SWEET SORGHUM FERMENTABLES AS INFLUENCED BY CULTIVAR AND PLANTING AND HARVEST DATES

by

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DEDICATION

To Mom, Dad, and

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I would like to thank my committee members: Dave Parrish, Dale Wolf, Harold Aycock, and Dr. Hutcheson for their assistance. Special thanks to Dale Wolf for giving me a home and a spot to call my own. Thanks to and for their wonderful help in the lab.

A very special thanks to for unending support and assistance in every possible way. Her friendship has made the past year much more bearable and even enjoyable.

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CONTENTS

INTRODUCTION

The conversion of biomass to ethyl alcohol has received much attention in the search for alternative fuel sources to reduce the dependency on foreign petroleum. Biomass is the above ground portion of the plant. Biomass includes the economically important plant parts such as grain. Crop residues like corn cobs may be used for energy production, but are not grown soley for that purpose. However, fuel crops are grown specifically for fuel production. The technology for alcohol production from plant materials is well known (Anon, 1978). Alcohol is produced from carbohydrate-rich materials by fermentation. Fermentation produces a dilute alcohol solution which must then be distilled to give concentrated ethanol.

Thompson (1979) suggested three major considerations in fuelcropping: yield per unit area, cost of production, and net energy ratio (total energy output to total energy input). Factors that can be of equal or greater importance in selecting a fuel crop for a particular region include continuity of production during the year, soil type and topography, relative value and demand for the crop as a food source, and climatic conditions.

Sugar and starch rich crops such as sugarbeet (Beta vulgaris), corn (Zea mays), and sugarcane (Saccharum officinarum) would be good fuel crops (Smith and Reeves, 1981); however, production of ethanol from these crops is limited because they are also important as food or feed. Sweet sorghum (Sorghum bicolor (L.) Moench) is not so limited, since it is grown on a limited basis and mainly for production of table syrup and livestock feed. Furthermore, sweet sorghum is adapted to many areas, grows relatively rapidly, has moderate water requirements, and develops a high carbohydrate content (Smith and Reeves, 1981). Sweet sorghum is a fibrous crop and thus might provide the considerable energy requirements for processing its own sugars to alcohol. With these desirable qualities, sweet sorghum is an excellent candidate as a fuel crop.

Sweet sorghum has a greater potential in the United States as a source of fermentable sugars than sugarcane or sugarbeet (Posler and Hill, 1980). Sweet sorghum can be grown as a summer annual crop over most of the United States, including the Great Plains, the Midwest, and the South (Marten et al., 1976). It requires less water and fertilizer inputs than sugarcane and sugarbeet; thus, it is cheaper and less energy intensive to produce (Posler and Hill, 1980). Tolerances to soil and climatic conditions are

wide ranged among cultivars (Ferraris and Stewart, 1979). Suitable soil conditions range from heavy clays to light sands with a pH from 5.0 to 8.5. Sorghum grows best under humid conditions with average temperatures between 20 and 35 C (Anon., 1973). It is more drought tolerant than other potential fuel crops. Its extensive fibrous roots contain a complete silica cylinder, which helps to prevent root collapse during drought stress (Lipinsky and Kresovich, 1980).

To make ethanol production from sweet sorghum economically feasible, there must be a long-term supply of fermentables. All traditional work done on sweet sorghum assumes it must be harvested before frost and near maturity when stem sugars are usually highest. A very short harvest or storage period would only permit alcohol production for about one month or less. Returns from alcohol production would not compensate for investments in fermentation equipment and crop production inputs. Sweet sorghum's fermentables must be stored or preserved in some way if the crop is to be a viable fuel crop. Sugars are difficult to store in juice form due to bulkiness and the potential for microbial attack. Cultural manipulation might effectively extend the period of availability of fermentables. Multiple planting dates may extend the harvest season in some areas of the United States. Harvesting could possibly be extended over several months to allow continuous alcohol production.

Elewad, Gascho, and Shih (1980) suggested sweet sorghum can be harvested only during a relatively short period of time. They concluded that, as a raw material for sugar or ethanol production, sweet sorghum would, therefore, have to be incorporated with other crops. They did not give any data to support this statement, but their research was conducted in Florida where they did find unprocessed sweet sorghum tends to spoil within 48 hr of harvest. They suggested that, in the Virginia highlands, cut, stripped sorghum could perhaps be stored for several months in the fall before it is processed for syrup.

I found no reports of research efforts to develop sweet sorghum as a fuel crop in Virginia. The research reported herein is an endeavor to examine various biological and cultural factors involved in production and preservation of sweet sorghum under Virginia conditions. The objectives of this research were:

1. to investigate the effect of two planting dates on the production of sweet sorghum fermentables,
2. to determine the effect of delaying harvest on sweet sorghum and yield of its fermentables, and
3. to examine respiratory physiology associated with freezing temperatures.

4. to compare several sweet sorghum cultivars and lines for production of fermentables

LITERATURE REVIEW

CHARACTERISTICS OF SWEET SORGHUM

Sweet sorghum is the same species as milo or grain sorghum and is closely related to broomcorn (S. dochna), johnsongrass (S. halepense) and sudangrass (S. sudanense). Sorghum is a perennial, but it does not overwinter where even moderate freezes occur, as in Virginia. Sweet sorghum cultivars usually have more height, more succulent stems, and a higher sugar content than the grain sorghums. They may produce comparable grain yields (Ferraris and Stewart, 1979).

Sorghum has several distinct physiological and agronomic characteristics that increase its potential as a multi-use crop over a wide geographic range. Sorghum exhibits the C4 (Hatch-Slack) pathway which is so efficient in assimilating carbon dioxide, partially due to the absence of photorespiration (Lipinsky and Kresovich, 1980). Total biomass production is relatively high compared to other crops. Yield differs among cultivars and is dependent on day length, solar radiation, and latitude (Hipp et al., 1970).

Smith and Reeves (1981) calculated that mature stalks of sweet sorghum contain 60 to more than 70 % of the total plant fresh biomass. The stalk provides the greatest quantity of fermentable carbohydrates, and the top portion of the stem contains proportionately less than the bottom. The relatively small contribution of stalk tops to total biomass and the uncertainty of seed yields due to insect or bird damage limit the use of tops as a source of fermentables.

Sweet sorghum's morphology and anatomy are also considerations in fuelcropping and preservation of the fermentables. The exterior of the mature internodes of most cultivars is covered with a continuous waxy bloom. The cortex, or rind, of the mature stalk internodes is hard and has many fibers to add strength. These rather impermeable structures surround the pith, which in the mature internodes is composed mainly of sugar-rich parenchymatous cells. As such, the internodes represent a fairly durable "container" for possible preservation of fermentables.

SWEET SORGHUM CULTIVARS

Sweet sorghums are separated into syrup and sugar sorghums based on the reducing sugar : sucrose ratio of the extracted juice. Sugar sorghums contain sucrose as the major sugar in their juice; while the juice of syrup types con-

tains higher levels of the more-soluble monosaccharide reducing sugars, fructose and glucose. Past sweet sorghum research has concentrated either on the production of crystalline sucrose or on high quality syrup. Sweet sorghum lines differ widely in carbohydrate distribution, total carbohydrate content, time to maturity, and yield (Smith and Reeves, 1981).

Very little work has been directed at developing cultivars specifically for fuelcropping. Such lines would presumably contain high levels of readily fermentable sugars (sucrose, glucose, fructose, etc.). Selection for such "high energy" types is relatively new. One major effort was initiated in 1976 by Food Crops Utilization Research (now Subtropical Products Chemistry Research), USDA, and the Texas Agricultural Experiment Station at Weslaco, Texas to determine the potential of sweet sorghum as a feedstock for ethanol (Smith and Reeves, 1979). The high sucrose requirement of cultivars suitable for crystalline raw-sugar becomes less important than the requirement for a high content of total fermentable sugars when selecting for fuel crop potential. (See the Appendix for more information on sweet sorghum cultivars).

PLANTING DATE

Delaying sweet sorghum planting until the soil is uniformly warm produces good germination, rapid early growth, and uniform stands (Anon., 1973). Sweet sorghum is sensitive to cool soils and will not germinate below 16 C. Adequate moisture is also essential for satisfactory germination and early growth. Prolonged delays in planting until rainfall is lower and soil warming has caused significant moisture loss is not advised.

In tests at Meridian, Mississippi (Anon., 1973), early plantings resulted in slow early growth, making weed control difficult. Later plantings grow faster but must be seeded in time to mature before the first killing frost. The range of average times to maturity is from 82 to 145 days for different cultivars (Ferraris and Stewart, 1979). In more northern areas of sweet sorghum's range, planting may be limited to a 10 to 15 day period near mid-May (Anon., 1973) as cool soils prohibit earlier planting, and frosts occurring before maturity drastically reduce sugar yield.

The effect of planting date on carbohydrate yield of sweet sorghum has not been studied extensively. Broadhead (1969) investigated three planting dates (15 April, 15 May, and 15 June) in Meridian, Mississippi. Yields of stalks and sucrose were similar for the 15 April and 15 May planting,

but lower for the 15 June planting. Sucrose yields do not necessarily reflect total available fermentables. Starch and other carbohydrates are also fermentable.

HARVEST DATE

Most research concerning sweet sorghum harvest date has been in the context of production of either sugar or syrup. Because these two different products require a rather specific ratio of reducing sugars to sucrose, harvest procedures have been developed along with specific cultivars to optimize the desired ratio. Very little attention has been devoted to harvesting for the maximum amount of fermentables. Some very recent studies are now monitoring total sugar (sucrose, glucose, and fructose) content at harvest (Gascho, 1983; Posler and Hill, 1983).

Harvesting sweet sorghum for syrup production requires skill at judging the proper harvest time. After stalks reach their full size, maturity of the entire plant advances at about the same rate as maturity of the seed head. Sucrose content of the juice increases progressively from early flowering stage through the ripe stage (Anon., 1973). More importantly, Ferraris (1981) found the highest stem fermentables concentration can generally be expected at or near grain maturity, though this is not true in all cultivars.

Thus, harvesting should be delayed to at least the ripe stage. Plants are generally considered mature for harvesting for syrup production when the seed are hard (ripe stage to past hard-dough stage) (Anon., 1973).

As maturity is reached, total sugars increase, the ratio of reducing to nonreducing sugars changes, and the quantity of starch in the juice increases (Ferraris and Stewart, 1979). Grain's starch production does not appear to reduce juice sugar yield. Sugar content may increase or remain constant between soft dough or ripe grain stages depending on the cultivar and ripening conditions. Stem sugar decreases somewhat with time after grain ripening. Ferraris and Stewart (1979) did not quantify the stem sugar loss or the length of time after grain ripening.

Broadhead (1969) harvested Rio sweet sorghum stalks weekly from time of maturity for 4 weeks in Meridian, Mississippi. He found that harvesting after the seeds were ripe had no effect on yield of stalks. Sucrose decreased slightly when harvested 4 weeks after ripe stage.

In another study, Rio was harvested at the soft dough stage, then biweekly for 4 weeks (Reeves et al., 1979). Dry matter increased consistently over the harvest period. The weight of total sugars in dry stalks, however, decreased over the harvest schedule, but this difference was not sig-

nificant. Percent sugar averaged 41 to 44, while starch ranged from 3 to 6 % for the four harvest dates; these values were not significantly different.

These harvest date studies taken together indicate sweet sorghum may lose stem sugars when cut stems are held past maturity. None of the studies monitored total stem sugars in uncut stems over an extended period of time.

Broadhead (1972a) evaluated sorghum samples harvested in flower, milk, dough, and ripe stages, and 1, 2, 3, and 4 weeks after full maturity. Stripped stalk samples from each harvest date were chopped into 10, 20, and 40 cm sections and compared with whole stalks in storage for 0, 24, or 48 hr. Broadhead (1972a) did not compare harvest dates for differences in fermentables but did conclude that unimportant differences in juice quality of chopped sections as compared to whole stalks of Rio sweet sorghum would be expected when stored up to 48 hr.

In a separate study, Broadhead (1972b) harvested Rio sweet sorghum in the flower, milk, dough, and ripe stages of seed maturity. Samples from each harvest were left in the open on the ground for 0, 24, or 48 hr before milling. Harvest date had no effect on fresh weight yield of stalks. Sucrose increased with maturity through the dough stage. Extensive sucrose inversion occurred in stalks harvested in

the flower and milk stages, while mature stalks showed little or no inversion during the first 48 hr after harvest. Inversion is probably not a limitation to fuelcropping since total fermentables are not reduced by inversion.

RESPIRATION, MICROBIOLOGY, AND CELL MORTALITY

Fermentables in sweet sorghum stems are produced by photosynthesis and subject to loss by respiration. Respiration is an oxidative process whereby complex molecules such as carbohydrates are broken down into carbon dioxide. The liberated free energy may be conserved as ATP and used to provide for cellular maintenance and growth (Goodwin and Mercer, 1983). No reports of sweet sorghum respiration studies are available, but some information is available for sugarcane and especially sugarbeet. Post-harvest storage of sugarcane has been researched extensively. Storage of sugarcane results in a reduction of sugar content and microbial deterioration within 24 hr of cutting (Anon., 1971; Bose et al., 1974; Egan, 1968).

Several researchers have focused on respiratory sucrose losses in sugarbeet (Mumford and Wise, 1976; Peterson et al., 1980). The loss of sugars during sugarbeet storage is economically important. Factors affecting the rate of respiratory sugar loss include temperature, mechanical injury,

and microbes (Dilley et al., 1968). Dilley et al. (1968) estimated sugar losses during storage to be between 0.5 and 18 kg per Mg of sugarbeets per day. Chemical treatments, such as fungicides and respiratory inhibitors, have been studied in efforts to reduce sugar losses (Mumford and Wyse, 1976).

Respiration in live tissue is largely mitochondrial though some respiration is possibly from mitochondria in parasitic microbes. Respiration would be expected in tissue that had not yet suffered a severe freeze and is therefore still living. Some respiration in live tissue may be caused by saprophytic, parasitic, or epiphytic microorganisms present. Freezing of sweet sorghum would be expected to cause cell death and disruption of the mitochondria. Loss of fermentables in dead tissue must be caused by other factors such as microbial respiration.

Few workers have focused on the microbiology of intact sweet sorghum tissue. Daeschel et al. (1981) found microorganisms to be abundant in juice freshly squeezed from sweet sorghum. The dominant bacterium identified was Leuconostoc mesenteroides. Other bacteria and yeasts were also cultured from the fresh juice. Daeschel et al. (1981) did not measure respiration by microorganisms when considering problems with juice spoilage and they were not at all concerned with

respiratory losses in the living sweet sorghum stem. Their work is of limited interest here, because the microbes were likely living on the outside of the stems and contaminated the juice only upon squeezing. However, in a post-freeze situation, breakdown of stem tissue might lead to microbial invasion from epiphytic organisms or spores.

Loss of fermentables after a severe freeze might occur by different processes. Cells must be alive to partake in long-term mitochondrial respiration. A light freeze may not be sufficient to cause death of all cells. All or part of the pith cells may survive depending on the temperature. No data have been found for sweet sorghum freezing injury, but Marten et al. (1976) reported light freezes (-3 C) killed sugarcane leaf tissue and stopped sugar accumulation. A freeze of -4.5 C damaged the upper part of the stalk and a freeze of -5 C killed the whole stalk causing deterioration. No further explanation concerning the type and degree of damage and deterioration was given.

Estimates of freezing injury can be made by staining with neutral red (toluylene red) (Levitt, 1980). Neutral red is a non-toxic, weakly-basic dye. The salt form is taken up and chemically altered by living cells causing a color change (Conn, 1946). Because this reaction occurs only in live cells, neutral red is considered a vital stain. Luyet

(1937) used the dye on plant tissue and found living cells stained cherry red, while dead cells stained intense orange. The reduction of colorless triphenyltetrazolium chloride (TTC) to the red pigment formazan (Gurr, 1960) also can be used to establish cell vitality (Parker, 1953). The reaction is catalyzed by a hydrogenase enzyme in mitochondria. In typical post-freeze viability determinations, tissue is allowed to thaw at least 2 hr at room temperature before sectioning and staining (Parker, 1953).

MATERIALS AND METHODS

PLANTING AND HARVEST DATES

Dale sweet sorghum was selected for planting due to its high germination, early maturity, and resistance to stalk red rot. Dale is not bird resistant; therefore, the grain is eaten and the stems are less susceptible to lodging (Anon., 1973).

Dale sweet sorghum was planted into a plowed and disced seedbed of Lodi soil (typic hapludult), a mixed mesic clay, on 15 May and 2 June, 1982 at the Turfgrass Research Center in Blacksburg, Virginia. All plots were fertilized with a preplant application of N, P, and K at 57, 50, and 94 kg/ha, respectively. Application of 2,4-D was used to control broadleaf weeds. Glyphosate was applied between rows to control grass invasion before the sweet sorghum was fully emerged. Additional N from ammonium nitrate was applied as a sidedress application at a rate of 48 kg/ha 6 weeks after planting. Plots were thinned to a 20 cm intrarow spacing when plant height was 10 to 15 cm.

A split-plot design included four blocks with planting dates as main plots and harvest dates as subplots. The main

plots were arranged in a randomized complete block design with the 15 May and 2 June planting dates randomly assigned within each block. Each main plot consisted of ten rows, 76 cm apart and 6 m long. One row on each end of the main plots was considered a border row. Each of the remaining eight rows was a subplot. The eight subplots in each main plot were randomly and independently assigned to one of the following eight harvest dates: 30 Sep., 15 Oct., 28 Oct., 15 Nov., 15 Dec. 1982, 15 Jan., 20 Feb., or 16 Mar. 1983. Thus, each subplot consisted of one row.

The center 4.5 m of each 6 m subplot row was harvested as the record portion. The sweet sorghum stalks were stripped, cut at ground level, and heads removed at the first node below the base of the inflorescence. The stripped, deheaded stalks from each subplot were weighed and plot weights were extrapolated to determine agronomic (fresh weight) yield.

Subsamples were taken to determine dry matter and total nonstructural carbohydrate (TNC). Ten stalks were randomly chosen from each plot. Two billets, about 15 cm long, were cut from each stalk, one from the top portion and one from the lower portion of the stalk. The twenty-billet subsamples from each plot were then weighed, split to facilitate drying, and dried with forced air at 70 C for 4 to 5 days. Percent moisture was calculated on a wet weight basis.

The dry subsamples were ground in a hammer mill and the pulverized material was subsampled for TNC analysis. Procedures of Smith (1969) as modified by Davis (1976) and Wolf (1975) were used to determine percent TNC on a dry matter basis. These procedures extract carbohydrates and analyze with a semi-automated reducing power test. In the procedure, all nonstructural carbohydrates are extracted and any nonreducing sugars (sucrose) and starch are converted to reducing sugars before being subjected to the reducing power test.

Dry sweet sorghum tissue was assayed for TNC after treating a 200 mg sample with 0.02 N sulfuric acid at 100 C for 15 min to gelatinize starches and hydrolyze nonreducing sugars. Twenty ml of acetate buffer (pH 4.5) and 20 ml of Clarase 40,000 enzyme (Miles Laboratories, Inc.) for starch hydrolysis were added. The solutions were incubated at 37 C for 44 hr. Subsamples of the hydrolysates were assayed with the Technicon auto-analyzer using the reaction of parahydroxybenzoic acid hydrazide with the reducing sugars to form a colored product (Davis, 1976). Readings were corrected for hysteresis and converted to percent TNC.

RESPIRATION

Dale sweet sorghum was grown at the Piedmont Research Center, Orange, VA during 1983. The sorghum was planted on 31 May 1983 in rows 76 cm apart. Rows were thinned to a 20 cm intrarow spacing when plants were about 13 cm high. The sorghum was fertilized with 57 kg/ha N, 50 kg/ha P, and 94 kg/ha K. The sweet sorghum was harvested on 27 October 1983.

Single stalks were selected as the experimental units for respiration measurements. The study was conducted as a completely randomized design with three replications. Each stem was cut in half and the two halves fastened together for respiration measurements. The stems were weighed and placed in a cylindrical PVC respiration chamber (1.8 m long with 5 cm inner diameter). Ambient air was passed into one end of the chamber at 2.5 l/min and a 0.8 l/min sample drawn from one of two ports at the opposite end. In this way a positive pressure air lock was maintained. The sample was fed into an Anarad infrared gas analyzer (IGA) giving a digital readout of ppm CO_2 differential between the exhausted air and the reference source. All measurements were made at 25±3 C.

Three of the six previously measured stems were placed in a freezer at -8±2C for 4 days to simulate an extended,

hard freeze. The remaining three stems served as the control and were left in ambient conditions (25±2C). After the 4 days, the frozen stems were allowed to thaw 2 hr at 25 C. Respiration was then measured on the stems that had been frozen as well as the stems stored at 25 C.

All six stems were again weighed and sampled for respiration rates 6 days later. The three stems that had been stored at room temperature were then placed in the freezer at -8±2 C for 5 hr to simulate a single diurnal freeze thaw cycle. The three stems were then tested for respiration after the 2 hour thaw period.

CELL MORTALITY

Fresh pith tissue obtained on 31 October was cut into sections about 120 um thick with a microtome. Some sections were soaked in 0.05% (W/V) aqueous neutral red (toluylene red) (Sigma Chemical) for 15 min (Luyet, 1937) and then mounted for microscopic observation. Other sections were soaked in 1% (W/V) TTC (triphenyltetrazolium chloride) (Sigma Chemical) for 24 hr at room temperature.

Tissue that had been frozen 4 days at -8C was tested for cell mortality. Sections were sliced and stained with neutral red and TTC as described above. Photographic documentation of the staining patterns was deemed appropriate for the all-or-none responses observed.

STATISTICS

The planting and harvest date study was analyzed using analysis of variance (ANOVA) under a split plot design. Duncan's multiple-range tests were used for mean separation.

All data were tested at the 0.05 percent level of significance. Results reported as different are significantly different at the 0.05 percent level.

The respiration data were analyzed using the paired-t test. Respiration values before and after freezing were used as pairs for testing.

RESULTS AND DISCUSSION

PLANTING AND HARVEST DATE

Analysis of variance revealed no differences between the the two planting dates for any of the parameters considered (Table 1). Mean agronomic, dry matter, and TNC yields tended to be higher for the second planting date (Table 2 and Table 3). Possible differences between planting dates may be somewhat masked by the large variability among harvest dates. All further analyses of the field study use data pooled across the two planting dates (Table 4 and Table 5).

Statistical analysis of the three yield parameters (fresh weight, dry matter, TNC) revealed differences among harvest dates. Agronomic yield declined significantly by 15 November. This decline at least partly reflected the dehydration of stems evident in declining moisture percentages. Weather data from Blacksburg show a heavy frost occurred between mid and late October, but the minimum temperature was -3C. This killed the leaves but the tissues inside the stalks probably did not freeze until later. Killing frosts occurred on 7 November with temperatures dropping to -9 C.

Table 1. Analysis of variance for total nonstructural carbohydrate yields in the planting-harvest date study.

Source of Variation	df*	SS+	MS≠	F
Subplots	63	42911312		
Mainplots	7	2372244		
Blocks	3	1202690	400897	2.14
Planting Date	1	968282	968282	5.18
Main Plot Error	3	561272	187091	
Harvest Date	7	34475063	4925009	45.69
Planting x Harvest Date	7	1176310	168044	1.56
Subplot Error	42	4527695	107802	

*df = degrees of freedom
+SS = sums of squares
≠MS = mean square

Table 2. Mean agronomic yield, percent moisture, and dry matter yields for Dale sweet sorghum planted on two dates in Blacksburg, VA in 1982. Means are averaged over eight harvest dates.

Planting Date	Agronomic Yield	Moisture	Dry Matter Yield
	Mg/ha*	%fw	Mg/ha*
15 May	14.0a	61	4.4a
2 June	15.2a	59	4.6a

*Values within a column followed by the same letter are not significantly different.

Table 3. Mean dry matter yield, percent TNC, and TNC yield for Dale sweet sorghum planted on two dates in Blacksburg, VA in 1982. Means are averaged over eight harvest dates.

Planting Date	Dry Matter Yield	TNC	TNC Yield
	Mg/ha*	%dw	Mg/ha*
15 May	4.4a	25	1.2a
2 June	4.6a	29	1.4a

*Values within a column followed by the same letter are not significantly different.

Table 4. Mean agronomic yield, percent moisture, and dry matter yield for Dale sweet sorghum harvested on eight dates in Blacksburg, VA in 1982 and 1983. Means are averaged over two planting dates.

Harvest Date	Agronomic Yield	Moisture	Dry Matter Yield
	Mg/ha*	%fw	Mg/ha*
30 Sep.	23.4a	75	5.8a
15 Oct.	22.6a	76	5.5a
28 Oct.	21.0a	77	4.9ab
15 Nov.	18.9b	74	5.0ab
15 Dec.	12.8c	68	4.1bc
15 Jan.	7.9d	54	3.7c
20 Feb.	5.6de	33	3.7c
16 Mar.	4.2e	25	3.1c

*Values within a column followed by the same letter are not significantly different.

Table 5. Mean dry matter yield, percent TNC, and TNC yield for Dale sweet sorghum harvested on eight dates in Blacksburg, VA in 1982 and 1983. Means are averaged over two planting dates.

Harvest Date	Dry Matter Yield	TNC	TNC Yield
	Mg/ha*	%dw	Mg/ha*
30 Sep.	5.8a	40	2.3a
15 Oct.	5.4a	38	2.0a
28 Oct.	4.9ab	41	2.0a
15 Nov.	5.0ab	32	1.7b
15 Dec.	4.4bc	23	1.0c
15 Jan.	3.7c	16	0.6d
20 Feb.	3.7c	13	0.5d
16 Mar.	3.1c	13	0.4d

*Values within a column followed by the same letter are not significantly different.

The sweet sorghum tissue likely began to break down after freezing ruptured and killed the cells.

Dry matter yields declined significantly by 15 December. TNC yields were significantly reduced after 28 October. Further significant drops occurred in November, December, and January. Analysis of these data show a positive correlation (r=0.88) between TNC and dry matter; as dry matter declines so does TNC. This correlation presents an interesting question. Where are the fermentables going?

RESPIRATION

Carbon exchange rates (CER) for Dale sweet sorghum before and after freezing 4 days are shown in Table 6. Also shown are CER's for fresh and frozen tissue 1 week later. The fresh tissues had a mean CER of 195 mg CO_2/kg fresh weight/hr . Because 1 mg CO_2 is produced stoichiometrically by the complete oxidation of 0.62 mg sugar (carbohydrates in general), this CER translates to a loss of 2.9 g carbohydrate/kg fresh weight/day. Such a rate of loss (20% of the fermentables per week) could much more than account for the declines in TNC yields for sweet sorghum left standing in the field. As noted earlier sugarbeets may lose between 0.5 and 18 g of sugar/kg of beets/day (Dilley et al., 1968).

Table 6. Influence of freezing on carbon exchange rates of sweet sorghum stems. One set of stems was left continuously at room temperature. The other was frozen at -8 ± 2 C for 4 days beginning after day 0.

Day	Room Temperature	Frozen
	----------mg CO_2/kg/hr*----------	
0	193a	196a
4	132b	22b+
11	128b	198a≠

*Values within a column followed by the same letter are not significantly different.

+Frozen 4 days, thawed 2 hr.

≠Stored at room temperature 7 days after thawing.

A decline in CER at 25 C occurred after stems were frozen 4 days. The CER decreased by 90% to 22 mg CO_2/kg fresh weight /hr. This corresponds to 0.3 g sugar/kg tissue/day. Respiration of the control tissue also declined by 30%, but this was not as dramatic as the decline in respiration of frozen tissue. Respiration of control tissue may have declined due to dehydration, disease, or a reduction to steady state.

A similar reduction occurred when pith tissue was frozen 5 hr. CER at 25 C decreased about 70% from 128 to 40 mg CO_2/kg fresh weight/hr (1.9 to 0.6 g sugar/kg fresh weight /day). The rates of respiration were different under the paired t-test. The decreased respiration rate appeared more severe when tissue was frozen continuously for the longer durations. More observations with multiple freeze durations are needed for confirmation.

These data indicate endogenous mitochondrial respiration is greatly inhibited by freezing presumably because of cell death. The neutral red and TTC tests indicate cell death is extensive in tissue frozen at -8 C for 4 days. The neutral red stained fresh tissue deep red (Fig. 1). Frozen tissue was pale pink (Fig. 2). The pink that did appear on the frozen section was probably due to the apparently high affinity of neutral red to the secondary cell walls.

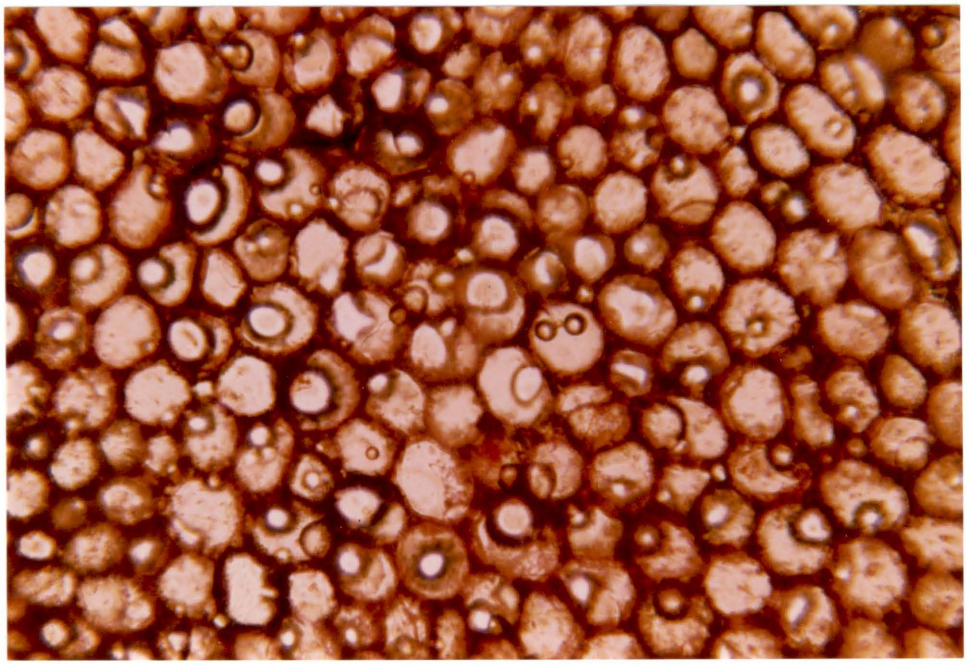

Fig. 1. Fresh sweet sorghum pith cells treated with neutral red (0.05% W/V) for 10 min (100x magnification).

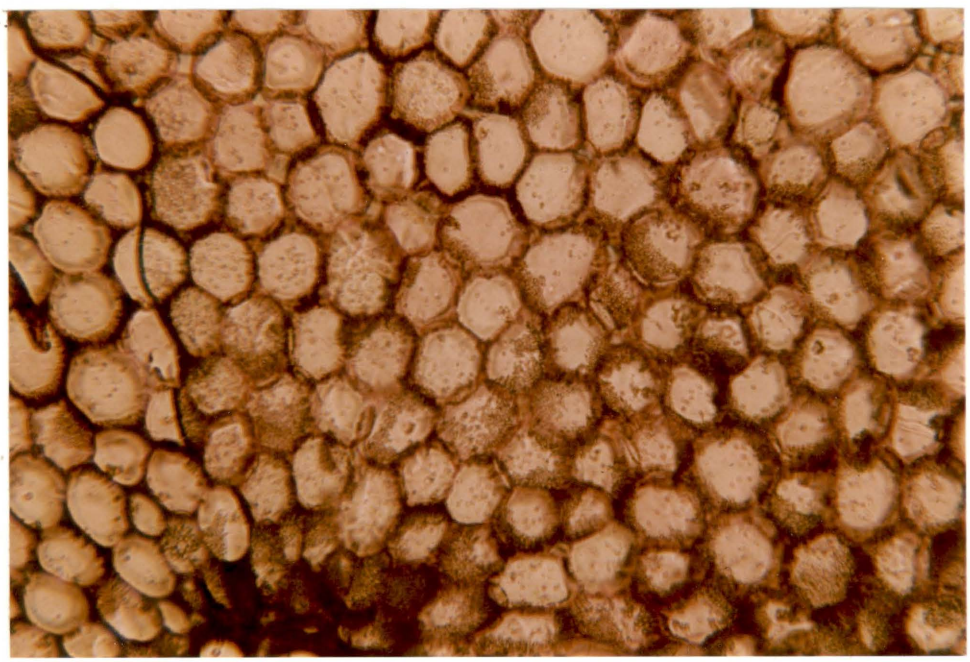

Fig. 2. Frozen sweet sorghum pith cells treated with neutral red (0.05% W/V) for 10 min (100x magnification).

The TTC test results were not so obvious, as the mature pith cells were apparently not highly active metabolically. TTC stains more effectively and rapidly in young, rapidly growing tissues (Parker, 1953). The unfrozen sections stained red-orange. On closer examination the stain was seen to be concentrated in the cytoplasm around the periphery of the fully developed vacuole where the mitochondria are located (Fig. 3). The frozen tissue showed no reponse to the TTC. Both stains show freezing at -8 C kills sweet sorghum pith cells.

All results indicate mitochondrial respiration ceases or becomes minimal after a hard freeze. The high rates of respiration seen after freezing seem likely, therefore, to be due to microbial activity in the sweet sorghum stems. Stalks that were frozen 4 days, then thawed and allowed to remain at room temperature for 1 week had about the same CER as before freezing. Microbes may be present in the pith prior to freezing and become temporarily dormant then later reactivate. Conversely, cellular death upon freezing may allow subsequent entry to saprophytic microorganisms.

In either case, the peak respiration rates observed in the laboratory would more than account for losses of fermentables observed in the field between 30 September and 16 March. An 80% decline in fermentables occurred between Oc-

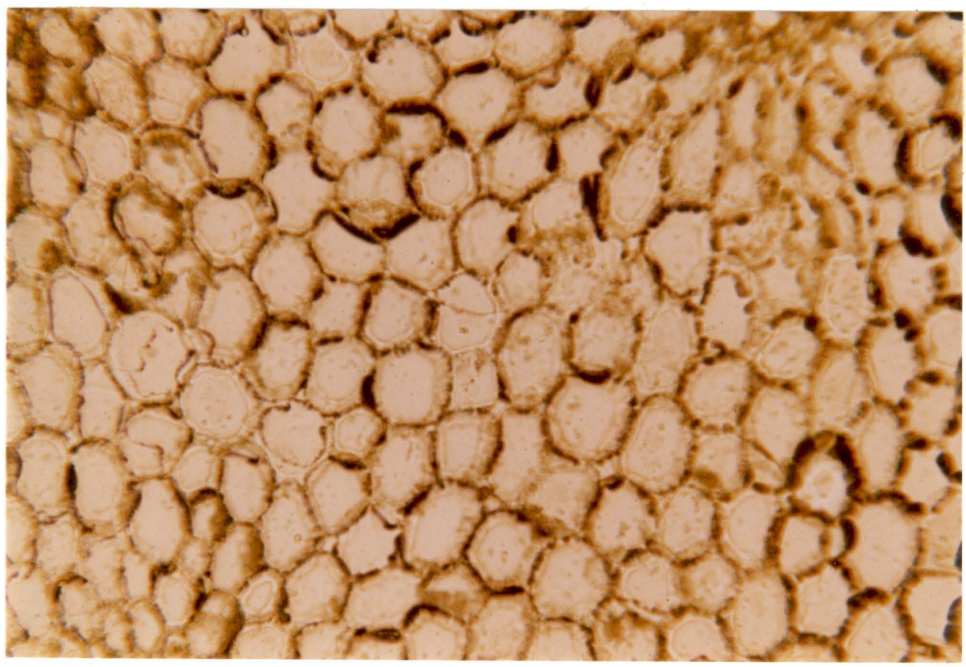

Fig. 3. Fresh sweet sorghum pith cells treated with triphenyltetrazolium chloride (1% W/V) for 24 hr (100x magnification).

tober and March. Respiration at 190 mg CO_2/kg fresh weight/hr would result in more than an 80% decline in fermentables. Such a discrepancy is probably due to temperature differences which occur in the field. The mean respiration rates in the field are probably lower than those measured in the laboratory. Respiration is temperature dependent. The rate of 198 mg CO_2/kg fresh weight/hr is probably not maintained when ambient temperatures drop below the experimental 25 C.

Respiration of fermentables may be relatively high before freezing with no net loss of fermentables due to offsetting rates of photosynthesis in the leaves prior to frost. Some loss of fermentables may have occurred through leaching of the tissue. The tough rind cortex probably prevents significant leaching losses of fermentables.

SUMMARY AND CONCLUSIONS

Multiple planting dates did not significantly affect the production of Dale sweet sorghum fermentables in Blacksburg in 1982. Multiple harvest dates, however, had an effect on fermentable yields. Fermentables are apparently respired at a rapid rate both before and after freezing kills the tissue.

Assuming an 80% efficiency of conversion of stem TNC to alcohol, about 1250 l/ha would have been obtained in September. The estimated alcohol yield droped to only 895 l/ha in November and 325 in January. Much higher yields might be expected in warmer production areas.

These data point to losses of potential fermentables that become quite severe under the climatic conditions that prevailed in Blacksburg in 1982-1983. For sweet sorghum to be an economically feasible feedstock, the cost of production obviously must not exceed the value of the product. In a system that results in declining production of fermentables, one must consider the increasing cost of alcohol production and decreasing net returns. It is necessary to determine when processing of the stalks is no longer

economically feasible under a given distillation system. One should plan the production schedule so as to utilize all of the plant material before the material surpasses a critically low level of fermentables.

Fuelcropping of sweet sorghum does not appear to be feasible in Virginia. However, sweet sorghum may be a viable fuelcrop in areas with longer growing seasons.

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APPENDIX: EVALUATION OF SWEET SORGHUM CULTIVARS AND LINES

LITERATURE REVIEW

SWEET SORGHUM BREEDING AND TESTING

Studies on sweet sorghum cultivars with both high biomass yield and sugar content for ethanol production are a relatively recent development in the United States. It is not clear whether energy research on sweet sorghum should be done with sugar or syrup cultivars. Total biomass yield of syrup cultivars is about 30 % more than sugar cultivars, but total soluble solids (Brix) content of sugar is greater (Elawad, Gascho, and Shih, 1980). The yield components desirable in a sweet sorghum cultivar to be used for biomass production differ from those in a cultivar for sugar production; total solids (brix plus fiber) replace sucrose in importance. This indicates that the conventional cultural and processing practices of sweet sorghum need to be modified in order to obtain maximum fermentable yields and to reduce the cost of fuel alcohol production.

The desirable characteristics of sweet sorghum cultivars used to produce fermentables include:

1. ability to produce a high biomass yield,
2. high percentage of fermentables,

3. strong erect habit of growth,
4. comparatively short growth period (in some locales)
5. resistance to diseases,
6. tolerance to water stress,
7. low fertility requirements.

Past research in varietal breeding has been conducted largely by a USDA group in Meridian, Mississippi. The now defunct group had a repository of over 4600 genotypes (Kresovich, Jackson, and Lawhon, 1980). Latest maturing cultivars mature in 135 to 145 days. Earliest lines can be grown in short seasons limited by rainfall or temperature. Photoperiod differences among cultivars allow flexibility in planting time, manipulation of harvest schedules, and can be used to adapt the crop to rotational cropping systems.

A large scale fuelcropping research program with various sweet sorghum cultivars and lines was conducted in Meridian, Mississippi; Belle Glade, Florida; Baton Rouge, Louisiana; Weslaco, Texas; and Columbus, Ohio (Lipinsky et al., 1979). Total dry biomass and total sugar yields were reported. Total sugars are reported as the sum of sucrose, glucose, and fructose because very litte energy differences among the three components is required for conversion to ethanol. Significant findings from the Lipinsky et al. (1979) report are summarized by state below.

No significant differences were found between 'Rio' and 'Wray' in Mississippi. The lowest yielding cultivar was 'Dale', while 'MN1500' was highest. Total dry biomass yields varied from 12.6 to 22.4 Mg/ha. 'Sart' amd 'Mer 60-2' were outstanding at 7.3 and 7.7 Mg of sugar/ha, respectively, while MN1500, 'Mer 64-7', and 'Mer 71-1' yielded 6.4, 6.6, and 6.6 Mg/ha, respectively.

In Florida the growing season is long enough to fit that they can fit in a second seeded crop, although the yields are lower on the reseeded crop. The first and reseeded crop together produced 14 to 38 Mg/ha of dry biomass for all cultivars. Wray was significantly inferior to the other cultivars when reseeded. The maximum sugar yield with reseeding was 8.9 Mg/ha.

Louisiana data were not provided but Wray was superior to Rio in dry biomass yields. Wray was also the top sugar producer at 8.5 Mg/ha. Sart produced the greatest biomass yield at 30 Mg/ha hectare in Texas. Rio was much lower than the other cultivars. At 9 Mg/ha, Sart sugar yield exceeded that of MN1500 and Mer 71-1. Rio was lowest at 4.8 Mg/ha.

In Ohio, Wray yielded 22 Mg/ha, while Sart was significantly lower at 12 Mg/ha and sugardrip was intermediate. Problems with early onset of maturity occurred, probably a response to different day-length patterns from those to

which the plants are adapted in the South. Mean sugar yields for Sart, Sugardrip, and Wray were 3.1, 4.5, and 6.0 Mg/ha, respectively, all significantly different.

Ferraris (1981a) evaluated 37 sweet sorghum cultivars under irrigation in Ayr, North Queensland, Australia. Highest sugar yields were from Rio, which produced 3.6 and 1.6 Mg/ha over 145 and 79 days from first and ratoon crops, respectively.

Reeves (1980b) reported Keller, Wray, and Rio produced 4.8, 4.3, and 4.3 Mg/ha of sugar per acre in Weslaco, Texas under irrigation. In a separate trial, also in Texas, Reeves (1980a) found Keller, Wray, Rio, and Sugardrip produced 3.6, 4.1, 3.4, and 0.7 Mg sugar/ha under irrigation.

Posler and Hill (1980) observed that Wray was significantly shorter than Dale or Rio, but produced significantly more total sugar. Wray produced 31.5 Mg/ha of dry biomass and 6.1 Mg/ha of total sugar. Dale had yields of 13.4 Mg/ha of dry biomass and 3.9 Mg/ha of sugar. Rio produced 13.8 Mg/ha of biomass and 3.9 Mg/ha total sugar.

CULTIVAR DESCRIPTION

Sugardrip, 'Brandes', Dale, 'Theis', and 'M81-E' are syrup type cultivars (Beatty, 1977). Wray and Rio are sugar types (Smith and Reeves, 1981; Beatty, 1977). No information is available on Keller, 'Sumac', 'Mer 76-6', and 'Mer 75-11'.

M81-E is a late maturing culitvar that matures about one week later than Theis (Broadhead et al., 1981). It is generally superior in yield of gross and stripped stalks. M81-E is resistant to leaf anthracnose and stalk red rot, both caused by Collletotrichium graminicola (Ces.) G. W. Wils.

Brandes is a late maturing cultivar and is resistant to leaf anthracnose, red rot, and other important diseases. It has a good resistance to lodging (Anon., 1973).

Dale is a midseason cultivar with superior disease resistance (Anon., 1973). Dale matures about three weeks earlier than Brandes.

Sugardrip is a midseason cultivar of unknown origin. It is susceptible to lodging and is very susceptible to most sweet sorghum diseases.

MATERIALS AND METHODS

Nine cultivars and two experimental lines of sweet sorghum were selected for testing in Virginia. The seed were kindly supplied by Dempsey Broadhead, with the Agriculture Research Service in Meridian, Mississippi. Field corn (Pioneer 3382) was chosen as a benchmark species with which the sweet sorghum responses could be compared. The sweet sorghum and corn were grown at the Piedmont Research Station at Orange, Virginia.

A preplant fertilizer was applied at 57, 50, and 94 kg/ha of N, P, and K, respectively, on 12 May 1982. The field corn was planted on 13 May 1982. Planting of sweet sorghum cultivars and lines was delayed from 1 June to 23 June 1982 due to wet soils. Plots were thinned to a 20 cm intrarow spacing when plant height was about 20 to 30 cm.

The experimental design consisted of a randomized complete block with four blocks. Eleven cultivars and lines and the one corn hybrid together made twelve treatments. Treatments were randomized across each block. Plots consisted of four 4.6 m rows 76 cm apart. Plots on the north and south ends of the experimental area had 5.5 m rows to allow 91 cm of border at the outer ends of these rows.

The center two rows of each sweet sorhgum plot were stripped, cut at ground level, and topped on 21 October 1982. The record portion was weighed and a twenty stalk subsample taken. The subsample was used to calculate agronomic yield (fresh stripped stalks). The twenty-stalk subsample was separated into two ten-stalk bundles.

One bundle of sweet sorghum was weighed and fed through a research-scale three-roller cane mill to determine juice yields and percent extraction. The mill is larger and sturdier than mills owned by most Virginia farmers for use in molasses production. Juice extraction was measured volumetrically to determine juice yields from the preweighed ten-stalk samples. Average percent extraction was based on the juice weights and fresh stem weights. Percent extraction is the weight of the juice extracted from the ten-stalk sample divided by the fresh weight of the ten-stalk sample, where the weight of the juice is 1.05 grams per ml of juice.

The other ten stalk bundle was used to determine dry matter and carbohydrate yields. A twenty billet subsample (two approximately 15 cm billets per stalk) was taken and weighed. The billets were split and dried at 70 C for 4 to 5 days and reweighed for moisture and dry matter determinations. The dry samples were ground in a hammer mill and subsampled for TNC analysis. Procedures of Smith (1969) as

modified by Davis (1976) and Wolf (1975) were used to determine percent TNC on a dry matter basis. See previous Materials and Methods for more detail.

RESULTS AND DISCUSSION

Analyses of these data showed differences among cultivars for agronomic yield, dry matter yield, juice yield and TNC yield (Tables 7 and 8). Wray was superior with respect to TNC yield, dry matter yield, agronomic yield, and juice yield. Theis, Mer 76-6, M 81-E, and Keller ranked consistently in the remaining top four when all yields were examined collectively. Sugardrip and Sumac were consistently ranked in the bottom for all parameters. Juice extraction did not differ among cultivars and lines.

Analysis of these data shows a positive correlation (.97) between dry matter yield and TNC yield. A correlation was also revealed between agronomic yield and TNC yield. These correlations are expected since bigger stems produce more sugars when %TNC values are higher.

Disease and lodging were not major problems with most cultivars. Rio did show 100% lodging in two out of four plots. Sumac showed some disease symptoms with purpled tissue and general stunting.

Sweet sorghum cultivars and lines were quite variable in yield responses, reflecting the large genetic differenc-

Table 7. Mean agronomic, dry matter, and TNC yields for sweet sorghum cultivars grown in Orange, VA in 1982.

Cultivar	Agronomic Yield	Dry Matter Yield	TNC Yield
	----------Mg/ha*----------		
Wray	48.9a	10.6a	4.2a
Theis	45.9ab	9.8ab	3.9ab
Keller	42.3abc	10.1ab	3.9ab
Mer 76-6	40.9abc	9.2ab	3.9ab
M 81-E	44.8ab	9.0ab	3.7abc
Mer 75-11	45.3ab	9.3ab	3.6abc
Rio	35.3cd	8.6bc	3.3bcd
Brandes	41.8abc	8.4bcd	3.3bcd
Dale	37.1bcd	7.0cd	3.1cd
Sugardrip	31.2de	6.7d	2.6d
Sumac	24.5e	2.4e	1.4e
Corn (grain)	11.6	9.8	9.0

*Values within a column followed by the same letter are not significantly different.

Table 8. Mean juice yield, percent extraction, and percent Brix for sweet sorghum cultivars grown in Orange, VA in 1982.

Cultivar	Juice Yield	Extraction+	Brix
	---kl/ha*--	----------%----------	
Wray	23.6a	44	17
Theis	21.2ab	42	14
Keller	19.1ab	41	16
Mer 76-6	18.8ab	42	16
M 81-E	20.4ab	42	12
Mer 75-11	20.1ab	41	14
Rio	16.5b	43	17
Brandes	18.0b	40	15
Dale	17.7b	43	14
Sugardrip	17.2	51	10
Sumac	11.9c	44	12

*Values within a column followed by the same letter are not significantly different.

+ Percent extraction=(weight of juice extracted/fresh weight of stems) x 100.

es. Further yield and cultural improvements may be possible through breeding. Cultivars and lines should be tested for the desired production area due to the genetic variability. Some cultivars are more suited to a particular climatic region. In the Piedmont of Virginia, based on these data, Wray would be the top choice among the cultivars tested. Theis, Keller, Mer 76-6, and M 81-E would be good alternative choices. Sumac and Sugardrip should be avoided in the Piedmont area.

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SWEET SORGHUM FERMENTABLES AS INFLUENCED BY CULTIVARS AND PLANTING AND HARVEST DATE

by

Mary Lynn Cooper Brinkley

(ABSTRACT)

Several crops are being touted as a renewable energy source. Sweet sorghum is adapted to many areas, grows relatively rapidly, has moderate water and nutrient requirements, and develops high levels of fermentables. A long term supply of fermentables is needed to make ethanol production economically feasible. Short term availability of fermentables results in unfavorable returns from investments in equipment and crop production.

Two planting dates (15 May and 1 June) and eight harvest dates (30 Sep. to 15 Mar.) were used in efforts to to extend the availability of sweet sorghum fermentables. Respiration of sweet sorghum tissue was measured with an infrared gas analyzer before and after freezing to quantify loss of fermentables associated with delayed harvests. Vital stains were used before and after freezing to estimate cell motrality.

No significant difference was found in the level of fermentables in sorghum from the two planting dates. Delay-

ing harvest caused fermentables to decline significantly (30%) by mid-November and to continue to drop through March.

Respiration dropped 90% after exposure to -8 C, but resumed the same rate after 1 week. Vital staining showed cells die upon freezing. Relatively high respiration rates in the dead tissue suggests saprophytic microbes are responsible for the large decline in fermentables after freezing.

Eleven cultivars and lines were tested for yield of fermentables. Wray was superior in yield of fermentables, while Sumac and Sugardrip were inferior.