# EVALUATION OF SWEET SORGHUM CULTIVARS AS A POTENTIAL ETHANOL CROP IN MISSISSIPPI

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Petroleum prices have made alternative fuel crops a viable option for ethanol production. Sweet sorghum [*Sorghum bicolor*] is a non-food crop that may produce large quantities of ethanol with minimal inputs. Eleven cultivars were planted in 2008 and 2009 as a half-season crop. Four-row plots 6.9 m by 0.5 m, were monitored bimonthly for °Brix, height, and sugar accumulation. Yield and extractable sap were taken at the end of season. Stalk yield was greatest for the cultivar Sugar Top (4945 kg $ha^{-1}$) and lowest for Simon (1054 kg $ha^{-1}$). Dale ranked highest ethanol output (807 L $ha^{-1}$) while Simon (123 L $ha^{-1}$) is the lowest. All cultivars peak Brix accumulation occurs in early October. Individual sugar concentrations indicated sucrose is the predominant sugar with glucose and fructose levels dependent on cultivar. Supplemental ethanol in fermented wort was the best preservative tested to halt degradation of sorghum wort.

# DEDICATION

This thesis is dedicated to the Department of Plant and Soil Sciences for furthering my education and preparing me for a future in agriculture.

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# CHAPTER I

# INTRODUCTION

Rising oil prices and concerns about the environment have given rise to an interest in renewable fuels. Much consideration has been given to cellulosic biomass crops, but with present technology this alternative fuel source is not a commercially viable option. Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a crop that can be grown for its fermentable sugars, and the crop is also widely adapted to different geographic locations and is very drought tolerant. The sweet sorghum plant is a very fast growing $C_4$ grass, which has a moderate response to nitrogen. These qualities make sweet sorghum a potentially viable crop for a sustainable alternative energy system.

Historically, sorghum in the United States is grown as a forage for cattle, feed for poultry, and as a high quality syrup. The types of sorghum grown for syrup are taller and juicier than the types grown for grain production, also the syrup produced from sweet sorghum should not be confused with molasses, which is a by-product from sugarcane juice crystallization. Kafirs and milos are types of sorghum grown for their grain, while sweet sorghums are grown for the juice contained in the stalk. As in any production system, the goal of the sweet sorghum producer is to obtain the highest level of return at the lowest input. Many factors such as cultural practices, insects, disease, weather, and processing can affect the potential yield of the sorghum, and subsequently the value of the syrup (Bitzer, 1997). Taking into account the final goal in a syrup production system, we can state that the ethanol goal of an energy production system is affected mostly by the same factors and the crop could be managed in a very similar manner.

Sweet sorghum has a major advantage over a cellulosic biomass crop as a feedstock for ethanol production. Sugars, in the form of sucrose and glucose, produced by the sorghum plant can be directly fermented by yeast; where as a cellulose crop must be hydrolyzed by a weak acid or enzymes before the fermentation process can begin. The current ethanol crop corn (*Zea mays*) requires an amylase treatment of the starch in the endosperm of the seed before it can be fermented into ethanol. Sugars in the sweet sorghum plant are removed by crushing the stalk and extracting the sap. The dominant sugars in the sap are: sucrose, glucose, and fructose, and of those only glucose and sucrose are directly fermentable by common brewers yeast (*Saccharomyces cervevisiae*). Stabilization of the wort with chemicals to stop bacteria and fungi growth from consuming the ethanol, and lowering the potential ethanol yield is also being investigated. This sap can be immediately “charged” with yeast, and the sugars of the sap are consumed and converted to ethanol and carbon dioxide. The resulting wort is distilled and the ethanol can be collected and used as fuel. Not all sugars are removed in this process, and some sugars remain in the stalk after sap extraction.

Evaluation of cultivars is one of the first steps in understanding how to impact the sorghum ethanol market. Cultivars may differ in maturity date, sugar concentration, standability, establishment, and a variety of other characteristics. Tracking sugar concentration in the plant throughout the growing season is one of the most important aspects of sweet sorghum production. Knowing when the plant is most laden with sucrose and glucose, would enable the producer to time his harvest when the maximum sugars are available for fermentation.  This in turn would determine the harvest for the greatest ethanol yield.

Another possibility being evaluated is two crops in a single season.  Either as two plantings or as a planting followed by a ratoon crop.  One planting taking place in late April and ratooned, the other in early July.  Rapid growth of sweet sorghum makes a July planting a feasible option.  It may be possible to use sweet sorghum as a rotation crop following a winter annual oil seed that would be harvested in June, to double crop and provide energy as well.  A two crop system could possibly double the amount of fuel produced by an ethanol farmer.  As markets emerge for this type of fuel, knowledge of locally adapted cultivars will be critical to the planting, harvest, and processing of sweet sorghum.

# CHAPTER II

# LITERATURE REVIEW

Historically sweet sorghum has been grown on a small scale in the United States. Its uses range from a forage for cattle to syrup production. The groups of plants known as sorghums are $C_4$ annual herbaceous grasses that are commonly grown for grain in the United States. Like grain sorghum, sweet sorghum can also be used for grain production, but also for its stalk rich in sugar. The grain production from sweet sorghum can reach 1500 to 1700 kg $ha^{-1}$, which can be used as feed. However, grain produced on sweet sorghum is difficult to harvest due to its height. Along with feed from the grain the whole plant makes high quality silage. The possibility of developing sweet sorghum as an energy crop could play a crucial part in the alternative energy problem (Dajue, 2008). A study by Knowles (1984) stated a possible ethanol yield of 6106 L $ha^{-1}$ which surpassed sugarcane at 4680 L $ha^{-1}$. The directly fermentable sugars in the sap, contained in the stalk of the plant, can be converted into ethanol using well understood primitive methods. This fact has increased sweet sorghum's potential to being adopted as an alternative feedstock for ethanol production (Lueschen, et al., 1991).

Sweet sorghum originated in the tropics, but is also cultivatable in more temperate regions (Lipinsky, et al., 1979; Smith et al., 1987; Wiedenfeld, 1984; Putnam, et al., 1991). When grown in the tropics the crop can be ratooned and two harvests removed from the field. When compared to the current ethanol crop of choice in the alternative fuel system, maize sweet sorghum has shown equality and even superiority in the South in terms of ethanol production (Smith et al., 1987) and in the Corn Belt (Putnam et al., 1991). Sweet sorghum sap and stalk is similar to that of sugarcane (*saccharum* sp.) and contains enough fiber to fuel the furnaces for distillation, but the crops differ greatly in the manner in which they are planted and managed. Sugarcane is a perennial normally grown on 18-24 month harvest intervals, and it's vegetatively propagated where as sweet sorghum is planted by seed and therefore normal seeding equipment can be used and the crop can be managed using regular row crop equipment (Smith et al., 1987).

*Sorghum bicolor* is divided into subspecies bicolor that encompasses the domesticated grain sorghums. These races of are a complex web of closely related annual taxa from Africa while the perennial types originated in Europe and Asia. The races are generally divided into: bicolor, caudatum, durra, guinea, and kafir. Bicolor is generally known as the sweet sorghums, while the kafirs, caudatum, durra, and guinea are grown for their grain (de Wet, 1978).

Historically, sorghum in the United States has been grown as forage for cattle, feed for poultry, and as high quality syrup. Kafir and milo are types of sorghum grown for their grain, while sweet sorghums are grown for the juice contained in the stalk. As in any production system, the goal of the sweet sorghum producer is to obtain the highest level of return at the lowest input. This low input system makes sweet sorghum an interesting possibility as an ideal cellulosic bioenergy crop combined with a sugar crop. Taking into account the final goal in a syrup production system, we can state that if the ethanol goal of an energy production system is affected by the same factors, then the crop could be managed in a very similar manner.

Sugar/Syrup Production:

The types of sorghum grown for syrup are typically taller and juicier than the types grown for grain production, also the syrup produced from sweet sorghum should not be confused with cane molasses, which is a by-product from sugarcane juice crystallization. Sweet sorghum has a major advantage over a crop grown solely for cellulose. Sugars, in the form of glucose fructose and sucrose, produced by the sorghum plant can be directly fermented by various yeasts; where as a cellulose crop must be hydrolyzed by a weak acid or cellulosic enzymes before the fermentation process can begin. Maize grain requires an amylase treatment of the starch in its endosperm before it can be fermented into ethanol. Sugars in the sweet sorghum plant are removed by crushing the stalk and extracting the sap. This sap can be immediately "charged" with yeast, which populate and consume the sugars of the sap and convert them to ethanol and carbon dioxide. The resulting wort, or fermented sap in this case, is distilled and the ethanol collected. The ethanol produced is a function of the high sugar content. Not all sugars are removed in this process, and some sugars remain in the stalk after sap extraction

Agronomics

Sweet sorghum is known to have a moderate response to nitrogen. Application of nitrogen above 2206 kg $ha^{-1}$ ha has been shown to reduce syrup quality, but this effect on ethanol yield has not been investigated. Higher nitrogen rates presumably would provide more above ground biomass desirable for ethanol production (both yeast and cellulosic), but potentially cause yield loss due to lodging. Because of its moderate response to nitrogen and water, sweet sorghums unusual qualities make it a potentially viable crop for a sustainable alternative energy system.

Crucial to the adoption of sweet sorghum is understanding the agronomics of the crop and how those agronomic practices effect sugar concentration and therefore subsequent ethanol yields. Previous research indicates sugars, especially sucrose, increase in the stalk as the plant reaches physiological maturity (Broadhead, 1972; Miller and Creelman, 1980) but when allowed to dry and deteriorate the free sugars declined due to microbial growth (Broadhead, 1969). This observation illustrates final sugar yield is firmly connected with harvest date. A contrasting study was conducted by Reeves et al. (1979) in which they concluded harvest date was not the predominant factor of sugar yield or concentration in Texas, but cultivar was the most important factor. Climatic differences may account for the different findings.

A long term study in Mississippi, between 1967 and 1972, explored the potential of maximizing sugar and stalk yield by varying combinations of inter- and intrarow spacings. Four intrarow combinations were combined with two interow combinations of the cultivar of sweet sorghum Rio. The results indicate plots of the narrower row at 52.5 cm distance produced higher yields per hectare when compared to conventional rows of 105 cm. In contrast, it was also observed that the plants from narrow rows were less suitable with respect to: °Brix, sucrose purity, and sugar yield per Mg of stalk. Other observations indicated that the narrow row plants weigh less, and contain less juice than conventional- row plants, and lodged more readily. Intrarow competition had no significant affect on gross stalk yield, stripped stalk yield, sugar per Mg of stalk, sugar per hectare, Brix, sucrose, and purity. Only stalk weight was significantly affected by width of row and plant arrangement within the row. Stalk weight on the narrow- row spacing was not affected, but the stalks gain weight on conventional rows as the spacing between plants increased. Based on this work the authors concluded the way to maximize total sugar yield per unit of area is by planting on narrow- rows (Broadhead and Freeman, 1980).

Smith et al. (1987) conducted studies between 1980 and 1983 at up to eight locations. Each year as many as six cultivars were evaluated, including three utilized in out study. Average fresh weight ranged from 66 Mg $ha^{-1}$ at Meridian, MS, to 115 Mg $ha^{-1}$ at Davis, CA. Of the total fresh weight; 79% can be attributed to water/sap, and dry matter was 24% of the stalk. Values for lignin and cellulose were taken from the stalk samples indicate at 1.4% lignin and 5.2% cellulose. Smith et al. also examined sugar concentration of the sap. There they observed sucrose as the dominant sugar, but also noted that glucose and fructose contributed significantly to overall sugar yield. Sucrose ranged from 1.4 Mg $ha^{-1}$ at Logan, UT in 1980 to 7.3 Mg $ha^{-1}$ at Davis, CA in 1980. The test site in Hawaii produced 12 Mg $ha^{-1}$ of sugar much higher that other test sites. The remaining sugars detected were glucose from 0.2 Mg $ha^{-1}$, at Beltsville, MD, in 1980 to 1.8 Mg $ha^{-1}$ at Fort Collins, CO in 1981, and fructose at 0.2 Mg $ha^{-1}$ at Beltsville, MD, in 1980 to 1.7 Mg $ha^{-1}$ at Davis, CA, in 1980, as well. It should be noted that in this study the hand refractometer was "not highly correlated with any individual sugars or with total sugar." Since total sugar yield is known, it was possible for the authors to calculate a theoretical ethanol yield. Ethanol production from this experiment was estimated at 2129 L $ha^{-1}$ for the Michigan test site in 1981 and up to 6388 L $ha^{-1}$ in Hawaii in 1981.

A three year study conducted by Tsuchihashi and Goto (2004) in Indonesia was conducted to observe ideal harvest time of sweet sorghum under tropical conditions. They tested three cultivars, but only reported stem yield of the cultivar, Wray. They found that during the 1996/1997 growing season they obtained a yield of 6593 g $m^{-2}$, a yield of 5296 g $m^{-2}$ was achieved in 1995/1996, and in 1994/1995 the crop produced 4790 g $m^{-2}$. However the growing season with the most biomass yield did not correspond to the highest sugar yield. The 1995/1996 season produced the most sugar at 401 g $m^{-2}$ followed by 293 g $m^{-2}$ in 1996/1997 and 286 g $m^{-2}$ in 1994/1995. The remarkably higher sugar content in 1995/1996 was attributed to the fact that birds consumed the grain during seed fill. Broadhead (1979) found that removing the seed head increased the stem Brix and sucrose concentration.

Seed is a well known sink for sugar and would be expected to lower plant sugars during development. Tsuchihashi and Goto (2004) also measured stalk growth characteristics. They found that the stalk grew rather slowly until around 36 days after planting and increased 5.8 cm $d^{-1}$ until the average height was around 320 cm at 80 days after planting (DAP). Of the three cultivars tested no difference was observed in height. On a different note, stem diameter increased quickly after emergence at a rate of 0.56 cm $d^{-1}$ and remained stable after 36 DAP, of the cultivars tested stem diameter was largest in Wray followed by Rio then Keller, respectively. Total sugar concentration was also studied, and the authors found sugar concentration increases at or near anthesis, with the maximum at 12 days after anthesis. The cultivar Wray had a maximum °Brix at 26 DAP, while Keller and Rio peaked at 33 days after anthesis.

A study in Weslaco, Texas was conducted on Rio sweet sorghum to understand the impact of planting date on yield (Hipp, et al. 1970). To test the effect of planting date the authors planted once a month from March through September. They observed a linear relationship between yield and the solar irradiance the plants received during the grain filling stage. Highest irradiance at the test sight occurs in June, July, and August; so a sorghum crop planted in May was able to take advantage of the high light before seed began to set. Broadhead (1969) conducted experiments in Meridian, Mississippi, near the same time. He also used Rio, as the cultivar, but he focused on sugar production. Broadhead observed a significantly higher yield from an April and May planting, also °Brix of the April planting was significantly higher that the June planted crop. °Brix was also shown to decline when harvest was delayed three and four weeks.

Putnam et al. (1991) compared the production of ethanol from a corn feedstock and a sweet sorghum feedstock in the northern Corn Belt. The test site was in Waseca, Minnesota in 1987 and 1988, thirteen sweet sorghum cultivars were compared to a maize hybrid popular in that region. The authors noted a significant difference between sorghum cultivars in relation to dry matter production, °Brix , fermentable carbohydrates, and ethanol yield. When compared to the ethanol yield of maize the cultivars; Keller, Dale, and Smith produced more ethanol per hectare that the maize hybrid DK524 in 1988, and were similar to maize in 1987. The authors contribute the difference between years to the fact that 1988 was a much dryer year than 1987, giving the more drought tolerant sorghum a yield advantage. The three public sorghum cultivars mentioned previously were superior to most other cultivars and hybrids used in this study, but also had the highest tendency to lodge. Lodging ranged between 94 and 100% in 1987 and 93 and 98% in 1988. Lodging is a problem not only to harvest, but in sugar accumulation. Lodged plants activate new growth. This growth acts as a sink, causing sugars stored in the stem to drop, therefore decreasing °Brix . The authors also examine other advantages to sweet sorghum in relation to energy costs at processing, and favored sweet sorghum because it "produces a readily convertible product which can be converted to ethanol without extensive processing." Another advantage the authors mention is the possibility of lower nitrogen requirement of sweet sorghum compared to maize, and the lack of a pre-fermentation treatment that would be required with maize. The major problems observed in this study in regard to sweet sorghum were the losses due to lodging, difficulty in harvest, transportation costs, and the absence of knowledge of a technology to fully extract a fermentable product.

It is well known that maize is a crop that responds well to irrigation. Researchers in North Dakota investigated the factors possibly affecting the sugar yield of sweet sorghum at temperate zone locations. They tested four sweet sorghum cultivars for fermentable sugar production under irrigated and non-irrigated conditions and two nitrogen rates (84 and 186 kg $ha^{-1}$) and control. Ethanol yields when averaged over the two years of the study are greater than 3100 L $ha^{-1}$. As expected the irrigated portion of the test produced more fresh weight with a value of 89.9 Mg $ha^{-1}$ compared to the non-irrigated with a value of 65.0 Mg $ha^{-1}$, but total sugar yield and theoretical ethanol yield were not significantly different. Also the investigators noticed little discernible effect with additional nitrogen in relation to fermentable sugar production (Smith and Buxton, 1993).

Sweet sorghum has also been viewed as a crop that can be planted on fallow sugarcane lands in Louisiana. At certain times of production as much as 25% of sugarcane production areas may lie fallow. As a possible source of income from these fallow fields, researchers at the USDA- ARS Sugarcane Research Laboratory in southeast Louisiana, investigated the possible potential of sweet sorghum as a complementary sugar bioenergy crop. They tested five sweet sorghum cultivars: Dale, M81-E, Rio, Theis, and Topper. They observed increases in sugar content between 85 and 138 DAP, but only Dale, M81-E, Theis, and Topper increased incrementally between 85 and 101 DAP and again between 101 and 119 DAP. Theoretical ethanol yields were calculated and reported as; Theis (6060 L $ha^{-1}$), Topper (5780 L $ha^{-1}$), and M81-E (5680 L $ha^{-1}$) however the differences due to cultivar were not significant. The authors believe that the fast growing annual sweet sorghums can play a significant role in the production of ethanol across the United States, and in southern Louisiana as long as it is possible to harvest the sweet sorghum before the sugarcane is planted (Tew et al. 2008).

In a study investigating strategies for future biofuel crops in the Mississippi Delta (western alluvial plain of Mississippi) data was presented from the University of Tennessee, Mississippi State University, Oklahoma State University, and University of Minnesota. Each plot consisted of two rows 9.1m in length, replicated three times, and harvested with a commercial silage harvester. These trials were conducted in 2007 and 2008 and the cultivars Dale, Della, Keller, M81-E, and Theis. Total dry matter yields were reported in dry tons per acre and were concluded to be 13.6 Mg $ha^{-1}$, Dale; 9.9 Mg $ha^{-1}$, Della; 15.7 Mg $ha^{-1}$, Keller; 14.9 Mg $ha^{-1}$, M81-E; and 9.7 Mg $ha^{-1}$, Theis. Information on planting dates and cultural practices was not available. Data presented for Mississippi was obtained from foundation seed at Mississippi Agriculture and Forestry Experiment Station (MAFES). The Mississippi data was collected for a four year period (2004-2008) for the cultivars M81-E and Theis while the data collected for Topper was for two years (2007-2008). Data indicates that M81-E and Theis yield 20.4 and 20..1 Mg $ha^{-1}$ of dry matter yield respectively and Topper produces 23.2 Mg $ha^{-1}$. The University of Minnesota conducted studies over two years (1987, 1988) on cultivars Dale, Keller, and M81-E, and found them to produce 10.0, 10.1 and 10.0 Mg $ha^{-1}$ of dry matter (Spain, 2009).

Another study conducted at Oklahoma State University tested cultivars at five locations in Oklahoma: Altus, Fort Cobb, Haskell, Lane, and Stillwater. Four cultivars were tested at each location: Dale, M81-E, Theis, and Topper, with the exception of Haskell and Stillwater where only three were tested: Dale, M81-E, and Topper. Two of the locations (Fort Cobb and Lane) there were irrigated and non irrigated treatments. In regard to the Fort Cobb site the results for the irrigated portion of the study were as follows: Dale, 9.88 Mg $ha^{-1}$; M81-E, 10.42 Mg $ha^{-1}$; Theis, 8.69 Mg $ha^{-1}$; Topper, 8.56 Mg $ha^{-1}$. In the non irrigated portion of the study the dry matter tonnage for the same cultivars were 7.07, 6.12, 4.33, and 4.48 Mg $ha^{-1}$ respectively. All further test sites were produced under non irrigated conditions. At Altus Dale, M81-E, Theis, and Topper were reported to yield 4.1, 5.01, 6.13, and 4.95 Mg $ha^{-1}$. The cultivars Dale, M81-E, and Topper produced results of 10.24, 7.93 and 13.91 Mg $ha^{-1}$ at Haskell, and 11.80, 10.93, and 8.7 Mg $ha^{-1}$ at Stillwater (Spain 2009).

The Texas Agricultural Experiment Station composed a study evaluating sorghum cultivars for potential ethanol yield. The authors compared stems of six cultivars for actual ethanol yield from yeast fermentation, ethanol yield from the stem compared to the pith, conversion ratios from fermentation of sap, and energy combustion of the bagasse. Here the scientists state that the cultivar Rio produces significantly more dry matter tonnage and ethanol per $ha^{-1}$ at 402 Mg and 3418 L, respectively than the other varieties tested (McBee, et al. 1988).

In Ohio researchers investigated the agronomic potential of sorghum production for ethanol in a temperate region. They observed the total biomass and potential sugar yield, and attempted to estimate the harvest season under normal conditions. Dry matter yields were concluded to range from 13 Mg to 19 Mg $ha^{-1}$, and fermentable sugars from 2.9 to 3.8 Mg $ha^{-1}$. If pith along with rind was hydrolyzed the total fermentable sugars exceeded 8.0 Mg $ha^{-1}$. Xylose produced from the hydrolization exceeded 2.0 Mg $ha^{-1}$, and will also be available to ferment by *Pachysolen tannophilus*. It was also indicated that sorghum stalks deteriorate rapidly following a frost, and the effective harvest window in this region was between 40 and 50 days. This being stated new technologies need to be developed to hydrolyze and convert all sugars from the stalk to ethanol and extend the processing season (Kresovich and Henderlong, 2003).

Table 1 Sweet Sorghum cultivars used in experiments, 2008 and 2009, indicating maturity and origin.

| Cultivar | Days to Maturity | Origin |
|---|---|---|
| Bale All | 90 | Kentucky |
| Bundle King | 90 | Texas |
| Dale | 115 | Mississippi |
| Della | 105 | Virginia |
| Keller | unknown | Kentucky |
| M81-E | 130 | Mississippi |
| Simon | 90 | Unknown |
| Sugar Drip | 100-110 | Unknown |
| Sugar Top | unknown | Kentucky |
| Theis | 130 | Mississippi |
| Topper | 120 | Georgia |

# CHAPTER III

# MATERIALS AND METHODS

The site chosen for this experiment is located on the South Farm of Mississippi State University's campus. The experiment was arranged as a randomized complete block with four replications, eleven treatments, and two years. Eleven cultivars of sweet sorghum chosen for the experiment: Bale All, Bundle King, Dale, Della, Keller, M81-E, Pace Setter, Simon, Sugar Drip, Sugar Top, Theis, and Topper (Table 1). The cultivars Bale All, Bundle King, Della, Keller, Simon, Sugar Drip, and Sugar Top were obtained from the University of Kentucky. Mississippi Agriculture and Forestry Experiment Station (MAFES) provided the remaining four cultivars.

## Field Experiment

Prior to planting a burndown application of glyphosate and 2, 4-D was applied at a rate of 2.34 L $ha^{-1}$ each followed by conventional disking and harrowing. Immediately following planting atrazine was applied at the rate of 1.16 L $ha^{-1}$ and the test area was irrigated with a traveling overhead sprinkler to incorporate the herbicide and initiate germination. Previous observations of sweet sorghum receiving 32 kg $ha^{-1}$ grew rank and lodged early in the growing season, so Ammonium nitrate was applied as a split application on 1 Jul and 11 Aug 2008, and 20 Jun and 15 Aug 2009 at a rate of 16 kg N $ha^{-1}$ each. Nitrogen was applied in a split application for better nitrogen use efficiency, and to curtail previous observations of logding. No lime was needed, but 27 kg K $ha^{-1}$ per year was applied. No crop deficiency symptoms were observed in any plot during the two year experiment. Fall armyworm (*Spodoptera frugiperda*) (identified by J.E. Smith) infestation observed late August made an application of Baccilus thuringensis (Bt), an insecticide, necessary in 2008.

The planting was conducted using a John Deere 71 four-row planter on 5 Jun 2008, and 1 Jun 2009. Seed of the cultivars were planted at a density of 13 seed $m^{-1}$ on 0.5 m row centers. Plots were 6m long and contained four rows. The narrow row spacing and decreased nitrogen rates used in this experiment was to attempt to curtail lodging observed in previous studies at Starkville, MS. Initial plant population and number of stalks were taken when the plants reached one m tall, on 21 Jul 2008 and15 Jul 2009. First height and °Brix data was also taken on these dates. The row in each plot containing the greatest number of plants was designated the sacrifice row, for subsequent destructive data collection. Data was taken every other week throughout the growing season up to harvests on 27 Oct 2008 and 26 Oct 2009. On these same dates heights to the whorl were also taken. After harvest data was taken monthly to observe degradation of sap over time. At each sampling time, three stalks were randomly cut at a height of one meter from the ground and °Brix data was taken. A 50 mL sample of sap was taken from each sampling row and frozen for further sugar analysis.

Harvest consisted of hand cutting five randomly selected stalks from within a randomly designated harvest row at a height of 0.08 m from the ground. The numbers of harvestable stalks per row were recorded and wet weight of leaves and stalks were recorded independently. Sap was extracted from the stalk using a sugarcane press, and the pressed stalks were also weighed. The leaves and stalks were then dried in a 66°C (150°F) oven until, no further weight loss was observed to obtain a dry weight. The volume and weight of the sap was taken along with a final °Brix reading. The frozen

50mL samples taken throughout the year were delivered to the Mississippi State University Chemical Laboratory for fractional sugar analysis via HPLC.

Fermentation and Ethanol Preservation

At harvest the extracted juice of each cultivar was bulked and placed in a two L bottle that was immediately charged with one g $L^{-1}$ of baker's yeast. The yeast was stirred into 10 mL water (35°C) to activate the yeast prior to being added to the sap. After the yeast was added into the wort, rubber bladders were placed tightly on the lid of the bottles to capture escaping $CO_2$ and to ensure an anaerobic environment. The bottles were fermented at room temperature until fermentation ceased, when $CO_2$ ceased to fill the bladders. The fermented wort was measured with a graduated cylinder and separated into four 400/mL alloquots for distillation. Each alloquot for each cultivar was poured into a 1L Erlenmeyer flask and distilled on a fractional still at a temperature of 80° C. If the temperature inside the vapor column approached 83°C on the thermometer attached to the vapor column then tap water was sprayed from a hand held sprayer onto the vapor column to reduce the temperature back to approximately 77-80°C to ensure that only azeotrope vapor was passing to the condenser. The condensed ethanol was captured in a graduated cylinder and the volume was recorded.

Data Analysis

Data was analyzed using Proc GLM (SAS, 2010). Since data between cultivars was analyzed over two years, differences between years due to environmental conditions were expected. Since year is a random effect, year and cultivar by year interaction were pooled together in the residual as suggested by (McLean et al. 1991) and Schabenberger and Pierce, (2002). Doing this takes the year out of the equation and by doing so we can observe “real” differences in cultivar using this method, differences observed by mean separation (LSD) can be expected in any year.

# CHAPTER IV

# RESULTS AND DISCUSSION

ANOVA for cultivars at each °Brix sampling date indicated significant differences between cultivars due to sampling date. On the late August sampling date °Brix measurements for the early season cultivars Simon (11.03) and Sugar Drip (11.33) were significantly greater than Bale All (9.20), which is another early season cultivar, and all other cultivars tested, except Keller, whose maturity classification is not noted in the literature (Table 1 & 2, lower case letters). It is interesting to note that Della is another early season cultivar, but it ranked with other mid-season cultivars on this date. Progressing to the early September sampling, the measure of high °Brix associated with earliness continued with Simon (13.62), Sugar Drip (14.62), and Keller (13.75) remaining significantly greater than all cultivars on this sampling date. The mid-September sampling date occurred at what would be time for mid season ripening. Again, Della ranked with mid and late season cultivars. This sampling date produced °Brix values for Simon (15.87), Sugar Drip (15.91), and Keller (15.91) that are significantly greater than the tested cultivars, except for Dale (14.33) (Table 2, lowercase letters). The significance of Dale in the high °Brix group on this sampling date indicated that it is a mid- maturing cultivar and would be expected to begin accumulating sugars later in the season than the earlier cultivars were significantly better than Bale All (3.68), but Dale (12.18), Della (12.26), Keller (13.40), M81-E (10.93), Sugar Top (10.88), and Theis (13.39) were not

Table 2 Two-year mean °Brix over time for eleven sweet sorghum (*Sorghum bicolor*) cultivars during the 2008 and 2009 growing season.

| Cultivar | Mean °Brix | | | | | |
|---|---|---|---|---|---|---|
| | Sampling Date 1[†] | Sampling Date 2 | Sampling Date 3 | Sampling Date 4 | EOS | Days to Maturity |
| Bale All | 9.20 bc[‡] AB[§] | 11.18 b A | 11.25 c A | 9.62 d AB | 7.68 e B | 90 |
| Bundle King | 5.68 e C | 8.21 c BC | 11.74 c A | 13.37 c A | 12.09 d A | 90 |
| Dale | 7.33 de D | 11.20 b C | 14.33 ab B | 17.04 ab A | 15.87 ab AB | 115 |
| Della | 8.30 cd D | 11.53 b C | 12.66 bc C | 16.08 abc A | 14.78 abc AB | 105 |
| Keller | 10.18 ab D | 13.75 a C | 15.91 a B | 18.33 a A | 17.06 a AB | Unknown |
| M81-E | 6.26 e E | 9.66 bc D | 12.79 bc B | 15.74 abc A | 15.31 ab A | 130 |
| Simon | 11.03 a C | 13.62 a AB | 15.87 a A | 15.45 bc A | 11.08 d C | 90 |
| Sugar Drip | 11.33 a CD | 14.62 a AB | 15.91 a A | 14.28 bc ABC | 12.75 cd BC | 110-115 |
| Sugar Top | 7.18 de E | 11.50 b CD | 13.91 b AB | 14.62 bc A | 14.81 abc A | Unknown |
| Theis | 6.37 e E | 8.58 c D | 11.49 c C | 16.66 ab A | 14.53 bc B | 130 |
| Topper | 6.33 e E | 9.70 bc D | 12.58 bc BC | 15.20 bc A | 15.53 ab A | 120 |

[†]Sampling date 1, 19 Aug 2008 &20 Aug 2009; sampling date 2, 2 Sep 2008 & 4 Sep 2009; sampling date 3, 16 Sep 2008 &18 Sep 2009; sampling date 4, 30 Sep 2008 & 2 Oct 2009; EOS (end of season), 26 Oct 2008 & 26 Oct 2009.

[‡]Different lower case letter following mean °Brix denotes significant differences within a column (cultivar effects) ( $\alpha \leq 0.05$).

[§] Different upper case letter following mean Brix denotes significant differences within a row (seasonal effects) ($\alpha \leq 0.05$).

Table 3 Two-year mean °Brix over time post season for eleven sweet sorghum (*Sorghum bicolor*) cultivars from late October 2008 to early January 2009. Post season indicates the period after killing frost until January following.

| Cultivar | Mean °Brix | | | | |
|---|---|---|---|---|---|
| | EOS † | Post season 1 | Post season 2 | Post season 3 | Days to Maturity |
| Bale All | 7.68 e ‡ B§ | 8.86 d AB | 3.81 e C | 3.68 d C | 90 |
| Bundle King | 12.09 d A | 12.42 c A | 8.26 cd B | 7.60 c BC | 90 |
| Dale | 15.87 ab AB | 15.13 b B | 11.80 ab C | 12.18 a C | 115 |
| Della | 14.78 abc AB | 13.47 bc BC | 12.33 ab C | 12.26 a C | 105 |
| Keller | 17.06 a AB | 17.55 a A | 13.56 a C | 13.40 a C | Unknown |
| M81-E | 15.31 ab A | 15.55 b A | 11.86 ab BC | 10.93 ab CD | 130 |
| Simon | 11.08 d C | 12.46 c BC | 8.18 cd D | 6.75 c D | 90 |
| Sugar Drip | 12.75 cd BC | 13.34 bc ABC | 6.81 d E | 9.18 bc DE | 110-115 |
| Sugar Top | 14.81 abc A | 12.35 c BC | 10.00 bc D | 10.88 ab CD | Unknown |
| Theis | 14.53 bc B | 13.40 bc BC | 12.57 ab C | 13.39 a BC | 130 |
| Topper | 15.53 ab A | 14.05 bc AB | 12.16 ab C | 10.90 ab CD | 120 |

† EOS (end of season), 26 Oct 2008 & 26 Oct 2009; Post harvest 1, 19 Nov 2008 & 12 Nov 2009; Post harvest 2, 19 Dec 2008 &17 Dec 2009; Post harvest 3, 6 Jan 2008 & 5 Jan 2009.

‡ Different lower case letter following mean °Brix denotes significant differences within a column (cultivar effects) ( $\alpha \leq 0.05$).

§ Different upper case letter following mean °Brix denotes significant differences with a row (seasonal effects) ($\alpha \leq 0.05$).

significantly different than each other (Table 3, lowercase letters). Based on these data it appears that Keller behaved as a long season cultivar.

Early season cultivars for this project were described as cultivars that mature at 90 days; mid season cultivars mature at 100 to 115 days, and late season cultivars mature at 120 to 130 days (Table 1). The three early season cultivars are: Bale All, Bundle King, and Simon. Examining these cultivars across dates it was observed that as sampling begins Simon (11.03) (Table 2, uppercase letters) is significantly higher in °Brix values, but sampling date 3 (mid September) Simon (15.87) and Bundle King (11.74) were significantly higher in °Brix than they are during the middle of August. As the season progressed for Bale All, no significant differences for °Brix values are noted, until the mid December sampling date when Bale All drops from 8.86 to 3.81 °Brix. Also at mid-December Bundle King (8.26) and Simon (8.18) showed a significant drop in sugar content as measured by °Brix from the previous sampling dates. It is important to note that sampling dates at the initial stages of sampling were not significantly different than some later stages of sampling, but the significance observed indicated the varieties peak sugar accumulation. This decline following the peak is significant, since maximum sugar is required for optimal ethanol output. In regard to mid season cultivars (Dale, Della, and Sugar Drip) a significant increase in °Brix measurements was noticed from the onset of sampling to the beginning of September; however, Dale increased in °Brix significantly every sampling date and peaked in early October with a value of 17.04 mean °Brix (Table 2, uppercase letters). This observation is in sync with the literature, which indicates Dale is the longest of the mid-season cultivars. No significant difference were noted from early September to mid-September for Della (11.53, 12.66), respectively. Della peaked °Brix in early October (16.08), which is significantly greater than the previous sampling date. For Sugar Drip peak °Brix was observed during the middle of September (15.91), this sampling date is not significant from its previous (14.62) or latter (14.28) observations, but was denoted as a significant increase from the beginning of observations (11.33) in August (Table 2, uppercase letters). Sugar Drip significantly declined from its peak readings at the EOS (12.75). While not different from the late August and early September sampling dates, harvestable sugar is a function of both Brix and total stalk fresh weight. While Brix was not increasing, the cultivars' biomass was increasing over this time. If no sugar were being produced, Brix would decline due to dilution by additional accumulated biomass. So some concurrent sugar accumulation must be occurring, but at a rate equal to the growth rate. Late season cultivars of M81-E, Theis, and Topper begin the season with relatively low °Brix readings (6.26, 6.37, 6.33) respectfully, compared to all other cultivars, except Bundle King (5.68) (Table 2, uppercase letters). As expected these late season cultivars did not reach a peak °Brix reading until early October. Significant increases in °Brix concentrations were observed over the sampling date for all late season cultivars until they reached their respective °Brix observations (Table 2, uppercase letters). Significant decreases in °Brix can be noted for Theis from early October (16.66) and the EOS (14.53) before frost.

Table 4 Two-year mean height in meters over time for eleven sweet sorghum (*Sorghum bicolor*) cultivars during the 2008 and 2009 growing seasons.

| Cultivar | Mean Height (m) | | | | | |
|---|---|---|---|---|---|---|
| | Sampling Date 1[†] | Sampling Date 2 | Sampling Date 3 | Sampling Date 4 | Sampling Date 5 | Days to Maturity |
| Bale All | 2.31 b[‡] A[§] | 2.22 d A | * | 2.49 c A | 2.24 c A | 90 |
| Bundle King | 2.31 b C | 2.72 c B | 3.18 bc B | 3.04 b A | 2.88 b B | 90 |
| Dale | 2.62 a D | 3.15 ab C | 3.76 bc B | 4.36 ab A | 3.18 ab C | 115 |
| Della | 2.84 a B | 3.16 ab A | 3.22 bc A | 3.29 ab A | 3.21 ab A | 105 |
| Keller | 2.79 a B | 3.02 ab AB | 3.10 c AB | 3.25 ab A | 3.08 ab AB | Unknown |
| M81-E | 2.32 b C | 2.93 bc B | 3.29 abc A | 3.21 ab A | 3.23 ab A | 130 |
| Simon | 2.11 b A | 2.06 d A | 2.25 d A | 2.31 c A | 2.19 c A | 90 |
| Sugar Drip | 2.11 b AB | 2.30 d A | 2.29 d A | 2.33 c A | 2.10 c AB | 110-115 |
| Sugar Top | 2.76 a B | 3.32 a A | 3.54 ab A | 3.50 ab A | 3.45 a A | Unknown |
| Theis | 2.77 a C | 3.16 ab B | 3.59 a A | 3.56 a A | 3.39 a AB | 130 |
| Topper | 2.20 b D | 2.65 c C | 2.96 c AB | 3.10 ab A | 2.84 b BC | 120 |

[†]Sampling date 1, 19 Aug 2008 &20 Aug 2009; sampling date 2, 2 Sep 2008 & 4 Sep 2009; sampling date 3, 16 Sep 2008 &18 Sep 2009; sampling date 4, 30 Sep 2008 & 2 Oct 2009; sampling date 5, 26 Oct 2008 & 26 Oct 2009.

[‡]Different lower case letter following mean height denotes significant differences within a column (cultivar effects) ( $\alpha \leq 0.05$).

[§] Different upper case letter following mean height denotes significant differences within a row (seasonal effects) ($\alpha \leq 0.05$).

* Missing data.

The significant decrease in °Brix for M81-E came between mid-November (15.55) and mid-December (11.86) sampling (Table 3, lower case) only after the killing frost. Topper followed the same full season pattern as M81-E, with the significant decrease in °Brix occurring from the November sampling (14.05) and the December sampling date (12.16). Keller and Sugar Top are not listed in the literature as having a definite seasonal length. Keller increased significantly until early October, and has a significant decrease from the mid-November (17.55) to mid-December (13.56) (Tables 2 & 3, lower case). This would seem to indicate these cultivars behave as a full season cultivar, but in fact Keller's early accumulation of sugar makes it fall into the range of early and mid season cultivars as well. Sugar Top increased, in regard to °Brix, significantly through mid-September (13.91), but its peak is not observed until EOS (14.81) (Table 2, lower case). A significant decrease in °Brix for Sugar Top is noted in mid November (12.35) (Table 3, lower case). These data would indicate Sugar Top falls into the category of a full season cultivar.

Height data was also collected for the 2008 and 2009 growing season. These data were recorded on the same dates as °Brix collection. Variance around the mean was substantial between cultivars for the mid-August sampling date, but the observations only fall into two categories with Dale (2.62 m), Della (2.84 m), Keller (2.79 m), Sugar Top (2.76 m), and Theis (2.77 m) being significantly taller than all other cultivars (Table 4, lowercase letters). Such variation around the mean is expected because the crop is often cross pollinated and cultivars are a mixture of genotypes. As the season progresses to the beginning of September, Sugar Top (3.32 m) ranked tallest among cultivars, but is only significantly taller than Bale All (2.22 m), Bundle King (2.72 m), M81-E (2.93 m), Simon (2.06 m), Sugar Drip (2.30 m), and Topper (2.65 m) (Table 4). During the middle

Table 5 Two-year fresh weight (kg $ha^{-1}$), mean extractable sap (L $ha^{-1}$), mean EOS (end of season), °Brix, mean ethanol distilled from fermented wort (ml $400ml^{-1}$)and mean ethanol (L $ha^{-1}$) of 11 sweet sorghum (*Sorghum bicolor*) cultivars during the 2008 and 2009 growing season.

| Cultivar | Fresh Weight (kg $ha^{-1}$) | Mean extractable sap ( L $ha^{-1}$) | Mean EOS °Brix | Mean ethanol (ml 400ml $^{-1}$) | Mean ethanol ( L $ha^{-1}$) |
|---|---|---|---|---|---|
| Bale All | 1804.02 CD† | 1717.10 D | 7.68 E† | 13.37 F | 137.70 C† |
| Bundle King | 2985.23 BC | 2487.80 CD | 12.09 D | 25.50 E | 409.03 ABC |
| Dale | 3724.43 AB | 3817.40 BC | 15.87 AB | 38.12 A | 807.14 A |
| Della | 3586.76 AB | 3893.40 BC | 14.78 ABC | 33.37 BC | 706.02 A |
| Keller | 3623.47 AB | 3918.30 BC | 17.06 A | 27.70 DE | 561.85 AB |
| M81-E | 4141.48 AB | 5174.90 AB | 15.31 AB | 33.37 BC | 800.60 A |
| Simon | 1054.91 D | 1634.50 D | 11.08 D | 25.80 E | 123.30 C |
| Sugar Drip | 1399.09 CD | 2388.00 CD | 12.75 CD | 32.50 BC | 273.06 BC |
| Sugar Top | 4945.69 A | 5997.90 A | 14.81 ABC | 31.00 CD | 788.35 A |
| Theis | 3600.89 AB | 4024.80 BC | 14.53 BC | 33.37 BC | 602.75 AB |
| Topper | 3657.80 AB | 4557.80 AB | 15.53 AB | 35.5 AB | 735.05 A |

† Upper case letters signify significant differences between cultivars at the level of ($\alpha \leq 0.05$).

of September data collection, Theis (3.59 m) was the tallest cultivar, and is similar to Sugar Top (3.54 m) and M81-E (3.29 m), all are significantly taller than the other cultivars tested on that date; Sugar Top and Simon were shortest (Table 4). In early October additional significant differences in height were observed, with Bale All (2.49 m), Simon (2.31 m), and Sugar Drip (2.33 m) having significantly lower heights than all other cultivars on that date. This is not un-expected as these are early season cultivars and have finished growth after 100 days. At EOS, cultivars followed the same pattern as in sampling date four with Bale All (2.24 m), Simon (2.19 m), and Sugar Drip (2.10 m) being significantly shorter than all other cultivars. Retention of this pattern indicates that the plants are mature having produced flowers and seed, and cannot grow any taller. Post season heights were not recorded.

Early season cultivars showed no significant increase in mean height from the mid-August sampling date to the beginning of September with the exception of Bundle King (Table 4). Bundle King increased significantly from the first observations (2.31 m) to the early September sampling (2.72 m), and does not increase significantly again until beginning of October (3.04 m) (Table 4 uppercase letters). The mid-season cultivar Sugar Drip does not significantly increase in mean height after the initial sampling date at mid-August (2.11 m), but significant differences are clear in the remaining two mid-season cultivars Dale and Della (Table 4, uppercase letters). Della increased significantly after initial sampling with mean height going from 2.84 to 3.16 m, and then does not increase significantly again during the growing season; while Dale increased significantly from mid-August (2.62 m) to early September (3.5 m) (Table 4). Dale also increased in height on mid September sampling date (3.76 m) significantly, and again on the early October sampling (4.36 m). This would indicate Dale may behave more like a full season cultivar with regard to growth/height. The late season cultivars of M81-E, Theis, and Topper all followed the same general trend increasing significantly until mid-September indicating maximum biomass had been achieved (Table 4).

Height is of importance because increased height is highly correlated to increased fresh weight. If the fresh weight is correlated to height, then sugar yield will be high as well, leading to high ethanol potential. At EOS harvest a five stalk sample of each designated harvest row was taken to give an indication of fresh weight. This fresh weight would be an indicator of the tonnage that a ethanol producer would have to handle from his field to a centralized crushing (sap extraction) facility. This sample was taken weight recorded and sap extracted. Two year mean fresh weight (kg ha-$^{1}$) per hectare was calculated. Sugar Top ranked highest yielding among tested cultivars with a mean fresh weight of 4945.69 kg ha$^{-1}$ (Table 5). While, Sugar Top ranks the highest, it is not significantly different from Dale (3724.43 kg ha-$^{1}$), Della (3586.76 kg ha-$^{1}$), Keller (3623.47 kg ha-$^{1}$), M81-E (4141.48 kg ha-$^{1}$), Theis (3600.89 kg ha-$^{1}$), or Topper (3657.80 kg ha-$^{1}$). These cultivars did however produce significantly more fresh weight than Bale All (1804.02 kg ha-$^{1}$), Bundle King (2985.23 kg ha-$^{1}$), Simon (1054.91 kg ha-$^{1}$), and Sugar Drip (1399.09 kg ha-$^{1}$) (Table 5). The early season cultivars performed poorer in the time allotted for this test in terms of fresh weight with Bale All and Simon having significantly lower yields than other cultivars. This may be expected because there was a single EOS harvest well past the optimal harvest date of these early season cultivars. Bundle King is not significantly lower than mid and full season varieties with the exception of Sugar Top (Table 5). Odd among the mid-season cultivars, Sugar Drip is significantly lower in terms of two year mean fresh yield than Dale or Della. The cultivars whose season lengths are unknown (Keller and Sugar Top) when compared to late season cultivars are not significantly different, further indicating they may behave as full season cultivars.

At the EOS, sap was collected by crushing the five stalk fresh weight harvest sample. Knowing each stalk's contribution to volume of sap, along plant populations, can give us the amount of sap produced per hectare. Sugar Top ranked the highest in mean extractable sap per hectare with 5997.90 L $ha^{-1}$, (Table 5) and was significantly higher than Bale All (1717.10 L $ha^{-1}$), Bundle King (2487.80 L $ha^{-1}$), Dale (3817.40 L $ha^{-1}$), Della (3893.40 L $ha^{-1}$), Keller (3918.30 L $ha^{-1}$), Simon (1634.50 L $ha^{-1}$), Sugar Drip (2388.00 L $ha^{-1}$), and Theis (4024.80 L $ha^{-1}$). M81-E (5174.90 L $ha^{-1}$) ranked higher than Topper (4557.80 L $ha^{-1}$), but neither were significantly higher in mean extractable sap than Sugar Top. Topper and M81-E ranked higher, but were not significantly higher producers of sap than Dale, Della, Keller, or Theis.

The EOS °Brix readings were taken at harvest (26 Oct 2008, 26 Oct 2009). Keller had the highest mean observation over the two-year experiment with a reading of 17.06. This observation was significantly higher than Bale All (7.68), Bundle King (12.09), Simon (11.08), Sugar Drip (12.75), and Theis (14.53). Bale All was the lowest ranking cultivar, and was significantly lower in terms of mean Brix than all other cultivars. While Keller ranked highest among cultivars, it was not significantly different than Dale (15.87), Della (14.78), M81-E (15.31), Sugar Top (14.81), or Topper (15.53 (Table 5). Sugar Top was also not significantly different than Della, Sugar Drip, or Theis. It should be noted that year had an effect on Brix. The drier 2008 year induced a higher in Brix for all cultivars than in the wetter 2009 growing season, but because environment is part of the error term, data is pooled for these mean separations.

A two liter sap sample of each cultivar was taken to the lab, fermented and distilled. After distillation of the initial samples significant differences between cultivars were observed (Figure 1). Dale ranked the highest in terms of ethanol output with a value of 38.12mL/400mL sample, or 9.53% ethanol; it is significantly higher than all cultivars, except Topper with an observed product of 35.5mL/400mL or 8.87% ethanol. Bale All was the lowest ethanol producer with 13.37mL/400mL or 3.34% ethanol. This was not unexpected as the EOS harvest was well past Bale All's maturation date of early October (Table 2). The other early season varieties of Bundle King (25.5 mL/400mL, 6.37%) and Simon (25.8 mL/400 mL, 6.46%) were significantly better than Bale All, but not significantly different from each other or Keller (27.7 mL/400 mL, 6.93%). The earliness of these varieties could account for the lower ethanol yields in the fact that wild yeast and other adventitious microorganisms would have been present for longer periods within the senescing plants, hence field degrading the sugars needed for fermentation. Mid and late season cultivars such as Dale and Topper would have been actively growing and producing sugars and biomass later in the season, and therefore not been exposed to degrading microorganisms for as long a period of time as the early season cultivars. The ethanol yield from the distillation experiments indicated how much ethanol can be distilled from the fermented wort. The ethanol yield can in turn be multiplied by the amount of sap per hectare of each cultivar. Calculating these data indicated that Bale All and Simon ranked lowest with ethanol per hectare values of 137.70 and 123.30 L $ha^{-1}$ respectively (Table 5). Dale (807.14 L $ha^{-1}$) ranked the highest and was significantly better than Bale All, Simon, and Sugar Drip (273.06 L $ha^{-1}$). Late and mid-season cultivars continued to be significantly greater yielding than early season cultivars with the continued exception of Bundle King (108.21 L $ha^{-1}$) which was not significantly different from any cultivar and the unknown maturity of Sugar Top (208.56 L ha$^{-1}$) which ranks third among the tested cultivars.

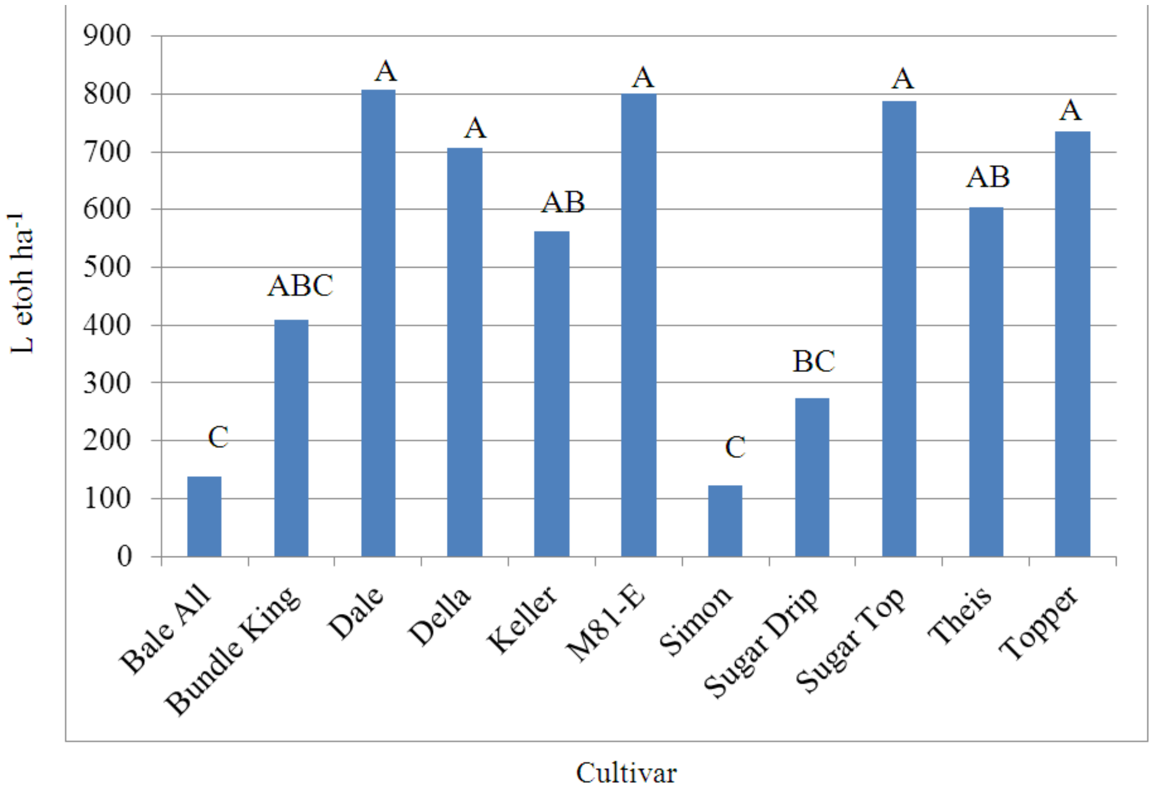


Figure 1 Ethanol output in l ha$^{-1}$ of sweet sorghum (*Sorhum bicolor*) wort at the end of season. Different letters signify significant differences between cultivars.

In a study by Smith et al. in (1987) a theoretical ethanol yield of 2129 L ha$^{-1}$ was reported for a sweet sorghum site in Michigan and 6388 L ha$^{-1}$ were reported in Hawaii; both theoretical ethanol yields were substantially higher that any cultivar tested in this study. Smith's observations over two years were greater than 3100 L ha$^{-1}$ in North Dakota, also more than any cultivar tested in Starkville. The experiment conducted in Louisiana (Tew et al. 2008) reported Theis producing 6060 L ha$^{-1}$, Topper 5780 L ha$^{-1}$, and M81-E with a value of 5680 L ha$^{-1}$. These values are theoretical yields but are

Table 6 Mean ethanol from four replications of 400 mL samples distilled after stabilization of wort to test for possible ways to keep fermented wort from degrading over time in 2008.

| Preservation of fermented wort (1 year data) | | |
|---|---|---|
| Treatment | Mean ethanol distilled (mL) | ETOH as % of 400 mL sample |
| Potassium hydroxide 4.25 | 29.75 CD | 7.4% |
| Potassium hydroxide 6.55 | 34.00 C | 8.5% |
| Potassium hydroxide 8.5 | 33.12 C | 8.2% |
| Hydrochloric acid 2.3 | 28.75 CD | 7.1% |
| Hydrochloric acid 3.37 | 29.25 CD | 7.3% |
| Acetic acid 3.508 | 26.75 D | 6.6% |
| Acetic acid 4.00 | 30.50 CD | 7.6% |
| Calcium hydroxide 5.5 | 30.50 CD | 7.6% |
| Calcium hydroxide 6.5 | 33.00 C | 8.2% |
| Calcium hydroxide 7.5 | 27.50 D | 6.8% |
| Calcium hydroxide 8.5 | 33.12 C | 8.2% |
| Supplemental ethanol 1% | 32.91 C | 8.2% |
| Supplemental ethanol 2% | 33.56 C | 8.3% |
| Supplemental ethanol 5% | 41.56 B | 10.3% |
| Supplemental ethanol 10% | 49.72 A | 12.4% |
| Control | 29.50 CD | 7.3% |

†Upper case letters signify significant differences between cultivars at the level of ($\alpha \leq 0.05$).

greater than the true observations in this experiment where Theis (603 L $ha^{-1}$), Topper (735 L $ha^{-1)}$, and M81-E (801 L $ha^{-1)}$. In Tew et al.'s study the differences in cultivar were not significant, and the same observation is seen in this study. The cultivar Rio was tested for actual ethanol yield and a value of 3418 L $ha^{-1}$ was reported by McBee, et al. (1988), but while Rio was not tested in this study no cultivar tested was as high as those reported by McBee et al.

Table 7 Two-year mean sucrose in mg ml of sweet sorghum cultivars Bale All, Dale, M81-E, and Simon on three respective dates during the 2008 & 2009 growing season.

| Cultivar | Days to Maturity | mg sucrose/ ml sap | | |
|---|---|---|---|---|
| | | Sep 2 | Oct 2 | Oct 26 |
| Bale All | 90 | 7.02 B† | 8.86 B | 7.28 B |
| Dale | 115 | 9.55 B | 16.21 A | 15.75 A |
| M81-E | 130 | 8.56 B | 16.66 A | 17.31 A |
| Simon | 90 | 14.15 A | 15.71 A | 11.01 B |

† Upper case letters signify significant differences between cultivars at the level of ($\alpha \leq 0.05$).

Table 8 Two-year mean glucose in mg/ml of sweet sorghum cultivars Bale All, Dale, M81-E, and Simon on three respective dates during the 2008 & 2009 growing season.

| Cultivar | Days to Maturity | mg glucose/ ml sap | | |
|---|---|---|---|---|
| | | Sep 2 | Oct 2 | Oct 26 |
| Bale All | 90 | 2.29 BC† | 3.58 A | 2.11 B |
| Dale | 115 | 4.37 A | 3.46 A | 4.43 A |
| M81-E | 130 | 2.91 B | 2.99 A | 4.00 A |
| Simon | 90 | 1.85 C | 2.52 A | 2.12 B |

† Upper case letters signify significant differences between cultivars at the level of ( $\alpha \leq 0.05$).

Table 9 Two year mean fructose in mg/ml of sweet sorghum cultivars Bale All, Dale, M81-E, and Simon on three respective dates during the 2008 & 2009 growing season.

| Cultivar | Days to Maturity | mg fructose/ ml sap | | |
|---|---|---|---|---|
| | | Sep 2 | Oct 2 | Oct 26 |
| Bale All | 90 | 2.19 AB† | 2.24 A | 1.27 C |
| Dale | 115 | 3.11 A | 2.58 A | 3.41 A |
| M81-E | 130 | 1.90 B | 1.87 A | 2.30 B |
| Simon | 90 | 1.27 B | 1.64 A | 1.01 C |

† Upper case letters signify significant differences between cultivars at the level of ( $\alpha \leq 0.05$).

A one year study was conducted to observe the effects of the addition of certain chemicals to the fermented wort. These chemicals may have the potential to preserve the fermented wort volume until distillation could take place. The addition of 95% ETOH at 10% of the volume of wort was significantly the best option given a rate of 12.4% return of ethanol distilled (Table 6). It was followed by the 95% ETOH at 5% wort volume given a yield of 10.3% which was significantly higher in return than all treatments; both provided more ethanol (after stabilizing ethanol was subtracted from ethanol yield) than other treatments and the control. Following the trends of this data only the addition of ethanol at a rate of 5 and 10% of the fermented wort actually had positive effects on preserving ethanol within the fermented wort.

Sucrose was tested, and it is observed that the early season cultivar of Simon with an observation of 14.15 mg sucrose $ml^{-1}$ is significantly higher in sucrose accumulation than the other tested cultivars. As the growing season progresses to the October 2 testing date Dale (16.21 mg sucrose $ml^{-1}$), M81-E (16.66 mg sucrose $ml^{-1}$), and Simon (15.71 mg sucrose $ml^{-1}$) were not significantly different from each other, but were significantly higher in sucrose than Bale All (8.86 mg/sucrose $ml^{-1}$). The final testing date of October 26 it is observed that Dale (15.75 mg sucrose $ml^{-1}$) and M81-E (17.31 mg sucrose $ml^{-1}$)

were not significant from one another, but were significantly higher than Bale All (7.28 mg sucrose $ml^{-1}$) and Simon (11.01 mg sucrose $ml^{-1}$) (Table 7).

Glucose was also tested for on the same respective dates as fructose. Dale was significantly higher than all other tested cultivars on September 2 with an observation of 4.37 mg glucose $ml^{-1}$. Sap on this testing date M81-E (2.91 mg glucose $ml^{-1}$) was significantly higher than Simon (1.85 mg glucose $ml^{-1}$), but not better in terms of glucose accumulation than Bale All (2.29 mg glucose $ml^{-1}$). On the October 2 testing date glucose followed the same pattern as fructose in the fact that no significant differences were noticed between cultivars. As we progress to the final testing date on October 26 Dale again ranked highest (4.43 mg glucose $ml^{-1}$), but is not significantly higher than M81-E (4.00 mg glucose $ml^{-1}$), but both are significantly higher than the other two cultivars tested Bale All (2.11 mg glucose $ml^{-1}$), and Simon (2.12 mg glucose $ml^{-1}$) (Table 8).

# CHAPTER V

## SUMMARY AND CONCLUSIONS

The importance of this being a half year study must be kept in mind. The sweet sorghum crop in an alternative energy system would be planted behind a winter annual. The data in the literature are reported for full season crops. This fact would help to explain the lower yields reported in this study. The tracking of sugar accumulation is critical to understanding the harvest window of this crop. Previous research by Reeves et al. (1979) concluded sugar yield was not affected by harvest date in Texas. This conflicts with observations made by Broadhead (1969) in which he found that sugars deteriorate and decline due to microbial growth as the season progresses. This study would agree with the observations made by Broadhead. All cultivars tested in this study showed that °Brix observations peaked and declined as the season progressed. This decline can be attributed to deterioration of the sugar concentrations by microbial organisms.

Maturity of the cultivars tested follow a maturity pattern noted in previous work. The observations in this study can be used to assign a maturity class to Keller and Sugar Top, whose maturities are not listed in the literature. Keller's early accumulation of sugar content as measured by °Brix would make it fall into an early or mid-season cultivar, °Brix does not significantly decrease until mid-December. This late drop in sugar would indicate that Keller behaves as a full season cultivar. Sugar Top's Brix climbs significantly throughout early September, but its peak comes at EOS with a significant decrease in mid-November. These observations would indicate Sugar Top is a full season cultivar.

Height data for these cultivars is not present in the literature, but heights can be correlated to overall fresh weight yields of the cultivars. Values for fresh weight were lower in Starkville than any reported in the literature, but this is a half-season late planted test. Fresh weights ranged from 1054 kg $ha^{-1}$ to 4945 kg $ha^{-1}$. Smith et al. (1987) reported observations between 66 and 115 Mg $ha^{-1}$ for full season. The low fresh weight yield would contribute minimal amounts of total sugar which in turn is a reason that the ethanol yield observed in this study are low compared to other literature.

In Smith's study he reported that sucrose was the dominant sugar present in the sap. The same was true in the sugar concentrations reported in this study. Sucrose is also reported to increase as the crop reaches physiological maturity (Broadhead, 1972 and Miller and Creelman, 1980) which concurs with observations from this experiment. Glucose and fructose observations were not present in the literature, and further investigations into these sugars contribution to sugar concentrations are vital to understanding the role they play in fermentation.

Ethanol yields from this study were less than those reported in the literature. The possibility of 6106 L $ha^{-1}$ reported by Knowles (1984) was not realized in this study. The highest ethanol yield was from the cultivar Dale (807 L $ha^{-1}$). The ethanol yield of Dale was also below the possible ethanol yield of sugarcane (4680 L $ha^{-1}$) (Knowles 1984).

If sweet sorghum is adopted as an energy crop the knowledge of preservation of the fermented sap or wort will be critical to a centralized distillation facility. An immediate charge of extracted sap with yeast is critical. The one-year work conducted on this problem is unique in that it is not present in any of the literature available. The data from this study would suggest that the addition of supplemental 95%ethanol at a rate of 10 and 5% of the total solution is the best option for preserving the wort.

As with any crop trying to integrate itself into the marketplace, more research is needed to fully understand the role it can fill. Additional information is needed to understand actual ethanol yields, sugar concentrations, and best agronomic practices to suite an alternative energy system. The minimal ethanol yields from this study compared to previous research could be attributed to the low yield which in turn is due to the fact that this testing was set up to predict half-season (catch crop) production methods following a winter crop. Additional knowledge of fermentation and more advanced distillation possibly could help higher ethanol yields be realized in the Southeast Region of the United Sates.

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# APPENDIX A

# FIGURES

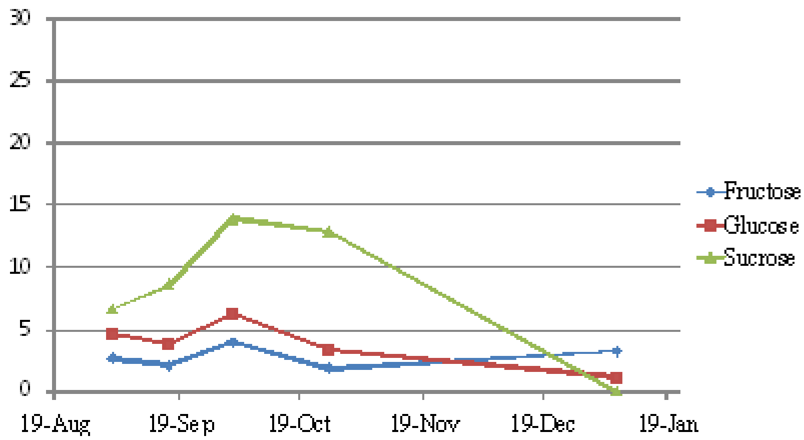


Figure 2 Bale All sugar composition and concentration in mg $ml^{-1}$ per 50 mL sample of sap for the 2008 growing season. Samples were taken on five dates.

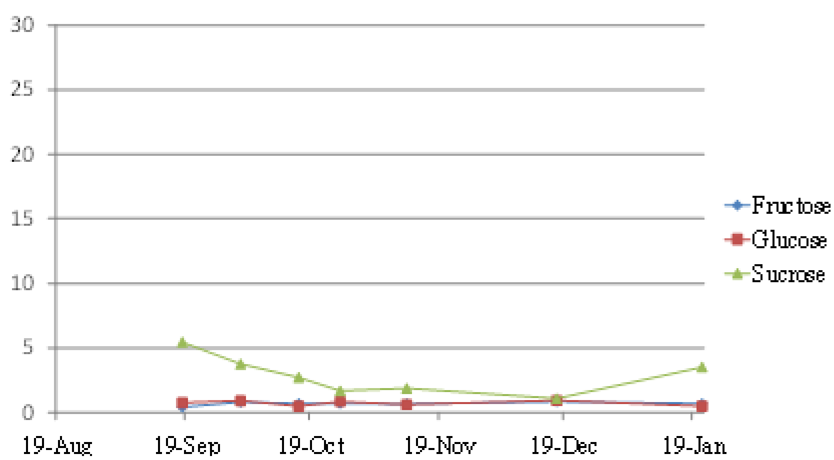


Figure 3 Bale All sugar composition and concentration in mg $ml^{-1}$ per 50 mL sample of sap for the 2009 growing season. Samples were taken on seven dates.

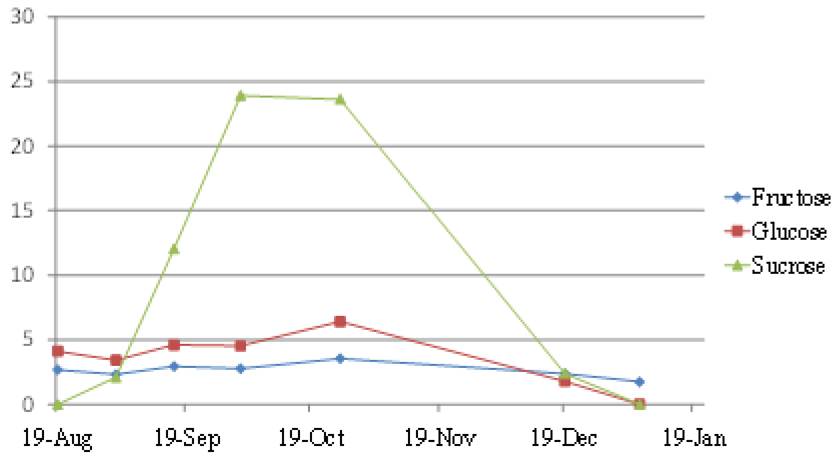


Figure 4 M81-E sugar composition and concentration in mg $ml^{-1}$ per 50 mL sample of sap for the 2008 growing season. Samples were taken on seven dates.

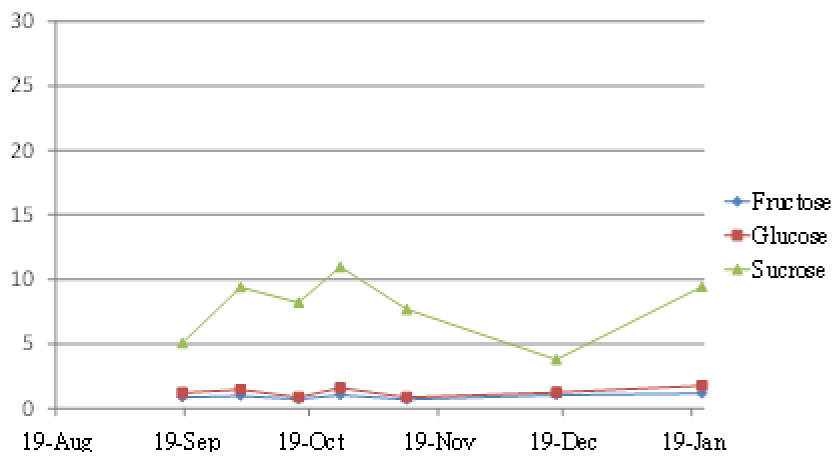


Figure 5 M81-E sugar composition and concentration in mg $ml^{-1}$ per 50 mL sample of sap for the 2009 growing season. Samples were taken on seven dates.

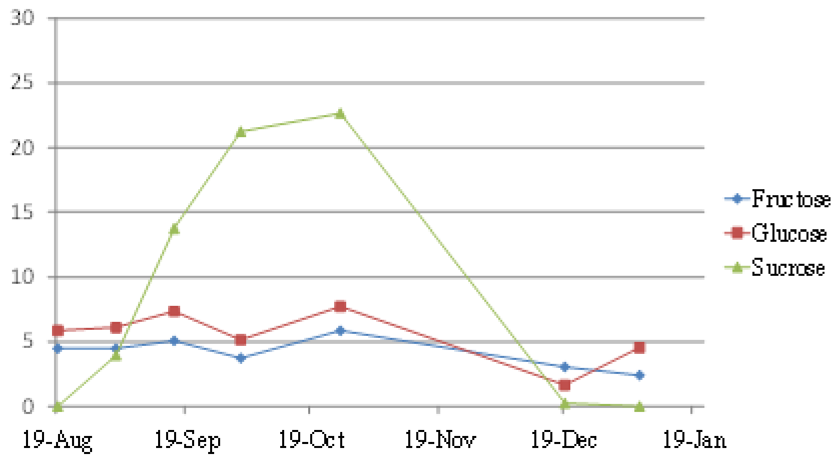


Figure 6 Dale sugar composition concentration in mg $ml^{-1}$ per 50 mL sample of sap for the 2008 growing season. Samples were taken on seven dates.

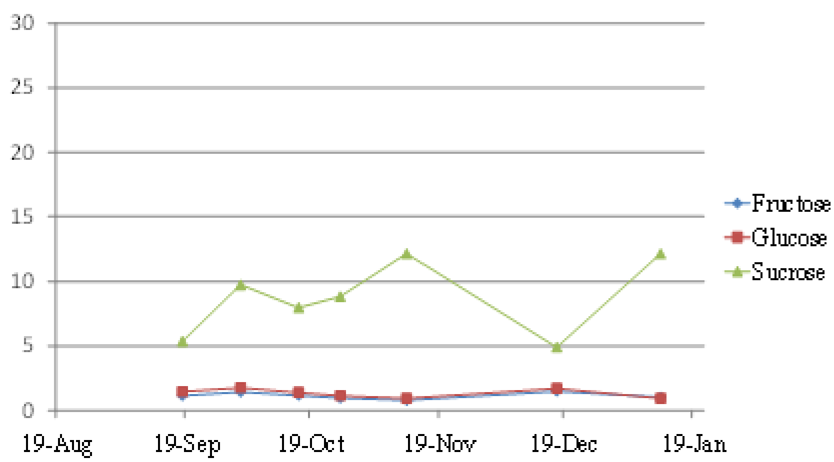


Figure 7 Dale sugar composition and concentration in mg $ml^{-1}$ per 50 mL sample of sap for the 2009 growing season. Samples were taken on seven dates.

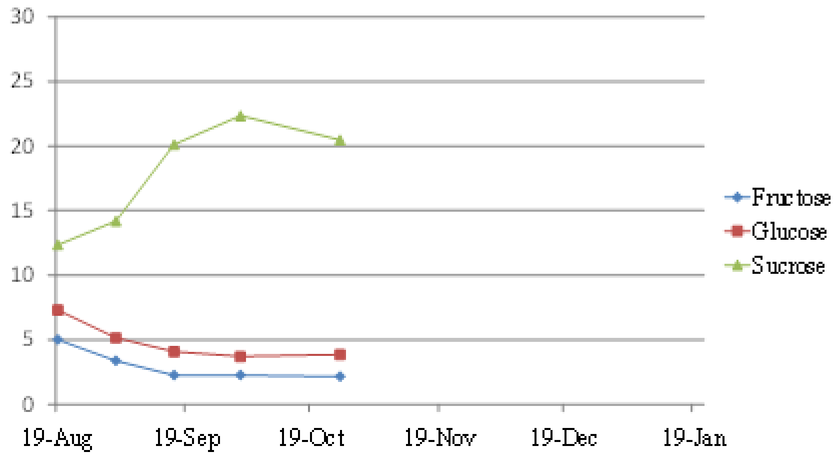


Figure 8 Simon sugar composition concentration in mg $ml^{-1}$ per 50 mL sample of sap for the 2008 growing season. Samples were taken on five dates dates. This data does not go to January because of total loss of these plots as the season progressed.

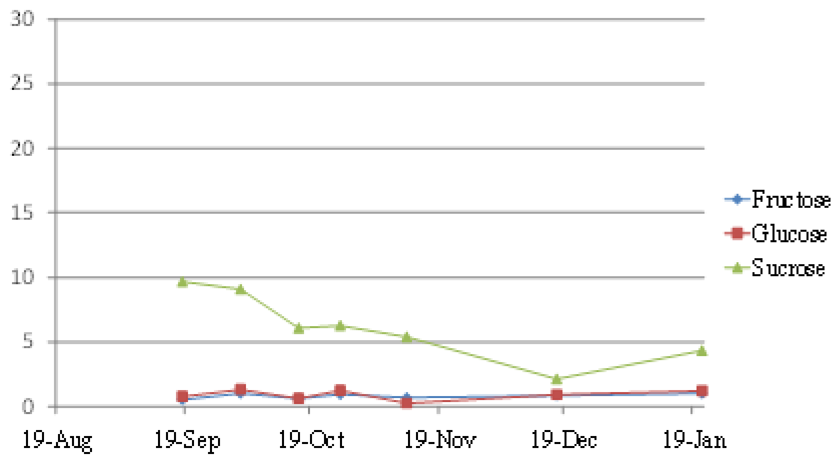


Figure 9 Simon sugar composition and concentration in mg $ml^{-1}$ per 50 mL sample of sap for the 2009 growing season. Samples were taken on seven dates