SUGAR STABILITY OF SWEET SORGHUM EXPOSED TO CLIMATE CONTROLLED AND AMBIENT STORAGE CONDITIONS

A Thesis

by

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ABSTRACT

Historically, crop based ethanol has predominantly been achieved in the United States through starch-based and sugar-based conversions. With corn being one of the leading food and feed crops in the United States, and sugarcane's inability to adapt to U.S. production regions, Sorghum (*Sorghum bicolor* L. Moench) has been identified as a potential alternative biofuel feedstock. The objective of this study was to evaluate the stability of non-structural carbohydrates (sugar) present in sweet sorghum juice, by tracking the sugar degradation of 'Dale' and 'M81E' while exposed to extended periods of climate controlled and ambient conditions after peak sugar accumulation. The data from both genotypes indicated that sugar yields can be sustained for weeks without significant losses. The plants left in the field for the ambient treatment continued sugar accumulation until photosynthesis and transpiration halted, causing immediate loss in sugar. Samples under the controlled treatment retained sugar yields for 3-4 weeks with minimal losses in yield, followed by a steady reduction for the remainder of the evaluations. However, the overall sugar loss after 70 days was comparable between treatments, which leads to the conclusion that sweet sorghum has the potential to be stored up to four weeks before significant yield loss occurs, regardless of storage methods. Combining staggering sweet sorghum plantings with short-term storage to sugarcane productions makes sorghum a suitable alternative or complementary feedstock to current sugar-based ethanol refineries.

DEDICATION

This thesis is dedicated to my loving wife Danielle Herb, my parents Matt and Rachel Herb, and my brother Brandon Herb, in addition to my family, friends, and past educators who have influenced and encouraged me over the years.

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NOMENCLATURE

CS	College Station
WE	Weslaco
WF	Weslaco Fall
Agro	Agronomics
Comp	Composition
SY	Sugar Yield
SOLU	Soluble
STRU	Structural
CELLU	Cellulose
LIGN	Lignin
SUCR	Sucrose
GLUC	Glucose
FRUC	Fructose

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CHAPTER I

INTRODUCTION

In the past ten years, the average national price of oil has increased by 760%, which has resulted in record high prices of gasoline at $3.695 per gallon (United State Energy Information Administration, 2012; Hoffman, 2012). The increased demand for fuel is the result of rising population growth and global economic expansion. Assuming population and economic growth trends continue, it is unlikely that oil prices will drop significantly in the future. Furthermore, environmental concerns related to CO_2 emissions provide political impetus to identify carbon neutral fuels. Such sources include, but are not limited to, wind, solar, geothermal, hydrogen, and biomass. To reduce our dependency on foreign oil and to assist in meeting the biofuels production goal for 2030, the Energy Independence and Security Act (EISA) was signed in December of 2007. The goals of EISA are to reduce greenhouse gas emissions by 20%, increase the production of biofuels through conventional fermentation and cellulosic conversion, and improve the knowledge on renewable fuel products through research (Energy Independence and Security Act of 2007). Under EISA, the Renewable Fuel Standards (RFS) has expanded its previous goal of producing 9 billion gallons of renewable fuels in 2008 to 36 billion gallons in 2022 (United State Energy Information Administration, 2012). Meeting these standards will require a continuous increase in renewable fuel production, of which over half must be derived from advanced biofuels. An advanced biofuel is defined as any non-starch based conversion such as ethanol from

sugar fermentation, cellulosic biofuels, and from biomass-based diesel (United State Energy Information Administration, 2012). Even without this mandate, it is not possible to further increase ethanol production solely on starch conversion alone, due to the finite amount of starch and sugar production from primary ethanol crops such as corn and sugarcane (Rooney et al., 2007). Therefore, bio-refineries must utilize alternative feedstocks in order to meet production goals. In order to accomplish these goals, alternative biomass sources are essential, and sweet sorghum poses as an alternative or complementary feedstock to current ethanol refineries. One issue faced by all biofuels crops is the ability to supply continuous inputs. The focus of this study is to evaluate sorghum's ability to integrate into the established logistical models of ethanol refineries to supplying continuous feedstock for year round energy conversion. Specific objectives are to 1) determine the effect of ambient temperature and storage duration on sugar yields in existing sweet sorghum varieties, 2) determine the effect storage has on sweet sorghum sugar concentration under controlled and field conditions, 3) identify the maximum days sugar yields can be stored before significant losses are observed, 4) determine the influences of season length, climate, and weather on sugar accumulation, and 5) determine if storage of sweet sorghum can complement sugarcane production and sugar processing logistics.

CHAPTER II

LITERATURE REVIEW

Bioenergy Crops

Biofuel is a collective term for liquid fuels (ethanol, biodiesel, and other) derived from renewable sources such as municipal solid waste, agricultural byproducts, and biomass from crops and timber (United State Energy Iinformation Administration, 2012). These energy sources supply energy through electrical generation and/or liquid fuels. Current biofuel production (primarily ethanol) is starch (corn) - or sugar-based (sugarcane) (Murphy, 2003).

In 2011, ethanol production was 13 billion gallons (U.S.) and 7 billion gallons (Brazil) and these two countries account for 88% of the world's production (United States Energy Information Administration, 2012; United States Department of Agriculture, 2011). Corn (*Zea mays* L.) accounts for the majority of U.S. production (United States Energy Information Administration, 2012), and in Brazil, sugarcane is commonly used to produce ethanol. In both countries, ethanol production from these sources is nearing their maximum threshold, due to economic demands for the utilization of these crops as feed or food commodities. Thus, alternative crops are required to meet the EISA's goals (United States Energy Information Administration, 2012; Pimentel et al., 2003).

Crop residues and timber byproducts are also potential biomass sources for biofuel production, but the quantities of these products are limited and too widely

distributed. In addition, not all crop residues are available, as some are needed to maintain soil tilth and quality. Consequently, residues represent only a portion of the biomass required to meet biofuel production mandates.

Ultimately, dedicated bioenergy crops are needed to meet biomass production requirements. A dedicated bioenergy crops is grown specifically for conversion to biofuel or bioproducts, and is not used as a feed or food commodity. Thus, while these crops compete for land use, they do not contribute directly to the food versus fuel debate. These crops must possess key characteristics, which include high yield potential, wide adaptation, and resistance to biotic and abiotic stresses. High yields are essential to the reduction of land required to support a conversion facility and make production economically viable. Wide adaptation is important for placement of commercial production fields in less than ideal conditions while still remaining productive. This allows for more producible acres that do not compete for arable land with primary food crops currently established, alleviating some of the food versus fuel production issues (Rooney et al., 2007). To protect the inherent yield and quality of the crop, resistance to various stresses is an essential factor in productivity.

There has been considerable debate on the use of annual crops versus perennial crops. An annual crop allows for the production of a harvestable crop the first year of establishment and is easily integrated into crop rotation systems, but requires more inputs than perennial crops. Perennial crops take longer to become established and have a biological lag period before production of a harvestable commodity, but they are

considered more sustainable over time and do not require continual establishment for the same production field.

Based on recent work, several potential bioenergy crops have been identified. These include miscanthus (*Miscanthus* x *giganteus*), switchgrass (*Panicum virgatum*), willow (*Salix ssp.)*, and hybrid poplar (*Populus ssp.)* used for lignocellulosic conversion (Lewandowski et al., 2000; McLaughlin and Adams, 2005), and sugarcane and sorghum (*Sorghum bicolor* L. Moench) as a sugar-based bioenergy crop adaptable to both cellulosic and fermentation conversion methods (Alexander, 1985). Other crops used or having the potential to be used as a bioenergy crop are corn, soybean [*Glycine max* (L.) Merr], and canola (*Brassica napus).*

Sorghum as Biofuel Feedstock

Sorghum is the fifth most important food crop in the world, and has been widely produced for both human and livestock consumption (Smith et al., 2000). Traditionally, sorghum has been produced as a grain and forage crop with the majority of production being in Texas and Kansas (National Agriculture Statistics Service, 2011). In recent years, sorghum has been identified as a bioenergy crop (Rooney, 2007) and approximately 30% of the U.S. grain sorghum is already used to produce ethanol (Sorghum Growers League, 2011).

Compared to most other bioenergy crops, sorghum has a long-established breeding and improvement history (Rooney, 2004). The drought tolerance and ability to produce under water-stressed conditions in sorghum ensures wide adaptation (Beadle et

al., 1973). Physiologically, it is a C4 grass that enhances carbon capture and photosynthetic efficiency. These characteristics have made sorghum an important seed commodity, and because of this sorghum can be readily applicable to existing seed industries.

There are several different types of sorghum, each used for distinct purposes. Forage sorghums have been selected for yield and quality, and are influenced by traits such as leafiness, the ability to ratoon, digestibility and palatability. Sweet sorghum cultivars are defined by the production of sugar through juicy stalk and high sugar concentrations in the stalk. Biomass sorghum is produced for high biomass, but the bulk of the biomass is represented by the stalk with less weight being contributed from the leaves (Rooney et. al, 2007).

Sorghum is unique in that different types can be used in the starch, sugar or cellulosic conversion approaches. Grain sorghum is used for ethanol production through starch-based conversion, and is commonly combined with corn grain at starch based ethanol mills. Grain sorghum can produce comparable amounts of ethanol per bushel of grain, compared to other commercially produced grain crops, while using one-third less water during growth (Sorghum Growers League, 2011). Energy and forage sorghum is used to produce ethanol through lignocellulosic degradation of the biomass, or as a combustion fuel that generates electricity; in either case, the whole plant is used for production. The high biomass production is influenced by its ability to remain in the vegetative stage of growth longer in the season, due to its photoperiod sensitivity, causing a delay of initiation of the reproductive phase until day lengths are reduced

below eleven hours (Rooney and Aydin, 1999). Sweet sorghum, containing high volumes of sugar, can be milled directly and distilled to ethanol from the extracted sugars, making sweet sorghum a primary candidate for mainstream ethanol production.

Sorghum Origins

Sorghum was domesticated in 4000 B.C. in (or around) the region of Northeast Africa, which is now the center of origin for sorghum, with wide diversity throughout the continent (Smith and Frederiksen, 2000). Sorghum has migrated with humans, and landraces are scattered throughout the tropical and subtropical regions of the world (De Wet et al., 1967). With migration and selection, cultivated sorghum has five distinct races (in addition to numerous sub races): Bicolor, Caudatum, Durra, Guinea, and Kafir (Smith and Frederiksen, 2000; De Wet et al., 1967).

Since the regions of domestication were tropical, most landrace accessions are photoperiod sensitive (PS). PS sorghum initiates reproductive growth based on decreasing day-length, and plants remain vegetative until this condition is met. (Rooney et al., 1999).

Sorghum Genetics

In domesticated sorghum, the two most important traits for adaptation are maturity and height. In sorghum, the *Dw* and *Ma* genes control height and maturity (which includes photoperiod sensitivity). Since these traits are complex, with both the qualitatively and quantitatively heritable components, the degree of their influence is

dependent on the allelic composition in specific lines. Six maturity loci are described in the literature: *Ma*$_1$, *Ma*$_2$, *Ma*$_3$, *Ma*$_4$ (Quinby, 1967), *Ma*$_5$, and *Ma*$_6$ (Rooney et al., 1999). *Ma*$_1$, *Ma*$_3$, and *Ma*$_6$ have been cloned, and the gene function and role in regulatory pathways is known (Murphy et al., 2011; Childs et al., 1997; Murphy et al., 2013). *Ma*$_1$, *Ma*$_5$, and *Ma*$_6$ are actually photoperiod sensitive response loci, while *Ma*$_2$, *Ma*$_3$, *Ma*$_4$ are associated with temperature effects. Length of maturity and PS response is a result of dominant allele action in the gene, and the epistatic interaction between genes. Another source that can influence maturity includes mutations at respective loci that control this trait (Quinby, 1967).

A hybrid derived from the cross of *ma*$_1$*Ma*$_2$*ma*$_3$*Ma*$_4$, a line that needs 49 days to reach anthesis, and *Ma*$_1$*Ma*$_2$*ma*$_3$*Ma*$_4$, which requires 102 days to reach anthesis, increased biomass yields by 245 g per plant due to the extended time in the vegetative stage of development (Quinby, 1967). Packer (2011) reported moderate levels of high parent heterosis (40%) for biomass when a grain type (*ma*$_1$, *Ma*$_5$, *ma*$_6$) was crossed to a photoperiod sensitive type (*Ma*$_1$, *ma*$_5$, *Ma*$_6$).

Characterization of the maturity genes is critical to diversified sorghum uses. These genes are the key to maximize biomass accumulation and manipulation is essential for the production of hybrid seed (Rooney et al., 1999). For example, crossing two genotypes that are *Ma*$_5$*Ma*$_5$*ma*$_6$*ma*$_6$ x *ma*$_5$*ma*$_5$ *Ma*$_6$*Ma*$_6$, produces a hybrid that is heterozygous at each locus and photoperiod sensitive (*Ma*$_5$*ma*$_5$*Ma*$_6$*ma*$_6$).

Sorghum Composition

For bioenergy production, composition of the plant biomass is important because it influences conversion efficiency. The majority of plant biomass is structural carbohydrates, which includes cellulose, hemi-cellulose and lignin. Cellulose is composed of a glucose molecule chain connected though hydrogen bonds. Hemicellulose is a branched polysaccharide, a more complex form of sugar than cellulose. Linked through hydrogen bonds to cellulose fibrils, hemicellulose has greater strength and stability than cellulose. Pectin, formed by uronic acids, suspends the cellulose-hemicellulose in a gel matrix (Somerville et al., 2004). Moreover, because these compounds are integrated to form the cell wall, therefore the biomass must be deconstructed and separated so that cellulose and hemicellulose can be reduced to simple sugars for conversion (DOE, 2012).

Lignin is found between cell walls and is a major component of plant vascular tissue (Hoffman, 2012). Lignin is a polymer in the vascular tissue with its primary roles being the strength and reinforcement of the cells, and lignin also influences water migration throughout the cell. The complex structure of this polymer resists degradation and is an important element in host plant resistance (Campbell et al., 1996), in addition to having involvement in digestibility of forage plants (Akin et al., 1986). In the context of biomass for energy, lignin inhibits the hydrolysis of cellulose and hemicellulose, reducing the efficiency of fermentation to ethanol (Vermerris et al., 2007), but lignin can be utilized in direct combustion or gasification to produce energy as an alternative to fermentation to ethanol (White et al., 1987).

Sorghum composition has been measured using a combination of Kjeldahl, crude fiber, and dietary fiber methods. Kjeldahl analyzes proteins that have been digested, revealing nitrogen values used to estimate the protein values of the sample (Wall and Blessin, 1970; Association of Official Analytical Chemists and Horwitz, 1980; Hoffman et al., 2012). Crude fiber analysis measures the neutral detergent fibers (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) through a neutral and acidic digestion period. This method is used widely for forage and silage analysis for animal feed. Dietary fiber, similar to crude fiber analysis, is analyzed by a gravimetric digestion process, but uses enzymes pepsin and pancreatin to replicate normal digestion, and is commonly used in the food industry (Association of Official Analytical Chemists and Horwitz, 1980; Olivier et al., 2005; Hoffman et al., 2012). These methods, though effective, are expensive and labor intensive, leading to the implementation of alternative analysis technology.

Near infrared reflectance (NIR) spectroscopy is a fast and inexpensive method to estimate sorghum composition (Sanderson et al., 1996; Roberts et al., 2011). NIR technology is based on the molecular bonds' ability to react to low frequency light via bond vibrations from stretching or bending molecules. The vibrations absorb near infrared light when encountering energy emitted from different wavelengths (800-2500 nm). This produces a spectrum that can be quantified and correlated to the composition of the sample exposed to the treatment (Hoffman et al., 2012). The composition correlation spectrum of the samples are calculated and reported by multivariate statistical tools. The NIR spectrums were plotted against a control predictive-calibration curve

developed by using between 30 to 100 samples submitted to conventional wet chemistry. Both of the sets of data are combined and analyzed by multilinear regression software, which results in a regression equation that can predict the composition of the samples using NIR spectroscopy data (Hames et al., 2003; Vermerris et al., 2007).

CHAPTER III

BACKGROUND

Ethanol production has risen substantially due to current demands for renewable energy integration into U.S. fuel consumption (United State Energy Information Administration, 2012), with the majority of this production quota being derived from corn, in addition to forestry products, municipal byproducts, and waste. Since corn is a dominant food and feed crop, partitioning large portions of grain to ethanol will lead to food versus fuel issues. The "push-pull" relationship between the corn industry and ethanol refineries limits the potential of corn-based ethanol being economically feasible. As more acreage of corn is converted to ethanol production, a shortage of feed grain results in the price of corn becoming too expensive for the refineries to produce the ethanol. Therefore there is a corn-to-ethanol threshold that limits the economical U.S. corn-based ethanol production. However, ethanol can be derived from other renewable crops. Sugar conversion systems have been widely utilized for ethanol production in Brazil using sugarcane. Other potential sources of fermentable sugars include sugar beets and sweet sorghum. Of these crops, sweet sorghum, for many reasons, has the most potential in U.S. production systems.

Energy Sorghum Characteristics

Bioenergy sorghum has been divided up into two distinct categories: energy and sweet sorghum types (Rooney et al., 2007). Energy sorghum lines and hybrids are

selected for high biomass yields wherein the bulk of the biomass is in the stalk. The lower frequency of leaf material is desirable because leaves contain higher ash and protein contents, both of which are not important in a biomass conversion facility. Unlike in forage sorghum, palatability is not important in energy sorghum cultivars (Rooney et. al, 2007). Sweet sorghum lines and hybrids are high in soluble sugar in the stalk, and produce large quantities of easily extractable moisture in the stalk.

Sweet Sorghum Characteristics

Sweet sorghum is propagated by seed and cultivated as an annual crop. Sweet sorghum is typically tall, reaching 2.4 to 3.0 meters in height, and is capable of producing a ratoon crop (Rooney et al., 2007). They are characterized by wide adaptability, drought resistance, waterlogging tolerance, saline-alkali tolerance, rapid growth, high juice content and sugar accumulation. The main selection criteria in modern breeding programs are extractable juice yield and sugar concentrations in the desired maturity and height combinations. Through selection, sweet sorghum germplasm of varying height, maturity and productivity have been developed, primarily for syrup production, although some industrial sweet sorghums have been developed.

Sweet sorghum produces a harvestable crop in approximately three to five months. On average, yields can be as high as 30 Mg ha^{-1} of biomass (Rooney et al., 2007) with sugar yields approximately 4 Mg ha^{-1} (Morris et al., 1994). When harvested, sweet sorghum is prepared for milling by removing the panicle and leaves from the stalk. This improves the extractability and purity of the juice (Broadhead, 1972). After milling,

the residual stalk or bagasse can be utilized as livestock fodder or in lignocellulosic ethanol conversion. However, sweet sorghum is not being grown for bagasse. Modifications to the bagasse composition are undesirable if it affects sugar concentrations. Ethanol yields from sweet sorghum are between 5.2 to 8.4 g of ethanol per 100 g of biomass (Sakellariou-Makrananaki et. al, 2007). These yields will differ based on weather conditions within the production region. Sweet sorghum grown in sub-tropical and tropical environments typically have ethanol yields ranging from 6500 to 8000 liters ha^{-1} (Sakellariou-Makrananaki et. al, 2007; Bennet et al., 2008; Dolciotti et. al, 1998) and in temperate climates reported yields range from 3000 to 4000 liters ha^{-1} (Keeney et al., 1992).

Sweet Sorghum Composition and Timing of Harvest

The composition of sweet sorghum juice is composed of simple nonstructural carbohydrates (starch and sugar) and the biomass is composed of complex structural carbohydrates (cellulose and hemicellulose). Within the juice, sucrose is the primary sugar, but glucose, fructose, and starch have been reported in significant quantities (Corn, 2009). The juice is usually 8% to 20% soluble sugars, which is similar to the concentrations found in sugarcane (Corn, 2009: Bradford et al., 2009). Sugar concentration will vary based on influences from the genotype and environment (Saballos 2008; Kundiyana et.al, 2006).

In sweet sorghum, maximum sugar accumulation is typically associated with the physiological maturity of the developing grain, but it is highly dependent upon genotype

and environment (Hunter et al., 1997; Almodares et. al, 2007; Lingle, 1987; McBee et.al, 1983). Some cultivars may not reach peak sugar accumulation until after physiological maturity, and others reach peak sugar as early as the milk stage of grain maturity (Bradford et al., 2009) (Figure 1). The duration of the peak sugar is highly influenced by environment and can last up to 3 weeks before significant losses are observed (Rao, personal communication). Typically, sugar yields last between 72 hours to a week under commercial dryland conditions. Regardless of the duration of the peak, sugar concentrations eventually decrease due to degradation and re-distribution within the plant as sorghum will resume growth from basal and auxillary buds (Tsuchihashi, 2004).

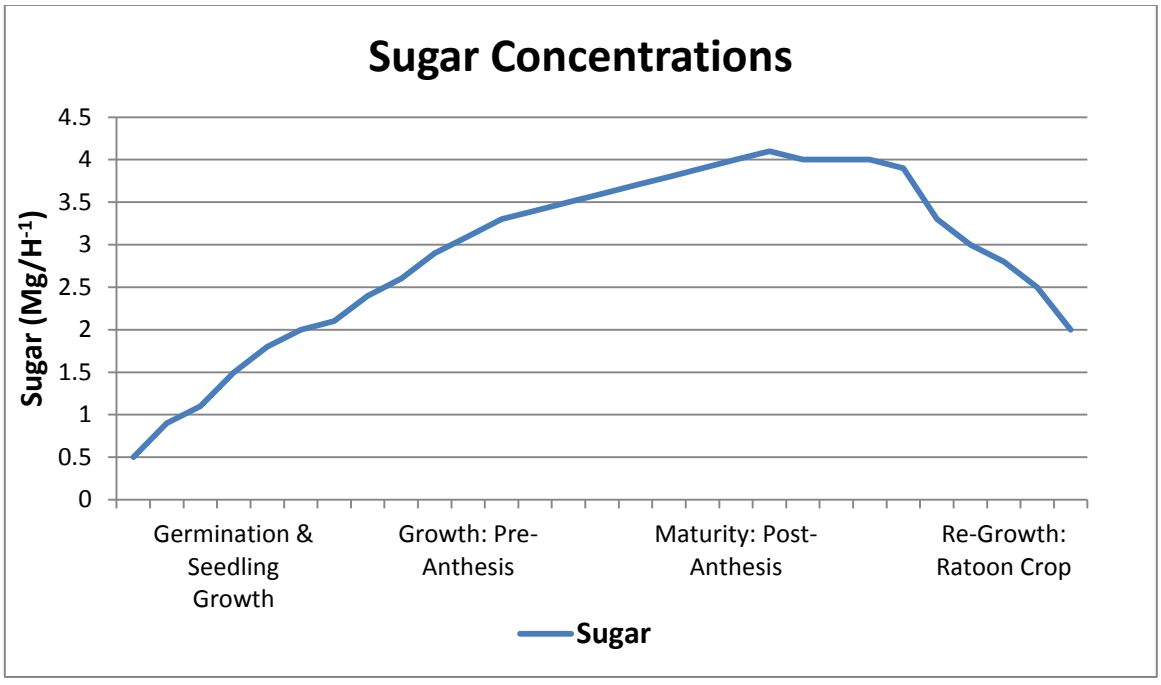

Figure 1.Illustration of sugar concentration throughout the sorghum plant development adapted from Tsuchihashi (2004) and Lingle (1987).

Provided with suitable moisture, sweet sorghum is productive across a wide range of environments, with high yields reported from the tropics to temperate production environments (Rooney, 2007). Like sugarcane, free sugars in sweet sorghum cannot be readily stored without significant processing; there is a need to harvest and process on a continual basis. Temperate environments do not have a long enough season to efficiently utilize sweet sorghum alone. In these environments, and in many tropical environments, complementation between two sugar crops (i.e. sweet sorghum and sugarcane) effectively uses industrial equipment over a longer period of the year.

Complementation to Sugarcane

Even for sugarcane produced in tropical environments, there are production seasons that maximize sugar yields. The addition of sweet sorghum to U.S. and Brazilian sugarcane production systems can extend the harvesting season for ethanol refineries between 30-100 days a year by staggering early, medium, and late planting dates, and by utilizing a range of maturity groups of sweet sorghum (Burks et al., 2013). Since sweet sorghum matures earlier than sugarcane, sweet sorghum is harvested in the month prior to the maturation of sugarcane (Bradford et al., 2009). Extending the mill season increases not only production but it reduces the cost of production on a per unit basis (Nguyen et al., 1996). This scheme is plausible for most sugarcane mills, because sweet sorghum conforms well to production practices of annual cropping systems and can use the same equipment as sugarcane (Rooney et al., 2007).

Even in a combined production system, there will be seasonal shut down of the mill. To minimize this time, storage methods have been proposed, but there are problems with this approach. Deterioration of sugars in the stalks of both sugarcane and sweet sorghum increases after harvesting and during storage (Bryan et. al, 1981). Eiland et al. (1983) confirmed that sugar deterioration decreased when sweet sorghum was stored as whole plants and billets. When stored as a chopped sample, the fermentable sugars were reduced by half within one week of storage, with the majority of the sugars being lost within the first 24 hours. The conditions in which the stalks are stored influence the availability of fermentable sugars. Ambient temperature can cause spoilage of sugars (Daeschel et. al, 1981), while freezing temperatures can reduce the overall sugar content, reduce ethanol yields, or cause failure during fermentation (Bennett et al., 2008).

The objective of this study was to determine the effect of ambient temperature and storage duration on sugar yields in existing sweet sorghum varieties by specifically addressing these objectives:

1) Determine the effect storage has on sweet sorghum sugar concentration under controlled and field conditions.
2) Identify the maximum days sugar yields can be stored before significant loses are observed.
3) Determine the influences of season length, climate, and weather on sugar accumulation.
4) Determine if storage of sweet sorghum complements sugarcane production and sugar processing logistics.

CHAPTER IV

MATERIALS AND METHODS

Gerplasm

Two sweet sorghum varieties, ‘Dale’ and ‘M81E’ were selected for this study. Dale was derived from a backcross between ‘Tracy’ and MN 1048 with Tracy being the recurrent parent (Broadhead, 1973). M81E is a moderately photoperiod-sensitive genotype that was selected from F_2 progeny in a cross between ‘Brawley’ x (Brawley x ‘Rio’) (Broadhead, 1983). Both of these varieties were developed for syrup production. They differ in relative maturity; Dale is earlier while M81 E is later in maturity. The exact difference in maturity between these varieties depends on the day length during the growing season.

Field Design

The varieties were planted in College Station and Weslaco, Texas at Texas A&M Agrilife Research Field Laboratories in the spring and fall for the 2012 summer (College Station, TX) and winter (Weslaco, TX) nurseries, and in the spring for the 2013 summer nursery. Planting of the varieties in each location was replicated 6 times across the field to account for spatial variation. The replications consisted of 3 ranges each with 6 plots per range. Plots were 5 to 6 meters in length and planted on 76 centimeter centers. Planting and stand densities were 370,000 plants per hectare for Dale and 245,000 plants per hectare for M81E. Standard fertilization and cultural practices were used for both

cultivars. In College Station fertilizer applications consisted of 168 kg of 11-34-0 + 4 zinc per hectare in January, and 131.19 Kg N_2 per hectare as urea 46-0-0 in May. Weed control used a pre emergence application of 3.51 L Atrazine 4L + 1.75 L Brawl + 2.34 L Roundup per hectare in March. In late summer, an aerial application of 1.75 L Lannate per hectare was used to control headworms, sorghum midge, and aphids. A pre-seeding nutrient supplement fertilizer applications in Weslaco consisted of 46.7 L 4-10-10 Quick Boost + 1.89 L Awaken per hectare in August, followed by an application of 150 kg N_2 per hectare as ammonium nitrate 32-0-0 in October. The preemergence herbicide Atrazine 4E (2.4 L per hectare) was applied just after planting.

Data Collection

To develop sugar yield curves, plants were harvested at regular intervals starting at 4 days post anthesis. Harvest dates were at 4, 7, 14, and 21 days after anthesis. On sampling dates, 5 randomly selected plants were harvested from the middle of the plot and these composed an experimental unit. Experimental units harvested within each replication were sampled, bulked, and blocked to account for sampling variation and reduced experimental error. This method was conducted for both genotypes in all environments.

Approximately 24 days after antithesis, when peak sugar concentrations were identified, the stored samples were harvested and the designated as Day 0. Peak sugar refers to the point at which maximum sugar yields occur before the yields start a slow but steady decline. Once initiated at each harvest, 5 plants were randomly chosen and cut

from each of the 6 replications. For each replication, total weight, panicle weight, leaf weight, stalk weight, juice brix, juice volume, and juice weight was recorded. The total sample refers to the weight of the stalk and leaf portion of the plant without any panicle. Leaves were then removed and stalks were weighed again and the difference between total weight and stalk weight is leaf weight. Stalks were passed through a corrugated three-roller mill (Ampro Sugar Cane Crusher model diamond) to extract the juice. The extracted juice was weighed, and volume was recorded using a graduated cylinder. Juice brix, the concentration of soluble sugars, was measured on extracted juice. The juice was then weighed, and the volume was measured using a graduated cylinder. After milling, bagasse samples were collected, weighed wet, then dried and weighed again.

At peak sugar, which occurred approximately 14 days after anthesis for both Dale and M81E, plants in the field were randomly classified into a storage or field treatment. On day 0, all plants for the storage treatment were harvested and all plants for the field treatment were tagged. A total of 270 plants were harvested on day 0 for the storage treatment. Those 270 plants were divided into 9 processing days consisting of 5 plants per replication. Each group of storage treatments plants were cut into 30 cm billets and placed in fiberglass bags with the leaves and panicles then labeled with an evaluation day and replication. These bags were then randomized and kept in a 10˚C cold vault with 57% humidity. The scheduled evaluation days for both treatments were 0, 4, 7, 14, 21, 28, 42, 56, and 70 days after peak sugar. The phenotypic data collected was the same as that collected prior to the application of treatments.

On each evaluation day, samples were harvested from the field and pulled from storage for analysis. For field and storage treatment, the samples were processed as previously described. Following juice extraction, 500 gram bagasse samples were washed in 2,000 ml of water (at air temperature) for 10 minutes. The goal was to remove any remaining sugar from the bagasse. Samples of bagasse and juice were collected from each milled replication, including the washed bagasse and the washing solution. A fresh weight of each bagasse sample was taken, and then dried in a forced air oven at 52˚C. Once dried, samples were re-weighed and dry matter concentrations were based on the differences. Non-milled chipped stalk samples were also harvested to obtain a maximum sugar concentration threshold for all evaluation days. These samples were then ground to pass through a 2 mm screen in a Wiley mill. The juice samples contained 13 ml of sampled juice from each replication, and 2 ml of an 8% aqueous solution of biocide (sodium azide) to stabilize and prevent spoilage of sugars while stored in a freezer until analysis. Figure 2 illustrates the outline and the flow of the study.

All samples were analyzed with near infrared reflectance (NIR) spectroscopy using a FOSS XDS and calibrations developed by Wolfrum et al (2013) to estimate biomass and juice composition. This process was repeated in each environment. NIR compositional analysis was based on correlations to the wet chemistry calibration algorithm of both biomass and juice samples developed by the National Renewable Energy Laboratory. Procedures for the NIR spectroscopy follow the outlined guidelines in Wolfrum et al (2013). The analysis gives composition estimates of structural (lignin, glucan, xylan, galactan, arabinan, acetyl, protein, structural inorganics (SI)) and non-

structural (sucrose, water extract, ethanol extract, nonstructural inorganics (NSI)) carbohydrates. Total composition estimates were comprised of all carbohydrate measurements, with exception to ash.

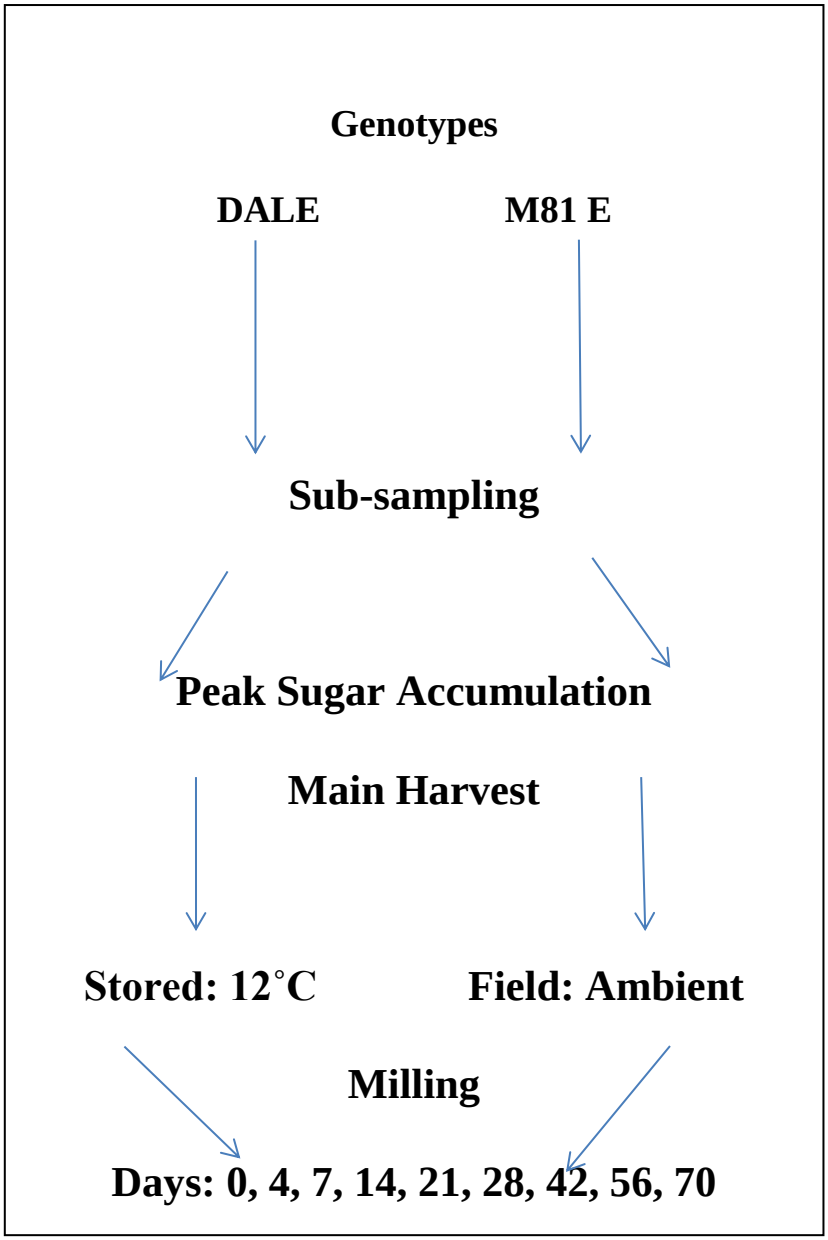

Figure 2. Step-by-step diagram of harvesting, storage, processing, and analysis procedures.

Data Analysis

Sugar yields were calculated and reported on a kilogram per stalk basis, allowing rough approximations of yield per hectare, based on the plant populations in the field.

The sugar yields were calculated using the following equation:

$$\boldsymbol{Sugar\ Yield} = \{[(\text{Extractable Wt.})/(\text{Stalk Wt.}) + (((\text{Wet Bagasse Wt.} - \text{Dry Bagasse Wt.})/(\text{Wet Bagasse Wt.}))/\text{Stalk Wt.})][(\text{Brix}/100)(0.873)(0.97)(0.95)]/(\text{Stalk Wt.})\}$$

The fixed values in the formula reflect an assumed .95 extraction efficiency like that observed in commercial milling facilities (Bennett, 2008). It is also assumed that .97 of all soluble sugar in the plant is extracted (Engelke, 2005). Finally brix ratings are typically .873 fermentable sugars (Corn, 2009).

The data was analyzed in SAS using Proc GLM, Proc Mixed, and Proc Reg (SAS Institute, v9.2). Using a randomized complete block design, a combined environment model was used for the analysis for each genotype separately. The model consists of treatment (in field v. cold storage); and evaluation date (time of harvest (or storage)) and environments (composed of locations and years) and replications (nested within environments) and all interactions. The main factors treatment and evaluation date were fixed effects while environments and replications were random effects.

CHAPTER V

RESULTS

Analysis of Variance for Agronomic Traits

In the combined analysis for M81E (Tables 1 and 2), all main effects location (LOC), Treatment (TRT), and length of storage (EVDA) for the agronomic traits (stalk weight, juice, brix, sugar yields) were significant, except for the treatment effect on sugar yield (Table 1). Interactions were highly significant for agronomic traits except stalk yield which was only affected by TRT x EVDA and sugar yields, only affected by LOC x EVDA interaction.

The combined analysis for Dale (Tables 3 and 4) detected significant effects for LOC and EVDA for all agronomic traits, whereas TRT was only significant for juice and brix (Table 3), Interactions in this analysis were mostly non-significant – only LOC x TRT, and TRT x EVDA were significant across all agronomic traits, except TRT x EVDA on juice yield (Table 3). For stalk yield, LOC x TRT x EVDA interaction was significant.

Analysis of Variance for Compositional Traits

In M81E, all the measured compositional traits were significantly affected by location, treatment, and evaluation date (Tables 1 and 2). Most of the interactions were also significant for all traits with the exception of TRT x EVDA which was lower across all traits and not significant for cellulose and glucose (Table 2).

In Dale, all the main effects significantly impacted compositional traits with the exception of treatment on structural carbohydrates and sucrose. First order interactions were significant for all traits, except for LOC x TRT on sucrose and fructose and LOC x EVDA on fructose. The degree of significance varies between biomass and juice measurements. For example, the effect of treatment on biomass sucrose was not significant compared to juice sucrose (Table 3). Overall, the significance of main effects and interactions on compositional traits (soluble and structural carbohydrates, sucrose, glucose, fructose, cellulose, and lignin) was higher than agronomic traits (Table 4), and more significant effects were detected in the M81E analyses than in the Dale analysis.

Effect of Environment

For both genotypes, stalk weight, juice, and reducing sugars (glucose and fructose) were higher in College Station compared to Weslaco (Tables found on pages 32-35). The yield stability of these traits was greater in cold storage and had less yield loss compared to field storage. Alternatively, brix and sugar yields were greater in Weslaco, and in field storage (Tables found on pages 32-35). Biomass compositional traits such as structural and soluble carbohydrates, including cellulose, lignin, and sucrose had yields comparable across environments (Tables found on pages 32-35).

Table 1. Mean squares of agronomic traits and biomass carbohydrate composition from the analysis of variance of 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

Source	Agronomic Traits				Biomass Carbohydrate Composition	
	Stalk yield	Juice yield	Brix	Sugar yield	Soluble	Structural
Location (LOC)	24.06**	2.16**	0.03**	7.3×10^{-3}**	540.21**	478.70**
Treatment (TRT)	2.23**	0.20*	5.7×10^{-5}*	3.7×10^{-5}	332.18**	376.79**
LOC*TRT	1.79**	0.87**	4.8×10^{-3}**	1.1×10^{-4}	29.40*	62.37**
Sample Day (EVDA)	0.39*	0.58**	7.1×10^{-3}**	3.2×10^{-4}**	71.37**	47.53**
LOC*EVDA	0.17	0.08**	4.4×10^{-4}**	8.1×10^{-5}*	31.65**	24.01**
TRT*EVDA	0.36*	0.18**	1.4×10^{-3}**	3.3×10^{-5}	26.62**	24.81**
LOC*TRT*EVDA	0.25	0.12**	4.6×10^{-4}**	1.1×10^{-5}	18.05**	7.88
Rep(LOC*EVDA)	0.35**	0.04	1.1×10^{-4}**	5.7×10^{-5}*	7.91	5.66
ERROR	0.16	0.03	6.2×10^{-5}	3.7×10^{-5}	6.57	5.64
MEAN	2.68	0.85	0.11	0.01	31.88	66.38
R2	0.88	0.85	0.96	0.88	0.86	0.85
CV%	14.92	22.65	6.75	32.69	8.04	3.57

* Significant difference at level of $\alpha = 0.05$ of probability.

** Significant difference at level of $\alpha = 0.01$ of probability.

Table 2. Mean squares of biomass and juice carbohydrate composition from the analysis of variance of 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

Source	Biomass Carbohydrate Composition			Juice Carbohydrate Composition		
	Sucrose	Cellulose	Lignin	Sucrose	Glucose	Fructose
Location (LOC)	694.09**	628.44**	154.52**	1667.99**	5661.51**	843.70**
Treatment (TRT)	255.18**	100.66**	47.70**	5896.79**	338.30**	219.37**
LOC*TRT	22.13*	28.13**	18.55**	1213.78**	34.58	40.05**
Sample Day (EVDA)	98.87**	28.57**	13.14**	4497.73**	659.95**	57.04**
LOC*EVDA	29.64**	11.08**	3.41**	634.93**	53.41	17.44**
TRT*EVDA	14.48*	3.13	4.17**	728.28**	68.8	17.16*
LOC*TRT*EVDA	10.21	4.23	2.95**	163.72*	57.32	7.88
Rep(LOC*EVDA)	8.26*	3.39*	1.07	155.35**	40.72	8.59
ERROR	5.68	2.45	1.13	75.71	36.58	8.05
MEAN	10.52	25.82	12.37	58.28	25.15	10.83
R2	0.88	0.9	0.87	0.92	0.86	0.82
CV%	22.65	6.06	8.59	14.92	24.04	26.18

* Significant difference at level of $\alpha = 0.05$ of probability.

** Significant difference at level of $\alpha = 0.01$ of probability.

Table 3. Mean squares of agronomic traits and biomass carbohydrate composition from the analysis of variance of 'Dale' grown in two Texas environments (2012 College Station and Weslaco).

Source	Agronomic Traits				Biomass Carbohydrate Composition	
	Stalk yield	Juice yield	Brix	Sugar yield	Soluble	Structural
Location (LOC)	8.04**	2.79**	1.1×10^{-2}**	7.9×10^{-3}**	74.86**	51.21
Treatment (TRT)	0.03	0.39*	8.6 x 1^{-3}**	$1. \times 10^{-4}$	49.19*	60.04
LOC*TRT	0.84**	0.79**	5.6×10^{-3}**	1.6×10^{-3}**	162.07**	93.31**
Sample Day (EVDA)	0.46**	0.27*	2.6×10^{-3}**	4.5×10^{-4}*	69.77**	59.35**
LOC*EVDA	0.09	0.07	3.9×10^{-4}	1.0×10^{-4}	32.26**	10.71*
TRT*EVDA	0.35**	0.18	1.2×10^{-3}**	7.2×10^{-4}**	80.44**	47.25**
LOC*TRT*EVDA	0.16**	0.08	4.9×10^{-4}	6.2×10^{-5}	16.32	8.83
Rep(LOC*EVDA)	0.06	0.09	1.6×10^{-4}	2.2×10^{-4}	6.93	5.08
ERROR	0.05	0.11	2.5×10^{-4}	1.7×10^{-4}	8.58	4.82
MEAN	1.45	0.37	0.16	0.05	36.85	62.04
R2	0.86	0.73	0.87	0.81	0.84	0.86
CV%	15.44	19.87	10	28.18	7.95	3.54

* Significant difference at level of $\alpha = 0.05$ of probability.

** Significant difference at level of $\alpha = 0.01$ of probability.

Table 4. Mean squares of biomass and juice carbohydrate composition from the analysis of variance of 'Dale' grown in two Texas environments (2012 College Station and Weslaco).

Source	Biomass Carbohydrate Composition			Juice Carbohydrate Composition		
	Sucrose	Cellulose	Lignin	Sucrose	Glucose	Fructose
Location (LOC)	31.09*	209.00**	9.06**	469.59*	486.81**	24.75
Treatment (TRT)	1.66	29.85**	13.72**	2833.17**	483.67**	408.78**
LOC*TRT	18.23	50.35**	13.17**	38.88	296.81**	24.61
Sample Day (EVDA)	121.64**	34.89**	16.31**	1250.29**	89.89**	31.75**
LOC*EVDA	21.06**	5.81*	4.19**	352.52**	61.54*	10.21
TRT*EVDA	50.28**	13.35**	11.47**	567.58**	63.47*	26.95*
LOC*TRT*EVDA	22.91*	1.2	1.29	176.39	23.01	3.91
Rep(LOC*EVDA)	6.17	3.20*	1.16	96.8	81.38**	23.23**
ERROR	7.07	2.1	1.02	99.84	24.2	10.54
MEAN	16.74	22.29	9.57	74.32	31.2	12.32
R2	0.86	0.9	0.83	0.88	0.88	0.85
CV%	15.89	6.5	8.91	13.45	15.77	26.35

* Significant difference at level of $\alpha = 0.05$ of probability.

** Significant difference at level of $\alpha = 0.01$ of probability.

Effect of Treatment

Treatment effects were detected in the analysis of both genotypes and the trends were generally the same (Tables 5, 6, 7, and 8). In cold storage, approximately 10% of the total yield loss was between 0 and 7 days of cold storage (Tables 5 and 7: Appendix Table A14). Lingle et al. (2012) reported the majority of yield loss was observed within the first 24 to 48 hours of post-harvest storage; however storage was under ambient conditions. Another significant yield loss of approximately 10% was detected between days 28 and 42 (Tables 5 and 7: Appendix Table A14). These two periods of loss were identified by Tukey's HSD mean separation as the most significant changes in trends over 70 days of cold storage and consistent for all traits. In field storage yields of agronomic and soluble composition traits decreased overall and losses were typically greater by day 70, but periods of yield recovery and peaks in trends were observed across many traits and were as high as a 30% increase in yields, as data indicates between day 42 to 56 in M81E field storage juice yields (Tables 6 and 8).

Effect of Storage Length

Yields for all traits trended downward across all environments from day 0 to day 70, with the greatest losses occurring after day 28, with exception to structural carbohydrates and reducing sugars, which increased with prolonged storage (Figures 3, 4, and 6). There was consistent loss in both cold storage and field conditions, but the rate of reduction and variability was less in cold storage (Tables 3 and 4). Relative to day 0, stalk weight in M81E dropped 20% loss over 7 days. In Dale, the reduction was 21%

over the same time. Stalk weight varied more under field conditions and yield peaks were observed on day 28 for Dale and day 21 and 56 for M81E across all environments (Appendix Tables A12 and A13).

Juice yield trends dropped throughout the evaluation period, but the trends varied between treatments. Juice yields in field conditions increased and peaked at day 28 across all environments for Dale and day 56 for M81E (Appendix Tables A12 and A13). In cold storage, the highest juice yields were recorded at Day 0 and then started a slow decline with significantly less variation in data from each evaluation period.

Brix concentrations gradually dropped over time in cold storage (Tables 5 and 7) and trended downward under field conditions, but they were highly variable. Under field conditions, brix peaked at day 21 and 28, and then decreased drastically until day 70 for Dale (Table 6). Similar trends were observed with M81E, which peaked later in College Station and day 21 in Weslaco (Table 8). Similar to yield, the variation with brix observed in field is due to variation in the weather, and the plants' ability to respond to those stimuli.

The soluble carbohydrate concentration in biomass includes all water and ethanol soluble extractives. The primary component of this category is soluble sugars, primarily sucrose, glucose and fructose. In both genotypes, there was a slow and steady decline in the soluble carbohydrates concentration, dropping from 40% at day 0 to 30% at the end of the study (Appendix Tables A6 and A8; Figure 3). In M81E similar trends were observed but the rate of loss was lower in the field storage samples (Figure 3).

Table 5. Combined environment trait means of 'Dale' cold storage treatment grown in two Texas environments (2012 College Station and Weslaco).

	Cold Storage											
EVDA	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	1.61	0.43	16.9	46.71	40.8	20.19	58.85	21.58	8.11	78.57	31.56	10.68
4	1.43	0.42	16.25	46.28	41.21	20.14	58.82	20.68	8.74	77.86	31.43	12.81
7	1.42	0.35	15.85	46.17	41.51	19.85	58.35	19.97	8.46	74.55	34.43	12.91
14	1.39	0.34	15.75	42.95	38.63	18.61	60.84	22.58	9.34	70.58	29.94	12.86
21	1.39	0.28	15.3	43.19	36.85	17.2	62.42	23.12	9.93	72.04	32.86	13.24
28	1.38	0.25	15.05	41.23	35.01	14.79	64.01	23.42	11.12	73.59	30.89	14.4
42	1.32	0.22	13.65	40.89	34.63	12.94	64.19	24.26	10.95	67.38	36.1	16.93
56	1.27	0.19	13.35	40.84	33.39	11.99	65.05	24.64	11.33	51.46	38.08	16.59
70	1.25	0.14	13.05	37.55	32.01	11.15	66.37	25.27	12.05	50.27	33.42	17.33
Mean	1.38	0.29	15.02	42.87	37.12	16.32	62.10	22.84	10.00	68.48	33.19	14.19
Avg. % Loss	0.22	0.67	0.23	0.20	0.22	0.44	-0.13	-0.17	-0.49	0.36	-0.06	-0.62
HSD	0.25	1.1	0.52	3.12	3.35	3.15	2.23	1.43	2.67	3.84	3.11	3.33

*Tukey's HSD mean separation analysis significant at level $\alpha = 0.05$

Table 6. Combined environment trait means of 'Dale' field storage treatment grown in two Texas environments (2012 College Station and Weslaco).

	Field											
EVDA	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	1.61	0.43	16.9	46.28	40.84	20.19	60.19	20.34	7.94	78.57	31.56	10.68
4	1.64	0.48	18.2	41.03	34.11	18.19	63.44	21.29	9.07	73.35	27.29	10.86
7	1.39	0.31	16.85	41.65	37.01	16.33	61.07	20.73	9.17	70.51	33.03	9.8
14	1.5	0.34	18.35	41.15	34.62	15.67	63.74	20.64	9.5	83.29	29.36	10.72
21	1.44	0.38	19.15	48.3	35.64	14.91	63.07	22.11	10.07	95.44	25.56	8.56
28	1.86	0.61	17.8	70.9	41.66	21.57	58.09	19.64	7.16	99.35	24.14	8.14
42	1.62	0.59	14.15	47.51	35.46	14.61	62.91	21.71	9.41	78.29	27.56	9.83
56	1.53	0.35	12.9	35.14	32.05	10.05	65.4	24.74	11.84	60.35	27.04	8.52
70	1.15	0.14	12	28.65	30.04	9.4	67.38	26.99	12.09	64.35	29.37	11.81
Mean	1.53	0.40	16.26	44.51	35.71	15.66	62.81	22.02	9.58	78.17	28.32	9.88
Avg. % Loss	0.29	0.67	0.29	0.38	0.26	0.53	-0.12	-0.33	-0.52	0.18	0.07	-0.11
HSD	0.25	1.1	0.52	3.12	3.35	3.15	2.23	1.43	2.67	3.84	3.11	3.33

*Tukey's HSD mean separation analysis significant at level $\alpha = 0.05$

Table 7. Combined environment trait means of 'M81E' cold storage treatments grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

	Cold Storage											
EVDA	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	2.59	1.03	14.2	26.02	36.26	15.27	62.55	23.12	10.49	53.86	30.27	12.94
4	2.6	0.87	13.2	25.01	35.99	15.31	63.06	23.74	10.4	55.66	32.1	14.16
7	2.48	0.82	12.95	21.95	33.23	12.92	65.14	24.46	11.35	62.22	27.25	12.44
14	2.37	0.66	12.75	21.91	33.04	11.85	65.6	24.88	12.02	60.78	26.81	11.42
21	2.27	0.62	12.05	29.33	32.28	11.28	66.14	25.32	12.6	55.1	28.88	13.01
28	2.2	0.59	11.95	21.12	31.84	9.89	67.36	26.33	12.37	53.25	29.54	12.85
42	2.16	0.55	11.7	19.65	29.88	8.82	67.33	26.1	12.87	50.99	30.14	14.24
56	2.14	0.47	11.05	19.64	29.63	8.15	68.17	26.78	13.43	39.34	32.15	14.08
70	2.03	0.35	10.65	19.31	27.3	6.65	70.16	27.27	14.03	34.58	41.93	16.16
Mean	2.32	0.66	12.28	22.66	32.16	11.13	66.17	25.33	12.17	51.75	31.01	13.48
AVG. % Loss	0.22	0.66	0.25	0.26	0.25	0.49	-0.12	-0.18	-0.34	0.36	-0.39	-0.25
HSD	0.25	1.1	0.52	3.12	3.35	3.15	2.23	1.43	2.67	3.84	3.11	3.33

*Tukey's HSD mean separation analysis significant at level $\alpha = 0.05$

Table 8. Combined environment trait means of 'M81E' field storage treatments grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

	Field											
EVDA	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	2.59	1.03	14.2	25.71	35.38	15.54	62.12	21.85	10.75	53.8625	26.6755	10.48
4	3.22	1.01	15.2	17.54	36.74	14.99	62.61	21.71	10.24	54.468	21.335	9.92
7	2.66	0.85	15	24.34	35.74	13.91	63	23.01	10.59	64.329	27.745	9.44
14	2.89	0.92	15.3	25.6	35.84	14.77	63.32	23.03	10.79	74.0105	24.557	8.62
21	2.69	0.99	15.1	30.35	35.92	15.37	62.87	22.58	10.56	84.9215	23.535	9.45
28	2.36	0.73	11.6	20.9	32.78	11.03	65.66	24.11	11.6	73.1035	27.0765	9.8
42	2.93	0.82	11	9.18	33.42	10.2	65.49	24.81	11.26	59.494	38.048	16.17
56	3.77	1.13	15.6	14.88	35.73	11.96	61.36	22.64	10.36	34.644	44.743	16.83
70	3.61	0.85	11	10.22	32.72	10.04	64.04	25.56	11.35	32.527	47.951	18.17
Mean	2.97	0.93	13.78	19.86	34.92	13.09	63.39	23.26	10.83	59.04	31.30	12.10
Avg. % Loss	-0.39	0.17	0.23	0.60	0.08	0.28	-0.03	-0.17	-0.06	0.40	-0.80	-0.73
HSD	0.25	1.1	0.52	3.12	3.35	3.15	2.23	1.43	2.67	3.84	3.11	3.33

*Tukey's HSD mean separation analysis significant at level $\alpha = 0.05$

Sucrose concentration in whole biomass also dropped in both Dale and M81E under cold storage (Tables 5 and 7). In the field treatment, peaks in biomass sucrose were observed on day 28 for Dale (Table 6; Appendix Table A7) and on day 21 and 56 for M81E (Table 8; Appendix Table A9). Across both treatments, a change in sucrose resulted in an inverse change in cellulose. As sucrose decreases, cellulose increases, following a similar trend as sucrose (Figure 4). Similar to other traits, yield losses in sucrose increased between 28-42 days of storage, regardless of treatment. Lignin increased slightly as sucrose decreased, which is expected, since it is negatively correlated with sucrose and soluble carbohydrates (Figures 4 and 5).

Sucrose concentrations in juice samples decreased during the study. Sucrose concentrations in juice dropped more in the field samples with an 8% loss in Dale and 39% loss in M81E (Appendix Tables A12 and A13.). The drop in sucrose is associated with a concomitant increase in fructose and glucose, which are precursors of sucrose and likely increase due to the reduction of sucrose to its component parts (Appendix Table A3; Figures 5 and 6).

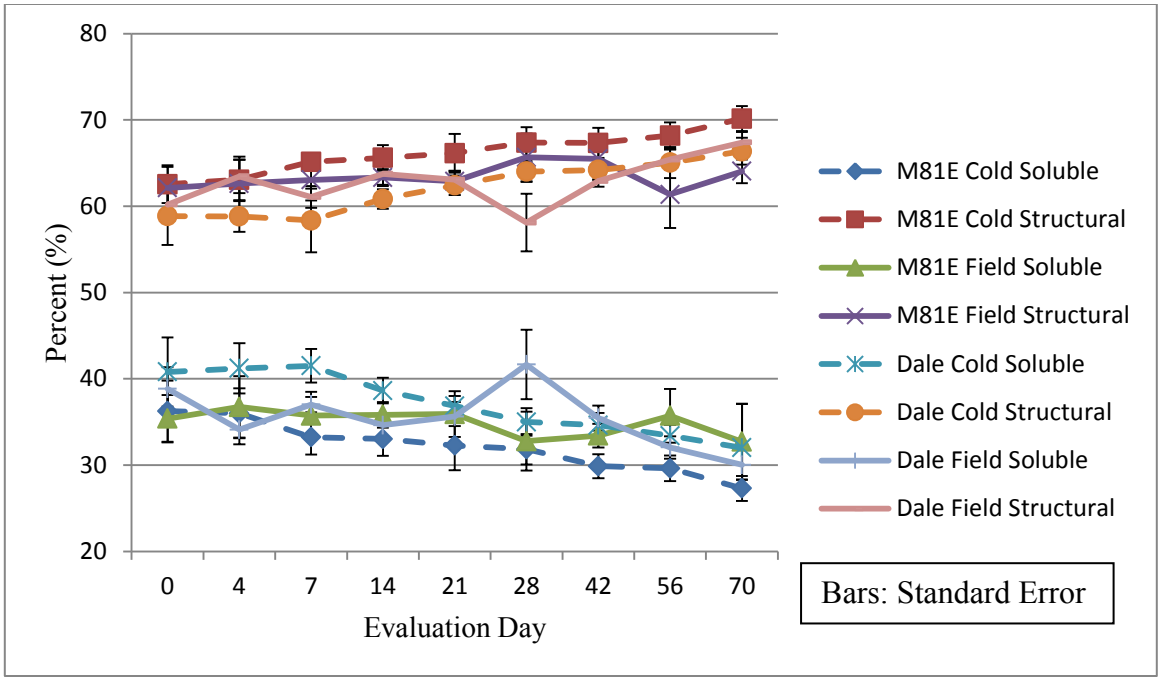

Figure 3. Plotted trends of combined biomass soluble and structural carbohydrates means of 'Dale' grown in two Texas environments (2012 College Station and Weslaco) and 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

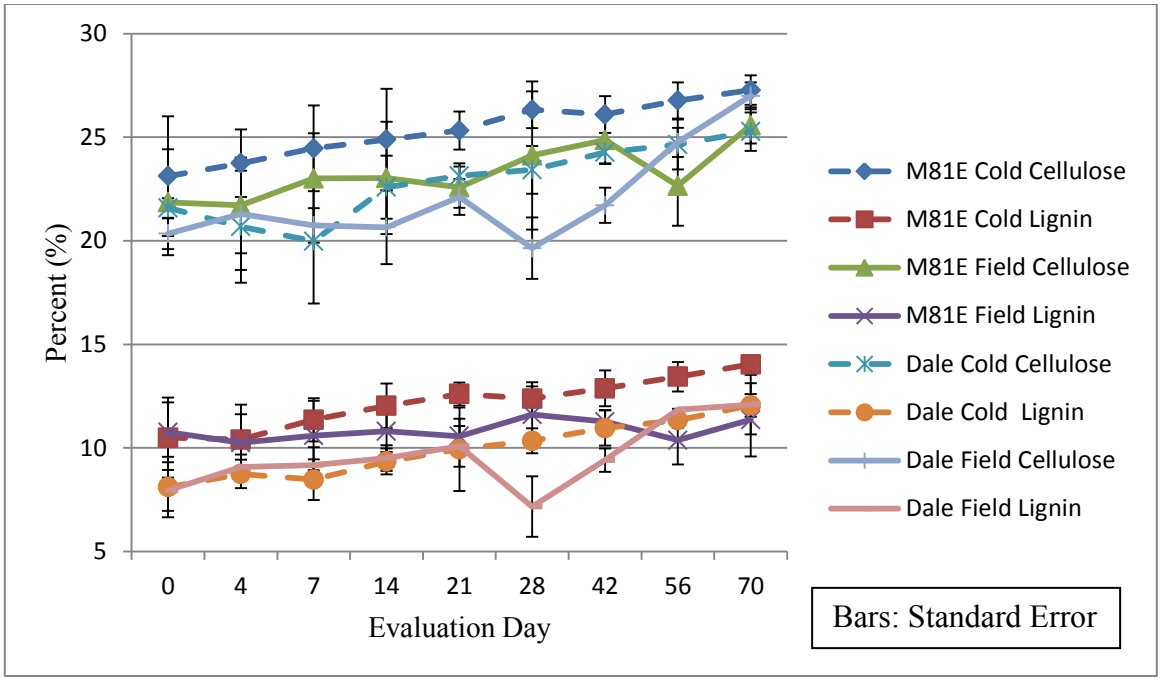

Figure 4. Plotted trends of combined biomass cellulose and lignin means of 'Dale' grown in two Texas environments (2012 College Station and Weslaco) and 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

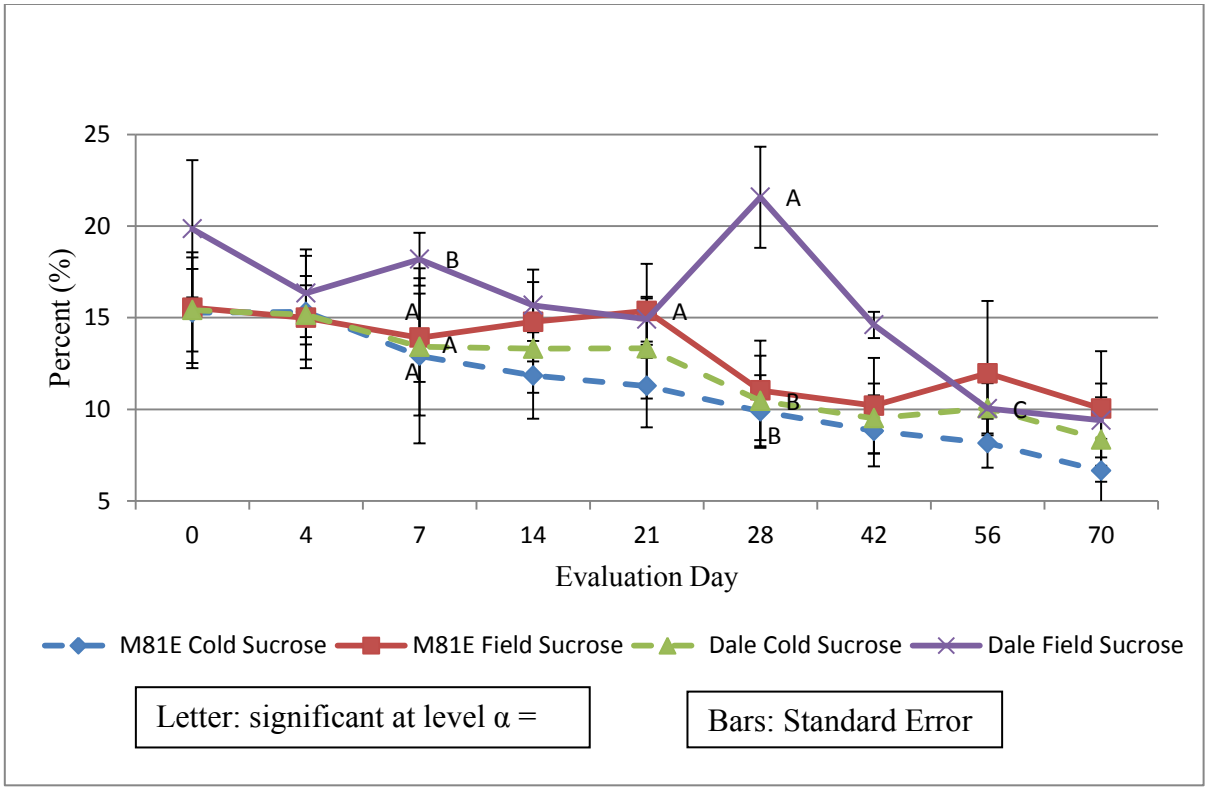

Figure 5. Plotted trends of combined biomass sucrose means of 'Dale' grown in two Texas environments (2012 College Station and Weslaco) and 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

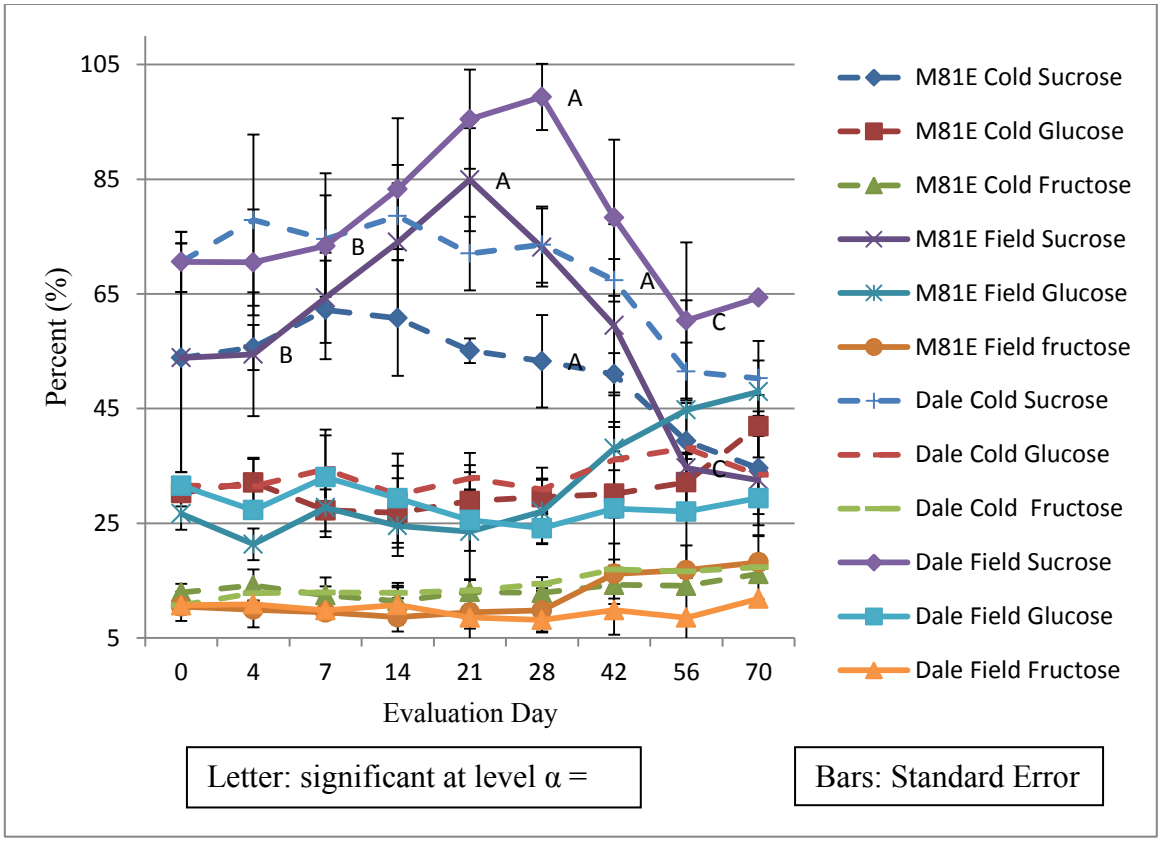

Figure 6. Plotted trends of combined juice sucrose, glucose, and fructose means of 'Dale' grown in two Texas environments (2012 College Station and Weslaco) and 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

In cold storage, sugar yields in both genotypes decreased over the evaluation periods and the rate of sugar loss was similar between Dale and M81E (Figure 7). Cold storage sugar yields in Dale dropped approximately 2% each evaluation period from day 0 through day 28, resulting in a 10% reduction in total sugar yield in a month of storage (Figure 7; Table 5). Sugar loss between day 28 and day 70 was approximately 9% of the

total sugar yield, resulting in an average total sugar loss of 19% for the 70 days of storage (Figure 7; Table 5). Sugar yields of cold storage samples of M81E reduced by approximately 5% each evaluation period until 28 days, totaling in a 19% reduction in sugar yields in a month of storage. Sugar loss between 28 days and 70 days was approximately 7% of the total sugar yield for an average sugar loss of 26% for 70 days of storage (Figure 7; Table 6).

Under field conditions, sugar yields increased in Dale from day 4 to day 28 before significant yield loss is observed (Appendix Table A12). M81E sugar yields in the field were similar, peaking twice at day 21 and 56 (Appendix Table A13). Over time, yield loss in the field was greater for M81E than in Dale (Tables 6 and 8). Overall, sugar yields dropped an average of 23% from day 0 to day 70 in cold storage, whereas field storage sugar yields were reduced by 49% over the same time (Figure 7). As with other traits, the rapid loss of sugar yield occurred in evaluation dates past 28 days.

Multiple Trait Correlations

Stalk weight was positively correlated with juice weight and was negatively correlated with sugar yields. Juice weight was also correlated with extractable sugar, although the relationship was weak (Appendix Table A3). Brix concentrations were positively correlated to extractable sugar, sucrose, and other soluble carbohydrates, but not as strong as was expected (Appendix Table A3). Furthermore, brix was negatively correlated to juice weight, stalk weight, and the structural carbohydrates. Sugar had similar correlations to both biomass and juice traits as brix, however the strength of

correlation was not as strong as brix. Sugar was also negatively correlated to juice weight, stalk weight, structural carbohydrates, cellulose, and lignin (Appendix Table A3). Compositional traits had both significant positive and negative correlations (Appendix Table A3). Negative correlations between structural and soluble carbohydrates (e.g. sucrose vs. cellulose/lignin) and sucrose and reducing sugars (e.g. sucrose vs. glucose/fructose) were expected and observed across environments and storage treatments (Tables 5, 6, 7, and 8; Appendix Table A3).

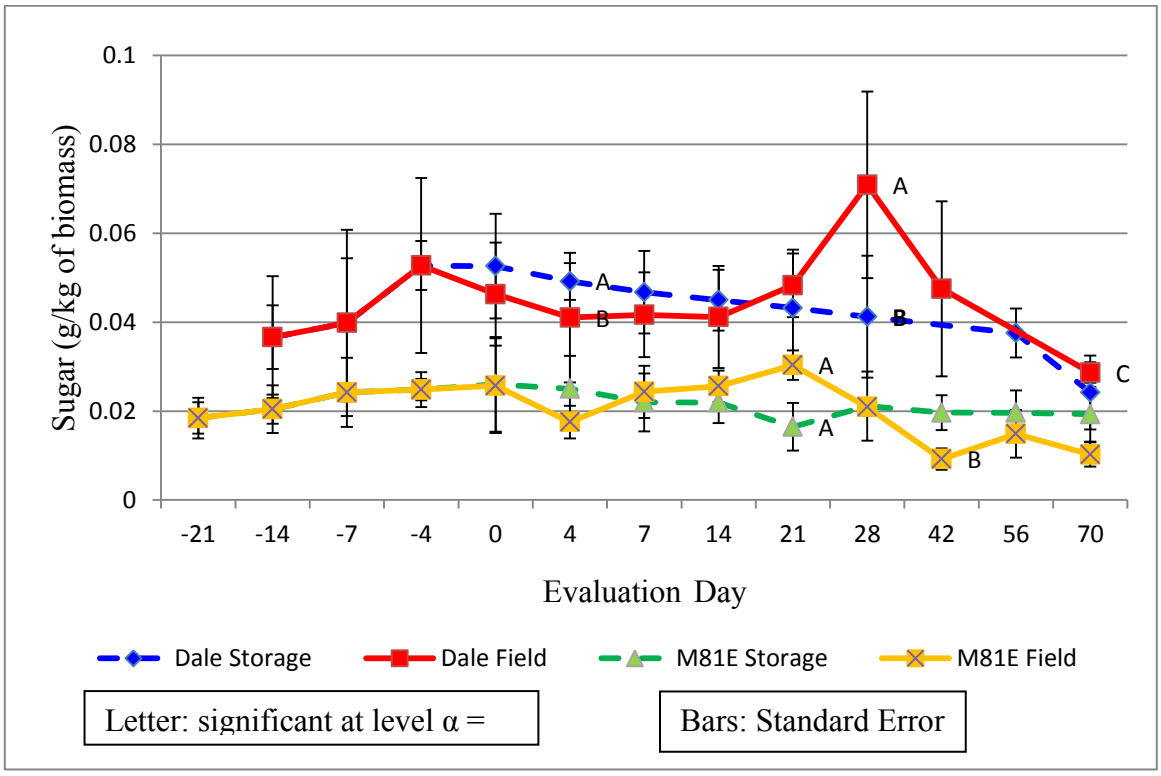

Figure 7. Plotted trends of combined sugar yield means of 'Dale' grown in two Texas environments (2012 College Station and Weslaco) and 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

Effect of Genotype

To determine the effect of genotype, a combined analysis of genotypes and environments was conducted. The genotypic effect on yields of both agronomic and compositional traits was highly significant (Appendix Tables A1and A2). Across environments and storage treatments, M81E had the highest yields for stalk weight and juice, whereas yields for brix, sucrose, and extractable sugar were greatest in Dale. Dale had the highest sugar yield of 44.1 g per Kg of biomass (4% of total weight) whereas M81E yielded 19.2 g per Kg of biomass (2% of the total weight) when harvested at day 0 (Table 9). As stated previously, there were differences in yield between College Station and Weslaco, however the ranking of genotypes in relation to yield performance remained consistent across environments.

Table 9. Sugar yields of 'Dale' and 'M81E' grown in three environments in Texas (2012 and 2013 College Station and 2012 Weslaco).

Environment	Treatment	Genotype	
		Dale	M81E
2012 College Station	Storage	(g / Kg biomass)	
	Day 0	44.18	19.2
	Avg	30.08	16.01
	Field		
	Day 0	44.18	19.2
	Avg	43.73	14.76
2012 Weslaco	Storage		
	Day 0	61.02	42.19
	Avg	55.01	29.3
	Field		
	Day 0	61.02	42.19
	Avg	54.05	36.55
2013 College Station	Storage		
	Day 0	N/A	15.73
	Avg	N/A	13.41
	Field		
	Day 0	N/A	15.73
	Avg	N/A	10.54

CHAPTER VI

DISCUSSION

Treatment Effect

In both field and cold storage there were eventual reductions in sugar concentration and yield. However, the variability associated with sampling date was less in the cold storage treatment, which is most likely because conditions in the cold room were stable and the plant was not actively growing. Furthermore, the cold temperatures slowed respiration and transpiration of harvested billets, as well as retarded microbial activity which reduced sugar yields. Watt et. al. (2009) reported an increase in respiration in the first 2 days after harvest, which resulted in a loss of sucrose overtime (Lingle et al., 2011). The moderate humidity level within cold storage helps maintain moisture content and keeps samples from drying out, which improves extractability during milling. The combination of consistent cool temperatures and moderate humidity played a key role in sugar retention in cold storage and allowed for observations on other factors that may influence sugar loss.

For both genotypes, sugar yields over 70 days of cold storage samples were relatively stable. Losses were linear with consistent downward trends, where the majority of the sugar loss was observed during the first week of storage between day 0 and day 7, and the fourth week between day 28 and day 42 (Figure 7).

Since the storage environment was constant, changes in trends may be attributed to sampling variation.

Under field conditions, plants responded to changes in environmental conditions, causing fluctuation in yields of sugar and associated traits over time. This interaction stimulated plant activity, increasing photosynthesis and production of carbohydrates. This variation in rainfall and temperature was significant in influencing the rate of juice and sugar loss within the stalk, resulting in peaks and valleys in the trend line (Figures 7). Combined field storage of Dale sugar yields decreased slightly from day 0 to day 14, but recovered yields similar to day 0 between day 21 to day 28 (Figure 7). In M81E field storage sugar yields decreased linearly, but peaked on day 21 and 56. Increases in sugar yields past day 0 could be a result of stimulated growth and increased extractability due to available moisture. However, after day 28 sugar yields steeply diminished with the loss of sustained growth for both genotypes. The ethanol yield potential for sweet sorghum is directly affected by sugar loss. As the length of storage increases, ethanol yields decrease (Figure 8).

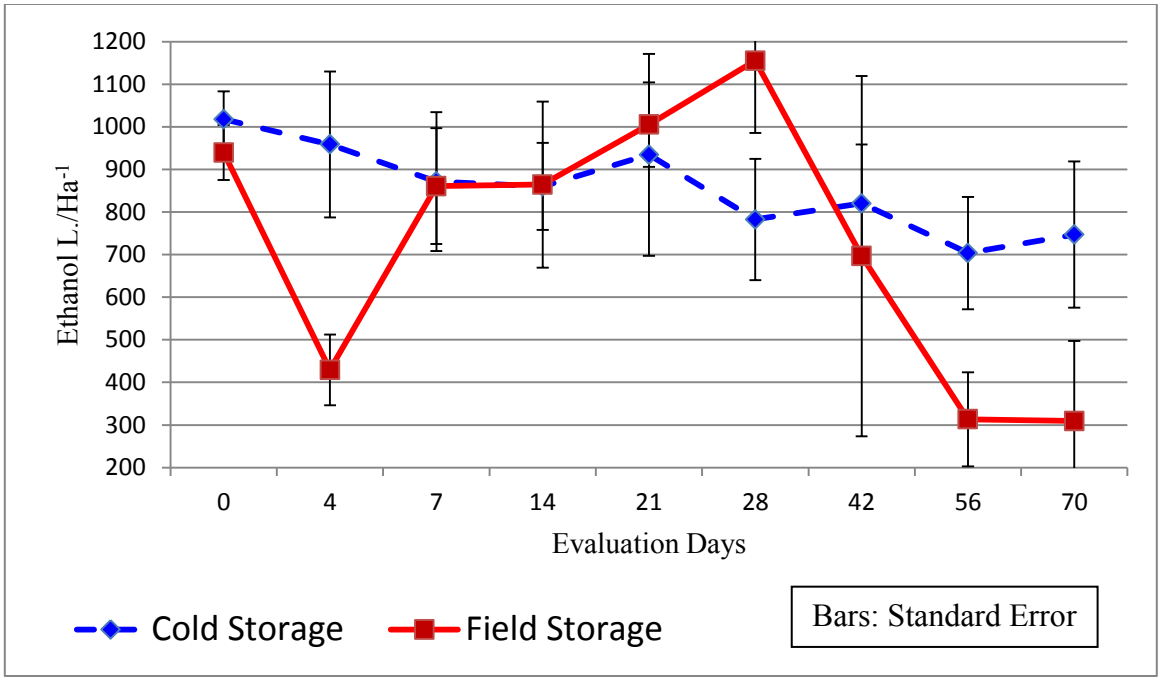

Figure 8. The effect of storage length on ethanol yields based on combined sugar yield from 'M81E' and 'Dale' grown in College Station and Weslaco 2012 and 2013.

Genotypic Effect on Yield

Differences in yield and performance were expected among the two genotypes. Photoperiod insensitive (PI) genotypes, such as Dale, mature in approximately 115 days whereas photoperiod sensitive (PS) genotypes like M81E reach maturity approximately 150 DAP depending on the precise planting date. The longer growing season of M81E allows for the accumulation of more biomass and also means that these two varieties do not mature in the same environment due to the vast differences in maturity time. Contrary to forage sorghum, the bulk of the biomass is contributed by increased stalk size, and differences in juice quantity and sugar concentrations were relative to the size of the plants. Murray et al (2008) reported that juice quantity is positively correlated to

stalk size whereas sugar concentrations were not significantly or weakly correlated to stalk size. As a longer growing season contributes to stalk size, it also contributes to increased juice and sugar accumulation. The shorter season in Weslaco resulted in lower yield in stalk and juice weights for M81E. However, sugar yields remained constant due to increased concentrations.

Genotypic effects were also significant for sugar stability during storage. Drops in sugar concentration were greater in Dale, possibly due its earlier maturity, smaller stalk diameter, and the environment being conducive for regrowth post-maturity. Larger stalks have more surface space, which may play a role in maintaining stalk moisture content used for sugar extraction. The protective properties of the thicker rind layer, consistent with larger stalks, may be due to higher concentrations of structural carbohydrates, such as cellulose and lignin, as seen in M81E (Appendix Tables A9 and A10). Additionally, wax layers on the surface of the rind layer are a reflective covering, which may contribute to the overall prevention of juice and sugar loss. Therefore, the smaller stalks of Dale may be a contributing factor to the juice evaporation and sugar loss. Since physiological maturity is reached at different times for Dale and M81E, they are subjected to different environments.

Earlier maturity promotes increased tillering during favorable environmental conditions, causing sugar to mobilize, redistribute, and be utilized for regrowth. As Dale moves from maturity to post-maturity in mid-July, growth slows, which in turn reduces the utilization of sugars, and initiates the storage of sugar in the main culm cell vacuoles (Tarpley et al., 1994; Lingle, 2012). However, there is still two months of active growing

conditions in which regrowth can occur. In this situation, sugar accumulation halts and even drops in the older main culm, as carbohydrates are redistributed and utilized for the new growth of new culms. Once established, sugar production and accumulation increases in tillers, just as the main culm prior to maturity. However, photosynthesis and sugar production may reoccur in main culm through the stimulation of growth during periods of rainfall (Figure 9).

M81E had less than a month of ideal growing conditions after maturity in mid-late August, limiting its ability to actively sustain growth as the evaluations extended into mid-November. The cooler weather slowed growth, and thereby reduced respiration and re-growth, which maintained sugar concentrations. Therefore, the environmental effect had less time with M81E, and its significance on yields was less in comparison to Dale. Hence variation within juice and juice brix was not as prevalent across the evaluation periods in M81E as it was in Dale (Figure 10).

Environmental Effect on Yield

During periods of rainfall in College Station, TX, Dale increased in juice weight, and brix values fell due to a dilution of soluble solids. In general, brix values increased in Dale until Day 28, wherein juice volumes dropped with a small increase in brix (Figure 9). In Weslaco, TX, periods of rainfall and increasing cooler temperature as the season progressed further into fall were prevalent in the remaining evaluation of Dale. As the season prolonged, the health of the plants dropped, and eventually growth ceased. At this point, brix levels plateaued from day 42 to 70.

In both environments, M81E did not have the same response to moisture and temperatures as Dale during post-maturity as the season prolonged. As M81E matured later in the season, deteriorating growing conditions (cooler and damper conditions) limited post-maturity growth and reduced the prevalence of tillers, resulting in more consistent downward trends (Figure 10). From these observations, environmental conditions after maturity will influence the amount and quality of sugar in the plants standing in the field.

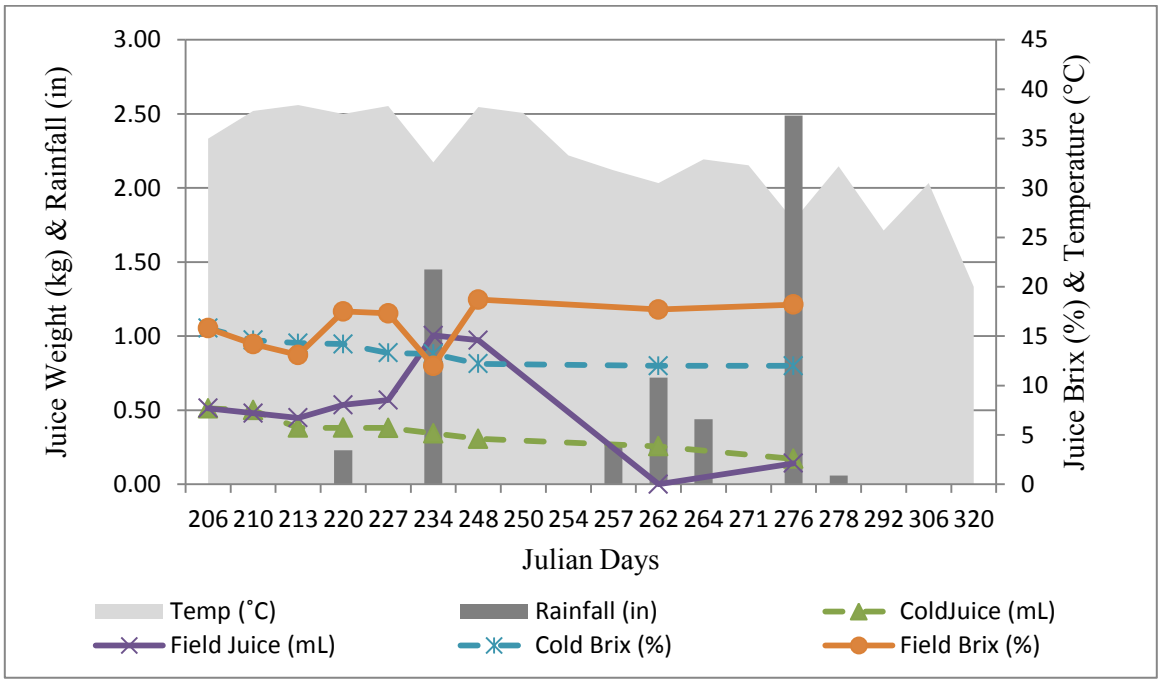

Figure 9. Combined regression of juice weight and brix trait means of 'Dale' field storage samples and weather patterns in 2012 College Station, Texas throughout the seventy day evaluation period.

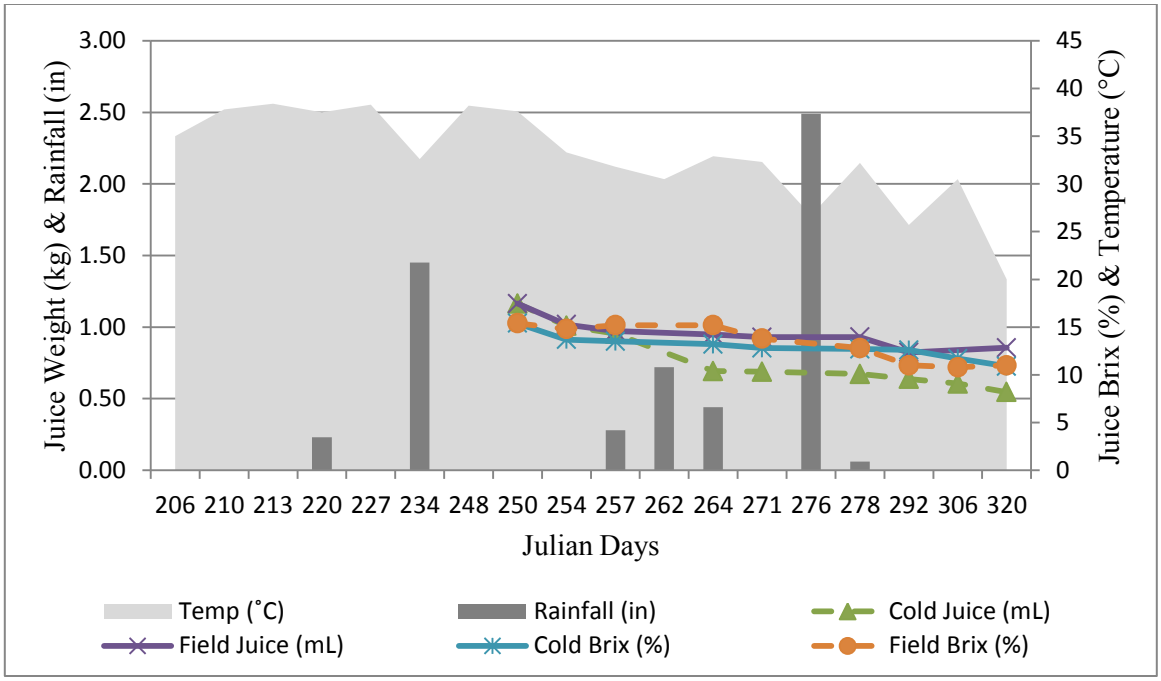

Figure 10. Combined regression of juice weight and brix trait means of 'M81E' field storage samples and weather patterns in 2012 College Station, Texas throughout the seventy day evaluation period.

Effect on Sugar Loss

Both genotype and environmental effects influence the growth of tillers. Longer seasons and plant stresses, such as increasing disease pressure, favor tiller development by initiating plant regrowth triggers (Bonnett et al, 2001). Reductions in commercial cane sugar (CCS) due to tillering range from 0 to 28% with an average loss of 5.8% (Crook et al, 1999). In general, mature stalks decrease in CCS by 1 unit for each 10% increase in tillering biomass of the harvested material. When reported CCS on a per hectare basis, inclusion of tillering biomass increases the total amount of harvestable

sucrose regardless of the concentration. However, CCS reported that per kg of biomass was diminished. Furthermore, the rate of tillering was dependent upon environment, but all plants harvested past evaluation day 28 had tillered prolifically. Increased tillering corresponded with reduced sugar concentration, leading to the conclusion that redistribution of sugars to tiller growth contributes to the sugar reduction. In Australia, average sucrose content is used to determine payment to growers, and inclusion of tillers would reduce grower profits. Additionally, more harvestable biomass adds extra cost to harvesting, transportation, milling, and processing. Jackson et al (2000) showed through modeling cost that a change from a high tillering (30%) to a low tillering (5%) genotype would be worth $4.9 million in recoverable sugars for the northern sugar growing regions in Queensland Australia alone.

Stalk size, quality, and quantity are important in the stability of extractible sugars. Since Dale and M81E differ in stalk diameter and plant height, the sugar accumulation and stability is influenced as well. Moreover, because the growing conditions of the two genotypes were different during crop maturation, the stability was different between the two. Dale encountered higher temperatures post-maturity than M81E (Figures 9 and 10). In addition, the smaller stalks of Dale may have reduced the ability of the rind layer to prevent juice evaporation, especially as stalk quality degraded overtime. Even though sugar concentrations rise as moisture concentration drops, sugar extractability is influenced by juice extraction. Juice evaporation may be less of a problem in M81E because prolonged vegetative growth results in larger stalks with a thicker rind. The larger plant may increase both juice quantity and retention. However,

as plants extend further into the post-maturity season, simultaneous reductions in brix and juice weight overtime leads to the conclusion that changes in dilution is not the only cause of sugar concentration loss, but rather it is sugar loss via deterioration (Bonnett et. al, 2001) as indicated by data (Appendix Tables A6 and A8).

It is well known that microbial activity reduces sugar stability and ultimately production. Daeschel et al (1981) reported 108 microorganisms per milliliter were present in fresh sweet sorghum juice (Lingle et al., 2011). Of these, *Leuconostoc mesenteriodes* was predominant, however *Lactobacilli*, yeast, and nonfecal coliforms bacteria were found. During sample processing and evaluations, the increasing disease incidence and severity on the sample led to the opinion that microbial degradation may play a role in increased sugar loss in prolonged storage. Juice samples from day 0, day 28, and day 70 in both treatments and genotypes where assayed using standard pathogen identification protocols (i.e. culturing, streaking, and gram staining). From this research, microorganisms such as *Fusarium verticillioides* and *Yeast* spp. were isolated in high concentrations. *Fusarium* spp. are responsible for numerous plant diseases, such as molds and stalk rot, which were frequently observed in biomass samples from both the storage and field treatments. Yeast, commonly associated with fermentation, increased in concentration from day 0 to day 70 in plated cold and field storage treatment juice samples. Fermentation is a metabolic process which converts sucrose to simpler carbohydrates such as glucose and fructose (reducing sugars) to make lactic acids and acetate. Herein, juice sucrose concentration decreased overtime while reducing sugars increased from day 0 to day 70 for both genotypes (Figure 5). This was expected, since

the longer the samples are stored, the more time the yeast has to metabolize sugars. Storage treatment had significantly higher concentrations of reducing sugars than the field treatment, since plants remaining in the field were still actively growing and not a conducive environment for yeast establishment.

Another form of sugar loss due to microbial activity is sucrose conversion to polysaccharides such as dextrans via bacterial synthesis (Solomon, 2009). Dextrans are commonly produced by the bacteria genera *Lactobacillus*, *Leuconostoc*, and *Streptococcus* and may cause substantial sugar loss. Clarke et al (1980) reported that sucrose loss as a result of dextran is 1.9 times the dextran formation and for every 0.1% of dextran produced there is a 0.04% loss in sucrose. Dextran is a gummy substance that impedes sugar processing and quality of sucrose (Solomon, 2009). United States sugar refineries dock biomass shipments of deteriorated biomass with high dextran concentrations. Dextran formation can result from (1) *Leuconostoc* spp. infection (2) prolonged time between harvesting and milling, (3) storage conditions, (4) billet size, (5) genotype and harvesting practices, (6) climate and ambient weather, (7) and poor sanitary conditions (Solomon, 2009). However *Leuconostoc* spp. were not found in the juice or biomass samples plated for this study. Since *Leuconostoc* spp. is commonly found in sugarcane and tropical environments, its absence in College Station is not unexpected. Additionally, juice samples were treated with biocide (sodium azide) to reduce microbial activity and this may have led to our failure to detect *Leuconostoc* spp. in plated samples. Our inability to isolate dominant sucrose reducing microbes indicates that inversion by endogenous invertase is likely the predominant factor reducing sucrose.

As sucrose content decreased, glucose and fructose increased in the billets so that the total sugar concentration remained static (Figure 6). Sucrose inversion is caused by acid and neutral invertases. These invertases are most prevalent at the boot stage of plant development and are highest concentrations in the upper internodes of the stalk. However invertases are also induced by wounding the plants to increase respiration (Lingle et al., 2011). Sucrose inversion during storage is known to reduce crystallization, and Smith et al. (2000) reported that sweet sorghum producers typically harvested stalks whole and stack in the field to reduce sucrose crystallization and increase inversion (Lingle et al., 2011).

Harvesting of excess biomass (i.e. leaves, panicles, weeds, and inorganic material) increases as field storage is extended. Larrahondo et al (2006) reported that for every 1% of plant residue (trash) added to clean biomass resulted in a sucrose loss of 0.18-2.3 units. Trash has a low sucrose and moisture content which absorbs extracted sucrose through osmotic pressure and reduces the total sugar yields (Larrahondo, 2006). In the current study, there was an increase in excess biomass and microbial activity after day 28, even though there are more leaves during the earlier dates. At those dates, the plant tissue is healthier and does not breakdown as easily when milled compared to older tissue beyond day 28. This was also evident in juice quality analyses. As samples were processed, the visual juice quality decreased, making extraction of clean pure juice difficult. The combination of continued plant respiration, microbial activity, sucrose inversion, and trash lead to the conclusion that sugar preservation methods are essential for long term storage.

Sugar Preservation

Retention of sugar concentrations could be increased by treating the biomass with sulfur dioxide (SO_2). Treatments of 3,000 ppm of SO_2 could preserve sugars of sweet sorghum for 2 months, whereas 4,000 ppm of SO_2 could preserve sugars for 4 months (Lingleet al., 2011; Eiland et al., 1983). Additionally, Eckhoff et al (1985) reported that SO_2 concentrations of 2,500 ppm and above could only preserve sugars a maximum of three months. The application of SO_2 prevents microbial activity by reducing the pH but the treatment requires that sorghum biomass be sealed in a container to retain the necessary SO_2 levels (Lingle et al., 2011). Prior to processing, the SO_2 is neutralized through the addition of lime. Because of the associated costs, this system is not economical for commercial processing.

Since sugar preservation treatments are expensive, commercial sugar retention methods are focusing on harvest logistics to limit the total sugar loss. Lingle et al (2011) evaluated sugar yields from chipped biomass, billeted and whole stalks and they found that sugar loss was greatest in chipped biomass samples and there was no significant difference between billeted and whole stalk samples. The increased sugar loss in chipped samples was attributed to increased microbial access to the biomass due to the increased surface area of the biomass.

The majority of sugar storage in sugarcane is in the vacuole of parenchyma cells; however 21% of sugar is stored in the apoplast (Lingle et al., 2011; Welbaum et. al, 1990). Since sorghum is closely related to sugarcane, it is expected that sugar storage sites are similar (Tarpley et al, 1994: Lingle et al., 2011). The vacuole is protected by the

cell plasma membrane and the vacuole membrane which both may prevent microorganisms infection (Lingle et al., 2011). For this experiment, plants were hand cut into billets for the storage treatment and cut as whole plants in the field treatment. There was little damage on the plants other than the cut ends. However, commercial producers utilize machine harvesters, then transfer the plants to processing facilities via trucks, which may cause more damage to billets and allow access to more interior sugars, resulting in increased sugar loss.

Complementation to Sugarcane

Post-harvest deterioration of sugars plays an important role in the sugarcane industry (Solomon, 2009), and the transition from whole stalk harvesting to billet harvesting is becoming more common. Complementation of sweet sorghum to sugarcane productions hinges on the ability to merge logistics between the two crops. Harvesting methods, planting schemes, and storage are key components to complementation because they impact the profitability of refineries. Sugar refineries need continuous feedstock to remain profitable. However, sugarcane requires 12 to 16 months to mature, and since sweet sorghum matures within three to six months (Rooney, personal communication), the use of staggered planting scheme while utilizing a range of maturity groups prior to sugarcane maturity can extend the processing windows of sugar refineries for 30 to 100 days (Burks et al., 2013). In addition, storage of biomass can extend the processing windows even further by maintaining the sugar yields for 30 days after maturity.

CHAPTER VII

CONCLUSION

Cold storage is effective in delaying sugar loss compared to ambient storage or post-maturity delayed harvest. The cold storage treatment allowed accurate observations on the "storage life" of sugars over 70 days by removing variation in climate. This also led to better hypotheses of what other factors could be influencing sugar stability and contributing to total sugar loss. The delayed harvest in the field treatment allowed for the continued biomass production through transpiration and photosynthesis, but most of the carbohydrates produced were redistributed to the ratoon and tiller growth and not captured as sugar.

Regardless of treatment, sugar yields are significantly reduced after 28 days, indicating that sugar yields can be maintained a maximum of one month after maturity. In addition to storage effect, genotypes also influence sugar yield and stability. The differences in the maturity of the genotypes allowed a longer harvest season, which delayed the need for storage. This also showed that the environmental effect was more varied than expected. Longer seasons increase the concentration of sugars, by prolonging vegetative growth. Periods of low temperatures and rainfall contributed to the ease of sugar extraction and increased overall yields. The processing window may be extended by 30 to 100 days by growing sweet sorghum with sugarcane, by staggering planting dates, and by utilizing diverse genotypes. Storage of biomass may only extend that window for an additional 30 days post-harvest. Due to the cost of cold storage under controlled conditions, this is likely not an economically viable option, unless

environmental conditions allow for it. Though sweet sorghum and sugarcane are closely related, we cannot be certain that the sugar stability of sugarcane will react similar to sweet sorghum, making it necessary to repeat this study using sugarcane, in order to determine storage potential. As a result, implementation of staggering planting dates, while maximizing potential sugar yields via accurate harvesting at peak sugar accumulation, is key for commercial production and processing.

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APPENDIX

Table A1. Combined analysis of variance of agronomic and composition traits from 'Dale' grown in two Texas environments (2012 College Station and Weslaco) and 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

SOURCE	AGRONOMIC				BIOMASS NIR	
	STALK (kg)	JUICE (kg)	BRIX (%)	SUGAR (kg)	SOLU (%)	STRU (%)
LOC	37.09**	4.01**	1.4×10^{-3}**	0.01**	7.369203	0.34
GENO	68.14**	9.94**	0.05**	0.03**	601.15**	419.19**
TRT	1.01**	0.99**	0.01**	1.2×10^{-4}*	20.9	71.10**
EVDA	0.49**	0.56**	4.5×10^{-3}**	3.6×10^{-4}**	125.38**	94.58**
EVDA*TRT	0.27*	0.13	1.2×10^{-3}**	3.5×10^{-4}**	50.04**	33.14**
EVDA*GENO	0.46**	0.18*	1.4×10^{-3}**	3.7×10^{-4}**	18.77**	21.42**
EVDA*LOC	0.13	0.07	4.6×10^{-4}**	1.4×10^{-4}	27.06**	13.88*
TRT*LOC	0.83*	0.41*	8.8×10^{-4}*	4.6×10^{-4}*	186.91**	191.19**
TRT*GENO	0.32	3.2×10^{-3}	2.7×10^{-4}	5.3×10^{-5}	247.64**	165.87**
GENO*LOC	6.41**	0.05	0.01**	3.8×10^{-4}	80.30**	19.34*
TRT*GENO*LOC	0.05	0.28	5.6×10^{-3}**	1.2×10^{-4}**	8.72	2.41
EVDA*TRT*GENO	0.34*	0.16	1.2×10^{-3}**	4.5×10^{-4}**	55.69**	29.79**
EVDA*TRT*LOC	0.2	0.09	3.6×10^{-4}	3.9×10^{-5}	12.39	4.11
EVDA*GENO*LOC	0.08	0.05	4.5×10^{-4}**	1.1×10^{-4}	13.96*	4.01
EVDA*TRT*GENO*LOC	0.07	0.01	7.2×10^{4}**	4.5×10^{-4}	29.29**	8.47
REP(EVDA*GENO*LOC)	0.22**	0.06	1.5×10^{-3}	1.5 x 10-5*	6.99	4.56
ERROR	0.12	0.07	1.6×10^{-4}	1.1×10^{-4}	6.74	4.46
MEAN	1.97	0.56	0.14	0.03	35.08	63.54
R2	0.93	0.83	0.92	0.89	0.87	0.88
CV%	17.76	48.04	8.87	30.99	7.39	3.32

* Significant at level of $\alpha = 0.05$ of probability.

** Significant at level of $\alpha = 0.01$ of probability.

Table A2. Combined analysis of variance of agronomic and composition traits from 'Dale' grown in two Texas environments (2012 College Station and Weslaco) and 'M81E' grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

SOURCE	BIOMASS NIR			JUICE NIR		
	SUCR (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
LOC	112.23**	0.68	28.52**	322.39	703.65**	282.49**
GENO	951.88**	270.02**	249.41**	18191.46**	114.97	4.05
TRT	74.13**	106.74**	45.63**	6175.71**	675.72**	628.11**
EVDA	194.92**	54.07**	25.84**	2582.31**	288.71**	77.74**
EVDA*TRT	25.05**	7.76**	8.01**	1030.77**	88.95*	20.12*
EVDA*GENO	35.91**	12.03**	2.64*	185.79	132.73**	9.91
EVDA*LOC	19.77**	5.75*	2.93**	180.43	42.68	8.51
TRT*LOC	64.51**	78.32**	35.98**	1364.99**	285.44**	49.98*
TRT*GENO	140.31**	10.31*	2.84	38.02	23.58	6.75
GENO*LOC	24.19*	460.42**	0.93	2332.65**	25.91	111.97**
TRT*GENO*LOC	16.31	0.15	2.25	1195.03**	26.59	0.18
EVDA*TRT*GENO	46.05**	6.78**	8.39**	64.08	86.09*	17.33
EVDA*TRT*LOC	13.19	2.36	2.13	197.85	16.33	3.34
EVDA*GENO*LOC	13.04*	2.95	2.22*	828.75**	69.60*	8.7
EVDA*TRT*GENO*LOC	23.78**	0.81	1.62	275.59*	25.02	15.63
REP(EVDA*GENO*LOC)	7.15	3.41**	1.14	146.94**	62.48**	15.93**
ERROR	6.02	2.18	1.03	94.47	33.68	8.79
MEAN	14.44	23.34	10.6	65.84	30.51	12.33
R2	0.9	0.91	0.91	0.92	0.84	0.85
CV%	16.98	6.33	9.58	14.76	19.01	24.05

* Significant at level of $\alpha = 0.05$ of probability.

** Significant at level of $\alpha = 0.01$ of probability.

Table A3. Combined trait correlations of 'Dale' grown in two Texas environments (2013 and 2012 College Station and Weslaco) and 'M81E' grown in three Texas environments (2013 nd 2013 College Station and 2012 Weslaco).

Traits	AGRONOMIC					BIOMASS COMP				JUICE COMP		
	SUGAR (kg)	BRIX (%)	JUICE (kg)	STALK (kg)	SOLU (%)	STRU (%)	SUCR (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
SUGAR	1**	0.67**	-0.52*	-0.71*	0.5**	-0.51**	0.62**	-0.50**	-0.52*	0.36**	0.2*	0.01
BRIX		1**	-0.28	-0.43	0.60**	-0.62**	0.69**	-0.71**	-0.68**	0.53**	0.17*	0.05
JUICE			1**	0.7**	0.24*	-0.02*	-0.06*	-0.03*	-0.01	-0.05	-0.37*	-0.27
STALK				1**	-0.12*	0.11*	-0.25**	0.3*	0.12	-0.15	-0.32*	-0.18
SOLU					1**	-0.89**	0.75**	-0.66**	-0.72**	0.47**	-0.11*	-0.19
STRU						1**	-0.75**	0.7**	0.75**	-0.46**	0.09*	0.17
SUCR							1**	-0.61**	-0.78**	0.51**	-0.12**	-0.24
CELLU								1**	0.66**	-0.53**	0.13*	0.13
LIGN									1**	-0.53**	0.05*	0.14
SUCR										1**	-0.14*	-0.18*
GLUC											1**	0.56**
FRUC												1**

*Significant at level of $\alpha = 0.05$ of probability
**Significant at $\alpha = 0.01$ of probability

Table A4. Trait means and Tukey's HSD mean separation analysis results for each evaluation day of the 2012 College Station and Weslaco 'Dale' agronomic data.

	College Station-Dale-Cold					College Station-Dale-Field				
EVDA	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	LOSS (%)	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	LOSS (%)
0	1.81	0.51	15.7	44.18	-----	1.81	0.51	15.8	44.38	-----
4	1.58	0.5	14.6	37.35	15.5	1.64	0.48	18.2	41.03	7.54
7	1.57	0.38	14.3	37.58	14.4	1.48	0.44	17.7	41.43	6.66
14	1.53	0.38	14.2	4.92	20.4	1.74	0.53	17.3	41.63	6.18
21	1.52	0.38	13.6	33.31	24.1	1.8	0.56	17.5	42.25	4.81
28	1.51	0.36	13.4	33.23	24.8	2.21	1	18.7	65.66	-47.93
42	1.48	0.31	12.1	N/A	N/A	2.02	0.97	14.2	44.8	-0.94
56	1.48	0.26	12	25.9	41.1	1.6	0.25	12.9	N/A	N/A
70	1.44	0.17	12	24.19	45.3	1.15	0.14	12	28.65	35.42
Mean	1.55	0.36	13.54	30.08	26.5	1.72	0.54	16.03	43.73	1.68
Avg. % Loss	0.2	0.67	0.24	0.45		0.36	0.73	0.24	0.35	
HSD	0.41	0.18	1.2	7.1	-----	0.73	0.35	1.3	15.03	-----
	Weslaco-Dale-Cold					Weslaco-Dale-Field				
0	1.4	0.35	18.1	61.02	-----	1.4	0.35	16.4	61.02	-----
4	1.27	0.35	17.9	61.89	-0.04	N/A	N/A	N/A	N/A	N/A
7	1.27	0.32	17.4	60.99	8.47	1.31	0.18	16	41.87	13.11
14	1.26	0.31	17.3	57.5	9.89	1.26	0.15	19.4	40.66	15.6
21	1.26	0.18	17.3	55.85	8.47	1.08	0.2	20.8	54.35	-12.79
28	1.24	0.17	16.9	54.98	9.8	1.48	0.21	16.9	76.14	-58.01
42	1.16	0.14	15.1	49.24	-1.42	1.22	0.21	14.1	50.23	-4.23
56	1.07	0.13	14.7	49.17	19.41	N/A	N/A	N/A	N/A	N/A
70	1.06	0.12	14.1	44.49	5.77	N/A	N/A	N/A	N/A	N/A
Mean	1.22	0.23	16.5	55.01	31.02	1.29	0.22	17.27	54.05	-9.26
Avg. % Loss	0.24	0.66	0.22	0.19		0.13	0.4	0.14	0.18	
HSD	0.37	0.16	1.2	5.5	-----	0.41	2.5	2.8	23.24	-----

*Means Separation via Tukey's HSD analysis

Table A5. Trait means and Tukey's HSD mean separation analysis results for each evaluation day of the 2012 College Station and Weslaco 'M81E' agronomic data.

EVDA	College Station-M81E-Cold					College Station-M81E-Field				
	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	LOSS (%)	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	LOSS (%)
0	3.15	1.16	15.4	19.2	-----	3.15	1.16	15.4	19.2	-----
4	3	1	13.7	17.44	9.16	3.22	1.01	15.2	17.54	8.61
7	2.98	0.95	13.5	16.26	15.29	3.24	0.97	15.2	16.92	11.82
14	2.84	0.69	13.2	16.48	14.12	3.12	0.94	14.8	16.39	14.61
21	2.65	0.68	12.8	16.47	14.19	3.15	0.93	13.8	15.84	17.45
28	2.59	0.67	12.7	15.94	16.98	2.97	0.93	12.8	12.66	34.04
42	2.69	0.63	12.6	14.85	22.11	2.93	0.82	11	9.18	52.17
56	2.67	0.6	11.7	14.21	25.95	3.77	1.13	15.6	14.88	22.47
70	2.63	0.54	10.9	13.21	31.15	3.61	0.85	11	10.22	46.74
Mean	2.8	0.77	12.94	16.01	18.62	3.24	0.97	13.87	14.76	25.99
Avg. % Loss	0.17	0.53	0.29	0.31		-0.15	0.27	0.29	0.47	
HSD	0.5	0.5	1.43	1.52	-----	0.95	0.3	2	1.02	-----
	Weslaco-M81E-Cold					Weslaco-M81E-Field				
0	2.03	0.91	13	42.19	-----	2.03	0.91	13	42.19	-----
4	2.21	0.73	12.7	32.85	0.22	N/A	N/A	N/A	N/A	N/A
7	1.97	0.7	12.4	32.58	0.23	2.07	0.74	14.8	31.76	0.25
14	1.9	0.62	12.3	27.65	0.34	2.65	0.89	15.8	34.8	0.18
21	1.89	0.56	11.3	27.34	0.35	2.24	1.05	16.4	44.86	-0.06
28	1.82	0.52	11.2	26.31	0.38	1.75	0.54	10.4	29.15	0.31
42	1.62	0.46	10.8	24.35	0.42	N/A	N/A	N/A	N/A	N/A
56	1.61	0.35	10.4	25.07	0.41	N/A	N/A	N/A	N/A	N/A
70	1.43	0.16	10.4	25.4	0.4	N/A	N/A	N/A	N/A	N/A
Mean	1.83	0.56	11.61	29.3	0.34	2.15	0.83	14.08	36.55	0.17
Avg. % Loss	0.3	0.82	0.2	0.4		0.14	0.41	0.2	0.31	
HSD	0.39	0.13	1.8	1.55	-----	0.61	0.35	1.8	15.5	-----

*Means Separation via Tukey's HSD analysis

Table A6. Trait means and Tukey's HSD mean separation analysis results for each evaluation day of the 2013 College Station 'M81E' agronomic data.

EVDA	College Station-M81E-Cold					College Station-M81E-Field				
	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	LOSS (%)	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	LOSS (%)
0	3.21	1.24	11.4	15.73	-----	3.02	1.16	11.4	15.73	-----
4	3.21	1.22	11.5	14.43	8.23	2.93	0.96	12.1	14.59	7.23
7	3.1	1.16	11.6	14.33	8.89	2.66	0.5	11.8	10.78	31.45
14	3.1	1.16	11.1	13.43	14.64	3.01	1.1	9.4	12.46	20.75
21	3.01	1.04	10.7	13.56	13.79	2.76	1.02	9.3	13.78	12.74
28	3.02	1.07	10.6	12.73	19.05	2.69	0.69	7.3	8.27	47.43
42	2.96	1.01	10.4	12.56	20.15	3.33	1.17	6.7	7.38	53.08
56	2.87	0.97	9.4	12.16	22.7	3.44	0.91	5.9	5.21	66.87
70	2.77	0.85	7.6	11.76	25.25	2.6	0.73	5.1	6.65	57.72
Mean	3.03	1.08	10.48	13.41	16.59	2.94	0.92	8.78	10.54	37.16
Avg. % Loss	0.08	0.16	0.33	0.25		0.14	0.37	0.55	0.58	
HSD	0.4	0.3	2	0.8	-----	0.75	0.81	2	2	-----

*Means Separation via Tukey's HSD analysis

Table A7. Trait means and Tukey's HSD mean separation analysis results for each evaluation day of the 2012 College Station and Weslaco 'Dale' cold treatment composition data.

	College Station-Dale-Cold							
	BIOMASS					JUICE		
EVDA	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	42.11	20.40	57.59	21.03	6.45	64.69	35.28	12.45
4	41.94	19.92	58.15	21.29	8.59	72.59	35.21	14.41
7	40.10	19.19	59.35	21.02	8.29	65.62	40.20	13.21
14	39.08	18.50	60.35	23.61	8.80	74.50	33.05	13.61
21	37.92	17.26	61.43	23.33	9.31	66.42	38.17	14.57
28	36.12	15.57	62.93	23.55	10.35	79.55	32.42	16.20
42	35.32	13.20	63.31	25.25	10.41	75.61	40.81	16.90
56	33.01	11.49	64.89	25.81	10.85	53.00	37.33	16.64
70	31.45	9.96	66.66	26.40	12.32	53.94	34.73	17.11
Mean	37.45	16.17	61.63	23.48	9.48	67.32	36.35	15.01
Avg. % Loss	0.25	0.51	-0.16	-0.26	-0.91	0.17	0.02	-0.37
HSD	5.00	4.80	5.15	3.05	2.00	14.50	8.65	4.05
	Weslaco-Dale-Cold							
0	39.50	19.31	60.12	22.13	9.76	76.48	27.84	8.92
4	40.48	20.47	59.50	20.07	8.90	83.14	27.67	11.22
7	42.93	21.09	57.37	18.93	8.64	83.49	28.67	12.61
14	38.20	18.73	61.34	21.56	9.89	82.66	26.84	12.12
21	35.79	17.15	63.42	22.93	10.57	77.67	27.57	11.93
28	33.90	14.01	65.10	23.29	11.91	67.64	29.38	12.61
42	33.94	12.69	65.09	23.27	11.51	59.16	31.39	16.96
56	33.79	12.50	65.22	23.48	11.82	49.94	38.83	16.56
70	32.58	12.34	66.08	24.14	11.78	46.61	32.11	17.56
Mean	36.79	16.48	62.58	22.20	10.53	69.64	30.03	13.39
Avg. % Loss	0.18	0.36	-0.10	-0.09	-0.21	0.39	-0.15	-0.97
HSD	7.01	6.40	4.75	3.40	2.00	12.50	12.00	7.51

*Tukey's HSD mean separation analyss significant at level $\alpha = 0.05$

Table A8. Trait means and Tukey's HSD mean separation analysis results for each evaluation day of the 2012 College Station and Weslaco 'Dale' field treatment composition data.

	College Station-Dale-Field							
	BIOMASS					JUICE		
EVDA	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	37.12	19.19	61.43	21.59	7.51	64.69	35.28	12.45
4	34.11	16.34	63.45	21.29	9.07	70.51	27.29	10.87
7	33.78	16.08	63.06	21.89	9.47	65.59	35.26	10.12
14	34.90	16.39	63.40	21.61	9.48	80.97	30.36	10.41
21	35.15	17.19	63.80	21.88	9.74	96.87	26.47	8.21
28	42.11	21.83	57.59	20.60	6.45	100.11	23.67	8.05
42	33.18	11.13	64.56	24.27	10.52	68.90	26.85	9.27
56	32.05	10.05	65.41	24.75	11.85	-----	-----	-----
70	30.04	9.40	67.39	27.00	12.09	-----	-----	-----
Mean	34.72	15.29	63.34	22.76	9.58	78.23	29.31	9.91
Avg. % Loss	0.19	0.51	-0.10	-0.25	-0.61	-0.07	0.24	0.26
HSD	6.73	4.30	963.00	5.31	2.23	10.34	11.80	4.21
	Weslaco-Dale-Field							
0	40.57	20.51	58.96	19.09	8.38	76.48	27.84	8.92
4	-----	-----	-----	-----	-----	-----	-----	-----
7	40.24	20.31	59.09	19.59	8.88	81.13	30.81	9.49
14	34.36	14.97	64.08	19.68	9.54	85.62	28.36	11.05
21	36.13	12.65	62.34	22.35	10.41	94.03	24.65	8.91
28	41.22	21.31	58.60	18.68	7.88	98.61	24.62	8.23
42	37.75	18.09	61.28	19.15	8.30	87.68	28.29	10.40
56	-----	-----	-----	-----	-----	-----	-----	-----
70	-----	-----	-----	-----	-----	-----	-----	-----
Mean	38.38	17.97	60.73	19.76	8.90	87.26	27.43	9.50
Avg. % Loss	0.07	0.12	-0.04	0.00	0.01	-0.15	-0.02	-0.17
HSD	6.78	8.15	5.25	3.18	1.85	11.42	6.60	2.71

*Tukey's HSD mean separation analyss significant at level $\alpha = 0.05$

Table A9. Trait means and Tukey's HSD mean separation analysis results for each evaluation day of the 2012 College Station and Weslaco 'M81E' cold composition data.

	College Station-M81E-Cold							
	BIOMASS					JUICE		
EVDA	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	37.20	15.14	61.62	20.54	9.70	68.80	32.60	15.48
4	37.04	14.81	62.11	22.12	9.35	61.05	37.89	17.07
7	34.30	13.01	63.82	22.04	10.77	70.79	28.21	15.39
14	34.51	11.59	63.94	22.84	11.26	73.22	27.24	11.64
21	34.25	11.68	64.52	23.86	11.27	61.27	30.51	15.32
28	32.72	10.44	65.26	24.89	11.25	59.20	29.87	14.99
42	31.11	8.61	66.72	24.44	12.05	58.48	32.10	16.19
56	30.28	7.31	67.44	25.57	12.75	43.62	34.05	17.02
70	26.99	5.05	70.15	25.95	13.52	38.62	47.95	18.18
Mean	33.16	10.85	65.06	23.58	11.32	59.45	33.38	15.70
Avg. % Loss	0.27	0.67	-0.14	-0.26	-0.39	0.44	-0.47	-0.17
HSD	0.88	1.81	1.97	1.73	4.23	4.99	8.00	3.60
	Weslaco-M81E-Cold							
0	35.33	15.40	63.48	25.70	11.28	38.93	27.95	10.40
4	34.95	15.82	64.01	25.36	11.45	50.28	26.32	11.25
7	32.16	12.83	66.48	26.88	11.95	53.66	26.29	9.50
14	31.58	12.10	67.27	26.93	12.81	48.35	26.40	11.22
21	30.31	10.87	67.76	26.78	13.94	48.93	27.26	10.70
28	30.98	9.34	69.46	27.77	13.51	47.32	29.22	10.72
42	28.66	9.04	67.93	27.75	13.70	43.52	28.18	12.31
56	28.98	9.00	68.91	27.97	14.11	35.07	30.25	11.14
70	27.62	8.25	70.16	28.60	14.55	30.56	35.92	14.16
Mean	31.17	11.41	67.27	27.08	13.03	44.07	28.65	11.27
Avg. % Loss	0.22	0.46	-0.11	-0.11	-0.29	0.22	-0.29	-0.36
HSD	1.62	2.30	2.34	1.71	1.50	6.22	9.65	4.72

*Tukey's HSD mean separation analyss significant at level $\alpha = 0.05$

Table A10. Trait means and Tukey's HSD mean separation analysis results for each evaluation day of the 2012 College Station and Weslaco 'M81E' field composition data.

	College Station-M81E-Field							
	BIOMASS					JUICE		
EVDA	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	37.50	15.14	62.19	20.64	11.25	68.80	25.40	10.56
4	36.75	15.00	62.61	21.71	10.25	54.47	21.33	9.92
7	34.79	13.69	63.61	21.81	10.33	71.84	27.10	9.80
14	34.71	13.23	63.82	21.62	10.39	73.50	24.50	9.72
21	34.56	12.46	63.74	22.38	11.10	75.31	24.58	12.05
28	34.30	11.72	64.97	22.90	11.14	68.94	28.29	10.67
42	33.42	10.20	65.49	24.86	11.27	59.49	38.05	16.18
56	35.73	11.97	61.36	22.65	10.37	34.64	44.74	16.83
70	32.73	10.05	64.04	25.57	11.36	32.53	47.95	18.18
Mean	34.94	12.61	63.54	22.68	10.83	59.95	31.33	12.66
Avg. % Loss	0.13	0.34	-0.03	-0.24	-0.01	0.53	-0.89	-0.72
HSD	4.25	3.01	1.90	3.51	1.15	12.75	8.41	6.67
	Weslaco-M81E-Field							
0	33.27	15.95	62.06	23.08	10.26	38.93	27.95	10.40
4	-----	-----	-----	-----	-----	-----	-----	-----
7	36.70	14.13	62.47	24.20	10.86	56.81	28.39	9.10
14	36.97	16.33	62.84	24.45	11.22	74.52	24.61	7.54
21	37.30	18.29	62.01	22.78	10.02	94.53	22.49	6.85
28	31.26	10.35	66.37	25.34	12.06	77.26	25.87	8.94
42	-----	-----	-----	-----	-----	-----	-----	-----
56	-----	-----	-----	-----	-----	-----	-----	-----
70	-----	-----	-----	-----	-----	-----	-----	-----
Mean	35.10	15.01	63.15	23.97	10.89	68.41	25.86	8.56
Avg. % Loss	0.06	0.35	-0.07	-0.10	-0.18	-0.98	0.07	0.14
HSD	3.38	4.10	3.50	2.55	1.20	15.27	3.36	6.22

*Tukey's HSD mean separation analyss significant at level $\alpha = 0.05$

Table A11. Trait means and Tukey's HSD mean separation analysis results for each evaluation day of the 2013 College Station 'M81E' cold and field composition data.

	College Station-M81E-Cold							
	BIOMASS					JUICE		
EVDA	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	28.41	69.48	8.21	29.01	13.59	71.56	13.97	8.53
4	26.86	71.32	6.71	29.94	14.50	72.11	15.51	8.59
7	30.36	67.83	9.12	27.64	13.08	66.98	17.68	10.50
14	30.44	67.64	7.50	27.38	13.02	74.49	16.61	8.40
21	25.07	72.80	3.44	29.56	14.71	50.66	12.86	7.08
28	28.89	68.96	6.65	28.23	13.56	59.91	17.30	9.71
42	30.90	67.41	7.22	27.73	12.44	47.50	16.16	7.18
56	28.05	70.09	6.33	28.55	14.04	32.23	24.13	9.35
70	25.31	73.09	5.83	30.79	15.56	19.70	27.99	8.73
Mean	28.25	69.85	6.78	28.76	13.83	55.02	18.02	8.67
Avg. % Loss	0.11	-0.05	0.29	-0.06	-0.14	0.72	-1.00	-0.02
HSD	2.01	3.61	1.30	3.05	1.11	6.23	3.64	3.35
	College Station-M81E-Field							
0	28.41	69.48	8.21	29.01	13.59	71.56	13.97	8.53
4	32.84	65.78	10.90	26.67	12.67	69.57	15.27	6.93
7	34.01	64.66	11.39	25.45	12.91	75.01	16.67	8.72
14	28.74	69.26	6.89	28.40	14.05	76.23	11.83	6.89
21	26.94	70.32	5.37	29.03	14.59	77.31	13.85	5.54
28	30.10	67.73	7.60	28.32	13.88	79.74	10.74	6.80
42	29.82	68.21	6.05	28.95	13.01	57.75	17.76	7.77
56	32.25	65.99	10.33	27.88	12.43	41.39	20.31	8.89
70	30.12	67.71	8.01	28.53	14.34	33.69	24.96	10.10
Mean	30.36	67.68	8.31	28.03	13.50	64.69	16.15	7.80
Avg. % Loss	-0.06	0.03	0.02	0.02	-0.06	0.53	-0.79	-0.18
HSD	6.95	5.66	0.60	3.65	2.15	11.52	4.55	4.51

*Tukey's HSD mean separation analysis significant at level $\alpha = 0.05$

Table A12. Agronomic and compositional trait means of combined genotypes and treatments for College Station, TX.

EVDA	COLLEGE STATION											
	AGRONOMIC				BIOMASS COMPOSITION					JUICE COMPOSITION		
	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	2.49	0.84	15.58	31.74	38.48	22.96	60.70	20.95	8.73	66.74	32.14	12.74
4	2.36	0.75	15.43	28.34	37.46	22.07	61.57	21.60	9.32	64.66	30.43	13.07
7	2.32	0.69	15.18	28.05	35.74	20.79	62.45	21.69	9.72	68.46	32.69	12.13
14	2.31	0.64	14.88	27.36	35.79	20.63	62.88	22.42	9.98	75.55	28.79	11.35
21	2.29	0.64	14.35	26.97	35.46	20.27	63.37	22.86	10.35	74.97	29.93	12.54
28	2.33	0.74	14.35	31.87	36.31	20.44	62.69	22.99	9.61	76.95	28.56	12.47
42	2.28	0.69	12.50	22.98	33.26	16.41	65.02	24.71	11.06	65.62	34.45	14.64
56	2.13	0.56	13.05	18.34	32.77	15.92	64.77	24.69	11.45	47.90	35.79	14.75
70	2.21	0.43	11.48	19.07	30.30	14.10	67.06	26.23	12.32	47.36	40.00	16.32
Mean	2.30	0.66	14.09	26.08	35.06	19.29	63.39	23.13	10.28	65.36	32.53	13.33
Avg. % Loss	0.11	0.49	0.26	0.40	0.21	0.39	-0.10	-0.25	-0.41	0.29	-0.24	-0.28
HSD	0.25	1.1	0.52	3.12	3.35	3.15	1.43	2.67	3.84	3.11	3.33	2.64

*Tukey's HSD mean separation analysis significant at level $\alpha = 0.05$

Table A13. Agronomic and compositional trait means of combined genotypes and treatments for Weslaco, TX.

EVDA	WESLACO											
	AGRONOMIC				BIOMASS COMPOSITION					JUICE COMPOSITION		
	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	1.72	0.63	15.13	43.58	37.16	22.77	61.15	22.50	9.92	57.70	27.90	9.66
4	1.74	0.54	15.30	46.79	37.71	27.68	61.75	22.72	10.18	66.71	26.99	11.24
7	1.66	0.49	15.15	39.29	38.01	21.90	61.35	22.40	10.08	68.77	28.54	10.17
14	1.77	0.49	16.20	39.45	35.28	20.39	63.88	23.15	10.86	72.79	26.55	10.48
21	1.62	0.50	16.45	48.63	34.88	19.58	63.88	23.71	11.24	78.79	25.49	9.60
28	1.58	0.36	13.85	45.21	34.34	19.16	64.88	23.77	11.34	72.71	27.27	10.12
42	1.34	0.27	13.33	45.49	33.44	19.78	64.76	23.39	11.17	63.45	29.29	13.22
56	1.34	0.24	12.55	37.12	31.39	20.69	67.07	25.72	12.97	42.50	34.54	13.85
70	1.25	0.14	12.25	41.45	30.10	19.96	68.12	26.37	13.17	38.58	34.02	15.86
Mean	1.56	0.41	14.47	43.00	34.70	21.32	64.09	23.75	11.21	62.44	28.95	11.58
Avg. % Loss	0.27	0.78	0.19	0.05	0.19	0.12	-0.11	-0.17	-0.33	0.33	-0.22	-0.64
HSD	0.25	1.1	0.52	3.12	3.35	3.15	2.23	1.43	2.67	3.84	3.11	3.33

*Tukey's HSD mean separation analysis significant at level $\alpha = 0.05$

Table A14. Combined environment and genotypes trait means of cold and field storage treatments grown in three Texas environments (2012 and 2013 College Station and 2012 Weslaco).

EVDA	COLD STORAGE											
	AGRONOMIC				BIOMASS COMPOSITION					JUICE COMPOSITION		
	STALK (Kg)	JUICE (Kg)	BRIX (%)	SUGAR (g)	SOLU (%)	SUCR (%)	STRU (%)	CELLU (%)	LIGN (%)	SUCR (%)	GLUC (%)	FRUC (%)
0	2.10	0.74	15.55	39.32	38.53	17.56	60.70	22.35	9.30	62.22	30.92	11.81
4	2.02	0.65	14.73	37.09	38.60	17.76	60.94	22.21	9.57	66.77	31.77	13.49
7	1.95	0.59	14.40	34.34	37.37	16.53	61.75	22.22	9.91	68.39	30.84	12.68
14	1.89	0.50	14.25	33.44	35.84	15.23	63.22	23.73	10.69	69.68	28.38	12.15
21	1.84	0.45	13.68	36.27	34.57	14.24	64.28	24.23	11.27	63.57	30.88	13.13
28	1.80	0.43	13.50	31.18	33.43	12.34	65.69	24.88	11.75	63.43	30.22	13.63
42	1.74	0.39	12.68	40.77	32.26	10.89	65.76	25.18	11.92	59.19	33.12	15.59
56	1.71	0.34	12.20	28.60	31.52	10.07	66.61	25.71	12.38	45.41	35.12	15.34
70	1.64	0.25	11.85	30.08	29.66	8.90	68.26	26.27	13.04	42.43	37.68	16.75
Mean	1.85	0.48	13.65	34.57	34.64	13.72	64.14	24.09	11.09	60.12	32.10	13.84
Avg. % Loss	0.23	0.24	1.85	4.62	4.44	4.33	-3.78	-1.96	-1.87	9.90	-3.38	-2.47
HSD	0.25	1.1	0.52	3.12	3.35	3.15	2.23	1.43	2.67	3.84	3.11	3.33

*Tukey's HSD mean separation analysis significant at level $\alpha = 0.05$

Table A15. Weather data correlations to juice weights and juice brix of ‘Dale’ and ‘M81E’ grown in 2012 College Station.

	Weather		Dale Yields				M81E Yields			
			Cold Storage		Field Storage		Cold Storage		Field Storage	
Julian Day	Rainfall (in)	Temp (˚C)	Juice (mL)	Brix (%)	Juice (mL)	Brix (%)	Juice (mL)	Brix (%)	Juice (mL)	Brix (%)
206	0	35	0.51	15.8	0.51	15.8				
210	0	37.8	0.5	14.6	0.48	14.2				
213	0	38.4	0.38	14.3	0.45	13.1				
220	0.23	37.5	0.38	14.2	0.54	17.5				
227	0	38.3	0.38	13.3	0.57	17.3				
234	1.45	32.6	0.34	13.2	1.01	12				
248	0	38.2	0.31	12.2	0.97	18.7				
250	0	37.6					1.16	15.4	1.16	15.4
254	0	33.3					1.01	13.7	1.02	14.8
257	0.28	31.8					0.95	13.5	0.97	15.2
262	0.72	30.5	0.26	12	N/A	17.7				
264	0.44	32.9					0.69	13.2	0.95	15.2
271	0	32.3					0.69	12.8	0.93	13.8
276	2.49	26.7	0.17	12	0.14	18.2				
278	0.06	32.2					0.67	12.7	0.93	12.8
292	0	25.7					0.64	12.6	0.82	11
306	0	30.5					0.61	11.7	N/A	10.8
320	0	20					0.55	10.9	0.86	11