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The Effect of Drought on Lignin Content and Digestibility of Tifton-85 and Coastal Bermudagrass (*Cynodon dactylon* L.) Hays Produced in Georgia

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Abstract: Digestibility of “Tifton 85” Bermudagrass has been noted to be higher than most other Bermudagrass cultivars. However, the superior digestibility of Tifton-85 has not been verified based on samples from producers, nor is it known how water availability might affect this comparison. Recent past weather conditions in Georgia allowed this comparison. Much of Georgia was in severe drought in 2007 and 2008. In contrast, there was less/no drought in 2006 and 2009. In each of these years, producers submitted a substantial number of Tifton-85 and Coastal forage samples to our laboratory for lignin and “Digestible Neutral Detergent Fiber (dNDF48)” analyses. Over all years, Tifton-85 had lower lignin content than coastal. However, Tifton-85 had significantly lower lignin content only in drought free 2006 and 2009, whereas the lignin content of Coastal was unaffected by drought in 2007 and 2008. The lignin of Tifton-85 increased during these two drought years. Despite this, the dNDF48 for Tifton-85 was significantly higher than coastal in all four years, suggesting that drought had hardly any effect on the digestibility of Tifton-85. Apparently, the type of lignin in Tifton-85 is different from that in coastal. Higher dNDF48 for Tifton-85 has been attributed to its lower concentrations of ether-linked ferulic acid than in Coastal. Decreased ether bonding in lignin results in higher digestion.

Keywords: Bermudagrass, Coastal, Drought, Digestibility, Digestible Neutral Detergent Fiber Lignin Content, Tifton-85

1. Introduction

Warm-season perennial Bermudagrass (*Cynodon dactylon,* L) is grown extensively throughout the southeastern United States for pasture and hay [1]. The stands often persist and remain productive for more than 35 years if properly managed [2]. Most are tolerant to acidic soils, moderate to heavy grazing pressure, variable rainfall distribution, and differing management.

The hybrid bermudagrass “Coastal” released by USDA-ARS, Tifton, GA in 1943, is the most widely grown bermudagrass due to its wide adaptability, persistence, yield, and quality. Coastal is used as a standard for comparison with new bermudagrass selections and hybrids in most breeding program in the southern United States [2]. More than 4×10^6 ha of Coastal bermudagrass are grown in the United States.

Another bermudagrass “Tifton-85”, an F1 hybrid pentaploid, resulting from crossing a stargrass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) “Tifton-68” with a bermudagrass “PI 290884” (in the literature as “Tifton 292”) introduced from South Africa, was also released by USDA-ARS, Tifton, GA in 1993 [3]. The grass is darker green in color, taller, has larger stems with wider leaves, and produces more upright growth than Coastal [3, 4]. Notably, Tifton-85 offers hay production and heavy grazing potential in the year of establishment, whereas most other cultivars do not produce high yields until the 2nd year. In research trials, Tifton 85 has produced among the highest dry matter (DM) yields with greater quality when compared to other grasses under diverse management and environmental conditions [3,5,6,7,8], making it an ideal forage for dairy and beef in the southern US [2,7]. Since its release in 1993, Tifton 85 has been increasing in popularity among hay and cattle producers in the southern US, Central and South America, and Southern Africa. Notably, more than 1×10^6 ha of this grass has been established in Brazil [2].

Tifton 85 produced higher DM with better fiber digestibility than Coastal, resulting in superior animal performance in cattle grazing trials [2, 9, 10, 11]. Although NDF concentrations are often very high in Tifton 85, this grass has been highly digestible in beef and dairy cattle experiments [7, 11, 12, 13, 14]. Additional research has documented increased digestibility of Tifton 85 hay compared with Coastal hay harvested at differing maturity dates [15]. The consistently higher digestibility of Tifton 85 than coastal was attributed to its lower concentrations of ether-linked ferulic acid, because a decreased ether bonding in lignin results in enhanced ruminal microbial digestion of this forage [2, 11, 13, 15].

The reported superior digestibility of Tifton-85 has not yet been verified in on-farm use involving large number of samples grown under different climatic conditions. Global climate change is expected to cause an increase in frequency and severity of drought in the southeastern United States during the next century [16]. It is unclear whether Tifton-85 would maintain higher digestibility than coastal during severe to exceptional/extreme drought. Recent weather conditions in Georgia have granted a unique opportunity to evaluate this. The National Drought Mitigation Center's U.S. Drought Monitor [17] classified a large majority of Georgia in their extreme (D3) to exceptional (D4) drought categories during the 2007 growing season and in a severe (D2) drought category during 2008 (Figure 1). These years had been preceded by one year (2006) and succeeded by one year (2009) of relatively little or no prolonged drought stress. In each year of 2006-2009, a substantial number of Tifton-85 and Coastal forage samples were submitted by forage producers for forage quality analysis including dNDF48 at the University of Georgia's Feed and Environmental Water Lab (FEWL). As a result, a large and robust database exists that could provide insight into the digestibility of Tifton-85 and Coastal during drought years and in years when drought stress was absent or less pronounced. Thus, the objective of this work was to evaluate the digestibility of Tifton-85 and Coastal bermudagrass forage samples analyzed by FEWL during the drought years of 2007 and 2008 and in 2006 and 2009 when drought stress was minimal or absent.

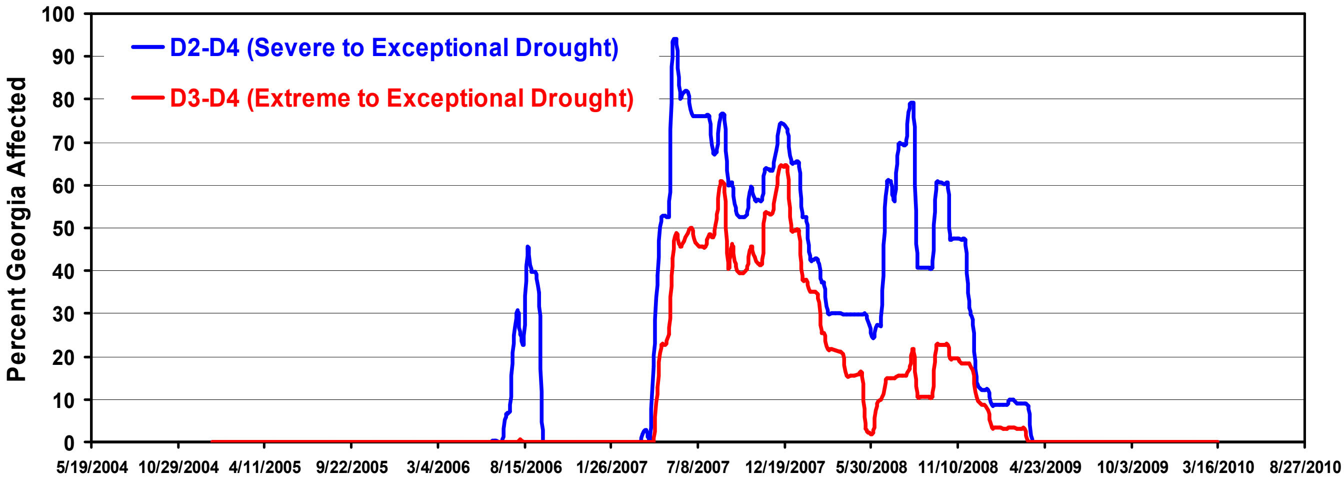

Figure 1. *The percent of the state of Georgia (USA) that was classified by the National Drought Mitigation Center's U.S. Drought Monitor (2009) as being in extreme to exceptional (D3-D4) and severe to exceptional (D2-D4) drought categories between 1 April 2005 and 16 March 2010.*

2. Materials and Methods

This study uses data obtained from the analyses of Tifton-85 and Coastal bermudagrass forage samples submitted to the University of Georgia's Feed and Environmental Water Laboratory (FEWL) from 1 July 2006 through 31 June 2010.

Upon arrival, forage samples were dried for 16 hrs. at 65°C and ground to pass a 1 mm sieve, and stored until further analysis. All samples were analyzed by near infrared spectroscopy (NIRS) using a FOSS NIRSystems model 6500 scanning monochromator (FOSSNIRSystems, Silver Spring, MD). Briefly, samples were packed into the sample holders and scanned from 400 to 2498 nm to collect NIR spectra every 2 nm in reflectance mode. Each sample was scanned 13 times and the results were averaged to produce a single spectrum. The spectral properties of the forage in the NIR regions (wavelength range from 1100 nm to 2500 nm) were used to predict forage quality using an updated version of the calibration equation for mixed hay "50mhy-2.eqa" originally developed and distributed by NIRS Forage and Feed Testing Consortium (http://nirsconsortium.org). The updating of "50gh-2.eqa" was done by the Feed and Environmental Water Laboratory (FEWL), University of

Georgia. As the routine forage analysis of FEWL encounters any unique samples (also called outliers), these samples are analyzed by wet chemistry procedure. The wet chemistry results and the NIR spectral features of the unique samples are incorporated to the consortium equation "50mhy-2.eqa". A modified partial least squares regression is then performed based on the added NIR spectral features and wet chemistry data, and an updated version of the equation is developed, which is then used for the subsequent routine forage analyses. Thus, updating the consortium equation to extend its applicability is a continuous process at FEWL. Range normalization, first derivative, standard normal variate, and detrending are used to remove the variation in spectra caused by unknown sources that tend to increase errors in the calibration model. The best calibration equation is judged by the lowest standard error calibration (SEC) and standard error of cross-validation (SECV), and the highest R^2.

According to the National Drought Mitigation Center's U.S. Drought Monitor [17], a large portion of Georgia experienced extreme (D3) to exceptional (D4) drought during the 2007 growing season and at least a severe (D2) to extreme (D3) drought during the 2008 growing season (Figure 1). These years had been preceded by one year (2006) and succeeded by one year (2009) of relatively little or no prolonged drought stress. The data were segregated into growing seasons based on sample submission date, using an assumption that bermudagrass from an individual growing season would be submitted between 1 June and 31 March of the following year. For example, bermudagrass samples submitted between 1 June 2007 and 31 March 2008 were categorized into the "2007" growing season category. All other observations falling outside of this time frame (i.e., with a sample submission date of 1 April through 31 May) were excluded from the analysis. Tifton-85 and Coastal bermudagrass samples submitted from the 2006, 2007, 2008, and 2009 growing seasons were utilized for this study. This allowed us to compare the forage quality of these two grasses from production situations in four consecutive years, as well as to study the effects of drought on their nutritional quality including lignin content and $dNDF_{48}$. Comparison between any pair of the data set was done based on the significant difference in their means as described below.

For each year, the data was tested for the the conformity to normal distribution using the Anderson-Darling procedure [18] using "QI Macros 2011" software on Excel 2007. All data sets were found to be normally distributed. For any paired comparison, for example between the two grasses in the same year or for the same grass in two different years, the sample sizes were not equal. It was assumed that for any pair of population under comparison, the variances were different and needed to be estimated separately. Therefore, we used Welch's t-test [19] to evaluate whether the population means were different. The t-statistic used was calculated as

$$t = \frac{\overline{X}_1 - \overline{X}_2}{s_{\overline{X}1 - \overline{X}2}} \quad \text{.....................(1)}$$

Where $\overline{X}$ is the sample mean, 1 = group one, 2 = group two.

$$s_{\overline{X}1-\overline{X}2} = \sqrt{\frac{s_1^{\,2}}{n_1} + \frac{s_2^{\,2}}{n_2}} \quad \text{...................(2)}$$

Where s^2 is the unbiased estimator of the variance of the two samples, n = number of samples, 1 = group one, 2 = group two. For use in significance testing, the distribution of the t-test statistic was approximated as being an ordinary Student's t-distribution with the degrees of freedom (*d.f.*) calculated using Welch–Satterthwaite equation [20, 21] as follows.

$$d.f. = \frac{\left(\frac{s_1^{\,2}}{n_1} + \frac{s_2^{\,2}}{n_2}\right)^2}{\frac{\left(\frac{s_1^{\,2}}{n_1}\right)^2}{(n_1 - 1)} + \frac{\left(\frac{s_2^{\,2}}{n_2}\right)^2}{(n_2 - 1)}} \quad \text{..................(3)}$$

3. Results

Drought and Lignin Content

As shown in Figure 1, in the 2007 and 2008 growing seasons, most areas in Georgia experienced severe (D2) to exceptional (D4) drought [18]. These years had been preceded by one year (2006) and succeeded by one year (2009) of relatively little or no prolonged drought stress. The lignin content of Coastal was more or less stable in the years 2006-2009 regardless of the presence (in 2007 and 2008) or absence (in 2006 and 2009) of drought (Figure 2). Such results indirectly support the reported unique adaptability and persistence of Coastal with stable yield, and quality, which established this grass as a standard check cultivar in the breeding trials in the southern United States [2].

In contrast Tifton-85 seems to have a trait of lower lignin content than Coastal. However, Tifton-85 could hold its trait of lower lignin content only in drought free years of 2006 and 2009. In the drought affected years of 2007 and 2008, the lignin content of Tifton-85 increased. But the lignin content of Coastal was more or less unaffected by drought in 2007 and 2008. Under experimental conditions with no soil moisture limitation, Mandebvu et al [11] observed 11% lower (statistically significant) lignin content of Tifton-85 than Coastal. So far no previous study compared the lignin content of these two grasses under drought and drought-free conditions. The outputs of t-test carried out to compare the mean lignin contents of Coastal and Tifton-85 in different years (Table 1) show that Tifton-85 had significantly lower lignin content than Coastal only in the drought free years of

2006 and 2009. In contrast, in the drought affected years of 2007 and 2008, the lignin content of Tifton-85 increased and became statistically identical to Coastal.

Table 1. *Outputs of t-test for comparison of mean lignin content (%) of coastal and Tifton-85 bermudagrass in different years.*

	Drought-free Year		Drought-affected Years				Drought-free Year	
	2006		2007		2008		2009	
	Coastal	Tifton-85	Coastal	Tifton-85	Coastal	Tifton-85	Coastal	Tifton-85
Mean	5.7505	5.2969	5.638	5.382	5.914	5.678	5.684	4.446
Variance	2.31253	2.14476	1.85395	1.993744	1.8706	2.13423	2.36391	1.39429
Observations	313	61	431	111	378	88	394	114
Degrees of Freedom	87		166		124		234	
t-Statistic	2.1958		1.7158		1.3809		9.1704	
P-Value	0.0308		0.088		0.1698		<0.00001	

Drought and dNDF48

The dNDF48 or "Digestible Neutral Detergent Fiber" is the 48-hour *in vitro* digestible fraction of Neutral Detergent Fiber (NDF) expressed as a percentage of the dry matter content. As discussed in the preceding section, in the drought affected years of 2007 and 2008, the lignin content of Tifton 85 increased and became similar to that Coastal (Figure 2 and Table 1). Despite this, dNDF48 of Tifton-85 was higher than that of coastal in all four years studied regardless of the drought situation (Figure 3). Table 2 shows the outputs of the t-test carried out to compare the mean dNDF48 of Coastal and Tifton-85 in different years. The results show that Tifton-85 had significantly higher dNDF48 than Coastal in each the four years considered in this study.

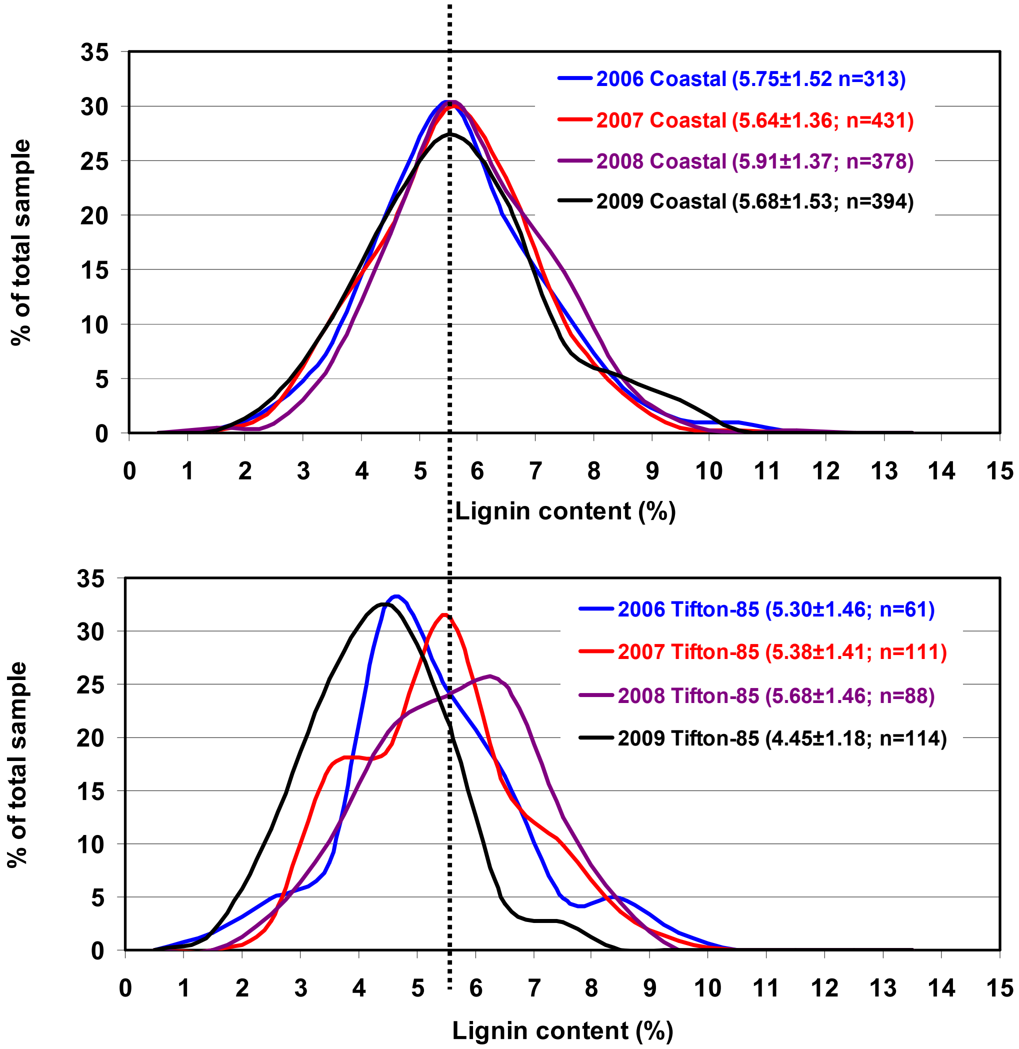

Figure 2. *Frequency distribution of lignin content for the Coastal and Tifton-85 bermudagrass samples analyzed in 2006, 2007, 2008, and 2009.*

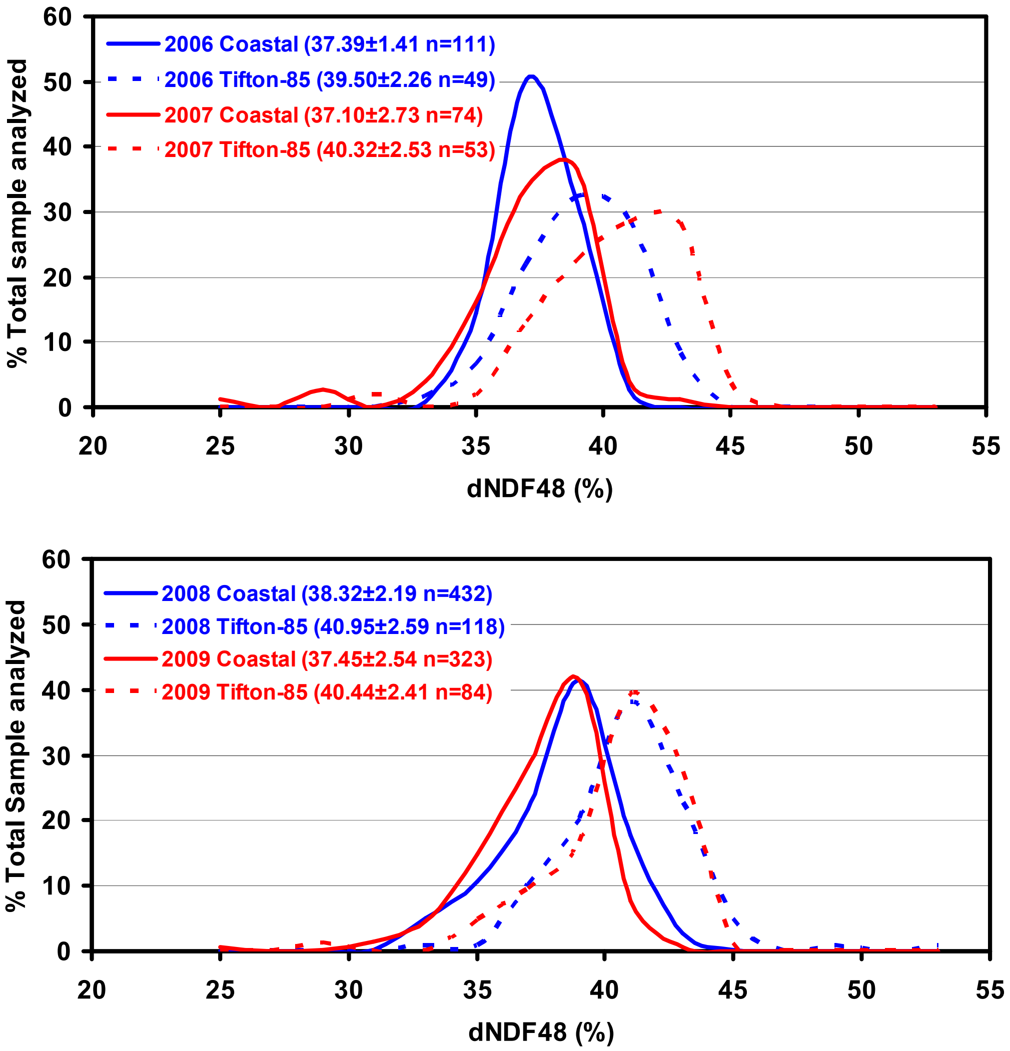

Figure 3. *Year-by-year comparison of dNDF48 between Coastal and Tifton-85 bermudagrass samples analyzed in 2006, 2007, 2008, and 2009.*

Table 2. *Outputs of t-test for comparison of mean dNDF*$_{48}$ *(%) of coastal and Tifton-85 bermudagrass in different years.*

	Drought-free Year		**Drought-affected Years**				**Drought-free Year**	
	2006		**2007**		**2008**		**2009**	
	Coastal	**Tifton-85**	**Coastal**	**Tifton-85**	**Coastal**	**Tifton-85**	**Coastal**	**Tifton-85**
Mean	37.3950	39.5040	37.1030	40.3200	38.3190	40.9460	37.4540	40.4450
Variance	1.9960	5.1261	7.4409	6.4455	4.82198	6.722612	6.4903	5.814368
Observations	111	49	74	53	432	118	323	84
Degrees of Freedom	65		116		165		135	
t-Statistic	6.0240		6.8258		10.0651		10.0067	
P-Value	<0.00001		<0.00001		<0.00001		<0.00001	

5. Discussion

In general, most plants tend to increase their cell wall components, particularly lignin to limit the transpiration during water stress due to drought. In this study we observed this effect of drought more on Tifton-85 and less on the coastal bermudagrass grown in Georgia, USA. Such a differential response to drought between these two cultivars was probably due to the fact the coastal bermudagrass is a native cultivar that is well adapted to environmental variations. Generally, a higher lignin content is associated with a lower digestibility of neutral detergent fiber (dNDF48). However, we found it to be opposite in case of Tifton-85. Despite the higher lignin build-up in Tifton-85 during the drought affected years as compared to coastal, Tifton-85 had a dNDF48. This result suggests that lignin composition in Tifton-85 is probably different from that in Coastal under real production situation as observed by the breeders under experimental conditions [7, 11, 13, 14, 15].

Higher dNDF48 for Tifton-85 was attributed to its lower concentrations of ether-linked ferulic acid content than Coastal. Decreased ether bonding in lignin of Tifton-85 results in higher ruminal microbial digestion of this forage [2, 11, 13, 15].

6. Summary

This study examined the effect of drought on the lignin content and dNDF48 under state wide growing conditions in Georgia in the years 2006, 2007, 2008, and 2009. The results demonstrated that Tifton-85 could hold its well-known trait of lower lignin content than Coastal only in the drought free years of 2006 and 2009. In the drought affected years of 2007 and 2008, the lignin content of Tifton-85 increased and became statistically identical to that of Coastal. However, Tifton-85 consistently documented its higher dNDF48 than Coastal regardless of the drought situation in the growing seasons. This supports the fact that lignin in Tifton-85 is compositionally different than that in Coastal as observed by earlier investigators under experimental conditions.

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