification scheme are both ease and certainty of identification, in some cases a more exacting multivariate procedure would be used, and in other cases a simplified key would be used.

St. Augustinegrass is vegetatively propagated in commercial sod production and cultivars are generally released as individual clones. Therefore, morphological variations of populations in cultivation should be fairly simple, depending on the number of cultivars present. The cultivar Floritam can be identified morphologically among unknown clones of St. Augustinegrass that are grown in a relatively uniform environment (Busey, 1979). A classification of worldwide germplasm (Busey et al., 1982) provides a framework for understanding morphological variations in cultivated populations, but not for the practical identification of cultivars. The objective of this study was to use morphology to measure the frequency of various St. Augustinegrass cultivars in Florida. Several improvements were made in identification criteria and in the delineation of cultivar relationships.

MATERIALS AND METHODS

Known Cultivars and Taxonomic Representatives

Clones of known origin, including cultivars and other representatives (Table 1) of St. Augustinegrass taxonomic groups (Busey et al., 1982) were propagated and grown in two containers per each of three blocks, among unknown lawn and sod samples (below section), using 7.6-L plastic containers filled with a potting mix (5:3:1, cypress shavings:Florida peat:sand). Plants were measured for internode length and thickness, leaf blade length, thickness, and width, floral region length (measured from the base of the lowermost spikelet to the tip of the inflorescence), number of branches per inflorescence, and length of lower spikelets in centermost branches. Vegetative measurements were

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made on the third to fifth fully expanded internodes and subtending nodes, and inflorescences measured were terminal and post anthesis. Measurements comprised three subsamples (multiple measurements from the same container), except for blade thickness and internode length, which comprised two and four measurements, respectively. Subsamples within containers were averaged prior to subsequent analysis, and derived traits were calculated: leaf volume (length × width × thickness), internode volume (length × thickness × thickness × π/4), internode attenuation (length/thickness) and distance between branches (floral region length/number of branches).

Data for known cultivars and taxonomic representatives were analyzed by analysis of variance (ANOVA) and means were separated by the Waller-Duncan *k*-ratio *t* test (SAS Institute, 1982). Means were based on individual clones, in contrast to those of Busey et al. (1982), which were overall averages of groups of several clones. When Bartlett's test for homogeneity of variances (Steel and Torrie, 1960) showed significant differences, log transformation of the original data was used and provided variances which were not significantly heterogeneous, and means comparisons were thereby obtained using a pooled residual variance applied to means of log-transformed values.

Discriminant functions (SAS Institute, 1982) were calculated for known clones, using taxonomic groups: Floratam Group (Floratam and Floratam=FA-108), Bitterblue Group (Bitterblue and Floratine), Roselawn-like (later called Longicaudatus Race) (Roselawn, Florida Common, FL-1811, and FL-2202), Gulf Coast Group (FA-87, FL-1926, and FL-1933), and Dwarf Group (Seville, FL-1721, FL-1817, FL-1867, and FL-2002). These groups were based largely on a previous classification (Busey et al., 1982). Discriminant functions were obtained from averages of the eight original variables across three blocks of each known cultivar and taxonomic representative, combining Container 1 in Blocks 1, 2, and 3, and combining Container 2 in Blocks 1, 2, and 3. This procedure resulted in two values for each cultivar standard, which, combined with other cultivars of the same taxonomic group, resulted in 4 to 10 observations for each taxonomic group. Discriminant functions were calculated assuming equal prior probabilities for each taxonomic group. Because within-group covariance matrices were homogeneous, they were pooled.

Unknown Lawn and Sod Samples

Lawn samples of St. Augustinegrass from southeast Florida (Dade, Broward, and Palm Beach counties) were collected using a geographic grid and random number table, so that all urban areas had an equal chance of being sampled. Sampling density averaged one site per 17.2 km², and each site was a quadrat of approximately 0.4 ha. St. Augustinegrass sprigs were collected from 107 of 143 accessible sites from October 1980 to February 1981. Another 51 samples were obtained similarly but at higher sampling densities July to September 1980 in Davie, Broward County, FL. Transects yielded 36 samples from Jacksonville, Orlando, and the Tampa Bay region. Of these 194 St. Augustinegrass samples, 20 were from adventive and unmowed sites, and 36 were from maintained lawns in which St. Augustinegrass was a secondary vegetation component. Additionally, 48 St. Augustinegrass sod samples were purchased from retail nursery outlets in southeastern Florida, March 1980 to October 1981. For each sample, five initial measurements of thickness, width, and length of leaf blades and stolon internodes were recorded. Lawn and sod samples were propagated outdoors in three complete blocks at

the same time and in the same manner as, and randomized among, known cultivars (above section).

Visual identification of all plants with exposed internodes was performed in October 1981 based on vegetative traits. Roselawn-like plants (long internodes, foliage not noticeably blue) were distinguished from polyploid-like plants (medium short internodes, with blue foliage). The polyploid-like plants were subdivided into Bitterblue-like plants (no conspicuous reddening of terminal leaf sheaths) and Floratam-like plants (conspicuous reddening of terminal leaf sheaths). The visual identification of an unknown clone was that which was recognized in at least two of three replicates (disputes among replicates were infrequent). Unknowns were then measured at the same time and in the same manner as known cultivars (above section).

Unknown lawn and sod samples were identified using discriminant functions calculated for known cultivars and taxonomic representatives (above section), and applied to overall averages for each unknown. Since averages for knowns and unknowns were each based on the averages of three containers, their variance and covariance estimates had the same model components. Clones that did not produce inflorescences were identified by separate discriminant analysis using vegetative traits only.

Unknown lawn and sod samples were identified to racial group by the following key, using container data, and the consistency of results was compared to that obtained from discriminant analysis:

- 1. Lower spikelets in centermost branches > 5.2 mm long Floratam Group
- 1. Lower spikelets in centermost branches ≤ 5.2 mm long, or else spikelets not available 2
- 2. Inflorescence branches ≤ 15; if absent, leaves < 65 mm long 3
- 3. Inflorescence branches ≥ 13 Gulf Coast Group
- 3. Inflorescence branches < 13 Dwarf Group
- 2. Inflorescence branches > 15; if absent, leaves ≥ 65 mm long 4
- 4. Internode attenuation (length/thickness) > 25 Longicaudatus Race
- 4. Internode attenuation ≤ 25 Bitterblue Group

Bivariate scattergrams of characteristics of visually identified lawn and sod samples were compared with 95% confidence ellipses (Sokal and Rohlf, 1969) for known cultivars and taxonomic representatives. Morphological variances among and within visually identified groups of unknowns were calculated by the ANOVA Procedure (SAS Institute, 1982).

Field Identification of Knowns and Unknowns

Field plots (75) were established representing the known cultivars Floratam, Floratine, Bitterblue, Roselawn, 'Florida Common', and 'Seville' and were maintained as turf for 0.8 to 5.0 yrs. Three stolons were removed from each plot, randomly assigned secret code numbers, and identified based on vegetative morphology. Stolons with narrow (<9 mm wide) or short (<6 cm long) leaf blades, or with internodes predominantly green, were identified as Seville. Among the remainder of clones, those with thin (<2.6 mm) internodes or without copious collar hairs, or without conspicuously blue foliage were identified as Roselawn-like (Roselawn or Florida Common), and those with thick (>2.7 mm) internodes, with copious collar hairs extending along the entire edge of the collar, and blue leaves were identified as polyploids. Among the latter, clones with some pink on some exposed leaf collars were identified as Floratam and those with no pink on any leaf collars were identified as Bitter-

blue-like (Bitterblue or Floratine).

Lengths of the floral region and of the spikelet were also measured in field-grown inflorescences of Floratam, Bitterblue, Roselawn, and Seville. Cultivar means of container (above section) and field measurements were analyzed as a combined experiment.

Unreplicated field plots 1.8×1.8 m were established of 240 unknown lawn and sod samples, and were maintained as turf. In February 1984, after 1.5 yrs, 212 field plots with 50% or more St. Augustinegrass coverage were inspected and one or two exposed stolon terminals were removed from the center of each plot. The clone in each plot was identified using the same vegetative field characteristics (above section), and the consistency of these identifications was determined by comparison of the results with those obtained from discriminant functions.

RESULTS AND DISCUSSION

Known Cultivars and Taxonomic Representatives

Floratam and Floralawn (FA-108), previously assigned to the Roselawn-Floratam Group (Busey et al., 1982) had significantly longer spikelets and shorter, thicker internodes than other members of the group (Roselawn, Florida Common, FL-1811, and FL-2202). Roselawn and other clones with attenuated internodes were therefore separated out as the Longicaudatus ("long-stemmed") Race (Table 1). Good floral diagnostic traits for this race were its intermediate-sized (4.6 to 4.8 mm) spikelets and long (>85 mm) floral regions.

Often, the confidence limits overlapped for characteristics of the Dwarf Group clones (Seville, FL-1721, FL-1817, FL-1867, and FL-2002) and Gulf Coast Group clones (FA-87, FL-1926, and FL-1933). In all cases, spikelets were less than 4.5 mm long. As a result, these groups were combined as the Breviflorus ("short-flowered") Race. Only the number of

inflorescence branches distinguished the Dwarf Group and the Gulf Coast Group in this study, although stigma color (Busey et al., 1982) is partly effective.

Inflorescence traits were more reliable than had been recognized previously. Spikelet length and floral region length separated ($P < 0.05$) cultivars and taxonomic representatives in 84 of 91 pairwise comparisons (based on univariate ANOVA's), whereas all eight vegetative characters (including derived characters) together separated cultivars in only 99 of 120 comparisons. Cultivars within races were generally not separable in more than one trait (Table 1). In some instances the cultivars within racial groups were recognized in the sod trade as synonymous (e.g., Bitterblue vs. Floratine), or performance differences were undocumented (Floratam vs. Floralawn). Roselawn and Florida Common could, however, be distinguished by two characters, yet few inflorescences were produced by Florida Common, making it difficult to use in later discriminant analysis. Discriminant analysis of known cultivars and taxonomic representatives resulted in a 99.99% or higher probability of group membership, which was consistent with the original known groups in 31 of 32 observations, but this was biased by the a posteriori nature of the comparison.

Unknown Lawn and Sod Samples

Of 242 survey clones, 62 did not produce inflorescences. Among the remainder 180 clones, for which all vegetative and floral characters were measured, 172 (96%) were confidently assigned by discriminant analysis to a racial group (prior $P > 0.95$). Clones not assigned to a racial group at $P > 0.95$ were in regions of overlap between racial groups. The latter could be due in part to a close proximity

Table 1. Morphological traits of 16 known St. Augustinegrass cultivars and taxonomic representatives.

Cultivar	Leaf blade			Internode		Inflorescence traits		
	Length	Width	Thickness	Thickness	Length	Floral region	Branches	Spikelet length
	mm						no.	mm
(Floratam Group)								
'Floratam'	92a*	11.3ab	0.233a	2.90a	62cd	111a	16.7b	5.58a
'Floralawn'	81ab	11.1ab	0.233a	2.81a	59de	103ab	17.3ab	5.57a
(Bitterblue Group)								
'Bitterblue'	80ab	11.2ab	0.216a-c	2.84a	56d-f	71d	17.7ab	4.84b
'Floratine'	88a	11.1ab	0.199b-d	2.84a	53f-h	81c	17.9a	4.80b
(Longicaudatus Race)								
FL-1811	80ab	11.6a	0.215a-c	2.43b	80a	96b	17.6ab	4.69b
FL-2202	99a	10.8bc	0.182de	1.92e	79a	77	18.4	4.72
'Roselawn'	88a	11.6a	0.184de	2.16cd	81a	103ab	17.5ab	4.66b
'Florida Common'	79ab	10.1c	0.192c-e	1.91e	72b	-	17.2	4.61
(Breviflorus Race, Gulf Coast Group)								
FA-87	63b-d	9.2de	0.178de	2.40b	54e-g	61e	14.3c	4.15d
FL-1926	55c-e	9.4d	0.190de	2.27bc	53e-g	74cd	14.1c	4.33c
FL-1933	45ef	8.6d-f	0.170e	2.04de	47i	70d	14.3c	4.21cd
(Breviflorus Race, Dwarf Group)								
'Seville'	66bc	9.3de	0.186de	2.36bc	50f-i	72d	12.6d	4.19cd
FL-1721	41f	8.1fg	0.187de	2.06de	48g-i	56e	11.8de	3.90ef
FL-1817	47ef	7.9g	0.177de	2.05de	47i	62e	11.5e	3.89ef
FL-1867	41ef	8.6e-g	0.223ab	2.01de	66c	49f	12.2de	3.79f
FL-2002	53d-f	8.4fg	0.180de	1.93e	47hi	71d	11.5e	4.04de

* Means of generally six containers. Means with a letter in common were not different by the Waller-Duncan k -ratio t -test ($k = 100, P = 0.05$). Several means were based on five containers, but those based on fewer are not followed by a letter. Means are means of individual clones, whereas Busey et al., 1982, reported means of groups of several clones.

between racial groups, and in part to errors in measurements. Alternatively, that could have been attributed to the ambiguously classified clones' membership in intermediate racial groups, for which no cultivar standard was used. The small proportion (4%) of plants which were not clearly assignable shows that both aforementioned sources of ambiguity were minimal. But, when discriminant analysis was reapplied, using only the five original vegetative traits, only 118 (49%) of plants could be confidently assigned to groups (prior $P > 0.95$). On the preceding basis, morphological variation of survey samples was distinctive and consistent with the cultivar standards, yet what identification problems occurred were attributable mainly to the choice of characters. Only 7% of all unknowns were assigned by discriminant analysis to either of the Breviflorus Race groups.

Among 134 unknown plants identified visually and for which a complete set of vegetative and floral traits was available, discriminant analysis was 88% consistent compared with visual identification. Most plants for which the two methods of identification were inconsistent were assigned by discriminant analysis to Dwarf and Gulf Coast groups (not considered in the visual identification), and consequently for plants and taxonomic groups considered by both identification methods, there was 95% consistency. When the 242 lawn and sod samples were identified using a simplified sequential key (see Materials and Methods), 225 (93%) of the plants were identified in a manner consistent with the discriminant analysis. Most discrepancies involved plants lacking inflorescences; the key was 96% consistent after those plants were excluded from the comparison.

The high consistency in morphology between lawn and sod samples and certain known cultivars may be seen graphically (Fig. 1). Lawn and sod samples that were visually identified formed clusters of morpho-

logical variation similar to the expected distributions for Floratam, Bitterblue, and Roselawn. (As mentioned previously, Floralawn and Floratine were virtually indistinguishable from Floratam and Bitterblue, respectively, and were not represented in Fig. 1). Florida Common produced only occasional seedheads, and also was not represented. Few lawn and sod samples from Florida corresponded to any other cultivar or taxonomic representative. The Gulf Coast and Dwarf Group (hence, Breviflorus Race) ellipses encompassed few lawn and sod samples (Fig. 1).

Based on ANOVA there was no significant variation in the visually identified Floratam-like plants, except for distance between inflorescence branches. Otherwise, the variation of this group was consistent with the hypothesis that only a single clone, Floratam, was present. The visually identified Bitterblue-like plants varied ($P < 0.05$) in most traits. About one-fourth of the clones identified visually as Bitterblue were outside the expected 95% confidence ellipses for Bitterblue floral traits (Fig. 1) and the probably synonymous Floratine. The most striking characteristics of the visually identified clones were their relatively narrow (mean 10.4 mm wide) leaf blades, and relatively slender (mean 2.61 mm thick) internodes compared with Bitterblue and Floratine. The Roselawn-like clones varied ($P < 0.01$) in all traits except for distance between inflorescence branches. The large morphological variation in this group suggested that it must be composed of more than one clone, possibly also including Florida Common or unnamed clones.

The frequency of St. Augustinegrass cultivars suggested historical and anthropogenic relationships. Sod samples were most often (77%) identified by discriminant analysis as Floratam. This frequency was considerably more than that in lawns (21%). Floratam, released in 1973, was substituted in new construction for older cultivars, but probably did not intensively penetrate preexisting lawns. The insignificant morphological variation of this group was consistent with Floratam's relatively recent release as a single clone. The frequency of Bitterblue (including Bitterblue-like plants) was 31% in lawns, and that of Roselawn-like plants (including Florida Common) was 43%.

Field Identification of Knowns and Unknowns

In 0.8- to 5.0-yr-old field plots of known cultivars, Seville was identified correctly 99% of the time. Stolon thickness was 89% accurate in separating known samples of Roselawn and Florida Common from the polyploids Floratam and Bitterblue, using 2.6 mm as the point of separation. The same separation of known cultivars was 98% accurate based on a qualitative observation of foliage color. A pinkish extension of the anthocyanin into the leaf collar occurred in Floratam and Floralawn, whereas in Bitterblue and Floratine the collars were yellowish white. Identifications were 91% accurate when based on this trait using individual stolons from many field plots of different ages. Thus, collar color appeared to be a relatively stable, qualitative aspect of vegetative coloration, although caution should be used in evaluating it under conditions (e.g., shade or nutritional stress) outside those

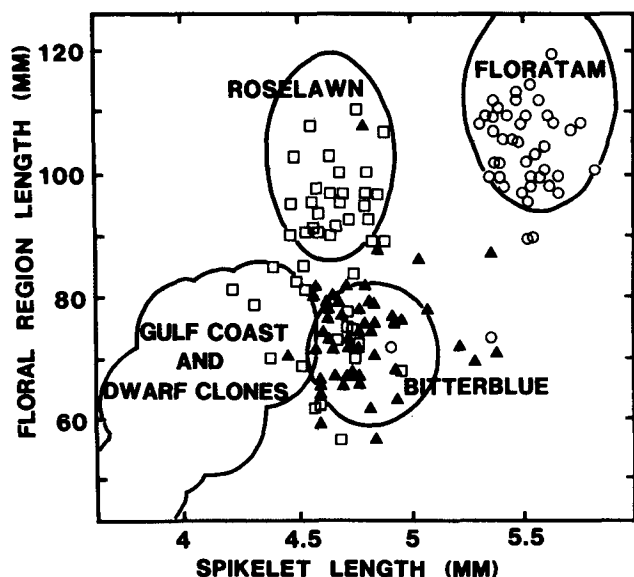


Fig. 1. Bivariate scattergram of floral traits in unknown St. Augustinegrass lawn and sod samples, Floratam-like (○), Bitterblue-like (▲), and Roselawn-like (□). Ellipses are the 95% confidence regions for the actual known cultivars and other taxonomic representatives.

in this study. Altogether, known cultivars were identified with 93% accuracy based on vegetative field characters.

When inflorescences were available on field samples (usually they are not), a more accurate identification was possible. Combined analysis of spikelet and floral region lengths of known cultivars in containers (Table 1) and field plots showed no significant environmental differences, and genotype \times environmental interaction was not significant.

In the initial on-site observations in lawns, the best quantitative vegetative trait, stolon thickness, provided a separation of the Longicaudatus Race vs. the polyploids, which was only 83% consistent with discriminant analysis. Other racial comparisons would have been considerably less consistent. When one or two stolons were examined qualitatively (for internode thickness and foliage color) in field plots of the lawn and sod samples, the Longicaudatus Race was distinguished from the polyploids in the same manner as discriminant analysis in 97% of the comparisons. Collar color was 93% consistent in distinguishing Floratam from Bitterblue and Floratine. Vegetative field identification was altogether 86% consistent with discriminant analysis in assigning lawn and sod samples to the racial groups.

Diagnosis for field samples of knowns and unknowns required an average of 36 s per sample, based on 1090 observations. This was an improvement over prior identification techniques (Busey, 1979), which required growing samples for 2 to 3 months in 5 to 10 replicates, beside a known cultivar.

CONCLUSIONS

Floral traits were extremely useful in St. Augustinegrass identification, when they were available, but qualitative vegetative traits permitted rapid identification of samples from a uniform environment. A multivariate approach, discriminant analysis, and sequential, monothetic keys were highly consistent and/or accurate in identifying unknowns or known cultivars, respectively. Simple keys could be used in sep-

arating broad groupings, but discriminant analysis was more powerful in picking out a few clones which did not belong to any known racial group. The vast majority of unknown Florida St. Augustinegrass samples fell within known racial groups.

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