EFFECT OF HARVEST DATES ON BIOMASS ACCUMULATION AND COMPOSITION IN BIOENERGY SORGHUM

A Thesis

by

DUSTIN ROSS BORDEN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2011

Major Subject: Agronomy

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Approved by:

Chair of Committee, William Rooney
Committee Members, Juerg Blumenthal
John Mullet
Head of Department, David Baltensperger

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ABSTRACT

Effect of Harvest Dates on Biomass Accumulation and Composition in Bioenergy Sorghum. (December 2011)

Dustin Ross Borden, B.S., Texas A&M University

Chair of Advisory Committee: Dr. William L. Rooney

Sorghum (*Sorghum bicolor*) has the potential to be used as a cellulosic feedstock for ethanol production due to its diversity and wide adaptation to many different climates. With a wide range of diversity, this crop could be tailored specifically for use as a feedstock for ethanol production. Other factors such as water use efficiency, drought tolerance, yield potential, composition, and established production systems also make sorghum a logical choice as a feedstock for bioenergy production. The objectives of this study were to better understand the biomass potential of different types of sorghum that may be used for energy production, and determine the composition of these sorghums over the season to better understand biomass yield and composition over time.

Six commercial sorghum cultivars or hybrids that represent sorghum types from grain to energy were evaluated near College Station, Texas during the 2008 and 2009 cropping years. An optimal harvest window (defined by maximum yield) was established for all genotypes, and significant variation was seen among the genotypes for fresh and dry biomass production. The later maturity genotypes, including the photo-period sensitive and modified photo-period sensitive type sorghums, produced the highest yields (up to 24 dry Mg/ha).

Compositional analysis using near infrared reflectance spectroscopy (NIR) for lignin, hemicellulose, and cellulose was performed on a dry matter basis for the optimal harvest window for each genotype. Significant differences were seen in 2009 between the genotypes for lignin, hemicellulose, cellulose, ash and protein; with the earlier genotypes having higher percentage of lignin, and the later genotypes having lower percentages of lignin. Genotype x Environment interactions were also seen, and show the significance that rainfall can have.

Based on this research, grain sorghum could be harvested first, followed by photo-period insensitive forage varieties, then moderately photo-period sensitive forage varieties followed by dedicated bioenergy sorghums (that are full photo-period sensitive), allowing for a more constant supply of feedstock to processing plants. Sweet sorghums would also allow the end user to obtain biomass when needed, however these types of sorghum may be much better suited to a different end application (i.e. crushing the stalks to obtain the juice).

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

As global population increases so does global energy consumption. With these increases, the demand for transportation fuel also rises, and petroleum stocks are finite resources. Thus, the world must identify and develop the alternative forms of energy that will eventually be used to fuel future societies. To meet future demand, the Department of Energy (DOE) recommends the use and development of biomass crops as raw material for transportation fuel production (DOE 2006). Their use is mandated in the Energy Security Act of 2007 which requires a minimum of 30 percent of fossil fuel be replaced by renewable fuel sources by 2030. More specifically, the DOE recognizes that our ability to produce alternative fuels from established sources (eg, corn starch) are limited; therefore, they have also required the production of ethanol (or other energy) from ligno-cellulosic biomass (DOE 2006). Of the 36 billion gallons of renewable fuel mandated by the act in 2022, 21 billion gallons must be derived from cellulosic sources (Sissine 2007). With such a large demand for renewable fuel, not only are conversion methods necessary, new sources of biomass will be needed. In addition, these sources must not compete with feed or food sources, and they must supply a sufficiently large quantity of biomass at a price low enough to compete with traditional fuel sources.

A billion ton study conducted by the DOE and USDA that used forest and agricultural resources as primary biomass sources indicated that the US is capable of producing approximately 1.3 billion tons of biomass annually (Perlack et al., 2005). Within the agricultural resources section of this study, ethanol production from corn grain is expected to plateau at 15 billion gallons; the remainder of ethanol production must be derived from non-starch carbohydrate sources. While crop residue will provide an estimated five billion gallons, dedicated bioenergy crops will be needed to meet the remaining expected renewable fuel demand (Sissine 2007).

This thesis follows the style of Agronomy Journal.

Given the diversity of environments, there is no single feedstock that can meet the remaining goal; many dedicated bioenergy crops will be needed to produce the required amounts of biomass to meet the goals specified in the Energy Independence and Security Act of 2007. In addition, these dedicated bioenergy crops must fit into established cropping systems and ideally, have the ability to utilize lands otherwise not suited for food and feed production.

Over several years, the DOE and USDA have evaluated numerous potential herbaceous feedstocks. Several factors, including but not limited to yield, composition and production logistics were considered and used to identify the species with the most promise for scalable production of biomass. The four most commonly mentioned energy crop species for the U.S. are energycane (*Saccharum spp.* L.), switchgrass (*Panicum virgatum* L), miscanthus (*Miscanthus* Andersson), and sorghum (*Sorghum bicolor* L. Moench) (DOE 2009). In addition, the mixed grasses from the Conservation Reserve Program (native grasses) are also mentioned in US DOE reports. Of these potential feedstocks, sorghum serves a unique niche; it is the only annual crop and it has a long established agronomic production history.

While technically a perennial that is killed by freezing temperatures, sorghum is managed as an annual crop and is currently grown for the production of grain and forage for feed and fodder systems. Sorghum is adaptable to many climates, and has traditionally been grown in semi-arid regions of the world where rainfall is limited. For these reasons, sorghum has a long history as a valuable food and feed crop, but it also has the potential to be used as a bioenergy crop. The factors that make sorghum an obvious choice for bioenergy production include; (1) yield potential and composition; (2) water use efficiency and drought tolerance; (3) established production systems; and (4) the potential for genetic improvement using both traditional and genomic approaches (Rooney et al., 2007). All types of sorghum (grain, sweet, forage, energy) have the ability to be used in the production of ethanol if the above factors are considered.

Much of the potential processing and modeling for sorghum as a bioenergy feedstock comes from the forage, and sugarcane industries. Forage sorghum cultivars and hybrids have been used and improved for more than 100 years; they now provide the

basis for breeding sorghum for biomass accumulation and energy conversion. Sweet sorghums are specific types that accumulate higher amounts of sugars in the stalks than grain and forage sorghums. These sugars are extracted with the juice by crushing the stalks using the same processes as those for sugarcane. In fact, the sugarcane industry provides a processing model and an opportunity for sweet sorghum to supplement sugar production of sugarcane for ethanol production. To date, sweet sorghum has been used for artensenal syrup production; future industrial use will likely be for sugar production in a crop rotation system with sugarcane. The baggasse that is produced from crushing the stalks can also be utilized as fuel for co-generation of electricity and/or for cellulosic ethanol production.

Biomass sorghums used for lignocellulosic biomass are photoperiod sensitive (PS) types that remain in a vegetative growth stage for most of the growing season in subtropical and temperate climates (Rooney et al., 2007). This vegetative growth habit is crucial to enhancing biomass yields. In these hybrids, the onset of floral development does not occur until day lengths shorten to a fixed length and this delay in flowering allows the crop to capture and convert solar energy throughout the growing season, resulting in higher biomass accumulation. In temperate environments, the parental lines must be developed using the Ma5/Ma6 genetic system employed in some forage sorghum hybrids (Rooney and Aydin, 1999). This system uses photoperiod insensitive (PI) lines as parents to produce PS hybrids. While yield of these hybrids is the primary focus, the structural composition of these PS sorghums is also important, but is not as influential as with forage sorghums used in feed and fodder systems.

Before wide-scale production of bioenergy sorghum hybrids can be implemented, there are significant management and production issues that must be addressed. Specifically, the optimum harvest time to balance productivity, composition, and nitrogen use are significant issues. With grain sorghum, harvest is based on physiological maturity of the grain. With forage sorghums, producers will usually allow the crop to reach the early stages of flowering to attain a maximum balance between yields and forage quality. An additional issue is the management and transport of biomass once harvested. While that is beyond the scope of this study, moisture content

does highly influence these logistics factors. At this time, relatively little is known about energy sorghum harvest optimization and how different harvest systems will influence total yield and quality.

With these factors in mind, the objectives of this thesis are:

1. To quantify biomass potential of energy sorghums over a growing season.
2. Compare dry matter and total biomass potentials over the growing season of different types of sorghum (grain, forage, sweet, and biomass).
3. To establish optimal time to harvest based on biomass accumulation and composition.
4. Compare and analyze sorghum biomass composition in different types of sorghum over the course of a growing season.

CHAPTER II

BIOMASS ACCUMULATION OF ENERGY SORGHUMS

Introduction

Sorghum is traditionally known for grain production; it is the fifth most widely grown and produced cereal crop in the world (FAO, 2006). However, in many regions of the world, sorghum is equally, if not more, important as a forage crop. While accurate statistics for forage use are not available, in the US the amount of commercial forage sorghum seed that is sold annually would indicate that the acreage of forage sorghum exceeds that of grain sorghum. In addition to forage and grain, there are sorghum types with high stalk sugar content, and extremely lignified types (for structural building).

Currently, almost 30% of the U.S. grain sorghum is used for ethanol production (USCP, 2009). Ethanol yields from grain sorghum are comparable to those derived from corn (Rooney et al., 2007). The stover from grain sorghum could also be used for production of ethanol through cellulosic conversion once the grain has been harvested. On average, grain sorghum will mature and reach its maximum dry matter content in 100 to 120 days after planting. Vanderlip and Reeves (1972) studied grain sorghum to define the stages of growth, and showed that in Kansas it took 95 days after emergence to reach physiological maturity and have maximum dry matter accumulation. This is a typical response in temperately adapted grain sorghums which are bred to produce grain.

Forage sorghums are grown and produced for grazing, hay and/or silage. For each use, there are specific hybrids that are recommended depending on end use. These sorghums are bred for higher total biomass yield, palatability and digestibility for use in a feeding system. For grazing and hay production, a higher leaf to stem ratio is desirable as leaf biomass is of higher quality and palatability. Protein concentrations are higher when plants are younger, therefore grazing should occur before plants are in the reproductive stage. Early grazing/haying will also allow for a ratoon crop to be cut later in the season. In systems where ensilage is the product, producers will prefer a hybrid with grain yields which amount to up to a third of total biomass, in order to maximize

feeding efficiency. Fermentation must occur when ensiling forage sorghum, thus carbohydrate and sugar concentration should be monitored to have adequate fermentation (McCormick et al. 1995).

Because of their growth pattern and biomass accumulation, it is logical to assume that forage sorghums serve as the initial energy sorghums. However, in an industrial processing system, palatability of the feedstock is unimportant and the definition of a high quality energy crop may be quite different than for animal feeding. In addition, low protein content in the biomass is highly desirable in energy sorghum; it means that less N has been removed from the soil profile on a per ton basis. Venuto and Kindiger (2008) evaluated hybrid forage sorghum and sorghum-sudangrass hybrids in a single and double cut system and they demonstrated that 28.3 Mg ha fresh weight could be acquired in a double cut system where the ratoon crop was harvested shortly after first frost. McCormick et al. (1995) reported that as harvest was delayed total dry matter yield increased. The highest dry matter yield of 7.9 Mg ha was reported at the bloom stage in a double cut system and 7.2 Mg ha at hard dough in a single cut system (McCormick et al. 1995).

Sorghum as a feedstock for bioenergy production is not a new concept. Monk et al (1984) evaluated 45 different cultivars of sweet sorghum, grain sorghum, and energy sorghum over three years to assess the biomass yield potential. Of this group, the sweet sorghums were shown to accumulate the highest fresh weight yields and this advantage was primarily attributed to maturity. Therefore Monk et al. (1984) concluded that higher yields could be obtained from high energy sorghums by increasing the length of the growing season. It is well known that, in the absence of stress, delayed maturity and increase height produces higher biomass yield. Miller and McBee (1993) demonstrated that fact, showing that 26 Mg ha or more could be produced by using the correct hybrid and harvest management plan.

Given its impact on total biomass yield, maturity (defined as mature seed production) is likely the single most important factor that influences biomass productivity and quality, in sorghum. Evolutionarily, most sorghums are photoperiod sensitive and this system presumably evolved to capture the benefits of seasonal weather

(Rooney, 2000). Because of the importance of this trait, there has been significant effort to characterize and utilize maturity genes in sorghum improvement programs. These locis are collectively known as the *Ma* loci. While both maturity and photoperiod sensitivity are designated as *Ma* loci, they have distinctly different actions and phenotype. Maturity genes, per se, influence days to flowering that do not involve photoperiod reactions, while photoperiod sensitivity genes are likely regulatory factors that react specifically to the day length. Six major *Ma* loci are highly heritable and easy to manipulate in a sorghum breeding program (Quinby, 1974; Rooney and Aydin, 1999). With these *Ma* loci, sorghum hybrids can be developed with an array of maturities.

By effective use of photoperiod sensitivity genes, it is possible to create very photoperiod sensitive sorghum hybrids from two photoperiod insensitive sorghum lines (Rooney and Aydin, 1999). This PS reaction is caused by the epistatic interaction of alleles at the Ma_5 and Ma_6 loci. This epistatic interaction maintains vegetative growth until day lengths drop below 12 hours and 20 minutes. This system and its phenotypes allow full season production and also maximize yield of ligno-cellulosic material while minimizing the effect of short droughts during the growing season (Rooney et al. 2007). In addition, the genetic system allows for production of PS hybrids to occur in areas where sorghum hybrids are currently produced such as the high plains of Texas.

Like PS hybrids and forage sorghum hybrids, sweet sorghum hybrids will also have a role in bioenergy production. While the concept of energy production from sweet sorghum is not new, the actual application of and use of sweet sorghum in an industrial setting is just beginning. For that reason, initial production of sweet sorghum for energy will likely be complementary to sugarcane production where it can be grown as a rotational crop that requires a shorter season and extended harvest season. Like sugarcane, sweet sorghum hybrids will be harvested with much higher moisture percentage and high sugar concentration; this biomass must be processed to prevent the loss of fermentable sugar. Shih et al. (1981) reported 34.4 and 31.6 metric tons/ha of fresh biomass from sweet sorghum, with 84.5 percent of this weight coming from the stalks. This high percentage shows that sweet sorghums are a viable option for rotation with sugarcane. However yields must be comparable to a single season of growth with

sugarcane to make it feasible to have rotation with sweet sorghum. Dolciotti et al. (1998) compared a sweet and a fiber sorghum and showed that the sweet sorghum accumulated more total fresh biomass (127.36 ton/ha) than did the fiber sorghum (100.21 ton/ha), however on a total dry biomass basis the two varieties were not different (27.59 and 27.57 respectively). This data shows that sweet sorghums do have the potential for high yields and still produce enough dry biomass to compete with fiber sorghums.

With these factors in mind, the objectives of this study are:

1. To quantify biomass potential of energy sorghums over a growing season.
2. Compare dry matter and total biomass potentials over the growing season of different types of sorghum (grain, forage, sweet, and biomass) accumulate.
3. To establish optimal time to harvest based on biomass accumulation.

Materials and Methods

The US DOE funded the Regional Biomass Energy Feedstock Partnership (RBFT) to quantify yield potential for many different types of herbaceous energy crops. Sorghum was identified as one of five crops for evaluation. Since 2008, sorghum trials are conducted annually at seven different environments across the U.S. This trial contains four replications, with six entries per replication. Entries were planted into four row strips through the field; varying in length depending on field location and size. All entries were planted using a vacuum planter to maintain proper seeding rates. Harvest timing for this trial was based on maturity, loosely defined as optimum yield for the type and management system utilized. Regrowth was harvested if seasonal conditions allowed.

Experimental Design

All six entries from the RBFT were planted in a randomized complete block trial with two replications (Table 2.1) adjacent to the primary RBFT trial in College Station in 2008 and 2009. Each variety was planted as a block three rows wide for the length of the field; in 2008 the length was 174 meters and in 2009 the length was 221 meters. Plant populations were controlled with a vacuum planter to maintain proper seeding

rates. The seeding rates for this trial were 75,000 seeds per acre for Graze All and 98456, while all other genotypes were planted at a rate of 60,000 seeds per acre. Row spacing for this trial was 0.76 meters and agronomic practices standard for sorghum were used. A total of 330 kg ha^{-1} of 10-34-0 fertilizer with an additional 22 kilograms of zinc was pre-plant incorporated; three weeks after planting, an additional 175 kg ha^{-1} of 32-0-0 fertilizer was side-dressed incorporated. The trial was grown under rainfed conditions. The entries in the test were the same in both years with the exception of 84G62, which was replaced by the bioenergy sorghum hybrid TAMX8001 in 2009 (Table 2.1).

Table 2. 1. **Entries used**

Genotype	Commercial Application	Growth Habit	Source
84G62†	Grain Sorghum	Photo Period Insensitive	Pioneer, Inc.
M81E	Sweet Sorghum	Modified Photo Period Sensitive	University of Kentucky
Sugar T	Silage Sorghum	Photo Period Insensitive	Advanta, Inc.
Graze All	Forage Sorghum	Photo Period Insensitive	Advanta, Inc.
98456	Forage Sorghum	Modified Photo Period Sensitive	Advanta, Inc.
22053	bmr Forage Sorghum	Modified Photo Period Sensitive	Advanta, Inc.
TAMX8001‡	Bioenergy Sorghum	Photo Period Sensitive	Texas Agrilife Research

† Planted first year only

‡ Planted second year only

Data Collection

To determine the optimum harvest times for each entry and to develop a growth curve, weekly sampling was initiated as soon as the first entry reached a height of 0.30 meters; in 2008 the initial harvest was on May 15th (48 days after planting), and in 2009 the initial harvest was on June 11th (55 days after planting). In 2008 hurricane Ike passed over the research farm on September 13-14, effectively ending the season early due to extreme lodging and plant destruction. In 2009 harvest were completed until a killing frost in late October. The 2008 final harvest was on September 12th (168 days after planting) and in 2009 the final harvest was on October 22 (188 days after planting).

At each harvest, 1.21 meters was harvested from the middle row (of three), and three additional stalks were randomly chosen from the row to determine moisture content and provide a sample for compositional analysis. All harvested plots were processed immediately in the following manner: (1) fresh plot weight recorded, (2) leaves were stripped from stalks, weighed, and sub-sampled, (3) panicles (if present) were cut from stalks, weighed, and sub-sampled, (4) stalks were weighed and then crushed in a three roller field sugar mill and baggasse was sub-sampled, (5) and a 15 ml juice sample was collected. From this juice sample, total soluble concentration was measured using an ATAGO digital refractometer (brix). In addition, the three stalk sample was processed in a wood chipper and a fresh grab sample was taken, weighed and dried in a forced air, convection dryer for three days at 48°C. Percent dry matter was determined by dividing oven dry sample weight by fresh sample weight and multiplying by 100. For all plots, plant height and days to anthesis were recorded as agronomic data. Plant height was measured at each harvest until flowering (anthesis); while days to anthesis was recorded as the date when half the plot had reached mid panicle flowering.

All measurements (fresh plot weights, leaf weight, panicle weight, stalk weight, etc.) were analyzed to find significance between genotypes, based on the optimal harvest window, and also to determine if processing procedures (i.e. stripping leaves, cutting panicles, etc) were relevant to the trial. Percent stalk is a combination of stalk weight and panicle weight, while leaf weight was calculated from this measurement.

To estimate ratoon crop yield, one-third of the trial that was not used for primary cut harvest was clear-cut with a self propelled forage harvester at a specific date in mid August. Clear cutting for ratoon crop was necessary for a consistent timeline comparison of all genotypes in the study and is not the optimum for any specific entry. In 2008, the clear-cut harvest was completed on August 11, and in 2009, it was performed on August 18. Harvest dates for the ratoon growth in 2008 were from 18 to 130 days after clear cutting, and 23 to 107 days after clear cutting for 2009. At each harvest, the process for data collection was the same as that used for the primary harvest.

Weather data was collected for both years from the USDA/ARS field 14 weather station. This station is located at Latitude 30° 31' 28.8192"N, Longitude 96° 24' 7.5888"W, more information about this weather station can be found at http://apmru.usda.gov/weather.

Statistical Analysis

For comparison purposes, optimum yield for each entry was based on the highest dry matter yield reported from the weekly yield data. This peak yield time was defined as the optimum harvest window for each genotype. Because harvest is likely to occur over a series of weeks due to weather issue and mechanical logistics, the optimal harvest window includes the yield data for one week before and after peak yield.

For comparison purposes, all data is reported in days after planting (DAP) for primary harvest and days after the clear cutting (DAC) for the ratoon harvest. DAP represents the number of days from planting to harvesting (for the primary harvest); while DAC represents the number of days after the primary harvest was clear cut, establishing an accurate starting date for all genotypes for ratoon growth. Peak data for each entry was analyzed by environment (each year). A combined analysis across both years was performed using the five entries grown in both years (84G62 and TAM8001 were not included). A student's *t* means comparison test was conducted to show significance between the genotype means.

To track the growth rate of the six entries, regression analysis was performed for each year (2008 and 2009), for both primary harvest and ratoon harvest. The independent variables used in the model include days after planting for primary harvest (DAP) (days after clear cutting for ratoon harvest (DAC)), Genotype, DAP (DAC) x Genotype, and DAP x DAP (DAC x DAC). The independent variable DAP x DAP (DAC x DAC) is the term for the type two regression used. Dependent variables include fresh and dry biomass for both primary and ratoon harvests.

Results and Discussion

Optimum Yields Based on Dry Biomass Yield

The optimum harvest time for the six entries grown in 2008 ranged from day 70-168 DAP (Table 2.2). At these times, the dry biomass yields ranged from 11.8 to 24.2 Mg ha^{-1} (Table 2.2). Significant variation was detected among genotypes for biomass yield (fresh and dry), panicle weight, moisture content and plant height (Table 2.3).

In general, optimum fresh yields and dry yields were consistent (i.e., optimum dry yield harvest corresponded with optimum fresh yield harvest), but there were several exceptions. For example, 84G62, a grain sorghum hybrid, produced optimal dry yield between 139-154 DAP. However, the maturity of this grain sorghum hybrid (defined as past black layer on the grain) occurred 35 days after flowering (105 DAP). The difference was likely due to biomass regrowth that occurred after grain maturity, resulting in slightly higher total yields than occurred solely at grain maturity. There was significant variation for moisture content among the entries, primarily due to lower moisture content in 84G62. Variation in panicle weight was expected as some of these hybrids do not produce panicles that compare to the high yield of a grain sorghum hybrid. Days to maturity were not analyzed as they were strongly influenced by optimum harvest date, but most of the entries had flowered by the time optimum dry weight was produced. The exception was M81E, a sweet sorghum, which is designed to produce sugar and was among the latest entries in the trial. Plant height at maximum yield varied from 1.2 to 2.9 meters in height (Table 2.2).

Table 2. 2. Days after planting (DAP), harvest month, growth stage, fresh biomass (Mg/ha), dry biomass (Mg/ha), % stalk, % leaf, panicle weight (Mg/ha), % moisture, height, and days to flowering. Numbers represent the means for the optimal harvest window for each genotype based on dry biomass yield in 2008. Letters designate significant differences between genotypes

Genotype	DAP	Harvest month	Growth stage†	Fresh biomass	Dry biomass	% Stalk	% Leaf	Panicle weight‡	% Moisture	Height	Days to Flower
98456	147-168	Aug./Sept.	M	65.25^{a}	24.23^{a}	79.14	20.86	0.05^{d}	62.52^{ab}	2.71^{b}	139
M81E	132-147	August	F	55.32^{ab}	19.29^{ab}	84.26	15.74	1.09^{cd}	65.52^{ab}	2.65^{b}	132
22053	139-154	August	M	45.18^{bcd}	16.61^{bc}	74.49	25.51	3.13^{bc}	62.92^{ab}	2.66^{b}	97
84G62	139-154	August	M	30.53^{d}	16.00^{bc}	75.43	24.57	9.44^{a}	47.09^{c}	1.21^{d}	70
Sugar T	139-154	August	M	46.54^{bc}	15.87^{bc}	82.19	17.81	3.81^{b}	60.21^{b}	2.89^{a}	104
Graze All	70-83	June	M	38.01^{cd}	11.84^{c}	82.83	17.17	5.47^{b}	69.28^{a}	2.34^{c}	76

† M = physiologically mature (defined as past black layer on the grain) , F = flowering

‡ % stalk includes panicle weight

Table 2. 3. Mean squares for ANOVA of fresh weight, dry weight, % stalk, % leaf, panicle weight, % moisture, height, and days to flower for the optimal harvest window for each genotype based on dry biomass yield in 2008

	Fresh weight		Dry weight		% stalk†		Panicle weight		Moisture		Height		Days to Flower	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	5	888.59**	5	111.36*	5	93.46	5	67.69**	5	345.88**	5	2.22**	5	25548.80**
Rep	1	340.45	1	178.34*	1	2.95	1	0.47	1	69.32	1	0.01	1	21.33**
Error	27	150.70	27	31.98	27	44.66	29	5.14	27	33.42	27	0.007	185	3.0

*,** Significant at p = .05 and .01, respectively

† % leaf numbers exactly the same as % stalk, therefore were excluded from table

In 2009 the optimum dates for dry matter yield accumulation for the six entries ranged from day 104-160 (Table 2.4). This was later than in 2008, presumably due to the increased rainfall during the summer months of 2009 (Table 2.5). During this period of time, the dry biomass yields ranged from 12.2 to 21.8 Mg ha^{-1}, and these numbers were very comparable to those observed in 2008 (Table 2.4). The increased moisture in 2009 and its distribution allow for a longer growing season; optimal yields for the genotypes were spread across the growing season and more indicative of each genotypes potential due to the increase and distribution of rainfall. Significant variation was detected among genotypes for biomass yield (fresh and dry), panicle weight, and moisture content and plant height (Table 2.6). All genotypes reached peak biomass yields ranging from early August to the middle of September; while TAMX8001 was the only genotype that had not flowered and was still in vegetative growth. TAMX8001 produced the largest amount of dry biomass overall, occurring late in the season and roughly 40 days before it flowered (Table 2.4). The hybrid is PS and grows vegetatively until very late in the growing season (mid-October).

Table 2. 4. Days after planting (DAP), harvest month, growth stage, fresh biomass (Mg/ha), dry biomass (Mg/ha), % stalk, % leaf, panicle weight (Mg/ha), % moisture, height, and days to flowering. Numbers represent the means for the optimal harvest window for each genotype based on dry biomass yield in 2009. Letters designate significant differences between genotypes

Genotype	DAP	Harvest month	Growth stage†	Fresh biomass	Dry biomass	% Stalk	% Leaf	Panicle weight‡	% Moisture	Height	Days to Flower
TAMX8001	125-146	Aug./Sept.	V	71.38^{a}	21.83^{a}	81.49	18.50	0.00^{d}	69.14^{ab}	2.11^{d}	188
M81E	132-160	Aug./Sept.	V/F	59.38^{ab}	16.23^{ab}	85.39	14.60	0.09^{cd}	72.97^{a}	2.03^{e}	146
98456	118-132	August	F	59.23^{ab}	16.13^{ab}	81.68	18.31	1.48^{abc}	72.62^{a}	2.05^{e}	125
Sugar T	104-118	August	M	52.31^{abc}	14.55^{b}	87.04	12.96	1.90^{ab}	72.25^{a}	2.92^{a}	97
Graze All	118-132	August	M	32.99^{c}	12.25^{b}	85.10	14.89	2.76^{a}	64.11^{b}	2.41^{c}	69
22053	132-160	Aug./Sept.	M	40.59^{bc}	12.15^{b}	83.96	16.03	0.70^{bcd}	70.02^{a}	2.51^{b}	105

† V = vegetative, F = flowering, M = physiologically mature (defined as past black layer on the grain)
‡ % stalk includes panicle weight

Table 2. 5. Year and inches of rainfall by month for College Station, Texas. Weather Data from the USDA/ARS Weather Station Located: Latitude 30° 31' 28.8192"N, Longitude 96° 24' 7.5888"W

Month	Year	
	2008	2009
	Inches	
March	3.46	4.21
April	2.24	5.47
May	4.63	2.13
June	0.68	0
July	0.14	0.48
August	6.11	1.05
September	3.83	6.62
October	1.36	8.79
Total	22.45	28.75

Table 2. 6. Mean squares for ANOVA of fresh weight, dry weight, % stalk, % leaf, panicle weight, % moisture, and height for the optimal harvest window for each genotype based on dry biomass yield in 2009

	Fresh weight		Dry weight		% stalk†		Panicle weight		Moisture		Height		Days to flower	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	5	1165.63**	5	76.32*	5	25.28	5	7.10**	5	66.95*	5	0.71**	5	44483.28**
Rep	1	114.63	1	19.12	1	75.36	1	1.87	1	3.42	1	0.01	1	4.34
Error	29	307.26	29	31.15	23	21.99	29	1.53	27	20.07	29	0.02	162	140.3

*,** Significant at p = .05 and .01, respectively

† % leaf numbers exactly the same as % stalk, therefore were excluded from table

Combined analysis of the five entries grown in both years revealed a significant Genotype x Environment interaction for % moisture and height; genotypes were different for fresh weight and days to flower while no differences were detected for environment (Table 2.7). Over the two years, fresh biomass yield averaged 49.4 and ranged from 35.5 to 62.2 Mg ha-1 (Table 2.8). The highest yielding entry was 98456 (TAMX8001 was higher yielding but it was included only in 2009 and thus not in the combined analysis), and yielded 71.3 fresh Mg ha-1 of biomass. Dry biomass yield averaged 15.9 and ranged from 20.2 to 12.1 Mg ha-1(Table 2.8). The highest yielding entry was 98456 (TAMX8001 was higher yielding but it was included only in 2009 and thus not in the combined analysis), and yielded 21.8 dry Mg ha-1 of biomass. The average yields reported herein are consistent with yields reported in previous studies that have evaluated sorghum as a bioenergy crop (Corn, 2009; Packer, 2010; (Propheter and Staggenborg, 2010)). In general there was good correlation between fresh and dry biomass yields, indicating that moisture content did not vary excessively (discussed further in the moisture content).

Table 2. 7. Mean squares for ANOVA of fresh weight, dry weight, % stalk, % leaf, panicle weight, % moisture, and height for the combined analysis of the 2008 and 2009 optimal harvest window for each genotype based on dry biomass yield

	Fresh weight		Dry weight		% Stalk†		Panicle weight		% Moisture		Height		Days to flower	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	Mean square
Genotype	4	1390.82*	4	118.2	4	62.58	4	26.12	4	20.41	4	0.21	4	8692.50**
Environment	1	20.25	1	163.74	1	239.1	1	26.26	1	544.34	1	0.12	1	21.6
GxE	4	94.09	4	31.6	4	29.03	4	8.39	4	130.84**	4	0.81**	4	410.1**
Rep(Environment)	2	185.26	2	71.26	2	63.63	2	1.34	2	8.36	2	0.007	2	48.00**
Error	48	187.29	48	30.11	48	37.92	48	3.42	48	25.61	48	0.40	48	4.50

*,** Significant at p = .05 and .01, respectively

† % leaf numbers exactly the same as % stalk, therefore were excluded from table

Table 2. 8. Fresh weight, dry weight, % stalk, % leaf, panicle weight, % moisture, height and days to flower. Numbers represent the means for the combined analysis of the 2008 and 2009 optimal harvest window for genotype and environment based on dry biomass yield

Genotype	Fresh weight	Dry weight	% Stalk	% Leaf	Panicle weight†	% Moisture	Height	Days to Flower
98456	62.25[a]	20.18	80.41	19.59	0.77	68.34	2.39	132.00[b]
M81E	57.35[ab]	17.77	84.83	15.17	0.60	69.25	2.34	139.00[a]
Sugar T	49.43[bc]	15.21	84.05	15.94	2.86	66.23	2.67	100.50[c]
22053	42.89[cd]	14.39	79.23	20.77	1.92	66.48	2.40	101.00[c]
Graze All	35.50[d]	12.05	83.97	16.03	4.12	66.70	2.37	72.50[d]
Environment								
2008	50.06	17.57	80.35	19.55	2.71	64.30	2.47	109.60
2009	48.90	14.26	84.63	15.36	1.39	70.34	2.38	108.40

† % stalk includes panicle weight

The overall yields in this study are competitive with other energy crops. Lemus et al. (2002) reported average yields of 9 Mg ha-1 of dry biomass from switchgrass grown over four years; while average yields of 30 dry Mg ha-1 for miscanthus were reported across three locations in the Midwest (Heaton, 2008). Under optimal growing conditions it is likely that sorghum will out yield both switchgrass and miscanthus when bioenergy sorghums are used; since these bioenergy sorghums will be used as an annual, rotation with other crops will be possible due to shorter maturation periods. The use of bioenergy sorghum as an annual crop, as opposed to perennials, will allow producers more flexibility while still producing comparable yields under optimal conditions.

Regression analysis was performed in 2008 and 2009 for both primary and ratoon harvests. In general, the consistency of regression was limited (reflected in low r^2 values) and this inconsistency minimizes the inferences that can be derived from them. Nevertheless, these curves do provide insight into the duration of yield accumulation, ie, how long is yield maximized in a particular variety and/or hybrid. As an example, the 2008 regression analysis of dry biomass detected significant differences for DAP, DAPxDAP (Table 2.9). The data herein on the optimal harvest dates implies that a suitably long harvest window exists for harvest; harvesting could be staggered throughout the growing season as peak yields across the genotypes are reached at different times. In evaluating yield performance over time, all genotypes stabilized in productivity or continued to increase, indicating that there was not a significant drop-off in the later portions of the season (Fig 2.1). While trends support this observation, it must be noted that even in this example, the r^2 value is 0.51 and this represented the best r^2 value seen in all the regression analysis (see Appendix). The variation in total moisture content played a large role in the variability from week to week sampling and ultimately made these estimates inconsistent for modeling purposes.

Table 2. 9. Anova mean squares for sources of variation affecting dry biomass for all one week harvest intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP) in 2008

Source	DF	Dry Biomass
DAP	1	2730.39**
Genotype	5	59.46**
DAPxGenotype	5	46.28*
DAPxDAP	1	113.24*
Rep	1	41.08
Error	178	17.63
		R^2= 0.51

*,** Significant at p = .05 and .01, respectively

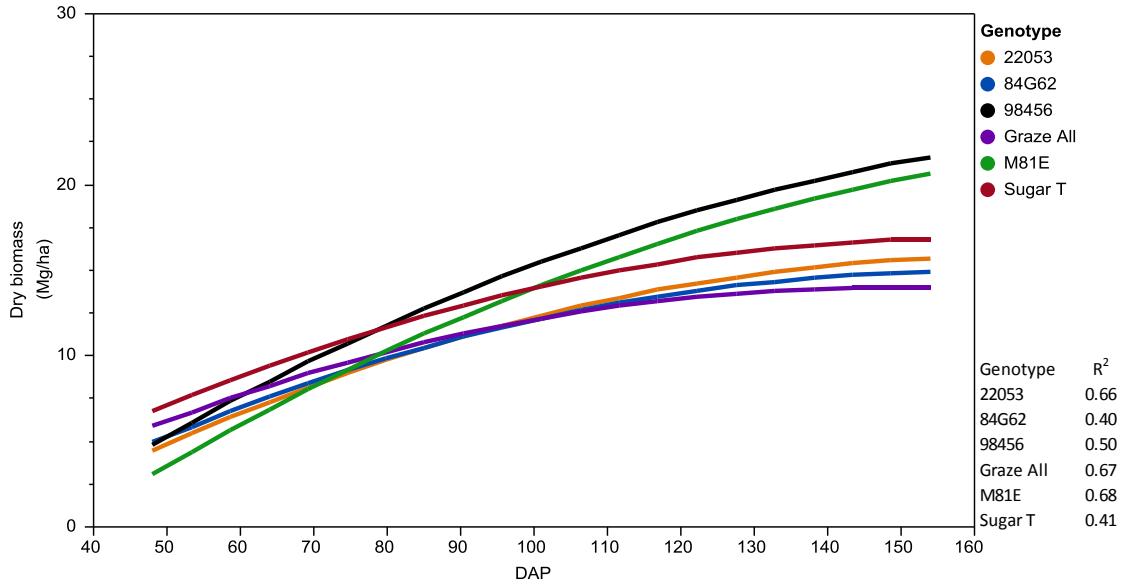

Figure 2. 1. Multiple regression of dry biomass (Mg/ha) for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

Consistency in the combined regression was limited (r^2= 0.51) The regression of individual genotypes also reflected this trend. There were several reasons for this including a limited number of replications (only two). In addition, the variation in total moisture content played a large role in the variability from week to week sampling and ultimately made these estimates inconsistent for modeling purposes.

Analysis of typical growth curves for each genotype indicates that the different genotypes differ in their growth patterns. Genotypes 22053 and 84G62 had steady and

consistent increases in biomass yield with time (Figs.2.2 and 2.3). Genotype 98456 had slow initial growth but a faster rate of increase later in the season (Fig. 2.4) while Graze All and Sugar T peak early and then slowed and eventually lost yield as the season progressed (Fig 2.5 and 2.6). Finally M81E produced steady and consistent gains over time with a slight drop off late in the season (Fig. 2.7).

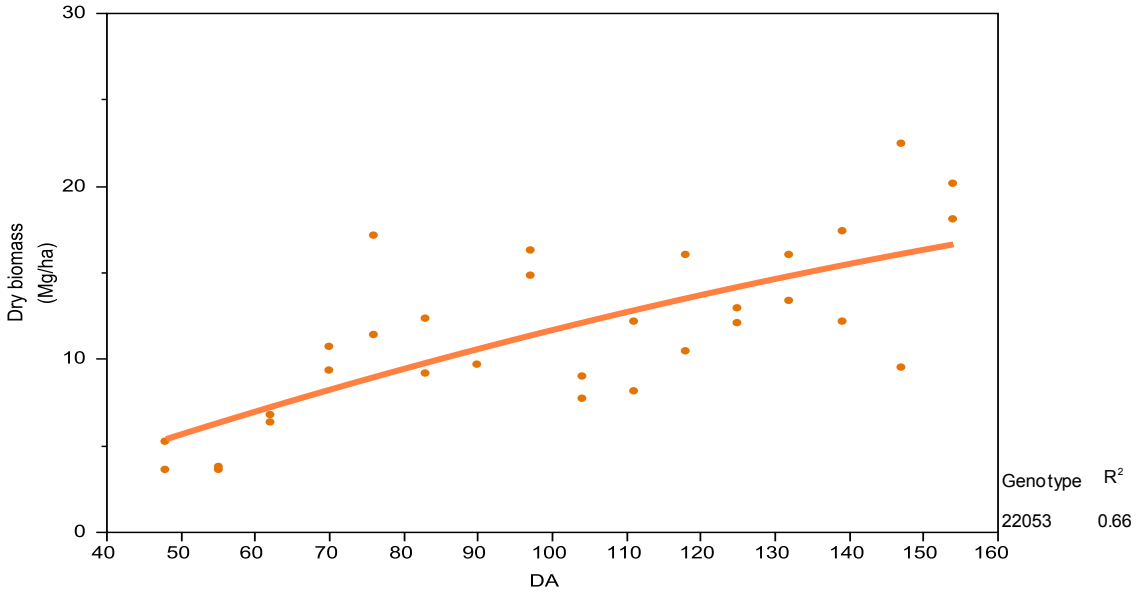

Figure 2. 2. Multiple regression of dry biomass (Mg/ha) for genotype 22053 grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

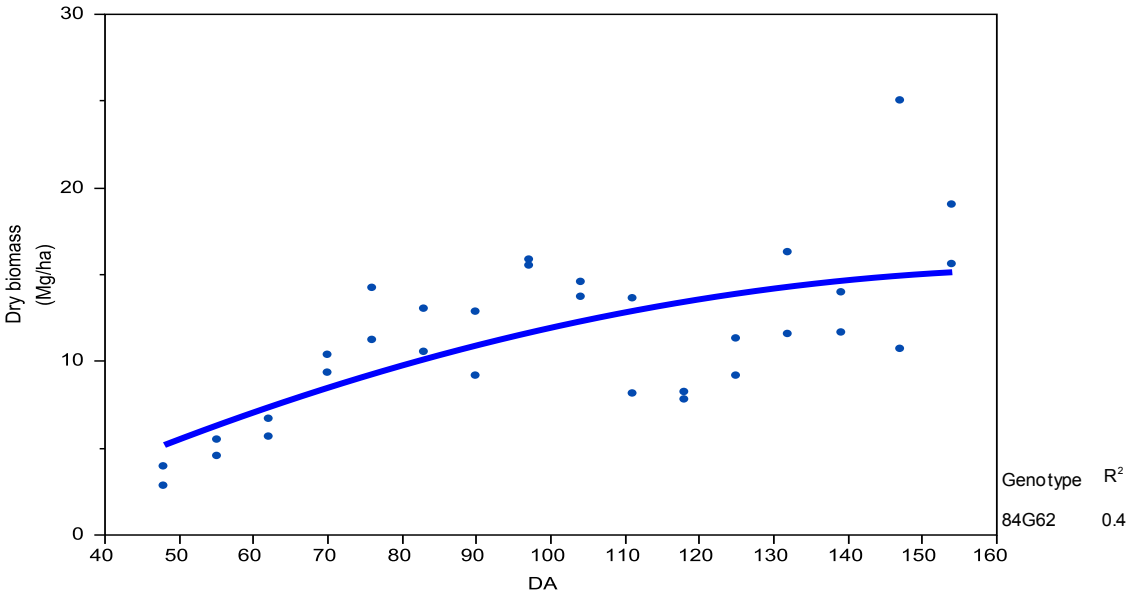

Figure 2. 3. Multiple regression of dry biomass (Mg/ha) for genotype 84G62 grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

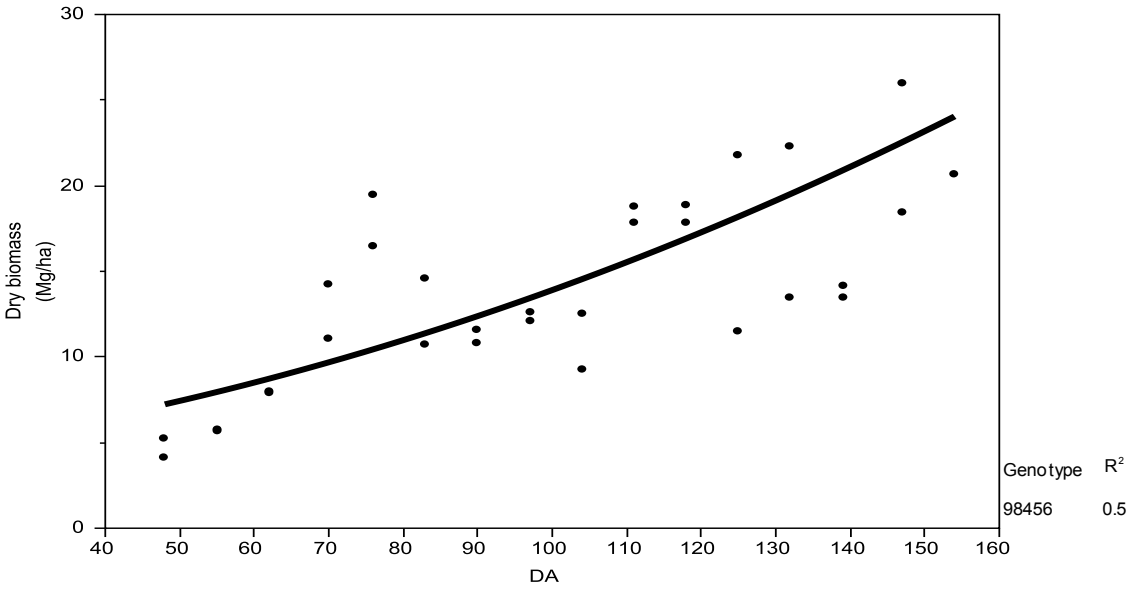

Figure 2. 4. Multiple regression of dry biomass (Mg/ha) for genotype 98456 grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

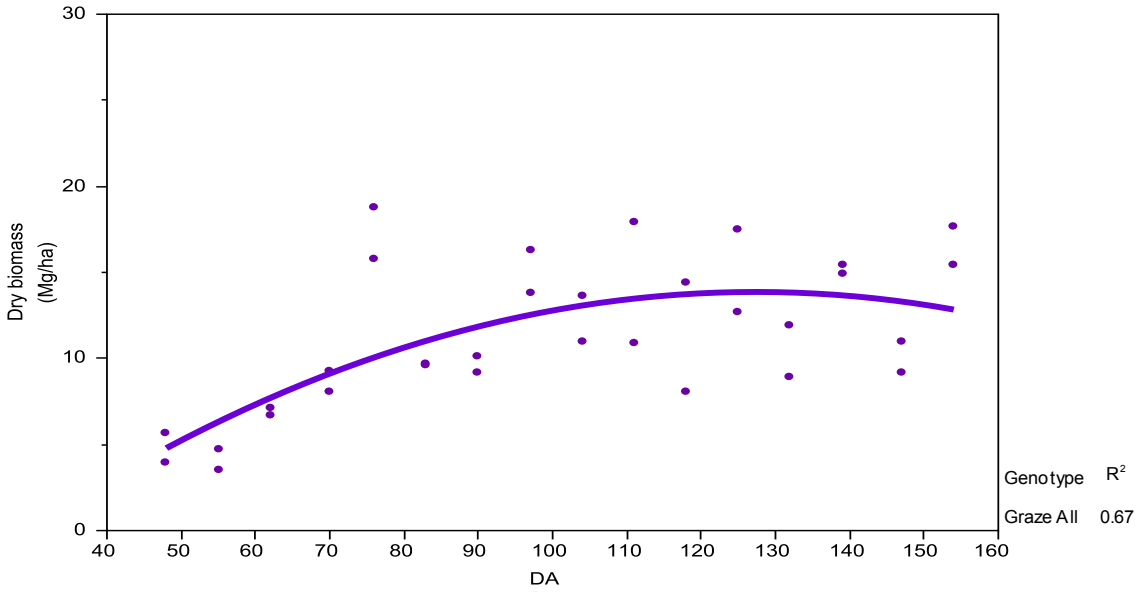

Figure 2. 5. Multiple regression of dry biomass (Mg/ha) for genotype Graze All grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

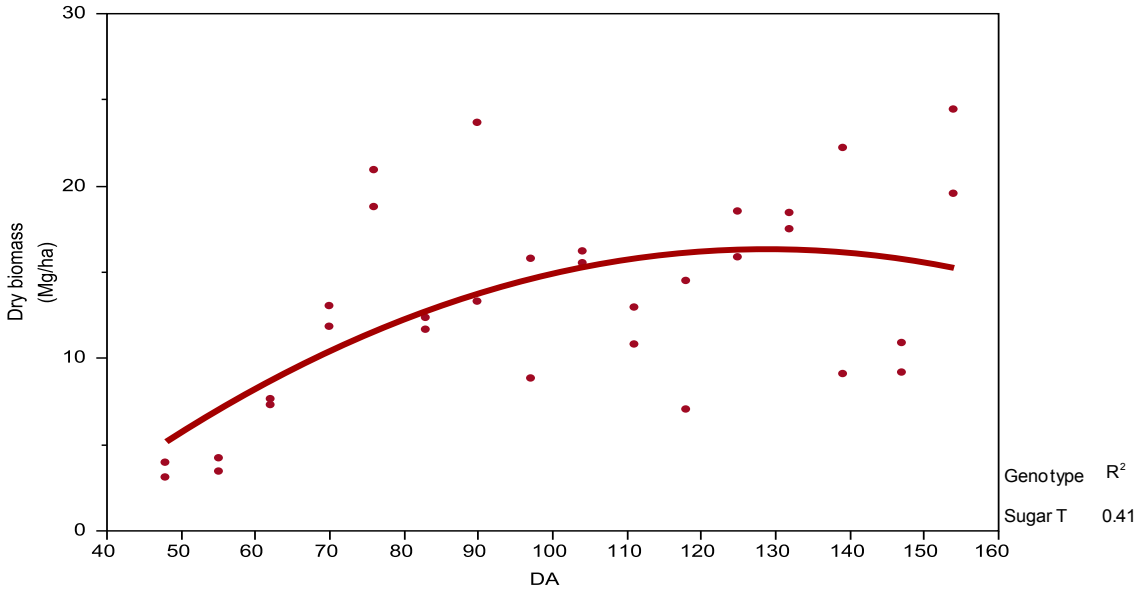

Figure 2. 6. Multiple regression of dry biomass (Mg/ha) for genotype Sugar T grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

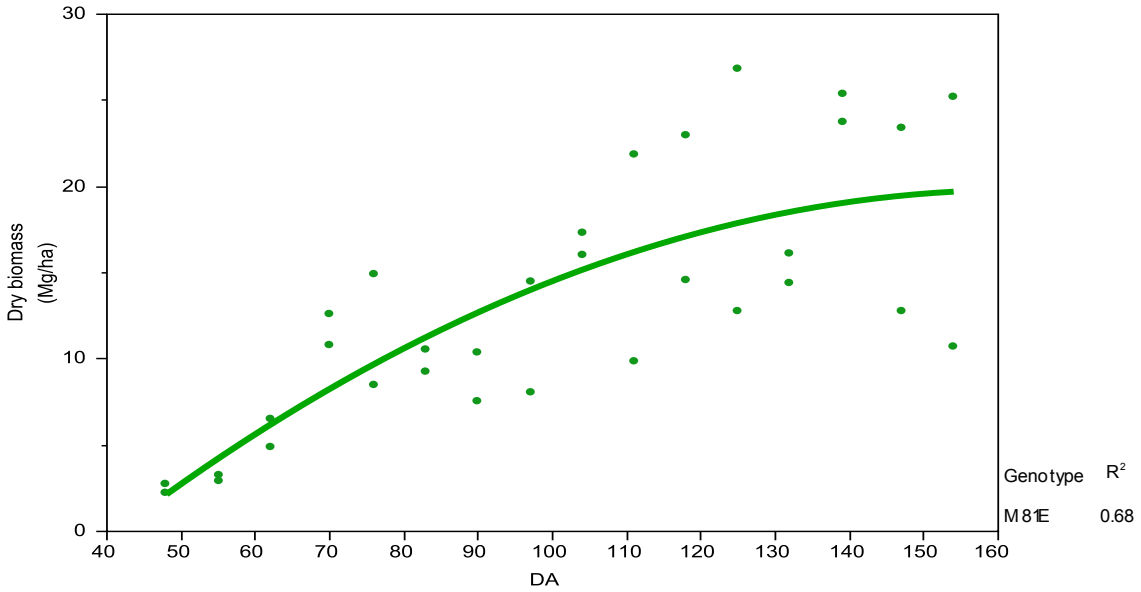

Figure 2. 7. Multiple regression of dry biomass (Mg/ha) for genotype M81E grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

Proportions of stalk and leaf varied very little between genotypes and environments (Table 2.2, 2.4, 2.8). The percentage of biomass in the stalk and panicle in 2008 averaged 79% and ranged from 74% to 84%, while in 2009 percent stalk and panicle was 84% and ranged from 81% to 87%. No significant difference was found for environments or genotypes. Studying the effects of environment on stalk and leaf percentages may be useful, as end users will likely prefer genotypes with higher proportions of stalk; leaf material is less dense and higher in protein content which increases production input costs and transportation (Propheter et al., 2010). Another critical component that must be considered is grain production, as higher amounts of grain increase the amount of starch input into the conversion process; though grain production was not specifically measured in this trial, panicle weights give good insight into grain production. Ultimately grain types produce the largest panicle weights and PS types produce the lowest (Table 2.2 and 2.4). At optimal harvest dates 84G62, grain sorghum, produced 9.4 Mg ha-1 of panicle weight while TAMX8001, PS sorghum, produced 0 Mg ha-1of panicle weight. Average panicle weight of the five common entries was 2.0 and ranged from 0.60 to 4.12 Mg ha-1. Grain types have the highest amount of grain production, but could be used as a dual purpose crop; with the grain

going to either food/feed or ethanol production and the biomass being used in a lignocellulosic conversion system. The amount of grain that is acceptable in the conversion process will need to set by the end user, while selection of different genotypes can meet these parameters.

Moisture content is important as it affects processing and storage logistics. Differential moisture content at harvest resulted in different responses in dry matter yields. At optimal harvest, the highest moisture contents occurred in M81E and 98456 (Table 2.4). The high moisture content was expected in M81E; sweet sorghums are selected for high moisture content in the stalk and 98456 is a thick-stalked forage sorghum that can be used for silage. In 2008, 84G62 had the lowest moisture percentage (47.09) of all the genotypes (Table 2.2). It is interesting to note that this moisture content occurred approximately 30 days post maturity and it realistically indicates the minimum moisture percentage that could be expected from any type of sorghum under field conditions. In forage or energy sorghums, the lowest moisture content observed in the plant at harvest was 60% to 64% across both years. If dry biomass is important to a processor, some form of drying will be required to facilitate further dry down.

Maturity classes ranged from photoperiod insensitive to several levels of photoperiod sensitive and it appears in this study and many others that maturity is a primary factor influencing total biomass yields (Corn, 2009; Packer, 2010 (Murray et al., 2008)). In 2008 the earliest and latest genotypes to flower and mature were 84G62 and 98456, respectively. 98456 is a moderately photoperiod sensitive genotype and it flowered late enough that grain production was minimized. In 2009, Graze All matured the earliest and produced the largest amount of grain (no grain hybrid was included in the trial in 2009), while TAMX8001 matured the latest of all the genotypes and produced no grain at any harvest date. Maturity not only plays a large role in grain production, it also allows for a much longer growing season when it is delayed (Rooney et al., 2007). Delayed flowering had a positive correlation with increased biomass ($r = 0.39$). Thus, the results of this study confirm the previous observation that photoperiod sensitivity is an effective mechanism for enhancing biomass productivity.

Optimal Dry Matter Yields in a Ratoon Harvest

The optimal ratoon harvest time for the six entries grown in 2008 ranged from 53-88 DAC (Table 2.10) and this resulted in optimum yields in September through November. At the optimal harvest dates, the dry biomass yields ranged from 9.0 to 13.0 Mg ha-1. Significant variation was detected among genotypes for % stalk, % leaf, % moisture and height (Table 2.11). M81E produced the largest amount of dry biomass while 84G62 produced the lowest (Table 2.10).

This would likely not be the case in a normal ratoon growth system, where the primary harvest was cut at optimal yields and the ratoon growth was allowed to have a longer growing season; Plant composition varied among entries (% stalk, % leaf and panicle weight) and the grain sorghum, 84G62 had the highest percentage of leaf material. This was likely because it was the shortest genotype and total biomass and stalk production was less than the other hybrids in the trial. Variation in Days to flowering in the ratoon harvest was minimal and the reduced range in flowering date was due to the shorter days initiating flowering earlier in the photoperiod sensitive types. Thus, all genotypes reached maximum yields at flowering or just before flowering. Thus, in ratoon cropping, photoperiod sensitivity will not be as important in total yield accumulation as it would be in the primary harvest.

Table 2. 10. Days after cutting (DAC), harvest month, growth stage, fresh biomass (Mg/ha), dry biomass (Mg/ha), % stalk, % leaf, panicle weight (Mg/ha), % moisture, height, and days to flowering. Numbers represent the means for the optimal harvest window for each genotype based on ratoon dry biomass yield in 2008. Letters designate significant differences between genotypes

Genotype	DAC	Harvest month	Growth stage†	Fresh biomass	Dry biomass	% Stalk	% Leaf	Panicle weight‡	% Moisture	Height	Days to Flower
M81E	67-88	Oct./Nov.	V/F	43.75	13.03	80.31^{bc}	19.68^{bc}	1.91^{a}	70.25^{a}	2.51^{a}	81
Graze All	53-67	October	F	37.83	12.79	82.16^{ab}	17.84^{cd}	1.72^{ab}	66.19^{b}	2.53^{a}	53
22053	60-81	October	F	37.40	12.18	86.64^{a}	13.35^{d}	2.20^{a}	66.25^{b}	2.57^{a}	60
98456	67-88	Oct./Nov.	V/F	38.55	11.97	75.16^{cd}	24.83^{ab}	0.73^{a}	68.90^{ab}	2.48^{a}	81
Sugar T	60-81	October	F	40.16	11.48	82.08^{ab}	17.91^{cd}	1.70^{ab}	71.36^{a}	2.40^{a}	60
84G62	53-67	October	F	29.76	9.08	70.46^{d}	29.54^{a}	2.15^{a}	69.84^{ab}	1.37^{b}	53

† F = flowering, M = physiologically mature (defined as past black layer on the grain)
‡ % stalk includes panicle weight

Table 2. 11. Mean squares for ANOVA of fresh weight, dry weight, % stalk, % leaf, panicle weight, % moisture, and height for the optimal harvest window for each genotype based on ratoon dry biomass yield in 2008

	Fresh weight		Dry weight		% stalk†		Panicle weight		% Moisture		Height		Days to flower	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	5	127.47	5	12.17	5	199.19**	5	1.71	5	27.66*	5	1.29**	5	1019.20**
Rep	1	82.29	1	21.24	1	64.50	1	3.18	1	23.76	1	0.15	1	1.00
Error	29	74.77	29	9.60	29	27.78	29	0.98	29	10.14	29	0.08	29	4.72

*,** Significant at p = .05 and .01, respectively
† % leaf numbers exactly the same as % stalk, therefore were excluded from table

The optimal ratoon harvest time for the six entries grown in 2009 ranged from 23-79 DAC, resulting in harvest times from September 10 to November 5(Table 2.12). All genotypes reached maximum dry biomass accumulation in a compressed range of days and were slightly earlier than in 2008 (September to October). The earlier maximum yields were likely due to the slightly earlier clean cut date (from which the ratoon crop was analyzed) and the excessive rainfall encountered in the fall of 2009 that effectively reduced growth in the late fall of 2009. At the optimal harvest dates the dry biomass yields ranged from 2.9 to 7.8 Mg ha-1. Significant variation was detected among genotypes for panicle weight, % moisture and height (Table 2.13). Interestingly, TAMX8001 (a PS sorghum) produced the largest panicle weight in the ratoon crop growth; occurring 51-79 DAC (October 8 to November 5). While the panicle weight was the largest, the weight is mostly panicle as grain development is limited due to the cool and wet conditions at the time and that this grain never fully developed or matured. Plant height ranged from 1.3 meters to 2.1 meters in the ratoon harvest, and was shorter than that observed in the primary harvest. As in 2008, variation in days to flowering in the ratoon harvest was minimal and the reduced range in flowering date was due to the shorter days initiating flowering earlier in the photoperiod sensitive types.

Table 2. 12. Days after cutting (DAC), harvest month, growth stage, fresh biomass (Mg/ha), dry biomass (Mg/ha), % stalk, % leaf, panicle weight (Mg/ha), % moisture, height (meters), and days to flowering. Numbers represent the means for the optimal harvest window for each genotype based on ratoon dry biomass yield in 2009. Letters designate significant differences between genotypes

Genotype	DAC	Harvest month	Growth stage†	Fresh biomass	Dry biomass	% Stalk	% Leaf	Panicle weight‡	% Moisture	Height	Days to Flower
TAMX8001	51-79	October	V/F	31.80	7.83	75.04	24.96	1.67[a]	76.14[b]	2.16[a]	65
98456	51-79	October	V/F	26.36	6.34	77.53	22.46	1.52[a]	76.50[b]	2.10[ab]	65
22053	51-79	October	V/F	19.40	4.67	76.25	23.74	1.53[a]	75.75[b]	1.91[ab]	51
Graze All	37-65	Sept./Oct.	F	21.09	4.65	75.94	24.06	1.63[a]	77.72[b]	1.86[b]	37
Sugar T	51-79	October	V/F	18.87	4.01	79.37	20.62	1.22[a]	75.84[b]	2.10[ab]	61
M81E	23-51	Sept./Oct.	V	19.09	2.97	73.32	26.67	0.06[b]	84.68[a]	1.35[c]	65

† V = vegetative, F = flowering, M = physiologically mature (defined as past black layer on the grain)
‡ % stalk includes panicle weight

Table 2. 13. Mean squares for ANOVA of fresh weight, dry weight, % stalk, % leaf, panicle weight, % moisture, and height for the optimal harvest window for each genotype based on ratoon dry biomass yield in 2009

	Fresh weight		Dry weight		% stalk†		Panicle weight		% Moisture		Height		Days to flower	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	5	164.83	5	18.11	5	22.21	5	2.27*	5	71.85*	5	0.52**	5	697.00**
Rep	1	129.27	1	11.54	1	9.16	1	0.02	1	32.49	1	0.02	1	4.00
Error	29	144.89	29	9.32	27	9.92	29	0.83	29	27.77	29	0.05	29	4.00

*,** Significant at p = .05 and .01, respectively
† % leaf numbers exactly the same as % stalk, therefore were excluded from table

Combined Analysis of the five entries grown in both years revealed significant Genotype x Environment interaction for % stalk, % leaf, panicle weight and height; environments were different for fresh weight, dry weight, % moisture and height while differences among genotypes were not detected except for days to flowering (Table 2.14). Over the two years, fresh biomass yield in the ratoon crop averaged 30.2 and ranged from 28.4 to 32.4 Mg ha-1. The highest yielding entry on average was 98456 (TAMX8001 was higher yielding in 2009, but it was not included in the combined analysis). Dry biomass yield averaged 8.4 and ranged from 7.7 to 9.1 Mg ha-1. The highest yielding entry on average was 98456 (TAMX8001 was higher yielding in 2009, but it was not included in the combined analysis). In general there was good correlation between fresh and dry biomass yields, indicating that moisture content did not vary excessively (discussed further in the moisture content).

In the combined analysis of the ratoon harvest genotype and environment effects were not detected leaf and stalk proportions, but there was a significant genotype x environment effect (Table 2.14). In the 2008 analysis the percentage of stalk and leaf were significantly different between genotypes. 84G62 (grain sorghum) had the highest percentage of leaves and 22053 (bmr forage sorghum) had the lowest percentage of leaves. In 2009, significant differences in stalk and leaf percentages were not detected. It is assumed that industrial biomass processors will prefer genotypes with higher proportions of stalk; leaf material is less dense and higher in protein content which increases production input costs and transportation (Propheter et al., 2010).Another critical component that must be considered is grain production, as higher amounts of grain increase the amount of starch input into the conversion process; though grain production was not specifically measured in this trial, panicle weights give good insight into grain production. However in a ratoon system grain is less important than overall biomass and grain production by genotype is largely driven by the short ratoon season. Average panicle weight of the five common entries was 1.5 and ranged from 1.1 to 1.8 Mg ha-1. The highest yielding entry was 22053, and out yielded the grain sorghum, 84G62, in 2008. In 2009, TAMX8001 produced 1.6 Mg ha-1 of panicle weight; and though this hybrid is a PS sorghum, day lengths were shorter during ratoon growth and

initiated reproductive development as is typical for the fall season. In all situations, grain maturation was limited by the cooler temperatures in the fall season. It is likely that most of the panicle weight was primarily biomass and did not have appreciable levels of grain per se.

Moisture content is important as it affects processing and storage logistics. The environment affected moisture content more than either genotype or the genotype x environment interaction (Table 2.15) and differential moisture content at harvest resulted in different responses in dry matter yields. Moisture contents in 2008 were lower than those observed in 2009 and the likely reasons for the differences are, (1) overall plant maturity was farther along in 2008 than in 2009 and (2) later harvests (October and early November vs. late September and early October) caused some dry down due to cooler weather. At optimal harvest times, the highest moisture contents in 2008 occurred in Sugar T (71.3 %) and M81E (70.2 %); and in 2009 the highest moisture contents occurred in M81E (84.6 %) and Graze All (77.7 %). M81E is expected to have the highest moisture content and forage sorghums such as Sugar T and Graze-All also can be high in moisture. The ratoon growth season had higher moisture content than the primary harvest because they are harvested earlier and in cooler fall weather then the harvests in the primary crop which reduces the total potential evapotranspiration rates.

While the entries were the same, maturity was not a major factor in productivity or classification in the ratoon crop. Because the ratoon crop started growth in mid-August, day lengths were already declining and thus, the photoperiod sensitive effects were minimized as all entries flowered between 37 to 81 days after cutting the primary growth. This will always be a consideration in fall grown regrowth as PS entries will flower, but in most U.S. latitudes, growth in the fall will not be limited by reproductive growth but rather by cool temperatures that slow growth rate.

Table 2. 14. Mean squares for ANOVA of fresh weight, dry weight, % stalk, % leaf, panicle weight, % moisture, and height for the combined analysis of the 2008 and 2009 optimal harvest window for each genotypes based on ratoon dry biomass yield

	Fresh weight		Dry weight		% Stalk†		Panicle weight		% Moisture		Height		Days to flower	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	4	32.44	4	3.77	4	59.44	4	1.62	4	74.71	4	0.24	4	1634.1**
Environment	1	5177.87**	1	902.87*	1	327.68	1	3.17	1	1356.41*	1	6.15*	1	3042.6**
GxE	4	66.73	4	7.6	4	69.58**	4	2.76*	4	42.92	4	0.33**	4	80.1**
Rep(Environment)	2	85.93	2	16.26	2	21.85	2	2.85*	2	35.18	2	0.02	2	15.00*
Error	48	89.91	48	7.91	46	16.51	48	0.77	48	20.68	48	0.05	48	3.75

*,** Significant at p = .05 and .01, respectively

† % leaf numbers exactly the same as % stalk, therefore were excluded from table

Table 2. 15. Fresh weight, dry weight, % stalk, % leaf, panicle weight, % moisture and height. Numbers represent the means for the combined analysis of the 2008 and 2009 ratoon optimal harvest window for each genotype based on dry biomass yield

Genotype	Fresh weight	Dry weight	% Stalk	% Leaf	Panicle weight†	% Moisture	Height	Days to flower
98456	32.46	9.16	76.35	23.64	1.31	72.71	2.29	73.00[a]
Graze All	29.47	8.72	79.05	20.94	1.68	71.96	2.20	45.00[d]
22053	28.40	8.43	81.45	18.54	1.87	71.00	2.24	55.50[c]
M81E	31.42	8.00	76.82	23.17	1.14	77.47	1.94	70.50[b]
Sugar T	29.52	7.75	80.73	19.27	1.47	73.61	2.25	55.50[c]
Environment								
2008	39.54[a]	12.28[a]	81.27	18.72	1.77	68.59[b]	2.50[a]	67.00[a]
2009	20.96[b]	4.53[b]	76.71	23.28	1.43	78.10[a]	1.86[b]	52.80[b]

† % stalk includes panicle weight

Overall ratoon growth was low compared to primary; maximum dry yields seen in the primary were 22 Mg ha-1, while in the ratoon maximum yields were 13 Mg ha-1. There are several reasons for the reduced productivity. First, the growing season is shorter and the plants have less time to grow and accumulate biomass. Second, while all entries did regrow, the grain, energy and sweet sorghum types were never selected, developed or even proposed for ratoon cropping. Hence, they have reduced ability to regrow. For regrowth, the forage sorghums are the best option as they were selected for improved performance and tillering ability. They also produced yields comparable to the primary cut; Graze All produced 11 Mg ha-1 at optimal yields in the 2008 primary and 13 Mg ha-1 in the ratoon. Harvesting could occur earlier (than the optimal time) on the forages with a reduced yield penalty in the primary cut to make ratoon cropping more feasible, and extend biomass production.

The current study was designed to compare relative growth rates in the different entries within the test, but it does not necessarily reflect the correct timeframe for rationing specific genotypes. For example, M81E is typically harvested in late August or early September; a harvest date would reduce regrowth time by at least two to three weeks compared to the system in place in the current study. For some of the forage sorghums the primary harvest was prior to or at the ratoon crop and therefore, ratoon cropping is recommended only for specific forage sorghum hybrids which were developed for that purpose.

Estimated Total Yields

Because yields were measured weekly, combining yield data from the week prior to clear cut in each with the optimum ratoon yield allows the total biomass yield to be estimated. In the combined total yield, 98456 had the highest total yields in both years as well as the highest combined average yield. In 2009 both forage sorghums, 98456 and Graze All, had the highest primary and ratoon yields; indicating that forage sorghums are well suited to a ratoon cropping system provided that optimum rainfall is available.

Production Logistics and Conclusion

Based on optimum yields, it appears possible to produce sorghum biomass for bioenergy production over a range of time in subtropical production environments. Based on the hybrids included in this trial, harvest could commence as early as mid July and continue unabated until the first part of October using a primary cut (Table 2.16). A comparison of the five common genotypes used in this study reveals that harvesting could begin with Graze All and Sugar T in the mid part of July; while utilization of longer season sorghums, M81E and 98456, can extend this harvest season into the first part of October. The addition of a PS hybrid sorghum, such as TAMX8001 can extend the harvest season into early November and possibly longer if the harvest continues after a killing frost. In addition, effective use of a ratoon crop could extend the season as well.

Table 2. 16. Harvest dates and mean dry matter yields (Mg ha-1) for five genotypes grown over two years in College Station, Texas. Numbers represent yields that could be expected during the harvest dates

	Harvest dates					
Genotype	15-Jul	1-Aug	15-Aug	1-Sep	15-Sep	1-Oct
	Mg ha-1†					
Graze All	11	**12**	**12**	9	7	7
Sugar T	13	**16**	**16**	15	14	7
22053	11	13	**14**	**14**	11	9
M81E	14	16	**18**	**18**	17	10
98456	15	15	18	**20**	**20**	13

† Numbers in bold represent optimal harvest time

Regardless of the sorghum hybrid that is used and when it is harvested, moisture content will be high and any processor must develop processing methodology that accounts for the moisture. In addition, the composition must be considered. At optimum yield, these entries showed surprisingly little variation in stalk and leaf proportions. This may be a positive development, but further testing is necessary to confirm that this is consistent over more environments. Nevertheless, differences in the chemical composition of the entries can be expected (both over time, environments and genotypes) and this will be important to processors.

Table 2.17 shows average means over two years across harvest dates from mid July to the first of October. Early maturing forage and silage sorghums (Graze All and Sugar T) will give optimal yields during the first part of August; silage sorghum 22053 and sweet sorghum M81E give optimal yields during the end of August. The use of later maturing forage hybrids such as 98456 extend the harvest season, giving optimal yields in the first part of September. Furthermore, with the addition of full PS lines such as TAMX8001 biomass accumulation and optimal harvest date can be extended in to October.

Table 2. 17. Primary, ratoon, total and average dry biomass yields by genotype for 2008 and 2009. Primary yields are from the harvest week prior to clear cutting and ratoon yields are the highest for the ratoon season. Clear cutting occurred on August 11 for 2008, and August 18 for 2009

	2008			2009			
	Primary	Ratoon	Total	Primary	Ratoon	Total	Avg.
Genotype	Mg ha-1						
98456	17.87	13.97	31.84	18.48	7.18	25.66	28.75
Sugar T	17.92	12.60	30.52	15.63	5.33	20.96	25.74
M81E	15.27	14.12	29.39	16.85	5.01	21.86	25.62
Graze All	10.40	16.40	26.80	19.05	5.11	24.16	25.48
22053	14.68	14.32	29.00	10.14	7.03	17.17	23.08
LSD(0.05)	5.68^{ns}	7.94^{ns}	15.49^{ns}	5.47^{ns}	5.03^{ns}	12.92^{ns}	

CHAPTER III

COMPOSITION OF SORGHUM FOR ENERGY PRODUCTION

Introduction

While total biomass yield is important, the manipulation and optimization of biomass composition can have profound impacts on the efficiency of conversion. For example, for some biochemical conversion processes, reduction in lignin minimizes or eliminates pretreatment requirements and results in higher conversion efficiency (Chang and Holtzapple, 2000). On a large scale these small differences amount to significant cost reduction and improvements in efficiency that cannot be ignored.

The basic composition of sorghum varies depending on the type of sorghum (grain, sweet, forage and bioenergy), and the method of measuring the composition. Grain sorghum produces larger amounts of starch with relatively lower levels of structural carbohydrates (Rooney et al., 2007). Sweet sorghum produces the largest amount of soluble simple sugars (sucrose, glucose and fructose). Forage sorghums are designed to produce primarily structural carbohydrates for a forage feeding system. Finally, in the new classification of bioenergy sorghums, the predominant compounds are the structural carbohydrates lignin, cellulose, and hemicellulose but also contain other components such as fat, ash and protein.

As with any biomass crop, the structural carbohydrates (lignin, cellulose, and hemicellulose) composition is the most important aspect of biomass sorghum. Lignin is a structural component of the plant which is composed of guaiacyl and syringyl (U.S. DOE, 2006). Lignin surrounds the cellulose and hemicellulose in the secondary cell walls, providing the plant cell the rigidity needed to grow upright and remain standing (U.S. DOE, 2006). Cellulose and hemicellulose are the main structural carbohydrates found in biomass in the form of polysaccharides; consisting of hexoses (mannose, glucose, and galactose) and pentose (xylose and arabinose) (Corredor et al. 2009). When deconstructed into the base units, both cellulose and hemicellulose can be converted to ethanol while the energy potential in lignin can currently be captured only through pyrolosis or burning. Sanderson et al. (1996) explains that the conversion of

lignocellulosic biomass to various end products to include microbial and enzymatic processes to produce ethanol or methane; thermochemcial processes (pyrolosis, gasification, direct liquefaction) to produce methanol, synthesis gas; and direct combustion for steam and electricity generation.

The quality of sorghum biomass is influenced by genotype, environment and relative maturity. In studies of forage sorghum quality, Siefers et al. (1997) found that specific genotypes significantly influence the forage quality of the crop. In addition, they determined that stage of harvest also strongly influenced forage quality; with younger forage consistently having better quality (as defined by forage parameters of highly digestible and high protein content). Factors in the genotype that influence quality are known and they include traits such as brown-midrib, which is known to reduce lignin concentration in the plant and increase animal performance (Oliver et al., 2005). Environment also influences quality and numerous studies have demonstrated that environment alone is responsible for the majority of variation within any sorghum (Oliver et al., 2005; Packer, 2011). While forage sorghum quality parameters are not necessarily the same as the bioenergy sorghum quality parameters, these results confirm the importance of establishing that relative genotypic, environmental and changes over maturity will occur.

Before the relative effects of genotype and environment can be measured on bioenergy sorghum, an accurate, reliable and efficient process must be identified to estimate structural carbohydrates within the plant at any given time. Currently, forage quality is quite accurately determined by detergent fiber analysis, but most chemical engineering processors rely on dietary fiber analysis for bioenergy feedstocks (Wolfrum et al., 2009). These two methods are based on different extraction methodologies and may not provide consistent results. A study of corn stover by *Wolfrum* et al. (2009) revealed moderate correlations between detergent fiber analysis and dietary fiber analysis but the correlations were largely driven by the extractives content of the corn. Therefore, a reliable correlation for structural carbohydrates between the two methods could not be established. Further work on the correlations between the two methods in being done with sorghum, and preliminary results indicate that detergent measures for

structural carbohydrates have a moderate to poor correlation with dietary fiber glucan content (Stefaniak et al., in review).

To facilitate screening of sorghum germplasm, Wolfrum et al. (submitted) described the development of an NIR calibration curve for dietary fiber composition in sorghum. The use of NIRS in designing feedstocks is not a new technology, and has mainly been used in the production of forages to monitor quality for animal feed. Stuth et al (2003) explains that the use of NIRS as an alternative to traditional analytical methods for determining nutritive value of forages is much more rapid, less labor intensive, and allows for timely decision making. However NIRS is not just employed in forage analysis, but can be tailored to a wide range of applications. Jin and Chen (2007) proved that NIRS was suitable for the rapid and accurate quantification of rice straw components such as ash, cellulose, hemicellulose, and Klason lignin. Plant material however is not the only product that can be analyzed with NIRS. Lyons and Stuth (1992) showed that the use of NIRS may be useful for nutritional profiling of free-roaming cattle on range lands through the use of fecal matter. With its broad range of uses and proven ability to quantify constituents within a broad range of materials, NIRS technology will be a very crucial tool in the continued perfection of bioenergy sorghums.

NIR technology can be used to quantify differences between sorghums, allowing breeders to establish which types will be best to use based on a set of parameters. Sorghum composition and the relative influence of the genotype, maturity and environment will be crucial to understand, as this crop evolves into a larger role in the ethanol industry. Initial requirement on the crop will likely be for yield, but composition and management thereof will become important as industrial plants look to first stabilize production and then improve efficiency. Given that there is little known regarding dietary fiber composition of sorghum biomass and how it changes throughout the growing season, there is a real need to assess it at this time. It is assumed that energy sorghums will perform much like forage sorghums; but with much different end uses in mind, energy sorghums must be studied in order to quantify plant constituents and how these constituents are accumulated over the growing season. With these factors in mind, the objectives of this study are to assess the relative composition of sorghum biomass

composition at harvest dates designed to maximize biomass production and to evaluate general trends in composition over the growing season.

Materials and Methods

Data Collection

In conjunction with the yield study conducted on the six hybrids (Table 2.1) in chapter II, NIRS analysis was performed on the whole plant sub-samples collected from this trial. The whole plant sub-sample was collected by chopping three random plants from within the row that were harvested for yield. All samples were dried in a forced air, convection dryer for three days at 48°C. Dried samples were ground in a Wiley mill to a 2mm particle size. All ground samples were scanned twice for NIR analysis on a Foss XDS near infrared spectrophotometer from a range of 400-2500 nm. Predictive curves for composition have been developed and were used to measure structural carbohydrate composition (cellulose, hemicellulose, and lignin), non-structural carbohydrates (starch, and sugar), protein and ash content (Wolfrum et al., submitted). This model generates relative percentages for plant composition and is a relatively new model; it is evolving and improving. Using the version in Wolfrum et al., (submitted), R square values for this model relating to lignin, hemicellulose, cellulose, ash and protein are 0.93, 0.70, 0.79, 0.82, and 0.72 respectively. While these values are not as high as they eventually will be in subsequent models, they are suitable for relative comparisons of constituents, environments and genotypes.

Statistical Analysis

The optimal harvest window for each hybrid was determined in the study described in Chapter II. Composition data (lignin, hemicellulose, cellulose, ash and protein) from samples taken from the optimum harvest window for each entry were analyzed for significance between genotypes using PROC GLM in SAS JMP. A student's *t* means comparison test was conducted to show significance between means when a significant effect was detected. A combined analysis across both years was

performed using the five entries grown both years (84G62 and TAM8001 were not included in the combined analysis as they were only evaluated in 2008 and 2009, respectively). In the combined analysis, the statistical model used in SAS JUMP was a mixed model with genotypes as a fixed effect while replication and environment were considered as random effects. A student's *t* means comparison test was conducted to show significance between the genotype means.

Theoretical Ethanol Yields

Using the U.S. Department of Energy theoretical ethanol yield calculator (http://www1.eere.energy.gov/biomass/ethanol_yield_calculator.html) (U.S. Department of Energy, 2011), theoretical ethanol yields were estimated for the entries at optimum yield potential based on the composition parameters estimated herein. Yield estimates from data collected in Chapter II were combined with composition estimates described herein to estimate total production of C5 and C6 sugars on a Mg ha-1 basis. Estimated ethanol yields are based on genotypic and environmental means and are presented for informational purposes only; statistical analysis was not and could not be completed. Per the equation used by the U.S. Department of Energy, C5 and C6 sugars are combined to express total ethanol yields. However, the model used to generate percertages herein does not express galactan, arabinan and mannan; therefore these numbers were not used in the calculation of theoretical ethanol yields.

Results and Discussion

Plant Composition Based on Optimal Yields for Dry Biomass

In 2008, significant variation was detected among genotypes for ash and protein content while genotypes were not a significant source of variation was detected for lignin, cellulose and hemicellulose (Table 3.1). Given the variation in optimum harvest dates (ranging from 70 – 168) and the range in different sorghum types, it is somewhat surprising that no variation among genotypes was detectable in 2008. The concentrations for lignin, hemicellulose and cellulose averaged 11%, 16% and 27% respectively, for the

six entries in the test and ash and protein content averaged 7 and 3%, respectively (Table 3.2). Ash concentrations ranged from a low of 6.1% in Sugar T to a high of 8.2% in Graze All while protein content was lowest in 98456 (at 2.5%) and highest in Graze All (4.4%).

In 2009, significant variation was detected among genotypes for all components that were estimated except protein content (Table 3.3). Concentrations for lignin, hemicellulose and cellulose averaged 11%, 15% and 25% respectively, for the six entries in the test and ash and protein content averaged 5% and 2%, respectively (Table 3.4). Lignin concentrations ranged from 10.17 to 12.91%; with the lowest lignin concentration in M81E and the highest in 98456. Hemicellulose concentrations, while different among genotypes had a relatively narrow range (14.9 to 16.%). A substantial range in cellulose was observed with a low of 21.28% (Sugar T) to a high of 29.25% (22053) (Table 3.4). Ash content varied among the genotypes with Sugar T being the lowest and 98456 the highest, approximately 2% higher than Sugar T (Table 3.4). Relatively speaking, protein contents as estimated with dietary fiber methods are low, but no differences were detected in the entries in 2009.

Table 3. 1. Mean squares for ANOVA of lignin, hemicellulose, cellulose, ash, and protein for the optimal harvest window for each genotype based on dry biomass yield in 2008

	Lignin		Hemicellulose		Cellulose		Ash		Protein	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	5	4.31	5	1.81	5	7.33	5	2.98**	5	3.31**
Rep	1	1.53	1	0.24	1	3.9	1	0.006	1	0.04
Error	28	1.96	28	0.84	28	8.48	28	0.69	28	0.72

Table 3. 2. Days after planting (DAP), harvest month, dry biomass (Mg/ha), lignin, hemicellulose, cellulose, ash, and protein. Numbers represent the means for the optimal harvest window for each genotype based on the dry biomass yield in 2008. Letters designate significant differences between genotypes

				Constituents†				
Genotype	DAP	Harvest month	Dry biomass	Lignin	Hemicellulose	Cellulose	Ash	Protein
Graze All	70-83	June	11.84^{c}	12.52	15.43	26.09	8.26^{a}	4.49^{a}
98456	147-168	Aug./Sept.	24.23^{a}	12.00	16.84	28.90	7.14^{bc}	2.57^{c}
Sugar T	139-154	August	15.87^{bc}	11.35	16.62	28.54	6.18^{c}	2.58^{c}
M81E	132-147	August	19.29^{ab}	11.07	15.95	26.64	6.70^{bc}	3.04^{bc}
22053	139-154	August	16.61^{bc}	10.50	15.62	26.99	6.81^{bc}	3.07^{bc}
84G62	139-154	August	16.00^{bc}	10.33	16.01	26.93	7.37^{ab}	3.74^{ab}

† predicted by Near Infrared Reflectance Spectroscopy

Table 3. 3. Mean squares for ANOVA of lignin, hemicellulose, cellulose, ash, and protein for the optimal harvest window for each genotype based on dry biomass yield in 2009

	Lignin		Hemicellulose		Cellulose		Ash		Protein	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	5	5.04*	5	2.61**	5	48.76**	5	3.91**	5	1.95
Rep	1	0.03	1	0.003	1	1.12	1	0.25	1	0.04
Error	28	1.55	28	0.68	28	7.15	28	0.48	28	0.79

Table 3. 4. Days after planting (DAP), harvest month, dry biomass (Mg/ha), lignin, hemicellulose, cellulose, ash, and protein. Numbers represent the means for the optimal harvest window for each genotype based on the dry biomass yield in 2009. Letters designate significant differences between genotypes

				Constituents†				
Genotype	DAP	Harvest month	Dry biomass	Lignin	Hemicellulose	Cellulose	Ash	Protein
98456	118-132	August	16.13^{ab}	12.91^{a}	15.65^{abc}	24.63^{bc}	6.23^{a}	2.73
Sugar T	104-118	August	14.55^{b}	12.42^{a}	14.78^{c}	21.28^{d}	4.07^{b}	3.07
22053	132-160	Aug./Sept.	12.15^{b}	12.01^{a}	16.56^{a}	29.25^{a}	6.15^{a}	1.51
Graze All	118-132	August	12.25^{b}	11.63^{ab}	14.94^{bc}	22.34^{cd}	5.93^{a}	2.98
TAMX8001	125-146	Aug./Sept.	21.83^{a}	11.46^{ab}	15.81^{ab}	25.33^{bc}	5.68^{a}	2.52
M81E	132-160	Aug./Sept	16.23^{ab}	10.17^{b}	15.90^{ab}	26.26^{ab}	6.05^{a}	2.28

† predicted by Near Infrared Reflectance Spectroscopy

Combined analysis of the five common entries revealed significant Genotype x Environment interaction for lignin, hemicellulose, cellulose, ash and protein contents (Table 3.5). Given the significant interaction and the mixed model, variation due to either main effect (genotype or environment) was detected only for ash content due to environments (Table 3.5).

The average genotype compositional concentrations observed herein are consistent with previous reports. Over both years, lignin, hemicellulose and cellulose averaged 11%, 15% and 26%, respectively(Table 3.6). Dahlberg et al (in press) reported average dietary fiber concentrations of lignin, hemicellulose, and cellulose at 12%, 15% and 25% in forage sorghum samples grown in Bushland, Texas. Stefaniak et al. (submitted) reported average dietary fiber concentrations of these same constituents at 13%, 16% and 32% in sorghum ranging from sweet to biomass. Stefaniak et al. (submitted) also indicated that the correlations between dietary fiber and detergent fiber concentrations are poor and that dietary fiber methods are much better for predicting plant constituents in sorghum biomass. Wolfrum et al (2009) reported similar results in corn stover and revealed some correlation between the two extraction methods, detergent fiber analysis and dietary fiber analysis; however correlations were thought to be largely driven by the extractives content of the corn, thus showing that a reliable correlation between the two methods could not be established.

Neither a genotype or environment effect for lignin was detected but a genotype x environment effect was detected, indicating that genotypes performed differently relative to the environments. The exact cause of the interaction appears to involve most of the genotypes as several responded differently in the environments. For example, 22053 which is a brown midrib hybrid was numerically the lowest for lignin in 2008 but in the middle of the entries for 2009. Brown mid-rib genotypes are consistently lower in lignin than non-brown midrib genotypes (McCollum et al., 2003; Sattler et al., 2010) and the absence of significance for lignin given that brown midrib genotypes are included is somewhat surprising. The environment difference was minimal in this study but others have reported differences. Packer (2011) reported that the environment was the primary source of variation in structural composition in the evaluation of 15 biomass sorghum

hybrids. Corn (2009) reported similar results in the evaluation of sweet sorghum hybrids. There are two possible explanations for these observations in this study. First, these estimates utilize the dietary fiber method; to date all other reports utilize the detergent fiber methods. Second, given the relatively small number of observation in the current test, the power of the ANOVA may not have the power to detect differences.

Similar results were observed for both cellulose and hemicellulose. In both cases, a significant genotype x environment interaction was detected implying that the genotypes performed differently relative to environments. In the case of hemicellulose, a significant shift in the hemicellulose concentration was observed for 22053 (second to lowest in 2008 and highest in 2009); all others were relatively consistent across years. Cellulose seems to vary across genotypes and years to a much greater extent than either hemicellulose or lignin and it indicates that management of this component may be more challenging in the future.

Genotype x environment interactions were detected for both ash and protein content and the relatively large interactions likely masked any main genotype or environment effects. Ash contents were similar to those reported by Wolfrum et al. (submitted) and Dahlberg et al., (in press). Protein content in the whole plant was similar to those reported for dietary fiber (Wolfrum et al. (submitted), Dahlberg et al. (in press)). Further research is needed to clarify the relationship between protein content estimates between the two methodologies; it main be confounding issues that are the basis. It is logical to expect that protein content would be lower in sorghum grown and managed for biomass as they are not harvested with forage quality as a consideration. In forage quality, high nutritive value (protein) and palatability is of substantial importance for animal feeding (Van Soest, 1967). However, for industrial use, the protein provides no advantage and in fact is better left in the field to reduce nitrogen requirements for future production. Further testing and evaluation of material is needed to confirm these initial observations. If these numbers are accurate, the lower numbers are actually beneficial and could reduce nitrogen requirements for crop production.

The Genotype x Environment interactions documents the importance of multiple environment characterization. For all traits in the current study, the shifts that are

observed are variable and not easy to explain. The potential explanations for these observations are lack of rain fall during the crucial times of the growing period and the fact that these hybrids were compared against each other on their optimal yields dates and not the same dates; while more significant differences may have been seen in a larger population, which could encompass a broader set of material. Plant constituent percentages are likely linked directly to maturity of the plant and the environment it is grown. The 2008 growing season received less rain than 2009 growing season, and allowed the genotypes to mature in a more consistent manner. This reinforces the fact that end users must have parameters for feedstocks while understanding that plant constituents will likely change from year to year as environments change. Regardless of the cause of the genotype x environment interactions, their presence underlies the importance in multi-environment testing to minimize their effect. Given the impact of even small shifts in composition, it is critical to understand and mitigate these issues.

The absence of genotypic and environmental effects has both positive and negative implications. First, the lack of an environmental or genetic effect indicates that the biomass available for conversion is consistent in composition, implying that the biomass supplied to a conversion plant would be consistent from year to year and for genotype to genotype. However, this result is not consistent with previous reports from Packer (2011) and Corn (2009) who both reported significant genotypic and environmental effects in biomass and sweet sorghum respectively. From an improvement standpoint the lack of variation from the genetic perspective minimizes potential improvement and this is an undesirable effect. It implies that sufficient variation does not exist within elite sorghum germplasm and that breeders will have to screen additional germplasm to find sufficient variation to make further improvements.

It is also likely that the statistical approach used herein affected the significance of the difference effects. The analysis conducted herein utilized a mixed statistical model with genotypes as a fixed effect and environments as a random effect. Therefore the tests of significance are more conservative with a mixed model than an all fixed model. In the current study, with a fixed model, both main effects would be significant

as well. Finally, the numbers of entries are relatively small and effectively reduce the power of the test and minimize our ability to detect differences.

Table 3. 5. Mean squares for ANOVA of lignin, hemicellulose, cellulose, ash and protein for the combined analysis of the 2008 and 2009 optimal harvest window for each genotype based on dry biomass yields

	Lignin		Hemi cellulose		Cellulose		Ash		Protein	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	4	6.03	4	4.39	4	29.18	4	6.63	4	3.41
Environment	1	1.64	1	2.14	1	112.94	1	29.44*	1	6.40
GxE	4	4.80*	4	3.29**	4	39.71**	4	1.73*	4	2.78**
Error	50	1.91	50	0.68	50	6.69	50	0.65	50	0.63

Table 3. 6. Lignin, hemicellulose, cellulose, ash and protein means for the combined analysis of the 2008 and 2009 optimal harvest window for each genotype based on dry biomass yields

Genotype	Lignin	Hemicellulose	Cellulose	Ash	Protein
98456	12.58	16.26	26.77	6.68	2.69
Graze All	12.08	15.19	24.22	7.10	3.74
22053	11.26	16.10	28.12	6.48	2.30
M81E	10.77	15.99	26.62	6.55	2.74
Sugar T	11.89	15.70	24.91	5.13	2.83
Environment					
2008	11.55	16.11	27.50	7.09[a]	3.18
2009	11.88	15.57	24.75	5.68[b]	2.53

Plant Composition Based on Optimal Yields for Ratoon Dry Biomass

In 2008, significant variation was not detected among genotypes for lignin, hemicellulose, cellulose, ash and protein (Table 3.7). The relatively small range of optimal harvest dates (53-88 DAC) likely minimized the opportunity for variation among genotypes. The concentrations for lignin, hemicellulose and cellulose averaged 10%, 15% and 26% respectively, and ash and protein content averaged 7% and 3% respectively (Table 3.8).

Table 3. 7. Mean squares for ANOVA of lignin, hemicellulose, cellulose, ash, and protein for the optimal harvest window for each genotype based on ratoon dry biomass yield in 2008

	Lignin		Hemicellulose		Cellulose		Ash		Protein	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	5	1.08	5	0.74	5	5.4	5	3.67	5	1.41
Rep	1	0.001	1	0.003	1	0.87	1	1.49	1	1.04
Error	27	0.61	27	0.31	27	3.77	27	1.46	27	1.12

Table 3. 8. Days after cutting (DAC), harvest month, dry biomass (Mg/ha), lignin, hemicellulose, cellulose, ash, protein. Numbers represent the means for the optimal harvest window for each genotype based on the ratoon dry biomass in 2008

				Constituents†				
Genotype	DAC	Harvest month	Dry biomass	Lignin	Hemi cellulose	Cellulose	Ash	Protein
22053	60-81	October	12.18	10.31	15.55	25.70	8.08	3.76
M81E	67-88	Oct./Nov.	13.03	10.25	15.46	25.97	6.14	2.51
Graze All	53-67	October	12.79	10.17	15.86	27.15	6.86	2.57
98456	67-88	Oct./Nov.	11.97	10.14	15.61	26.40	5.91	2.68
Sugar T	60-81	October	11.48	10.08	15.81	26.25	7.34	2.99
84G62	53-67	October	9.08	9.16	14.86	24.30	6.92	3.29

† predicted by Near Infrared Reflectance Spectroscopy

In 2009, significant variation was detected among genotypes for all components that were estimated (Table 3.9). Concentrations for lignin, hemicellulose and cellulose averaged 13%, 14% and 21% respectively, for the six entries in the test and ash and protein content averaged 8% and 6% respectively (Table 3.10). Lignin concentration ranged from 11.53 to 13.37%; with the lowest lignin concentration in 22053 and the highest in TAMX8001. These were logical as 22053 is a brown midrib hybrid and is expected to be lower in lignin. Hemicellulose concentrations ranged from 14.2% to 15.2% with the lowest concentration in M81E and the highest in TAMX8001. Cellulose concentrations ranged from 20.1% to 22.6% with the lowest concentration in M81E and the highest in TAMX8001. The ranges in both hemicellulose and cellulose were relatively narrow compared to Packer (2011) and are likely due to the narrower range of genotypes considered in this study. Ash concentration ranged from 6.49 to 10.05%; with the lowest ash concentration in Sugar T and the highest in M81E. Protein concentrations ranged from 5.67 to 7.72%; with the lowest protein concentrations in Sugar T and the

highest in M81E. Ash and protein concentrations, while significant among genotypes, did not have a wide range of variation and they were consistently higher than those observed in the primary harvest. The higher protein numbers were likely because the ratoon crop was harvested at a younger growth stage which is consistently associated with high protein content in forage sorghum (McCormick et al., 1995). The cause of the higher ash content is not known.

Table 3. 9. Mean squares for ANOVA of lignin, hemicellulose, cellulose, ash, and protein for the optimal harvest window for each genotype based on ratoon dry biomass yield in 2009

	Lignin		Hemicellulose		Cellulose		Ash		Protein	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	5	2.97**	5	0.78**	5	5.40**	5	10.11**	5	3.93**
Rep	1	0.003	1	0.06	1	2.34	1	0.31	1	0.25
Error	29	0.31	29	0.15	29	0.86	29	0.88	29	0.57

Table 3. 10. Days after cutting (DAC), harvest month, dry biomass (Mg/ha), lignin, hemicellulose, cellulose, ash, and protein. Numbers represent the means for the optimal harvest window for each genotype based on the ratoon dry biomass in 2009. Letters designated significant differences between genotypes

				Constituents†				
Genotype	DAC	Harvest month	Dry biomass	Lignin	Hemi cellulose	Cellulose	Ash	Protein
TAMX8001	51-79	October	7.83	13.37^{a}	15.25^{a}	22.62^{a}	7.20^{bc}	5.91^{bc}
M81E	23-51	Sept./Oct.	2.97	13.29^{ab}	14.22^{c}	20.08^{b}	10.05^{a}	7.72^{a}
98456	51-79	October	6.34	13.28^{ab}	14.70^{b}	21.10^{b}	7.58^{bc}	5.84^{bc}
Graze All	37-65	Sept./Oct.	4.65	12.63^{bc}	14.68^{bc}	20.93^{b}	9.01^{a}	6.65^{b}
Sugar T	51-79	October	4.01	12.59^{c}	14.47^{bc}	20.27^{b}	6.49^{c}	5.67^{c}
22053	51-79	October	4.67	11.53^{d}	14.39^{bc}	20.23^{b}	7.67^{b}	5.68^{c}

† predicted by Near Infrared Reflectance Spectroscopy

Combined analysis of the five common entries revealed significant Genotype x Environment interaction for lignin, ash and protein (Table 3.11). Significant variation due to environments was seen for lignin, hemicellulose, cellulose and protein (Table 3.11). No differences were detected due to genotypes for any of these traits. The environment effect on these traits was strong; these two fall seasons were dramatically different; 2008 was much drier than 2009 (Table 2.5).

Over both years, lignin, hemicellulose and cellulose averaged 11%, 15% and 23%, respectively (Table 3.12). The Genotype x Environment and Environment interactions documents the importance of multiple environment characterization. As seen in the primary harvest, lignin variation was linked strongly to genotype x environment; variation was also detected for environments in the ratoon harvest for lignin with 2009 having higher lignin overall. 22053, a bmr sorghum had the lowest lignin concentration across both years of the ratoon harvest. This outcome is to be expected since brown midrib sorghum genotypes are commonly associated with lower lignin concentrations (Oliver et al., 2005); while a compounding effect is likely due in part to sampling young plant material compared to the primary harvest.

Environmental variation was seen for all constituents except for ash concentrations. Hemicellulose, though effected by environment did not have large variation across years in total percentages. Cellulose, however varied approximately 6% from 2008 to 2009; proving that producers will need to understand and account for environmental variation when using feedstocks for ethanol production. Protein varied approximately 4% from 2008 to 2009, which is unexpected; protein in young plant material is usually higher and more consistent, but was controlled by environment as seen here. Protein in 2008 was not significant by genotypes, but in 2009 it was.

Plant constituent percentages are likely linked directly to maturity of the plant and the environment it is grown, and with such a short ratoon growth season it is not surprising that larger differences were not seen. The ratoon growth seasons were shorter than normal, mainly due to the way the cuttings were handled; and showed that variation in plant composition can still vary with such a short growth season. This reinforces the fact that end users must have parameters for feedstocks while understanding that plant constituents will likely change from year to year as environments and agronomic practices change. Regardless of the cause of the genotype x environment interactions, their presence underlies the importance in multi-environment testing to minimize their effect. Given the impact of even small shifts in composition, it is critical to understand and mitigate these issues.

Table 3. 11. Mean squares for ANOVA of lignin, hemicellulose, cellulose, ash and protein for the combined analysis of the 2008 and 2009 optimal harvest window for each genotype based on ratoon dry biomass yields

	Lignin		Hemi cellulose		Cellulose		Ash		Protein	
Source of Variation	df	mean square	df	mean square	df	mean square	df	mean square	df	mean square
Genotype	4	1.36*	4	0.38	4	2.57	4	5.19**	4	1.6
Environment	1	87.60**	1	21.51**	1	516.32**	1	26.81**	1	179.36**
GxE	4	1.78*	4	0.12	4	0.72	4	10.74**	4	4.67**
Error	50	0.51	50	0.23	50	2.15	50	0.79	50	0.67

Table 3. 12. Lignin, hemicellulose, cellulose, ash and protein means for the combined analysis of the 2008 and 2009 optimal harvest window for each genotype based on the ratoon dry biomass yields

Genotype	Lignin	Hemicellulose	Cellulose	Ash	Protein
98456	11.71[a]	15.16	23.75	6.75[b]	4.27
Graze All	11.41[ab]	15.27	24.05	7.94[a]	4.62
22053	10.92[b]	14.97	22.97	7.88[a]	4.72
M81E	11.77[a]	14.84	23.03	8.10[a]	5.12
Sugar T	11.48[ab]	15.21	23.50	6.82[b]	4.23
Environment					
2008	10.24[b]	15.69[a]	26.39[a]	6.82[b]	2.86[b]
2009	12.66[a]	14.49[b]	20.52[b]	8.16[a]	6.31[a]

Theoretical Ethanol Yields

Average ethanol yields were 4602 L/ha and 3439 L/ha in 2008 and 2009, respectively for the primary harvests. Because significant differences between genotypes for hemicellulose and cellulose were not seen in 2008 (Table 3.2), ethanol yields are more impacted by overall dry biomass production than by composition (Table 3.13). In 2009, even with differences in hemicellulose and cellulose composition among genotypes (Table 3.4), total biomass yield remained the most influential factor in estimating total ethanol yield.

Based on structural composition, the conversion efficiencies of the different genotypes ranged from 248 to 273 L/Dry Mg biomass. However with the addition of a true bioenergy sorghum (TAMX8001) in 2009, ethanol yields were increased approximately 1300 L/ha over the next highest ethanol producer (M81E) (Table 3.14). These estimates are based solely on structural carbohydrates and do not include potential ethanol derived from starch or sugar. As has been documented previously, these components are present in substantial quantities in grain and sweet sorghums, respectively. These results underlie the relative importance of yield and quality. It also should be noted that antiquality factors (crystalinity of cellulose) which prevents challenges in efficient cellulose breakdown are not accounted for in this model (U.S. DOE, 2006). Eventually they must be identified, evaluated and considered when evaluating sorghum or any other biomass crop. Ultimately, total biomass yield is of primary importance; once that is improved, then quality maintains or optimizes the efficiency of the system.

Table 3. 13. Theoretical ethanol yields for six sorghum genotypes grown in College Station in 2008. C5 and C6 sugars predicted from dry biomass samples with NIR, using means for hemicellulose and cellulose during the optimal harvest window (expressed in days after planting (DAP))

Theoretical Ethanol Yields								
Genotype	DAP	Hemicellulose %	Cellulose %	C6 sugars (L/Dry Mg)	C5 sugars (L/Dry Mg)	L/Dry Mg	Dry Biomass (Mg/ha)	Ethanol Yield (L/ha)
Graze All	70-83	15.43	26.09	154.88	93.75	248.63	11.84	2943.74
98456	147-168	16.84	28.90	171.36	102.34	273.70	24.23	6631.63
Sugar T	139-154	16.62	28.54	169.30	100.96	270.26	15.87	4289.04
M81E	132-147	15.95	26.64	157.97	96.84	254.81	19.29	4915.24
22053	139-154	15.62	26.99	160.03	94.78	254.81	16.61	4232.36
84G62	139-154	16.01	26.93	159.68	97.18	256.87	16.00	4109.89

Table 3. 14. Theoretical ethanol yields for six sorghum genotypes grown in College Station in 2009. C5 and C6 sugars predicted from dry biomass samples with NIR, using means for hemicellulose and cellulose during the optimal harvest window (expressed in days after planting (DAP))

Theoretical Ethanol Yields								
Genotype	DAP	Hemicellulose %	Cellulose %	C6 sugars (L/Dry Mg)	C5 sugars (L/Dry Mg)	L/Dry Mg	Dry Biomass (Mg/ha)	Ethanol Yield (L/ha)
Graze All	118-132	14.94^{bc}	22.34^{cd}	132.56	90.66	223.21	12.25	2734.38
98456	118-132	15.65^{abc}	24.63^{bc}	146.29	95.12	241.41	16.13	3894.02
Sugar T	104-118	14.78^{c}	21.28^{d}	126.37	89.63	216.00	14.55	3142.84
M81E	132-160	15.90^{ab}	26.26^{ab}	155.91	96.50	252.40	16.23	4096.52
22053	132-160	16.56^{a}	29.25^{a}	173.42	100.62	274.04	12.15	3329.57
TAMX8001	125-146	15.81^{ab}	25.33^{bc}	150.41	96.15	246.57	21.83	5382.54

As seen in the primary harvest, ethanol yields in the ratoon crop varied greatly by year. Average ethanol yields were 2850 L/ha and 1090 L/ha in 2008 and 2009 respectively (Tables 3.15 and 3.16). As in the primary crop, biomass yields were the single most important factor as excessive moisture in 2009 reduces growth and yields (Table 2.5). While composition differences were observed across the two years for the combined analysis, the differences were not so great that differences in biomass yield overrode any total production numbers. In 2008, higher constituent percentages were seen for hemicellulose and cellulose; dry biomass production was also higher in 2008 than in 2009. These two factors combined, contributed to the major differences in ethanol production between 2008 and 2009. These estimates however, do not include any potential ethanol derived from starch or sugar (which were not estimated in this model) and are present in substantial quantities in the grain sorghum and sweet sorghums, respectively. These results underlie the relative importance of yield and quality. Total yield is of primary importance; once that is improved, then quality maintains or optimizes the efficiency of the system.

Table 3. 15. Theoretical ethanol yields for ratoon growth of six sorghum genotypes grown in College Station in 2008. C5 and C6 sugars predicted from dry biomass samples with NIR, using means for hemicellulose and cellulose during the optimal harvest window (expressed in days after clear cutting (DAC) of primary harvest)

Theoretical Ethanol Yields

Genotype	DAC	Hemicellulose %	Cellulose %	C6 sugars (L/Dry Mg)	C5 sugars (L/Dry Mg)	L/Dry Mg	Dry Biomass (Mg/ha)	Ethanol Yield (L/ha)
Graze All	53-67	15.86	27.15	161.06	96.50	257.56	12.79	3294.13
98456	67-88	15.61	26.40	156.59	94.78	251.37	10.14	2548.93
Sugar T	60-81	15.81	26.25	155.91	96.15	252.06	11.48	2893.66
M81E	67-88	15.46	25.97	154.19	93.75	247.94	13.03	3230.65
22053	60-81	15.55	25.70	152.47	94.44	246.91	12.18	3007.36
84G62	53-67	14.86	24.30	144.23	90.32	234.55	9.08	2129.69

Table 3. 16. Theoretical ethanol yields for ratoon growth of six sorghum genotypes grown in College Station in 2009. C5 and C6 sugars predicted from dry biomass samples with NIR, using means for hemicellulose and cellulose during the optimal harvest window (expressed in days after clear cutting (DAC) of primary harvest)

Theoretical Ethanol Yields

Genotype	DAC	Hemicellulose %	Cellulose %	C6 sugars (L/Dry Mg)	C5 sugars (L/Dry Mg)	L/Dry Mg	Dry Biomass (Mg/ha)	Ethanol Yield (L/ha)
Graze All	37-65	14.68^{bc}	20.93^{b}	124.31	89.29	213.60	4.65	993.24
98456	51-79	14.70^{b}	21.10^{b}	125.34	89.29	214.63	6.34	1360.75
Sugar T	51-79	14.47^{bc}	20.27^{b}	120.19	87.91	208.10	4.01	834.50
M81E	23-51	14.22^{c}	20.08^{b}	119.16	86.54	205.70	2.97	610.93
22053	51-79	14.39^{bc}	20.23^{b}	120.19	87.57	207.76	4.67	970.24
TAMX8001	51-79	15.25^{a}	22.62^{a}	134.27	92.72	226.99	7.83	1777.35

Production Logistics and Conclusion

Table 3.17 shows average constituent percentage means over two years across harvest dates from mid July to the first part of October. Cellulose concentrations ranged from 23 to 30 percent across the genotypes for the sampling dates and this represented the largest shift in concentration. Small changes across the genotypes at optimal harvest dates were seen for lignin, hemicellulose, ash and protein. Given the limited shifts and lack of statistical significance due to genotype, these five genotypes do not impact plant composition. This clearly implies that dry biomass yield has a larger impact on production than composition at this stage of bioenergy sorghum development. Naturally, additional testing over multiple locations and environments will be needed to further understand plant composition over a growing season.

Table 3. 17 Harvest dates and mean constituent percentages for five genotypes grown over two years in College Station, Texas. Numbers represent constituent percentages that could be expected during the harvest dates

	Harvest dates					
Genotype	15-Jul	1-Aug	15-Aug	1-Sep	15-Sep	1-Oct
	Lignin†					
Graze All	13	**12**	**12**	13	13	14
Sugar T	12	**11**	**11**	12	13	15
22053	12	11	**11**	**13**	13	12
M81E	12	12	**12**	**10**	8	11
98456	12	12	12	**11**	**11**	14
	Hemicellulose†					
Graze All	15	**15**	**15**	17	18	17
Sugar T	16	**15**	**15**	17	17	18
22053	15	15	**15**	**17**	18	17
M81E	16	15	**15**	**16**	16	17
98456	16	15	16	**16**	**17**	17
	Cellulose†					
Graze All	24	**23**	**24**	29	31	30
Sugar T	25	**24**	**25**	27	29	30
22053	25	24	**25**	**30**	33	29
M81E	25	24	**25**	**25**	26	28
98456	25	24	26	**29**	**30**	29
	Ash†					
Graze All	7	**7**	**7**	7	7	7
Sugar T	6	**6**	**5**	6	6	6
22053	6	6	**6**	**6**	6	6
M81E	6	6	**6**	**6**	6	5
98456	6	6	6	**6**	**7**	6
	Protein†					
Graze All	3	**3**	**3**	3	3	3
Sugar T	3	**3**	**3**	3	3	3
22053	3	3	**3**	**2**	2	2
M81E	4	3	**3**	**2**	2	2
98456	3	3	3	**2**	**2**	2

† Numbers in bold represent optimal harvest time based on maximum dry biomass yield for that particular genotype.

CHAPTER IV

CONCLUSIONS

Biomass Accumulation

Based on the results reported herein, it is clear that sorghum provides a high yielding biomass source that can be harvested continuously from the middle of July through mid November. Given that industrial plants must process biomass continuously throughout the year, it is expected that complementary biomass crops can be used to fill other production times. For example, perennial crops such as Miscanthus and switchgrass are best harvested in the winter season when they are dormant (Heaton et al., 2004). Consequently using a sorghum/switchgrass system implies that biomass would be readily available from July through March. Given that length of time, it is assumed that stored reserves of both crops could be used to support processing in the April through June timeframe.

To produce enough biomass from sorghum, several different types (i.e. forage, sweet and bioenergy) will need to be utilized. Forage sorghum hybrids have good primary and ratoon biomass potential, and can be utilized as a primary source of biomass coming from the primary harvest and a secondary biomass supply coming from the ratoon harvest as needed to meet production demands. Sweet sorghums, though mainly used in ethanol production via juice, can also be utilized from a biomass stand point. These sorghums, such as M81E, can be semi photo-period sensitive and give producers late season production. With the addition of PS sorghum hybrids, which do not flower until late October to early November; biomass supplies can be extended into the later part of November until other crops are ready to be utilized.

Composition

Based on optimal harvest for the genotypes in this trial, it appears that plant constituent percentages will vary by genotype and also by environment. Significant genotype x environment interaction is also a major factor in the production of bioenergy sorghum. Further testing across environments and years will be needed to fully

understand the percentages and ranges that can be expected when producing feedstocks for ethanol production. The ethanol produced from these hybrids also varied by genotype and environment. Overall the highest ethanol producer of all the hybrids in this trial was TAMX8001, a PS bioenergy sorghum. Compositional factors did play a role in this, but higher ethanol yields were mainly attributed to overall biomass production. These results underlie the relative importance of yield and quality. Total yield is of primary importance; once that is improved, then quality maintains or optimizes the efficiency of the system.

REFERENCES

Chang V., and M. Holtzapple (2000) Fundamental factors affecting biomass enzymatic reactivity. Applied Biochemistry and Biotechnology 84-86:5-37.

Corn R.J. (2009) Heterosis and composition of sweet sorghum. PhD Dissertation, Texas A&M University, College Station, Texas

Corredor D., J. Salazar, K. Hohn, S. Bean, B. Bean, and D. Wang (2009) Evaluation and characterization of forage sorghum as feedstock for fermentable sugar production. Applied Biochemistry and Biotechnology 158:164-179.

Dahlberg J., E. Wolfrum, B. Bean, W.L. Rooney (in press) Composition and agronomic evaluation of sorghum biomass as a potential feedstock for renewable fuels.

DOE's Office of Science and Office of Energy Efficiency and Renewable Energy. 2006. A summary roadmap for making cellulosic ethanol a practical alternative to gasoline. http://genomicscience.energy.gov/biofuels/B2Bfactsheet.pdf Verified May 1, 2010

DOE's Office of Science and Office of Energy Efficiency and Renewable Energy. Executive summary high-yield scenario workshop series report. 2009.

Dolciotti I., S. Mambelli, S. Grandi, and G. Venturi (1998) Comparison of two Sorghum genotypes for sugar and fiber production. Industrial Crops and Products 7:265-272.

FAO. (2006). Commodities by country. http://faostat.fao.org/site/339/default.aspx Verified May 17, 2010

Heaton E.A., F.G. Dohleman, and S.P. Long (2008) Meeting US biofuel goals with less land: The potential of Miscanthus. Global Change Biology 14:2000-2014.

Heaton E.A., S.P. Long, T.B. Voigt, M.B. Jones, and J. Clifton-Brown (2004) Miscanthus for renewable energy generation: European Union experience and projections for Illinois. Mitigation and Adaptation Strategies for Global Change 9:433-451.

Jin S., and H. Chen (2007) Near-infrared analysis of the chemical composition of rice straw. Industrial Crops and Products 26:207-211.

Lemus R., E.C. Brummer, K.J. Moore, N.E. Molstad, C.L. Burras, and M.F. Barker (2002) Biomass yield and quality of 20 switchgrass populations in southern Iowa. Biomass and Bioenergy 23:433-442.

Lyons R.K., and J.W. Stuth (1992) Fecal NIRS equations for predicting diet quality of free-ranging cattle. Journal of Range Management 45:238-244.

McCollum, T., K. McCuistion, and B. Bean. (2003) Brown midrib and photoperiod-sensitive forage sorghums. Texas Ag. Exp. Stn. Amarillo. http://amarillo.tamu.edu/programs/agronomy/publications/Forage%20Sorghum/brownmidrib.pdf. Verified May 9, 2010

McCormick M.E., M.E. Morris, B.A. Ackerson, and D.C. Blouin (1995) Ratoon cropping forage sorghum for silage: Yield, fermentation, and nutrition. Agron. J. 87:952-957.

Miller F.R., and G.G. McBee (1993) Genetics and management of physiologic systems of sorghum for biomass production. Biomass and Bioenergy 5:41-49.

Monk R.L., F.R. Miller, and G.G. McBee (1984) Sorghum improvement for energy production. Biomass 6:145-153.

Murray S.C., W.L. Rooney, S.E. Mitchell, A. Sharma, P.E. Klein, J.E. Mullet, and S. Kresovich (2008) Genetic improvement of sorghum as a biofuel feedstock: II. QTL for stem and leaf structural carbohydrates. Crop Sci 48:2180-2193.

Oliver A.L., J.F. Pedersen, R.J. Grant, and T.J. Klopfenstein (2005) Comparative effects of the sorghum -6 and -12 genes. Crop Sci. 45:2234-2239.

Packer D.J. (2011) High-biomass sorghums for biomass biofuel production. PhD Dissertation, Texas A&M University, College Station, Texas

Perlack, R.D., L.L Wright, A.F Turhollow, R.L Graham, B.J. Stoles, and D.C. Erbach, Biomass as a feedstock for bioenergy and biproducts industry: The technical feasability of a billion ton annual supply. 2005. http://www1.eere.energy.gov/biomass/pdfs/final_billionton_vision_report2.pdf Verified May 10, 2010

Propheter J.L., S.A. Staggenborg, X. Wu, and D. Wang (2010) Performance of annual and perennial biofuel crops: Yield during the first two years. Agron. J. 102:806-814.

Quinby. J. R. Sorghum improvement and the genetics of growth. Texas A&M University Press, 1974. Print.

Rooney W.L. (2000). Genetics and cytogenetics. p. 261-308. In C.W. Smith and R.A. Frederiksen (ed.) Sorghum: Origin, history, technology and production. Wiley Press, New York.

Rooney W.L., and S. Aydin (1999) Genetic control of a photoperiod-sensitive response in sorghum bicolor (L.) Moench. Crop Sci. 39:397-400.

Rooney W.L., J. Blumenthal, B. Bean, and J.E. Mullet (2007) Designing sorghum as a dedicated bioenergy feedstock. Biofuels, Bioproducts and Biorefining 1:147-157.

Sanderson M.A., F. Agblevor, M. Collins, and D.K. Johnson (1996) Compositional analysis of biomass feedstocks by near infrared reflectance spectroscopy. Biomass and Bioenergy 11:365-370.

Sattler S.E., D.L. Funnell-Harris, and J.F. Pedersen (2010) Brown midrib mutations and their importance to the utilization of maize, sorghum, and pearl millet lignocellulosic tissues. Plant Science 178:229-238.

Shih S.F., G.J. Gascho, and G.S. Rahi (1981) Modeling biomass production of sweet sorghum. Agron. J. 73:1027-1032.

Siefers M.K., J.E. Turner, G.L. Huck, M.A. Young, S.A. Anderson, R.V. Pope and K.L. Bolsen. (1997) Agronomic and Silage Quality Traits of Forage Sorghum Cultivars in 1995. Kansas Agric. Exp. Sta. Rep. Prog., 783: 75-79

Sissine F. 2007. Energy independence and security act of 2007: A summary of major provisions. http://energy.senate.gov/public/_files/RL342941.pdf . Verified May 1, 2010

Stefaniak T.R., E. Wolfrum, J.A. Dahlberg, B.R. Bean, N. Dighe, and W.L. Rooney (in review) A comparison of detergent and dietary fiber analysis of sorghum samples for bioenergy components.

Stefaniak T.R., E. Wolfrum, J.A. Dahlberg, B.R. Bean, N. Dighe, and W.L. Rooney (submitted) Variation in biomass composition components among forage, biomass, and sweet sorghums.

Stuth J., A. Jama, and D. Tolleson (2003) Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. Field Crops Research 84:45-56.

USCP. Biofuels. Sorghum use in growing biofuels production. 2009. http://www.sorghumcheckoff.com/biofuels (accessed on May 17, 2010)

U.S. DOE. 2006. Breaking the biological barriers to cellulosic ethanol: A joint research agenda, DOE/SC-0095, U.S. Department of Energy Office of Science and Office of Energy Efficient and Renewable Energy (www.doegenomestolife.org/biofuels/).

U.S. Department of Energy. 2011. Theroretical ethanol yield calculator. http://www1.eere.energy.gov/biomass/ethanol_yield_calculator.html. Verified July 7, 2011.

Vanderlip R.L., and H.E. Reeves (1972) Growth stages of sorghum [Sorghum bicolor, (L.) Moench.]1. Agron. J. 64:13-16. DOI: 10.2134/agronj1972.

Van Soest P.J. (1967) Development of a comprehensive system of feed analyses and its application to forages. Journal of Animal Science 26:119-128.

Venuto B., and B. Kindiger (2008) Forage and biomass feedstock production from hybrid forage sorghum and sorghum–sudangrass hybrids. Grassland Science 54:189-196.

Wolfrum E., A. Lorenz, N. deLeon (2009) Correlating detergent fiber analysis and dietary fiber analysis data for corn stover collected by NIRS. Cellulose 16:577-585.

Wolfrum E., C. Payne, T. Stafaniak, N. Dighe, B. Bean, J. Dahlberg, W.L. Rooney (Submitted) Multivariate calibration models for sorghum composition using near-infrared (NIR) spectroscopy.

APPENDIX

Table A. 1. Anova mean squares for sources of variation affecting dry biomass for all one week harvest intervals from 55 (April 11th) days to 174 (October 8th) days after planting (DAP) in 2009

Source	DF	Dry Biomass
DAP	1	1224.08**
Genotype	5	14.24
DAPxGenotype	5	69.63**
DAPxDAP	1	495.62**
Rep	1	125.04**
Error	136	12.16
		R^2= 0.48

*,** Significant at p = .05 and .01, respectively

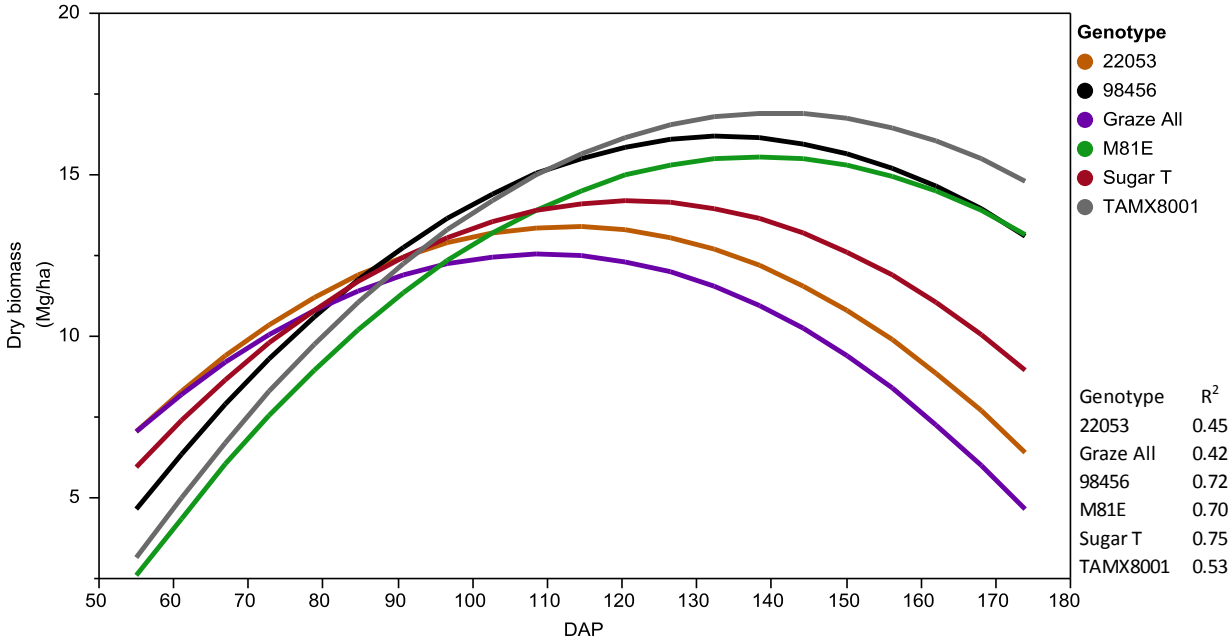

Figure A. 1. Multiple regression of dry biomass (Mg/ha) for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 55 (June 11th) days to 174 (October 8th) days after planting (DAP). Genotype fit is represented by the R^2 value

Table A. 2. Anova mean squares for sources of variation affecting ratoon dry biomass for all one week harvest intervals from 18 (August 18th) days to 130 (December 19th) days after cutting (DAC) the primary harvest in 2008

Source	DF	Dry Biomass
DAC	1	341.35**
Genotype	5	16.65
DACxGenotype	5	18.20
DACxDAC	1	268.81**
Rep	1	60.66**
Error	118	8.84
		R^2= 0.35

*,** Significant at p = .05 and .01, respectively

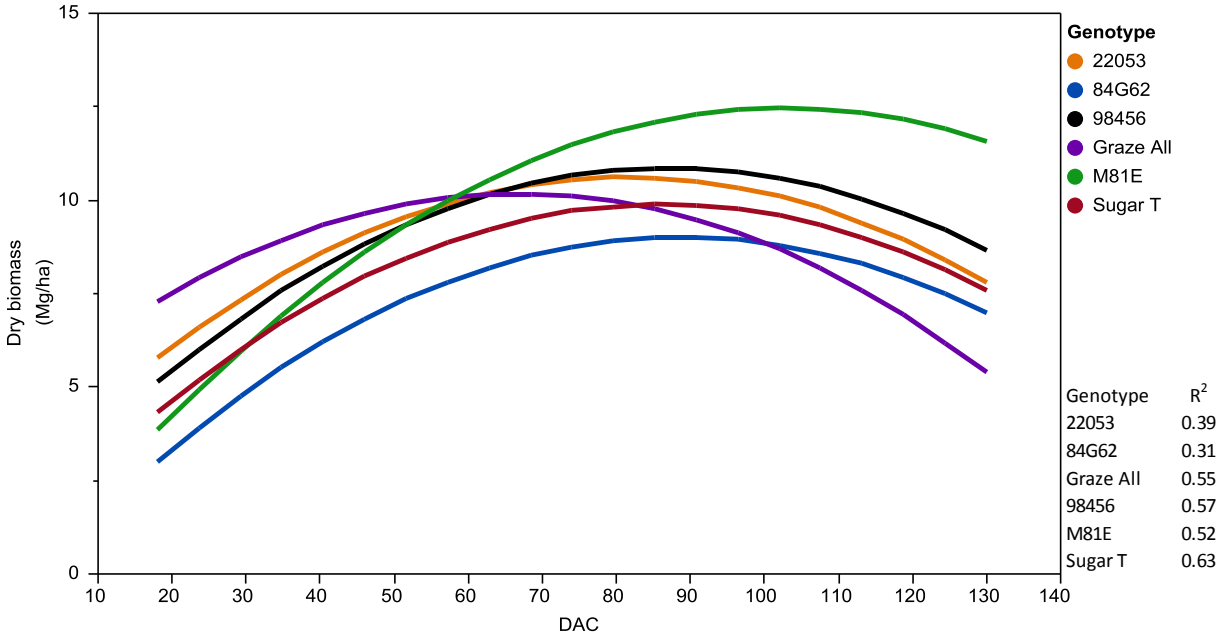

Figure A. 2. Multiple regression of ratoon dry biomass (Mg/ha) for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 18 (August 29th) days to 130 (December 19th) days after cutting (DAC) the primary growth. Genotype fit is represented by the R^2 value

Table A. 3. Anova mean squares for sources of variation affecting ratoon dry biomass for all one week harvest intervals from 23 (September 10th) days to 79 (November 5th) days after cutting (DAC) the primary harvest in 2009

Source	DF	Dry Biomass
DAC	1	25.31*
Genotype	5	17.14*
DACxGenotype	5	4.75
DACxDAC	1	90.77**
Rep	1	6.26
Error	46	6.26
		R^2= 0.43

*,** Significant at p = .05 and .01, respectively

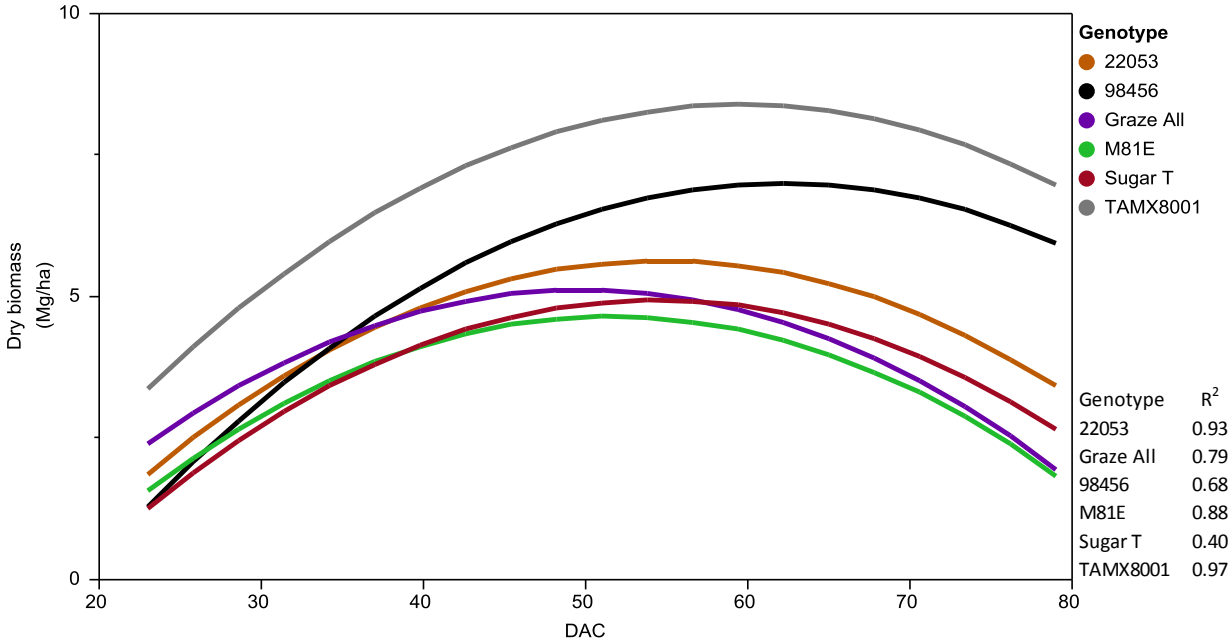

Figure A. 3. Multiple regression of ratoon dry biomass (Mg/ha) for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 23 (September 10th) days to 79 (November 5th) days after cutting (DAC) the primary harvest. Genotype fit is represented by the R^2 value

Table A. 4. Anova mean squares for sources of variation affecting lignin, hemicellulose, cellulose, ash and protein for all one week harvest intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP) in 2008. Model based on dry biomass yields

Source	DF	Lignin	Hemicellulose	Cellulose	Ash	Protein
DAP	1	166.31**	25.59**	0.09	870.60**	424.53**
Genotype	5	5.76**	2.96**	25.76**	1.59	2.36**
DAP*Genotype	5	0.42	0.13	0.54	0.85	0.56
DAP*DAP	1	18.14**	0.36	158.93**	317.72**	168.48**
Rep	1	0.03	0.35	4.60	1.93	1.33
Error	184	0.93	0.58	4.8	1.24	0.64
		R^2= 0.56	R^2= 0.29	R^2= 0.25	R^2= 0.84	R^2= 0.84

*,** Significant at p = .05 and .01, respectively

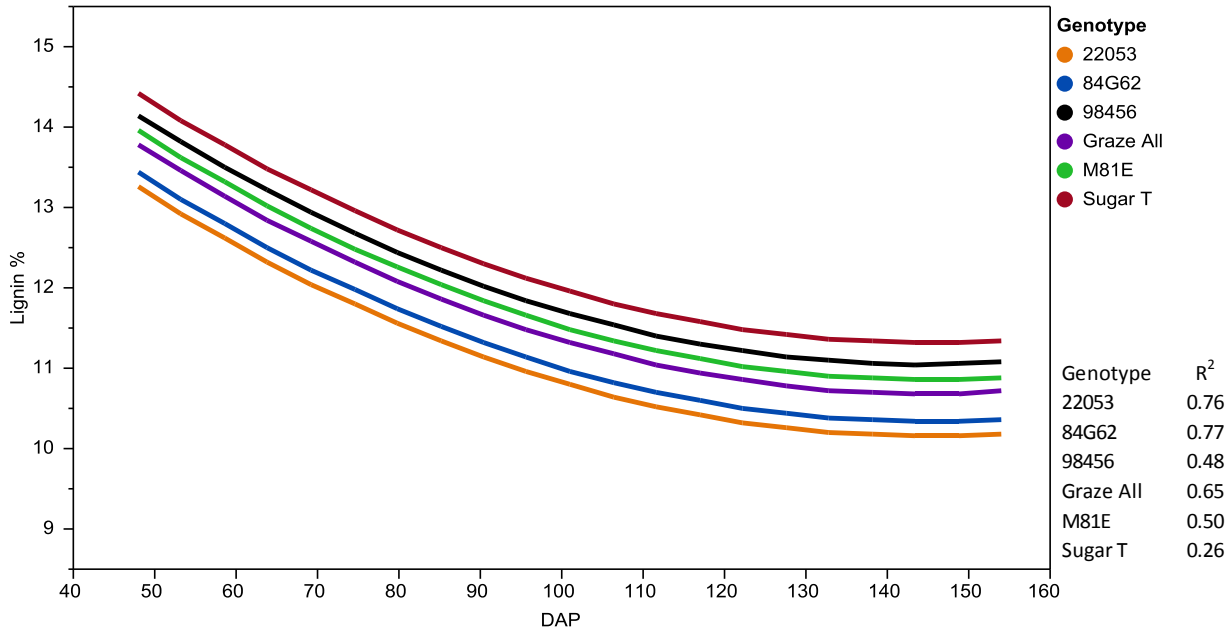

Figure A. 4. Multiple regression of lignin percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

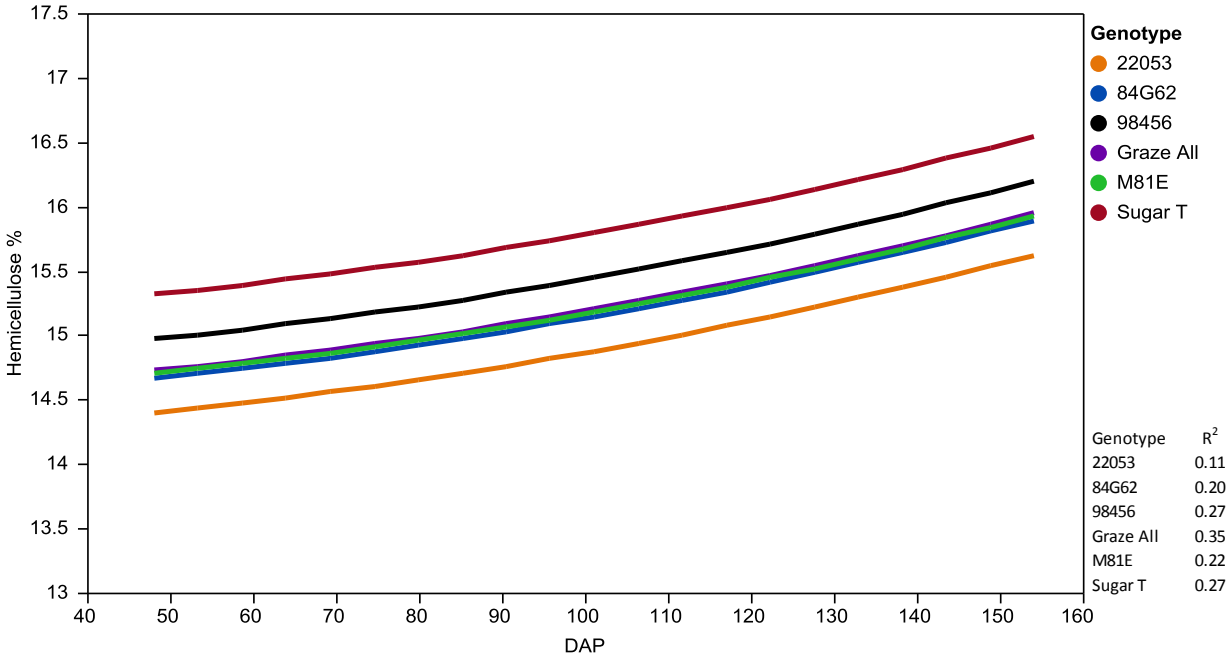

Figure A. 5. Multiple regression of hemicellulose percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

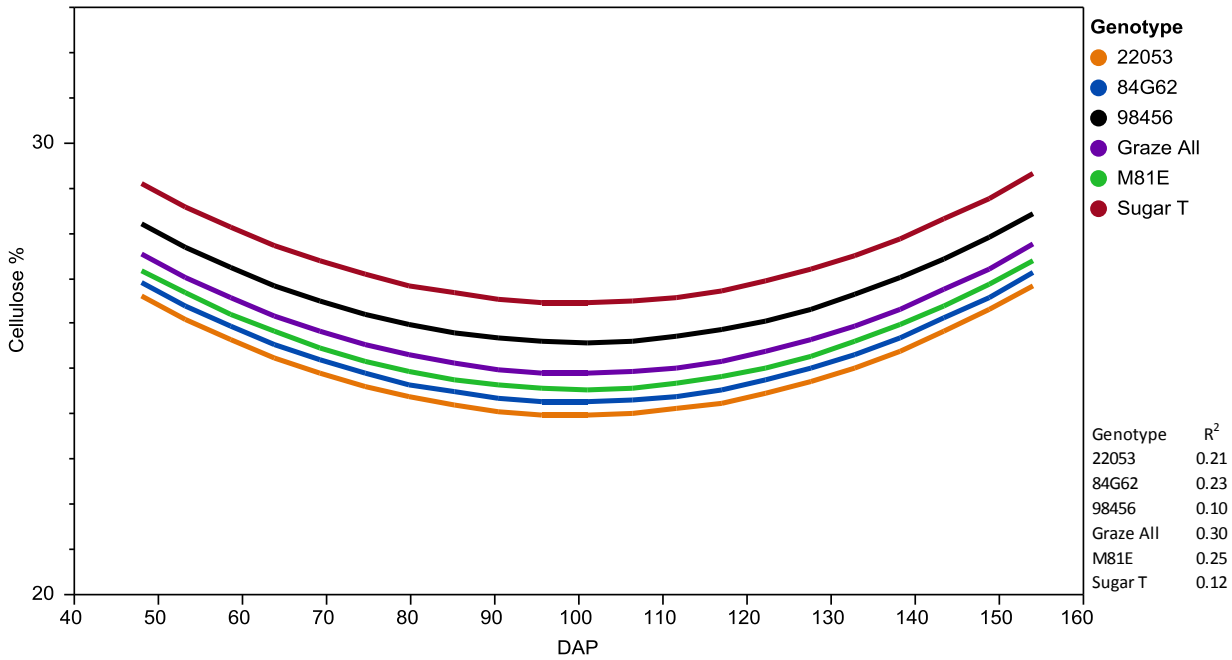

Figure A. 6. Multiple regression of cellulose percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

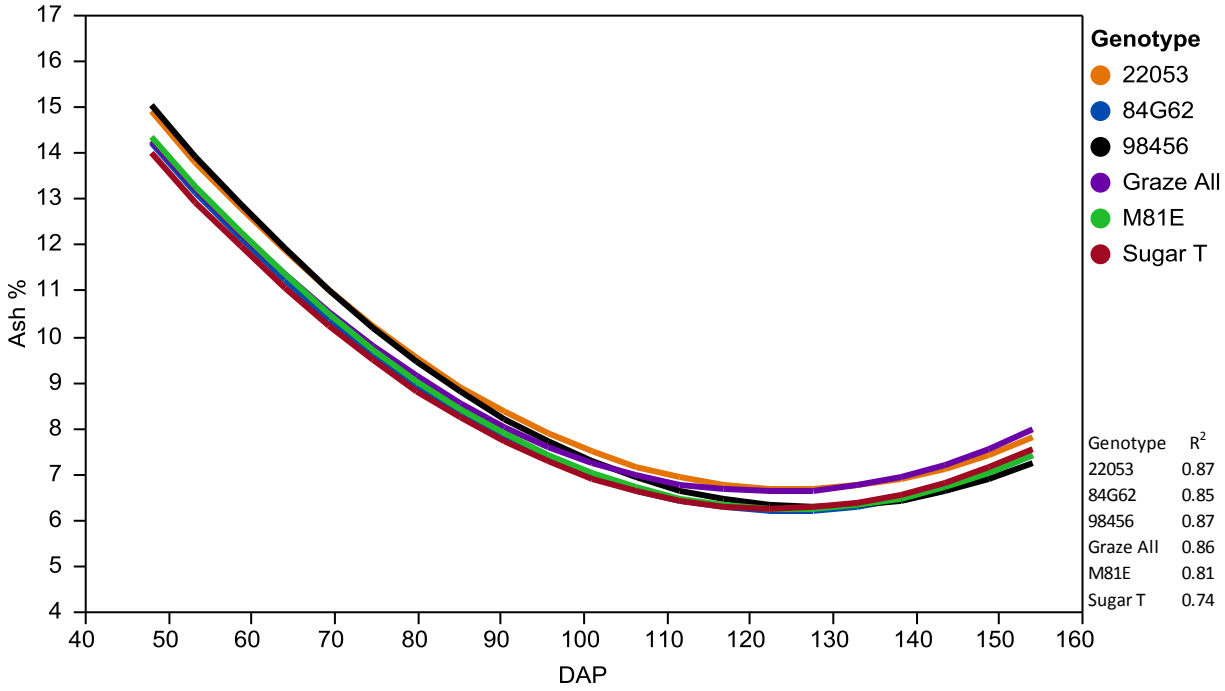

Figure A. 7. Multiple regression of ash percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

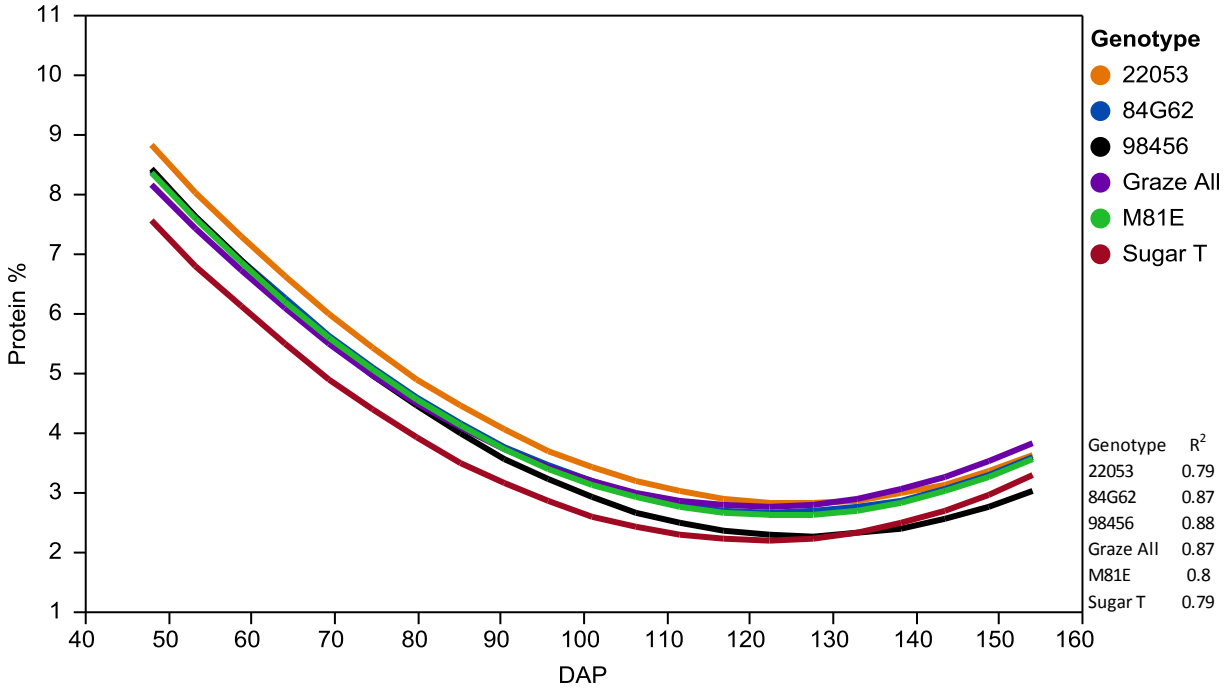

Figure A. 8. Multiple regression of protein percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 48 (May 15th) days to 154 (August 29th) days after planting (DAP). Genotype fit is represented by the R^2 value

Table A. 5. Anova mean squares for sources of variation affecting lignin, hemicellulose, cellulose, ash and protein for all one week harvest intervals from 55 (April 11th) days to 160 (September 24th) days after planting (DAP) in 2009. Model based on dry biomass yields

Source	DF	Lignin	Hemicellulose	Cellulose	Ash	Protein
DAP	1	23.76**	25.44**	271.50**	283.43**	465.04**
Genotype	5	3.96*	1.45**	12.16**	4.81**	1.20**
DAP*Genotype	5	2.80	0.36	4.14	2.89**	1.06**
DAP*DAP	1	7.17*	10.37**	197.47**	230.14**	56.50**
Rep	1	0.20	0.08	0.60	0.16	0.59
Error	149	1.36	0.44	3.69	0.73	0.39
		R^2= 0.22	R^2= 0.44	R^2= 0.55	R^2= 0.80	R^2= 0.89

*,** Significant at p = .05 and .01, respectively

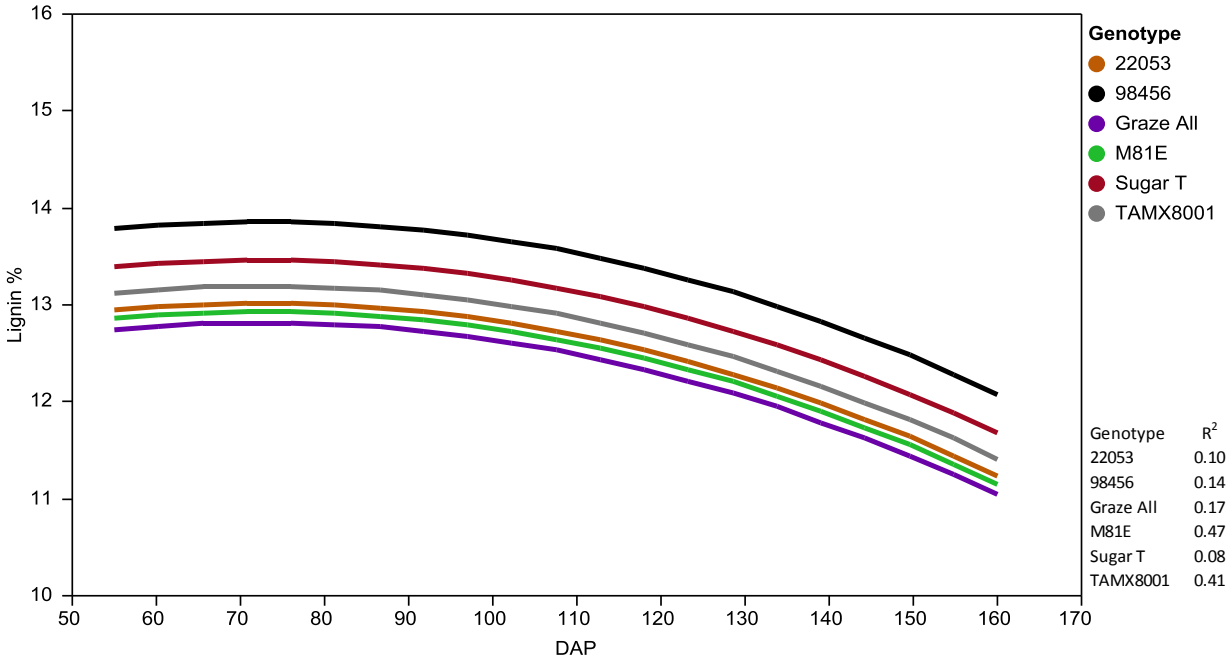

Figure A. 9. Multiple regression of lignin percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 55 (April 11th) days to 160 (September 24th) days after planting (DAP). Genotype fit is represented by the R^2 value

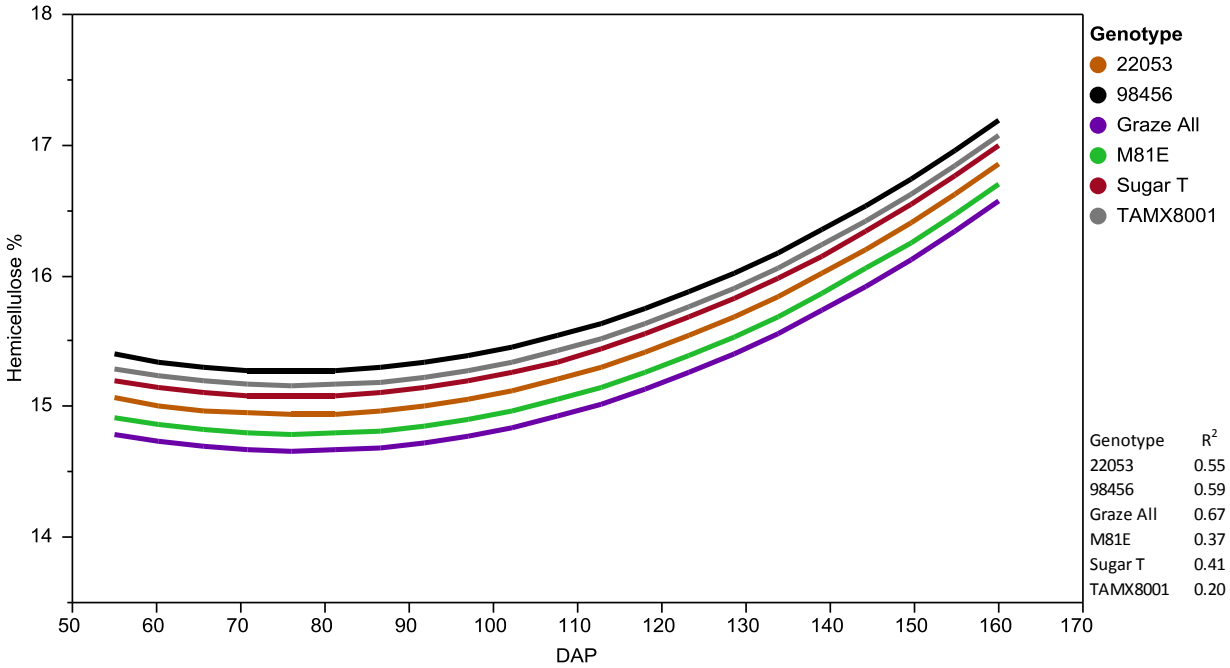

Figure A. 10. Multiple regression of hemicellulose percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 55 (April 11th) days to 160 (September 24th) days after planting (DAP). Genotype fit is represented by the R^2 value

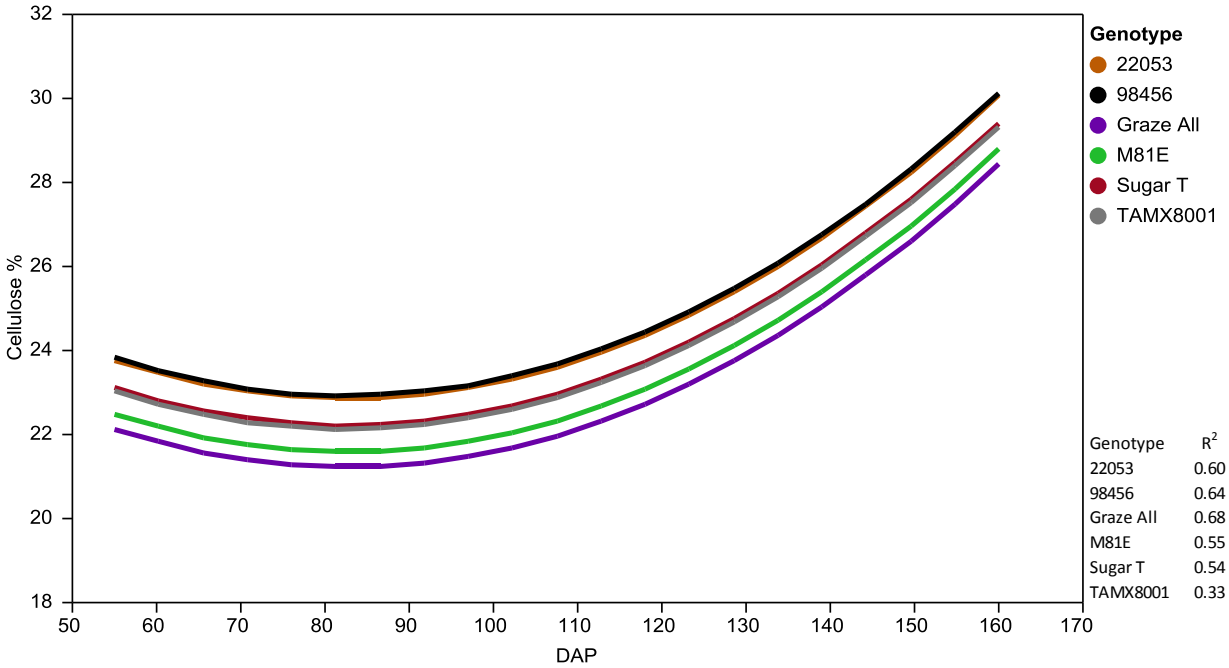

Figure A. 11. Multiple regression of cellulose percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 55 (April 11th) days to 160 (September 24th) days after planting (DAP). Genotype fit is represented by the R^2 value

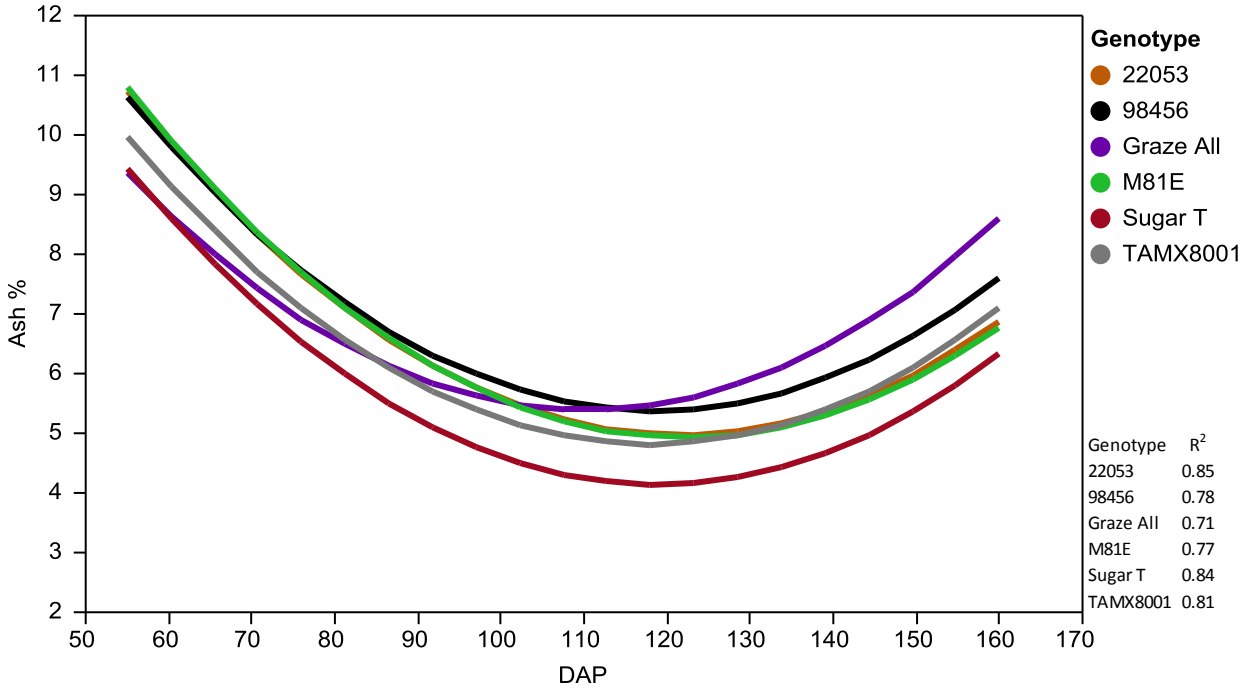

Figure A. 12. Multiple regression of ash percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 55 (April 11th) days to 160 (September 24th) days after planting (DAP). Genotype fit is represented by the R^2 value

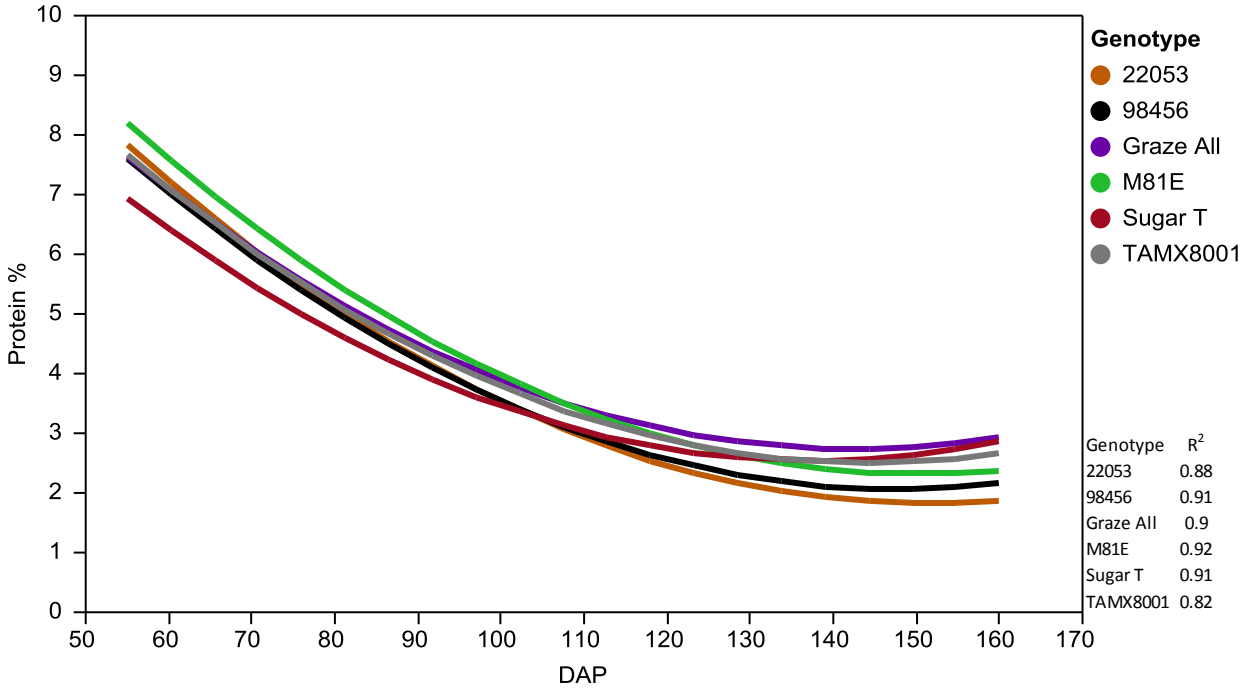

Figure A. 13. Multiple regression of protein percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 55 (April 11th) days to 160 (September 24th) days after planting (DAP). Genotype fit is represented by the R^2 value

Table A. 6. Anova mean squares for sources of variation affecting ratoon lignin, hemicellulose, cellulose, ash and protein for all one week harvest intervals from 18 (August 18th) days to 130 (December 19th) days after cutting (DAC) the primary harvest in 2008. Model based on dry biomass yields

Source	DF	Lignin	Hemicellulose	Cellulose	Ash	Protein
DAC	1	26.92**	58.08**	117.06**	748.74**	251.85**
Genotype	5	1.48	1.37*	13.79**	1.64	3.07**
DAC*Genotype	5	4.21*	0.68	5.65	1.76	1.24
DAC*DAC	1	73.98**	4.93**	75.12**	158.18**	66.30**
Rep	1	0.01	0.17	6.27	0.43	0.53
Error	116	1.81	0.45	3.83	1.23	0.93
		R^2= 0.26	R^2= 0.64	R^2= 0.45	R^2= 0.84	R^2= 0.71

*,** Significant at p = .05 and .01, respectively

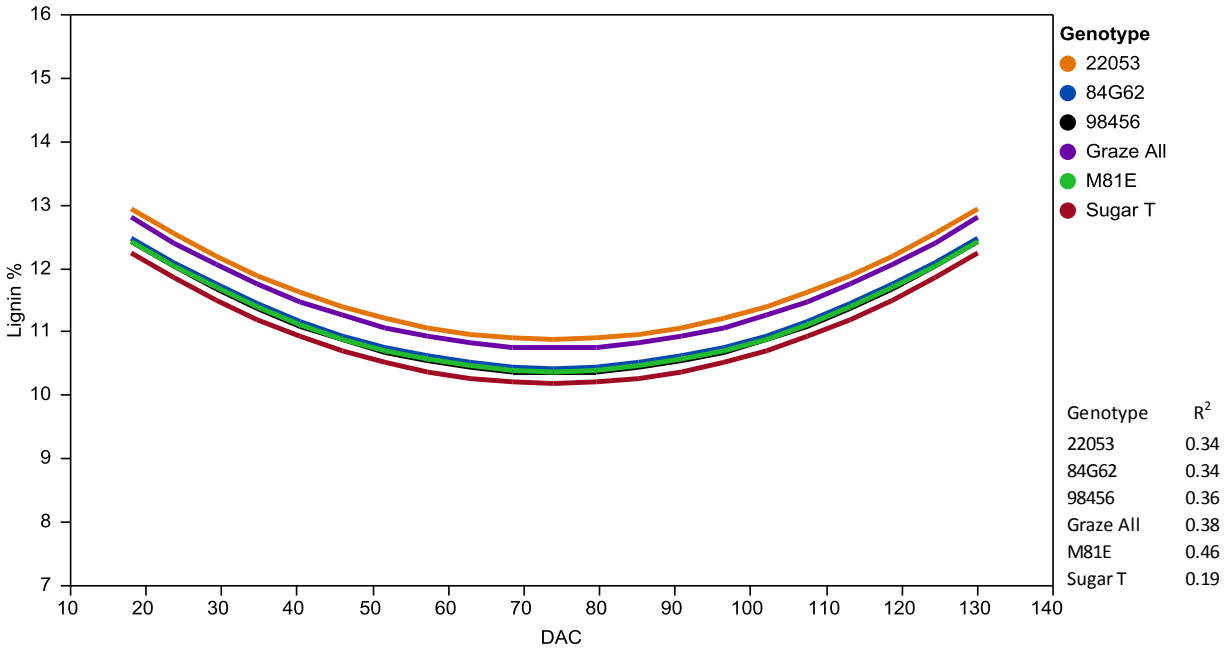

Figure A. 14. Multiple regression of lignin percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 18 (August 18th) days to 130 (December 19th) days after cutting (DAC) the primary harvest in 2008. Genotype fit is represented by the R^2 value

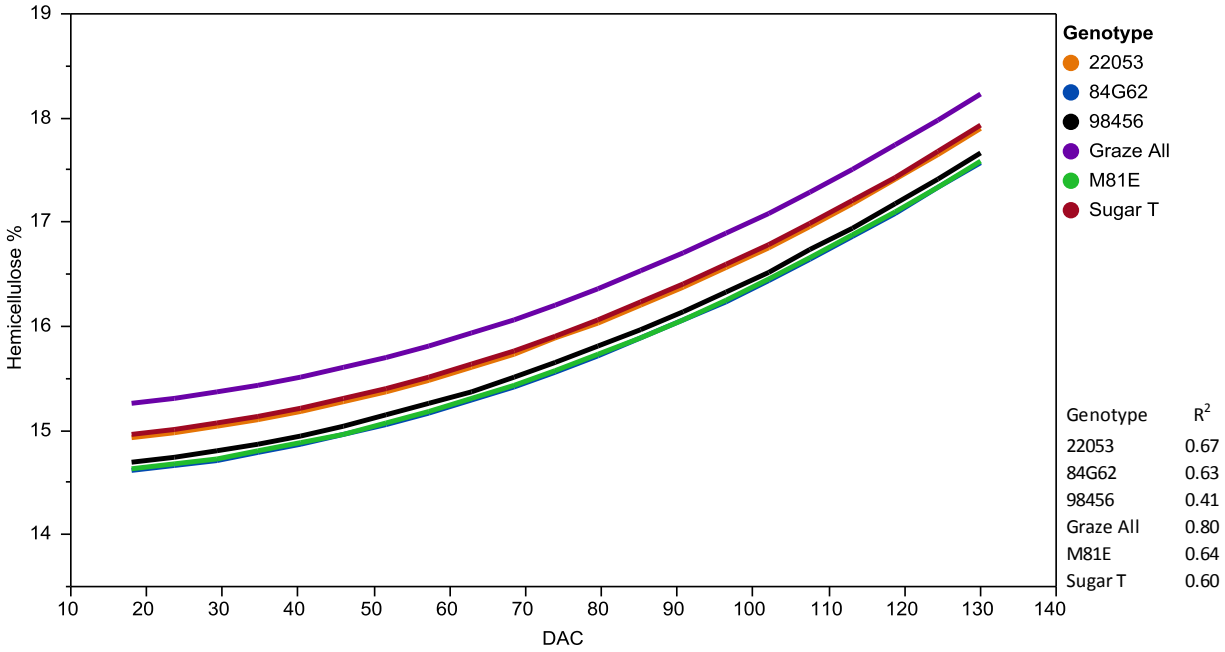

Figure A. 15. Multiple regression of hemicellulose percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 18 (August 18th) days to 130 (December 19th) days after cutting (DAC) the primary harvest in 2008. Genotype fit is represented by the R^2 value

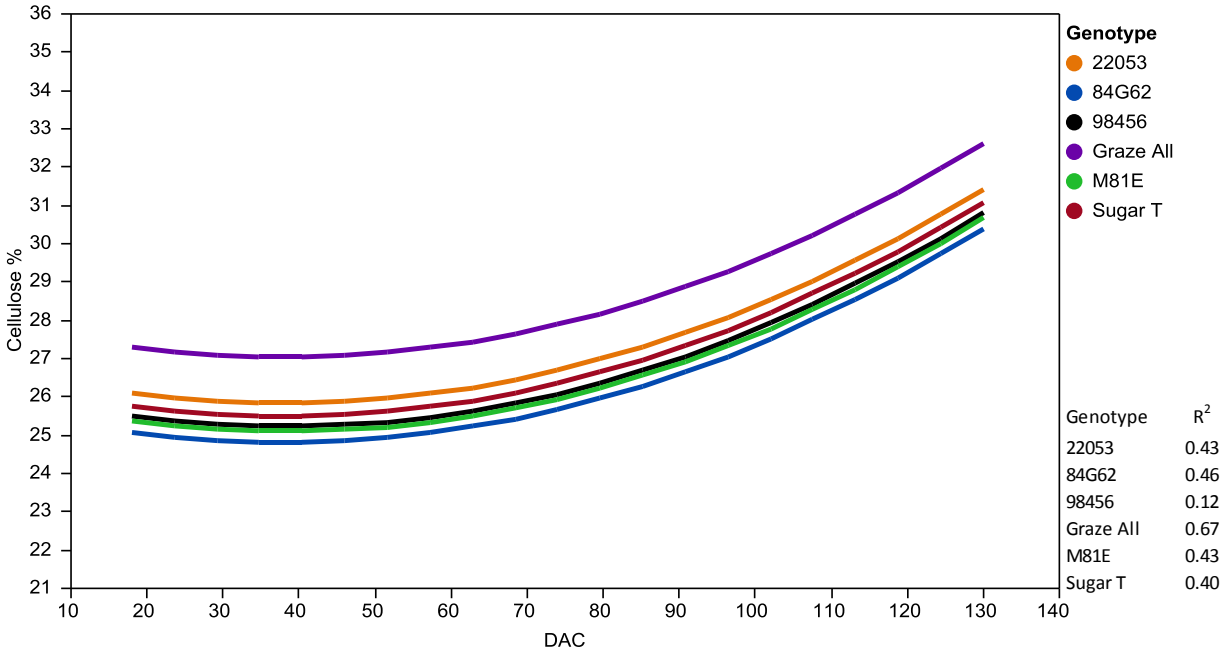

Figure A. 16. Multiple regression of cellulose percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 18 (August 18th) days to 130 (December 19th) days after cutting (DAC) the primary harvest in 2008. Genotype fit is represented by the R^2 value

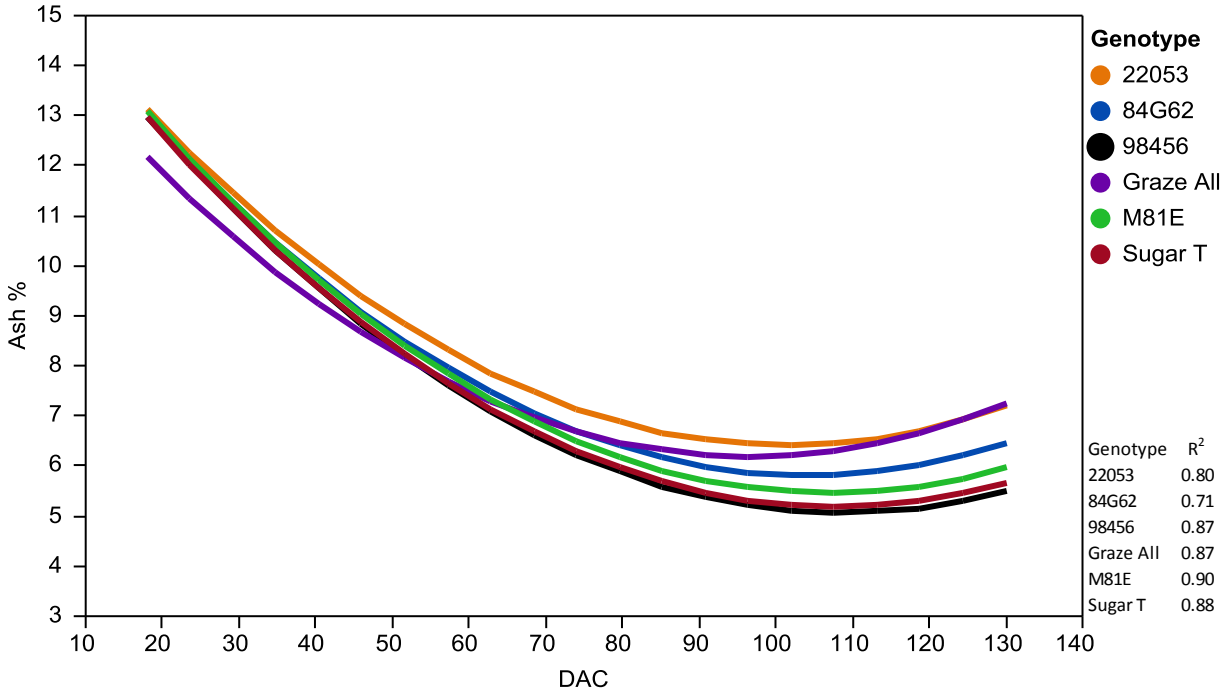

Figure A. 17. Multiple regression of ash percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 18 (August 18th) days to 130 (December 19th) days after cutting (DAC) the primary harvest in 2008. Genotype fit is represented by the R^2 value

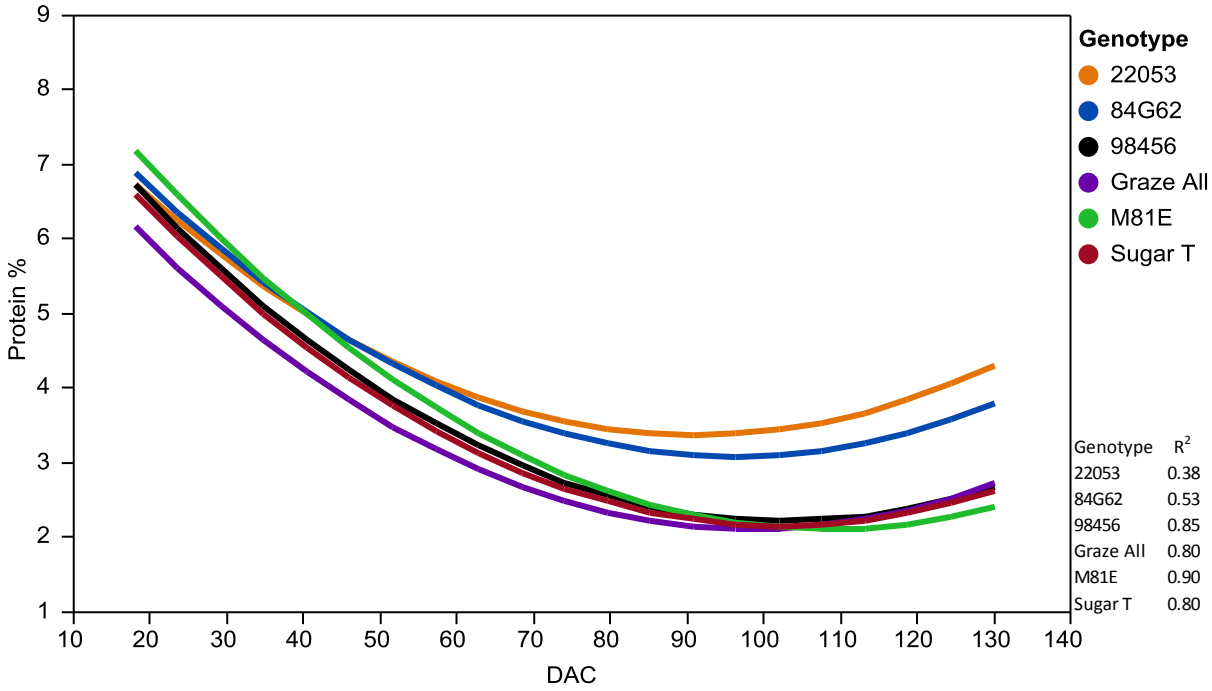

Figure A. 18. Multiple regression of protein percentage for six sorghum genotypes grown in College Station in 2008. Harvests were made at one week intervals from 18 (August 18th) days to 130 (December 19th) days after cutting (DAC) the primary harvest in 2008. Genotype fit is represented by the R^2 value

Table A. 7. Anova mean squares for sources of variation affecting ratoon lignin, hemicellulose, cellulose, ash and protein for all one week harvest intervals from 23 (September 10th) days to 107 (December 3rd) days after cutting (DAC) the primary harvest in 2009. Model based dry biomass yields

Source	DF	Lignin	Hemicellulose	Cellulose	Ash	Protein
DAC	1	9.97**	15.60**	106.30**	248.74**	163.64**
Genotype	5	3.14**	0.87**	6.12**	2.24**	0.42
DAC*Genotype	5	1.38*	0.35	1.84	1.05	0.75
DAC*DAC	1	9.10**	0.004	18.56**	42.18**	9.71**
Rep	1	0.15	0.17	0.01	0.36	0.05
Error	70	0.42	0.21	1.56	0.61	0.34
		R^2= 0.48	R^2= 0.54	R^2= 0.56	R^2= 0.87	R^2= 0.88

*,** Significant at p = .05 and .01, respectively

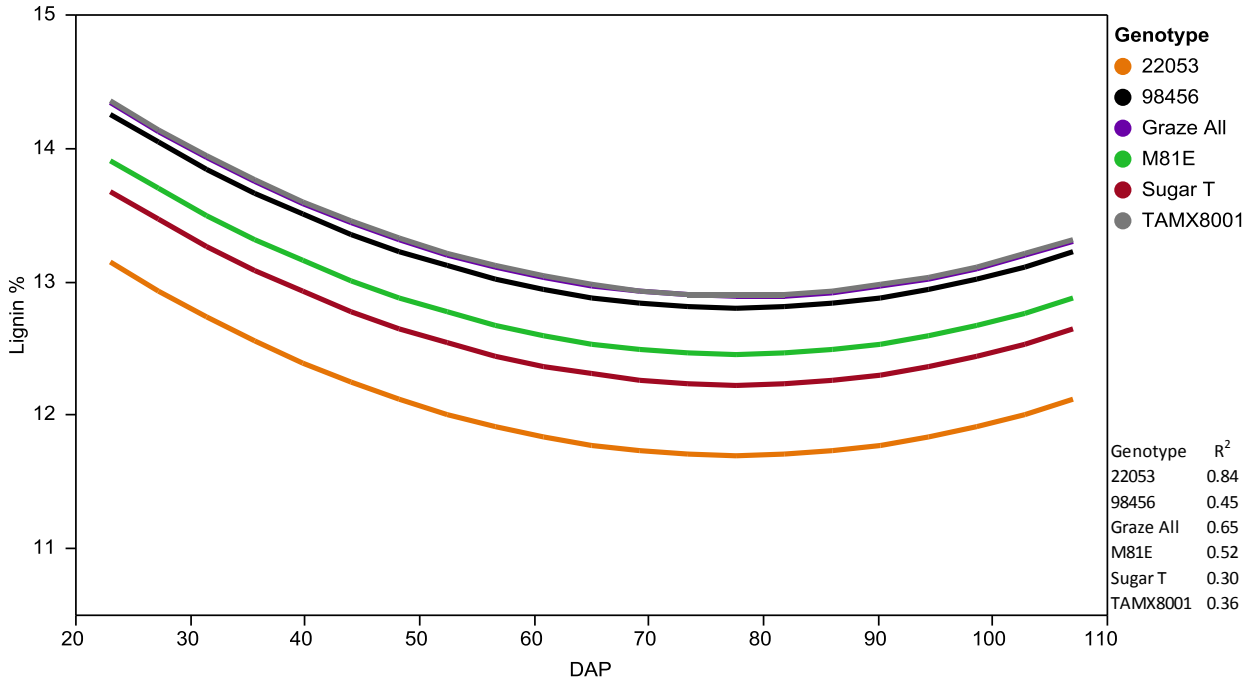

Figure A. 19. Multiple regression of lignin percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 23 (September 10th) days to 107 (December 3rd) days after cutting (DAC) the primary harvest in 2009. Genotype fit is represented by the R^2 value

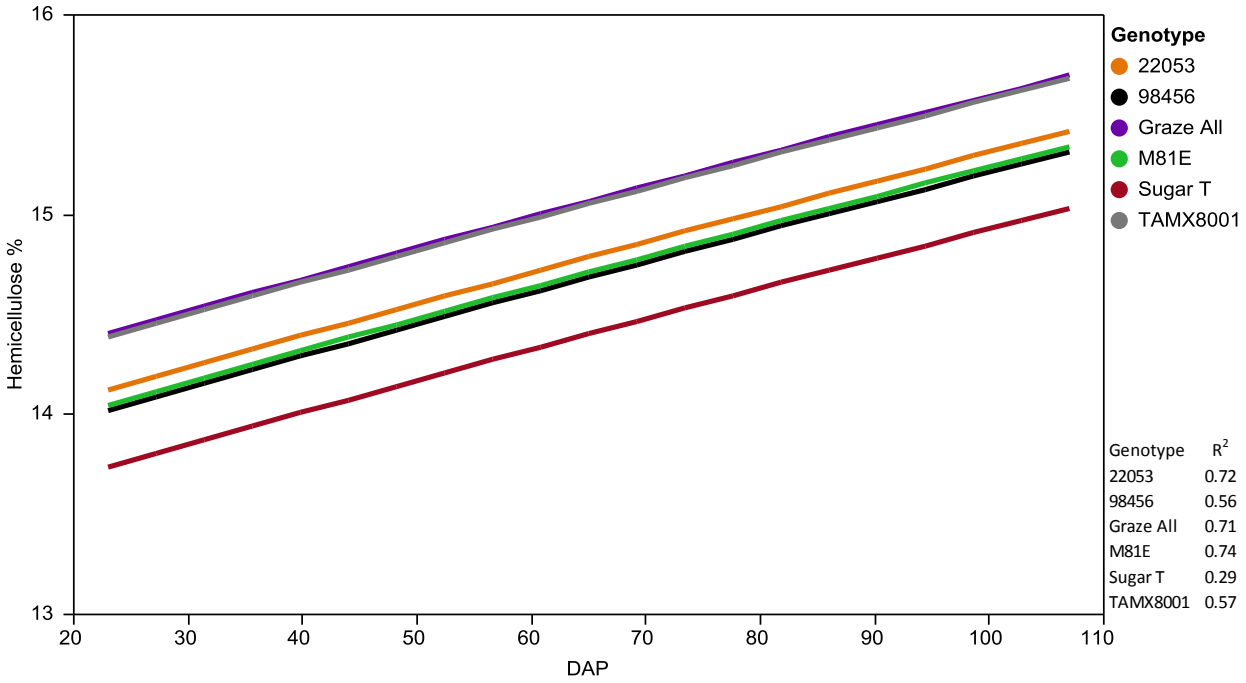

Figure A. 20. Multiple regression of hemicellulose percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 23 (September 10th) days to 107 (December 3rd) days after cutting (DAC) the primary harvest in 2009. Genotype fit is represented by the R^2 value

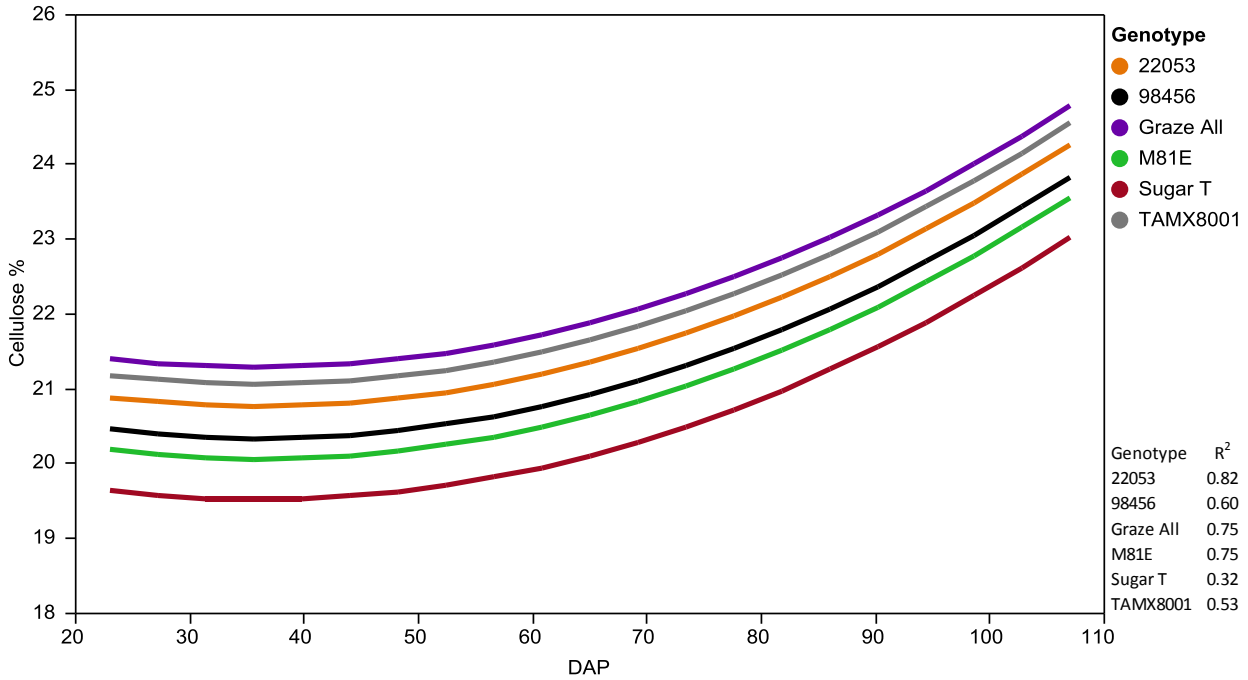

Figure A. 21. Multiple regression of cellulose percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 23 (September 10th) days to 107 (December 3rd) days after cutting (DAC) the primary harvest in 2009. Genotype fit is represented by the R^2 value

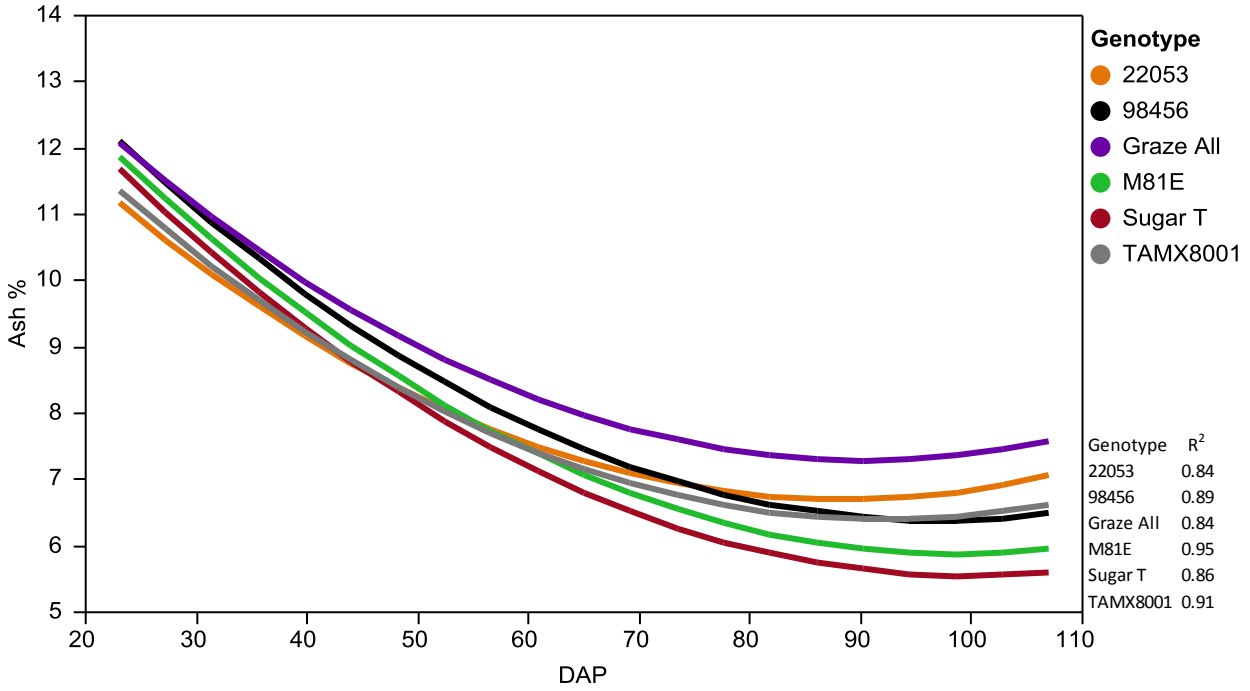

Figure A. 22. Multiple regression of ash percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 23 (September 10th) days to 107 (December 3rd) days after cutting (DAC) the primary harvest in 2009. Genotype fit is represented by the R^2 value

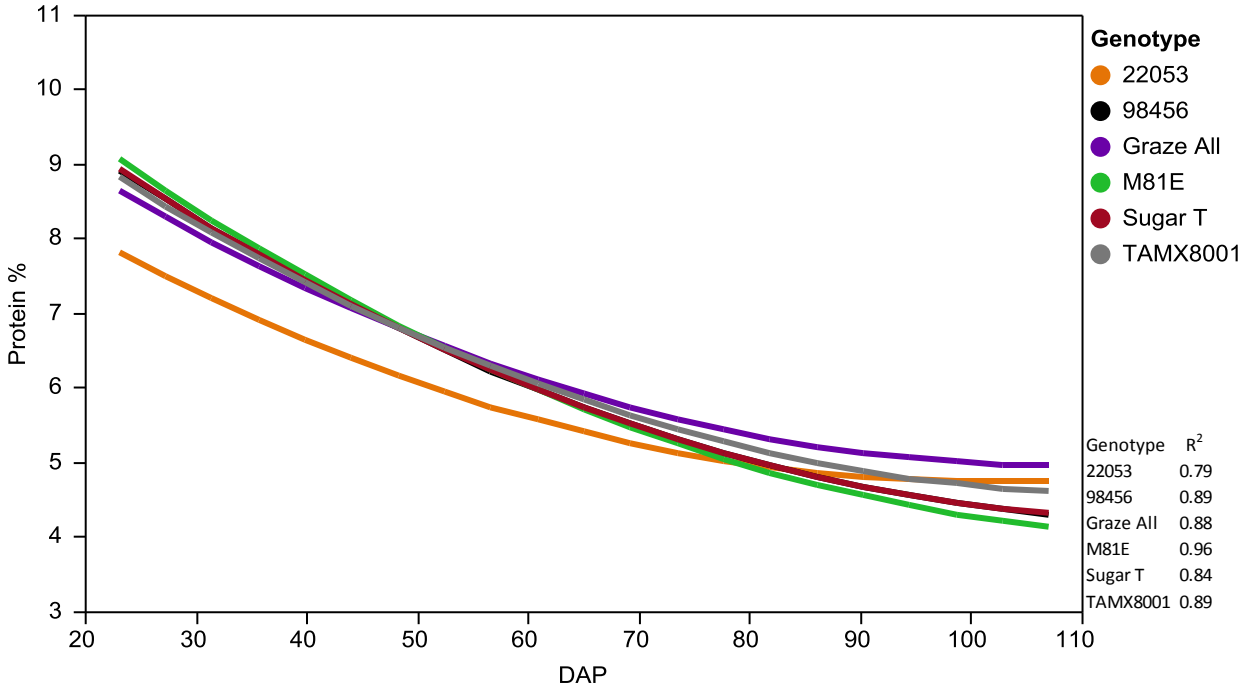

Figure A. 23. Multiple regression of protein percentage for six sorghum genotypes grown in College Station in 2009. Harvests were made at one week intervals from 23 (September 10th) days to 107 (December 3rd) days after cutting (DAC) the primary harvest in 2009. Genotype fit is represented by the R^2 value

VITA

Name:	Dustin Ross Borden
Address:	Department of Soil and Crop Sciences Mail Stop 2474 Texas A&M University College Station, TX 77845
Email Address:	DBorden@neo.tamu.edu
Education:	B.S., Agronomy, Texas A&M University, 2007 M.S., Agronomy, Texas A&M University, 2011