

**Investigation of management strategies for the production of sweet sorghum as a
bioenergy crop and preservation of crop residue by the ensiling process**

by

Todd Joseph Cogdill

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Crop Production and Physiology

Program of Study Committee:
Ken Moore, Major Professor
Rob Anex
Steve Fales

Iowa State University

Ames, Iowa

2008

Copyright © Todd Joseph Cogdill, 2008. All rights reserved.

TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION.....	1
CHAPTER 2. LITERATURE REVIEW.....	7
CHAPTER 3. MANAGEMENT OF SWEET SORGHUM FOR BIOMASS PRODUCTION.....	25
CHAPTER 4. ENSILING CHARACTERISTICS OF PRESSED SWEET SORGHUM RESIDUE.....	64
CHAPTER 5. SUMMARY.....	89
APPENDIX.....	90
ACKNOWLEDGEMENTS.....	103

CHAPTER I

General introduction

Sweet sorghum (*Sorghum bicolor* (L.) Moench) is a member of the Poaceae family, which includes all the important cereal crops produced worldwide. Sweet sorghum is an annual warm-season grass that utilizes the C₄ carbon fixation pathway. It is native to northern Africa and the first introduced variety 'Chinese Amber' was brought to the United States in 1853. In 1857, fifteen additional varieties that Leonard Wray had collected in South Africa were brought to the United States. Following its introduction, sweet sorghum quickly gained popularity for production of syrup from the sugary stem juice and as a forage crop. Land devoted to sweet sorghum production quickly increased to its maximum area of approximately 142,000 hectares in 1933 and steadily decreased thereafter (Coleman, 1970). Cultivation of sweet sorghum and the subsequent syrup production was predominately conducted in the southeastern region of the United States. Sweet sorghum was also grown to a limited extent throughout the Midwest. In fact, in 1964 Iowa was one of the top four syrup producing states including Alabama, Kentucky, and Tennessee (Coleman, 1970). As mentioned, production of sweet sorghum steadily declined throughout the 20th century and has not recovered.

However, interest in sweet sorghum was stimulated again during the 1970s in response to high petroleum prices and the search for alternative transportation fuel sources. Sweet sorghum juice, high in carbohydrates, was considered as a substrate for ethanol production. Considerable research was conducted to determine management strategies that would improve sugar content and yield of sweet sorghum for subsequent ethanol production. Additionally, breeding of cultivars that were high in sugar content began and numerous

cultivars developed for sugar production were released in the early 1980s. These cultivars differed from those released for syrup production in that total biomass production was generally lower than syrup cultivars but soluble sugar content was significantly higher thereby increasing total sugar production and potential ethanol yield. Unfortunately, further research was limited thereafter due to low petroleum prices in the mid-1980s and 1990s.

Recent increases in worldwide energy demand have increased energy prices and renewed interest in a wide spectrum of alternative energy sources. Demand for cheaper transportation fuel in the United States has increased domestic ethanol production levels from 1.63 billion gallons (6.17 billion liters) in 2000 to 6.5 billion gallons (24.61 billion liters) in 2007 (Renewable Fuels Association). The predominant substrate for ethanol production in the United States has been starch from corn (*Zea mays* L.) grain utilizing nearly 18% of the corn produced in 2007 (National Corn Growers Association).

Realizing the importance of alternative energy sources and the limitations of a grain-based ethanol industry the U.S. Government passed legislation in 2007 forming the Renewable Fuels Standard. The Renewable Fuels Standard mandates domestic biofuel production of 36 billion gallons (136.3 billion liters) by 2022. Additionally, it stipulates that 21 billion gallons (79.5 billion liters) must come from advanced biofuels including lignocellulosic ethanol and other forms of renewable fuel not produced from corn grain starch.

Support for lignocellulosic ethanol has necessitated the identification of high-yielding biomass crops for potential bioenergy feedstocks. Sweet sorghum has gained a lot of attention because it possesses numerous characteristics that make it an appealing bioenergy crop. As previously mentioned, sweet sorghum utilizes the C₄ carbon fixation pathway

which allows the plant to more efficiently capture and utilize the available light energy for production of biomass. When grown in northern latitudes sweet sorghum remains green and actively photosynthesizing until frost thereby capturing more light energy and accumulating more biomass throughout the growing season. Sweet sorghum is highly productive throughout the United States with documented dry matter yields of 18.3 Mg/ha in Ames, IA (42° N) (Hunter, 1994) and up to 43.8 Mg/ha in the southern climate of Weslaco, TX (26° N) (Reeves et al., 1979). Sweet sorghum has inherently high water use efficiency and remains productive even under drought conditions. Another important characteristic of sweet sorghum is low nutrient requirements especially nitrogen. Nitrogen fertilizer contributes significantly to the production cost of agricultural systems and lower requirements enhance the economic viability of biomass production from sweet sorghum. Additionally, extensive genetic diversity is available that plant breeders are utilizing to produce new bioenergy cultivars.

These characteristics are important because they provide sweet sorghum with a wide geographic and environmental range of adaption for biomass production. In fact, sweet sorghum has been successfully cultivated in the United States (Hipp et al., 1970; Broadhead, 1972; Caravetta et al., 1990; Anderson et al., 1995), South Africa (Balole, 2001), Italy (Amaducci et al., 2004), Iran (Almodares et al., 2006), India (Rattunde et al., 2001), Indonesia (Tsuchihashi and Goto, 2004), and Australia (Martin and Kelleher, 1984; Ferraris and Charles-Edwards, 1986).

Further research can improve upon the results of previous studies when applied to biomass production systems. Biomass yields mentioned above were obtained using traditional management practices for sugar production, which may be increased utilizing

more intense production methods. Additionally, determination of chemical composition of sweet sorghum has generally been limited and primarily focused on yield of soluble sugars rather than whole-plant carbohydrate fractions. Efficient production systems will also require the evaluation of storage systems for large quantities of biomass feedstocks. In order to utilize sweet sorghum as a biomass crop research must focus to improve biomass yield, determine biomass quality of sweet sorghum, and develop efficient storage of the biomass feedstock.

Thesis organization

Chapter one is an overview the history of sweet sorghum production in the United States and provides a summary of the organization of this thesis. Chapter two contains a two-part literature review. The first section of the literature review discusses the production of sweet sorghum and the effect planting date, row width, plant density, and nitrogen fertility have had in previous studies. The second section of the literature review discusses the principles of ensiling as it relates to a storage method for biomass feedstocks. Chapter three describes a management experiment of sweet sorghum for biomass production. Chapter four describes an ensiling experiment of pressed sweet sorghum residue. Chapters three and four will be modified for submission to peer-reviewed journals for publication. Chapter five contains the general conclusions of the experiments conducted and describes the significance of the results. An appendix of data and ANOVA tables for both experiments is provided.

References

- Almodares, A., R. Taheri, M.R. Hadi, and M. Fathi. 2006. The effect of nitrogen and potassium fertilizers on the growth parameters and the yield components of two sweet sorghum cultivars. *Pakistan Journal of Biological Sciences* 9:2350-2353.
- Amaducci, S., A. Monti, and G. Venturi. 2004. Non-structural carbohydrates and fibre components in sweet and fibre sorghum as affected by low and normal input techniques. *Industrial Crops and Products* 20:111-118.
- Anderson, I.C., D.R. Buxton, and A. Hallam. 1995. Performance of annual and perennial crops for forage and biomass energy production. USDA. U.S. Dairy Forage Research Center, 1995 Research Summaries.
- Balole, T.V. 2001. Strategies to improve yield and quality of sweet sorghum as a cash crop for small scale farmers in Botswana. Ph.D. dissertation, University of Pretoria.
- Broadhead, D.M. 1972. Effect of planting date and maturity on juice quality of Rio sweet sorghum. *Agronomy Journal* 64:389-390.
- Caravetta, G.J., J.H. Cherney, and K.D. Johnson. 1990. Within-row spacing influences on diverse sorghum genotypes: I. Morphology. *Agronomy Journal* 82:206-210.
- Caravetta, G.J., J.H. Cherney, and K.D. Johnson. 1990. Within-row spacing influences on diverse sorghum genotypes: II. Dry matter yield and forage quality. *Agronomy Journal* 82:210-215.
- Coleman, O. H. 1970. Syrup and sugar from sweet sorghum. p. 416-440. *In* J. S. Wall and W. M. Ross (eds.), *Sorghum production and utilization*. The AVI Publishing Company, Inc., Westport, CT.
- Ferraris, R., and D.A. Charles-Edwards. 1986. A comparative analysis of the growth of sweet and forage sorghum crops. I. Dry matter production, phenology and morphology. *Australian Journal of Agricultural Research* 37:495-512.
- Hipp, B.W., W.R. Cowley, C.J. Gerard, and B.A. Smith. 1970. Influence of solar radiation and date of planting on yield of sweet sorghum. *Crop Science* 10:91-92.
- Hunter, E.L. 1994. Development, sugar yield, and ethanol potential of sweet sorghum. M.S. thesis, Iowa State University.
- Martin, P.M., and F.M. Kelleher. 1984. Effects of row spacing and plant population on sweet sorghum yield. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24:386-390.

National Corn Growers Association. 2008. Ethanol & Coproducts [Online]. Available at <http://www.ncga.com/ethanol/main/index.asp> (verified 4 February 2008).

Rattunde, H.W.F., E. Zerbini, S. Chandra, and D.J. Flower. 2001. Stover quality of dual-purpose sorghums: genetic and environmental sources of variation. *Field Crop Research* 71:1-8.

Reeves, S.A., B.W. Hipp, and B.A. Smith. 1979. Sweet sorghum biomass. Part I: Agronomic data. *Sugar y Azúcar* 74:23-30.

Renewable Fuels Association. 2008. RFA - The Industry - Industry Statistics [Online]. Available at <http://www.ethanolrfa.org/industry/statistics> (verified 4 February 2008).

Tsuchihashi, N., and Y. Goto. 2004. Cultivation of sweet sorghum (*Sorghum bicolor* (L.) Moench) and determination of its harvest time to make use as the raw material for fermentation, practiced during the rainy season in dry land of Indonesia. *Plant Production Science* 7:442-448.

CHAPTER II

Literature review

Introduction

Production of sweet sorghum and the management strategies utilized have been extensively investigated. Previous research has primarily focused on management strategies to improve total sugar yield of sweet sorghum through increased biomass yield and improved sugar content. Additionally, determination of chemical composition relevant to lignocellulosic ethanol production has generally been limited. However, the management practices utilized in these studies are the foundation of nearly all management studies regardless of research objectives. Management factors important to the production of sweet sorghum include planting date, row width, plant density, and nitrogen fertility. This review will evaluate the effects these management practices have had on the production of sweet sorghum in previous studies. In addition, given the importance of an efficient system for the storage of biomass this review will detail the principles of the ensiling method of storage.

Management practices

Planting date

Planting date for sweet sorghum varies across production regions and is dictated by specific soil conditions. Sweet sorghum planting should occur after soil temperature has reached a minimum temperature of 23°C (Kanemasu et al., 1975) and sufficient soil moisture is available (Freeman et al., 1973). Under optimum conditions, rapid germination will occur and emergence can be expected in 3 to 10 days (Vanderlip, 1993). Timing of crop establishment can have a wide range of effects on the crop yield and composition. Planting sorghum prior to reaching optimum soil conditions can reduce percent germination, extend

the period between planting and emergence, hinder early season growth, and reduce potential yield (Freeman et al., 1973). Lipinsky et al. (1979) noted these effects when comparing planting dates in Columbus, Ohio. Additionally, delayed planting can reduce potential yield due to reduced time between growth stages and a shortened growing season (Freeman et al., 1973).

Studies conducted in Meridian, Mississippi (Broadhead, 1969; Broadhead, 1972) and Weslaco, Texas (Hipp et al., 1970) evaluated optimum planting dates for sweet sorghum. These studies found that plantings in early May had the highest biomass yields for the range tested. Specifically, these studies showed 20-30% reductions in biomass yield when planted in April and greater reductions when planted after May. In addition, Gascho et al. (1984) found that planting sweet sorghum in early June rather than early May reduced total biomass yield by 21%. Soluble sugar concentration was increased by 11% in the June planting date (Gascho et al., 1984). When the same cultivar, 'Wray', was grown in the subtropical environment of Queensland, Australia total biomass yield also decreased when subjected to later planting (Ferraris and Charles-Edwards, 1986). Additionally, Ferraris and Charles-Edwards (1986) noted that later planting reduced tiller number in both cultivars tested.

The biomass yield response of sweet sorghum to date of planting is well established; however, studies evaluating structural composition are limited. McBee and Miller (1990) noted an inverse relationship between nonstructural and structural carbohydrate composition in sorghum but the relationship was not evaluated in respect to planting date. Effects of planting date on forage quality have been evaluated in related crops such as corn (*Zea mays* L.). Darby and Lauer (2002) found that planting forage hybrids in late June rather than late April resulted in 10 and 30% reductions in concentrations of neutral detergent fiber (NDF)

and acid detergent fiber (ADF), respectively. Slight variations in NDF and ADF were noted in a similar study evaluating planting dates (Graybill et al., 1991).

The literature shows that planting date is an important determining factor in the production of sweet sorghum and optimum planting dates may vary by region. As shown by these studies planting prior to or after the optimum planting date causes reductions in biomass yield. Additionally, soluble sugar concentration tends to be increased with later planting but total sugar yields are generally reduced. Number of tillers is also reduced with later planting. Structural composition studies specific to planting date of sweet sorghum are limited but results in corn suggest that later planting may reduce the concentration of structural components. Further research on composition is needed in relation to sweet sorghum before a consensus can be made.

Row width

Changes in row width have significant effects on the spatial distribution of plants. In relation to sorghum, varying row width in previous research has shown effects on light interception, biomass yield, plant morphology, and carbohydrate partitioning. Percent incident light intercepted, an important factor for biomass accumulation, has been shown to increase substantially as row width decreases. Stickler et al. (1961) found that light interception of grain sorghum at heading was highest in the 25.4 cm row width compared to 50.8, 76.2, and 101.6 cm.

Experiments conducted by McGowan et al. (1991) on grain sorghum found total plant biomass and number of tillers increased when row width was narrowed from 150 to 50 cm. Similarly, Broadhead and Freeman (1980) found biomass yield of sweet sorghum increased by 21% when row width was decreased from 105 to 52.5 cm. The authors noted that plants

were thinner and tended to lodge more frequently in narrow rows (Broadhead and Freeman, 1980). Lipinsky et al. (1979) compared row widths of 46 and 102 cm on biomass and sugar yields of sweet sorghum grown in Ohio. Biomass yield increased by 45% in the narrow rows, total sugar yield showed a similar response, and the number of tillers increased by 72% (Lipinsky et al., 1979). Martin and Kelleher (1984) assessed the effects of 30, 75, and 105 cm row widths on numerous characteristics of sweet sorghum grown in New South Wales, Australia. Dry matter yield was increased by 17% when row width was narrowed from 105 to 75 cm and further increased by 13% when narrowed from 75 to 30 cm (Martin and Kelleher, 1984). Additionally, soluble carbohydrate yield (Mg/ha) was increased by 37% when row width was decreased to 75 cm and further increased by 23% when decreased to 30 cm (Martin and Kelleher, 1984). The magnitude of increase in carbohydrate yield is explained by both the increase in dry matter yield and a change in carbohydrate partitioning from structural carbohydrates to nonstructural carbohydrates (Martin and Kelleher, 1984). Plant height, stem diameter, and tiller number were also increased with each successive decrease in row width (Martin and Kelleher, 1984).

As discussed with date of planting, studies focused on sweet sorghum structural composition in relation to row width are also limited. Experiments with similar crops have suggested that row width affects biomass yield but may not affect structural composition. Koller and Scholl (1968) evaluated row width effects on biomass yield and plant composition in sudangrass (*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet and Harlan) and a sorghum-sudangrass hybrid. Similar to sweet sorghum biomass yield for both sudangrass and sorghum-sudangrass was highest in 17.8 and 35.6 cm row widths and significantly lower at 71.1 cm (Koller and Scholl, 1968). Plant composition was not affected

by changes in row width (Koller and Scholl, 1968). Evaluating the same sudangrass and sorghum-sudangrass varieties, Worker (1973) noted increased biomass yield with narrow rows and structural composition remained unaffected. When similar studies were conducted on corn a decrease in row width from 76 to 38 cm increased total forage yield by 4.2% (Cox et al., 1998) and 5% (Widdicombe and Thelen, 2002). Forage composition as measured by NDF and ADF concentrations was unaffected by row width in either experiment.

These results demonstrate that narrow row spacing provides an advantage in sweet sorghum biomass yield and soluble carbohydrate accumulation. Additionally, narrow row spacing increases plant height and tiller number. Stem diameter has been shown to both increase and decrease when subjected to narrow row spacing suggesting that other factors may have contributed. Given the results of these studies row width also appears to have little effect on structural composition regardless of species evaluated.

Plant density

Similar to row width, modifying plant density of sorghum also influences light interception, biomass yield, plant morphology, and carbohydrate partitioning. Percent incident light intercepted increases substantially as plant density is increased. Caravetta et al. (1990a) found as plant density of sorghum increased from 2.2 to 26 plants/m² that average light interception at boot stage increased from 68% to 95% interception. Studies on grain sorghum (Stickler and Laude, 1960; Stickler et al. 1961) and sweet sorghum (Ferraris and Charles-Edwards, 1986) have also noted increased light interception at higher densities.

Caravetta et al. (1990b) reported that biomass yield increased 34.6% as plant density increased from 2.2 to 26 plants/m². Additionally, number of tillers decreased, stem diameter decreased, and plant height increased with higher plant density (Caravetta et al., 1990a). An

experiment conducted by Worley et al. (1991) showed that an increase in plant density from 5 to 17 plants/m² increased biomass and soluble carbohydrate yield of sweet sorghum by 78% and 123%, respectively. Martin and Kelleher (1984) found that biomass yield was increased by 23% when plant population was increased from 8 to 16 plants/m². Soluble carbohydrate yield was also increased by 22% at the higher population (Martin and Kelleher, 1984). Average stem diameter was thicker in plots planted at 8 plants/m² than those planted at 16 plants/m² (Martin and Kelleher, 1984). McBee and Miller (1982) noted similar responses in stem diameter. Additionally, total nonstructural carbohydrates increased by 12.8% when plant spacing within a row was decreased (McBee and Miller, 1982). Though not specifically measured the authors postulate that the increase in nonstructural carbohydrates was at the expense of structural carbohydrates which is consistent with the explanation provided by Martin and Kelleher (1984) as well as McBee and Miller (1990).

Recently, Amaducci et al. (2004) compared sweet sorghum planted at densities of 10 and 20 plants/m² and noted that sucrose content was increased with the higher population however; structural carbohydrate composition was not affected. Rattunde et al. (2001) measured NDF and ADF concentrations in dual-purpose sorghum grown in India at 7.5 and 17.5 plants/m² and found no significant effects of plant density. Studies on sudangrass and sorghum-sudangrass showed increases in biomass yield up to the highest density when evaluated over a range of seeding rates from 8.4 to 53.8 kg/ha; yield increases were enhanced when seeded in narrow row widths (Koller and Scholl, 1968). As with sweet sorghum, structural composition of sudangrass and sorghum-sudangrass were not affected by increased plant density (Koller and Scholl, 1968). The yield response of corn to increased plant density is similar to that of sweet sorghum. Numerous studies (Widdicombe and Thelen, 2002;

Cusicanqui and Lauer, 1999; Karlen et al., 1985; Rutger and Crowder, 1967) have documented increased dry matter yield in corn when subjected to increasing plant densities. The response of structural composition of corn to plant density tends to differ from that of sweet sorghum, sudangrass, and sorghum-sudangrass. Widdicombe and Thelen (2002) measured the effects of plant density on NDF and ADF concentrations of corn forage and found both values increased over a range of plant densities from 6.42 to 8.89 plants/m². Cusicanqui and Lauer (1999) found similar results over a range of densities from 4.45 to 10.45 plants/m².

These studies showed similar results to increased plant density as narrow row width in sweet sorghum. Sweet sorghum responds to increases in plant density with increased biomass yields, soluble carbohydrates, and total nonstructural carbohydrates. Increased plant density also increases plant height, reduces number of tillers, and results in thinner plants. Given the increases in total nonstructural carbohydrates it would be expected that structural carbohydrates would be reduced, however, this may not be the case. Previous research has shown that structural composition of sweet sorghum and related *Sorghum* species is not affected by changes in plant density.

Nitrogen fertility

Nitrogen is widely recognized for its utility in cropping systems, however it contributes considerably to the production costs of agricultural systems. A proper application rate of nitrogen fertilizer will adequately stimulate photosynthesis and crop growth while minimizing losses to leaching or denitrification. Therefore, significant research has been directed toward optimizing application rates of nitrogen fertilizer.

Previous studies (Lyon, 1957; Matherne, 1970) determined that sweet sorghum grown in 1-meter row widths did not respond to nitrogen applications higher than 45 kg N/ha. However, Wiedenfeld (1984) found biomass yield increases in sweet sorghum grown in 68-cm row widths when nitrogen was applied at 112 kg N/ha compared to no nitrogen application but no further yield increase occurred at 224 kg N/ha. Yield increases for cultivars 'Grassl' and 'Rio' were 23 and 40%, respectively (Wiedenfeld, 1984). Sugar concentration, measured with a refractometer, was reduced by nitrogen application of 224 kg N/ha in "Grassl" while concentrations in 'Rio' were equally reduced by either application rate (Wiedenfeld, 1984). Anderson et al. (1995) compared nitrogen fertility on six potential biomass crops; fertilizer rates evaluated were 0, 70, 140, and 280 kg N/ha. Sweet sorghum showed a large biomass yield response at 70 kg N/ha, a marginal increase at 140 kg N/ha, and a decrease at 280 kg N/ha. However, structural composition was relatively unaffected (Anderson et al., 1995). Lueschen et al. (1991) applied rates of 0, 56, 112, 168, and 224 kg N/ha and found an increase in biomass at 56 and 112 kg N/ha rates above the control and no further increases above 112 kg N/ha. Smith and Buxton (1993) compared rates of 0, 84, and 168 kg N/ha and found marginal increases in total biomass at one location (Ames, Iowa) across nitrogen treatments; soluble carbohydrate concentration was not affected. Almodares et al. (2006) evaluated nitrogen fertilizer rates of 0, 41.4, and 82.8 kg N/ha on yield of sweet sorghum grown on nutrient limited soil. Dry matter yield increased by 43.2 and 71.9% with application of 41.4 and 82.8 kg N/ha compared to the control (Almodare et al., 2006). Hons et al. (1986) evaluated fertilizer rates of 84 and 168 kg N/ha on high energy sorghum (grain sorghum x sweet sorghum) which resulted in 40 and 60% increases in biomass yield, respectively, when compared to no nitrogen application. Amaducci et al. (2004) compared

nitrogen application rates of 60 and 120 kg N/ha for effects on biomass yield, soluble carbohydrate content, and structural carbohydrate composition of sorghum grown in Italy. Biomass yield was increased by 25% with the higher fertilizer rate in one year of the study, which was characterized as having more moisture available during early growth stages than the other years (Amaducci et al., 2004). Soluble and structural carbohydrate composition were unaffected by nitrogen application treatments (Amaducci et al., 2004).

The body of literature further supports the utility of nitrogen fertilizer application for sweet sorghum production systems. These studies have shown that sweet sorghum responds with increased biomass production up to application rates of 168 kg N/ha. Further increases in the rate of nitrogen fertilizer provide no benefit to biomass yields and generally reduce the concentration of soluble carbohydrates. Structural composition has been shown to be unaffected by rate of nitrogen fertilizer application.

Principles of ensiling

Ensiling is a method of preserving fresh plant material for extended periods with limited decomposition of the ensiled material. Traditionally, ensiling has been used to preserve forage material for feeding of animals after the cropping season. Recently, ensiling has been considered as a potential method of storing the large quantities of biomass feedstocks necessary for lignocellulosic ethanol production. Long-term preservation is possible once anaerobic conditions are obtained and silage pH is low enough to inhibit decomposition. A fundamental understanding of the ensiling process is necessary in order to be adapted for storage of biomass feedstocks.

Comprehensive reviews of the ensiling process (Bolsen, 1995; Jaster, 1995) and the biochemistry involved (McDonald et al., 1991) have been conducted. Reviews of the subject

generally characterize the process as having distinct phases that influence the ensiling process. The three common phases of ensiling are the aerobic, fermentation, and utilization phases.

Aerobic phase

The aerobic phase of ensiling is the period between harvesting the forage and the point at which oxygen within the stored silage has been utilized by enzymatic and bacterial activities. Plant material that will be ensiled is normally harvested at moisture concentration range of 40-85% (Jaster, 1995). The extremes of this range present some challenges to the ensiling process that must be addressed. Silages of low moisture (40-60%) have the potential for increased harvest losses, reduced fermentation, and increased heat damage (Jaster, 1995). Limiting oxygen penetration by packing the silage well, sealing with plastic, and chopping the forage to a finer particle size can resolve the latter two issues. High moisture (75-85%) silages tend to have higher clostridial populations, increased effluent, and reduced quality (Jaster, 1995). Plant material of high moisture would likely benefit from field wilting. Field wilting reduces the moisture content of the forage, improves fermentation, and reduces transportation costs.

During the harvesting process, enzymes within plant cells are released as the plant material is chopped. In the initial periods of storage, these enzymes and the naturally occurring bacteria on the forage utilize sugars and oxygen in the silage to produce carbon dioxide, water, and heat via respiration. The process of respiration can contribute to significant reductions in soluble sugar content if the silage is continually exposed to oxygen. However, given optimum storage conditions, oxygen content and permeability is reduced by compressing the forage and quickly sealing with plastic. Under these conditions, oxygen

content within the silage is very low and is quickly utilized thereby reducing the loss of soluble sugars and producing an anaerobic environment suitable for fermentation. This is important because soluble sugars are the primary substrate for lactic acid production by lactic acid bacteria. Lactic acid is the principal acid responsible for the reduction of silage pH and provides the conditions necessary for stable storage of the forage.

Fermentation phase

The fermentation phase is the most important phase concerning the potential of long-term storage of ensiled plant material. An anaerobic environment is essential for fermentation processes of ensiling that lower silage pH and preserves the ensiled material. As conditions within the silage become anaerobic, plant cells burst releasing sugars that will be available for fermentation by bacteria.

Lactic acid bacteria are essential for proper fermentation. The population of lactic acid bacteria rapidly increases in an anaerobic environment. Lactic acid bacteria are classified as either homofermentative or heterofermentative (McDonald et al., 1991). Homofermentative lactic acid bacteria use the available carbohydrates to produce only lactic acid. Heterofermentative lactic acid bacteria produce both lactic and acetic acid. Dry matter and energy content of silage is better conserved under homofermentative conditions. However, loss of dry matter and energy content due to the activity of heterofermentative lactic acid bacteria is generally low. Silage pH is dependent upon the activity of lactic acid producing bacteria and water soluble carbohydrate concentration available in the plant material. Lactic acid is a much stronger acid than acetic acid. Therefore, homofermentative lactic acid bacteria are more desirable for reducing pH and preserving silage. Provided an adequate supply of carbohydrates lactic acid production will quickly lower the pH of ensiled

material. Low pH preserves the ensiled material, promotes hydrolysis of hemicellulose, and inhibits the activity of deleterious microorganisms such as enterobacteria and clostridia.

Enterobacteria ferment sugars to lactic acid, acetic acid and ethanol but predominately produce acetic acid. Enterobacteria are of concern because acetic acid, as a weaker acid, cannot reduce the pH of ensiled material low enough to preserve ensiled material adequately. Dry matter and energy losses are of concern when acetic acid production by enterobacteria is high. The activity of enterobacteria is highest at the beginning of the ensiling process. However, when lactic acid production is rapid enterobacteria populations are significantly reduced, as survival at pH below 5.0 is not possible (Bolsen, 1995).

Clostridia are anaerobic bacteria that ferment both sugars and lactic acid to produce butyric acid. Clostridia populations increase to significant levels only if pH levels remain high enough for growth due to a lack of carbohydrate source for adequate lactic acid production (McDonald et al., 1991). Production of butyric acid by clostridia causes an increase in pH and significant losses in dry matter and energy that can approach 50% and 20% loss, respectively (Bolsen, 1995). As with enterobacteria, rapid production of lactic acid and the subsequent reduction in pH will suppress the activity of clostridia. However, clostridia are less sensitive to pH changes than enterobacteria and are suppressed at a pH around 4.6 (Bolsen, 1995).

Long-term preservation of ensiled material depends on lactic acid bacteria to reduce silage pH and prevent the activity of enterobacteria and clostridia. Provided an adequate supply of sugar, lactic acid production will continue until pH has reached 4.0, below which

only a few species of lactic acid bacteria can still grow (McDonald et al., 1991).

Fermentation by lactic acid bacteria is usually completed within 3 weeks (Jaster, 1995).

Once fermentation is complete, pH, sugar content, and organic acid content should remain relatively stable. Hydrolysis of hemicellulose due to the acidic conditions is common during the stable period following fermentation (McDonald et al, 1991). Hydrolytic activities can be quantified by measuring changes in the concentration of the common carbohydrate monomers (Ren, 2006). Monomers common to the structure of hemicellulose include arabinose, galactose, glucose, mannose, and xylose (Ren, 2006). Hydrolyzed sugars may be converted to lactic acid if previous sugar sources were limiting. In order to maintain stable, long-term preservation of silage the continued exclusion of oxygen is necessary. If the silage is directly exposed to oxygen or it penetrates the plastic seal, microbial respiration will occur resulting in deterioration.

Utilization phase

The final phase of the ensiling process is the utilization of the ensiled plant material. At this point, the protective seal has been disrupted and oxygen is in direct contact with the silage. Aerobic microorganisms present in the silage that had been dormant during anaerobic conditions resume respiration and cause deterioration of silage quality. The predominant microorganisms involved in respiration during silage utilization are yeast and mold (McDonald et al, 1991). The respiratory process can lead to large losses in dry matter due to heating and conversion of sugars and organic acids to less desirable products. This phase accounts for the largest losses in dry matter and reductions in quality of a properly ensiled forage (Bolsen, 1995). The extent to which these processes deteriorate the silage is best

controlled by rapidly utilizing silage exposed to oxygen and maintaining the compressed nature of the remaining silage.

Summary

Interest in sweet sorghum production has increased in recent years as a potential bioenergy crop for lignocellulosic ethanol production. Sweet sorghum is widely recognized for high carbohydrate content and biomass yields. Previous studies have shown that sweet sorghum responds to management strategies to increase sugar and biomass yields when grown for syrup production. The fundamentals of these management experiments can be adapted to the production of sweet sorghum as a bioenergy crop.

Additionally, the high water soluble carbohydrate content of sweet sorghum is of benefit when considering storage via the ensiling process. Adequate supply of water soluble carbohydrates and maintaining a stable anaerobic environment are necessary for the long-term preservation of plant material as silage. The carbohydrates available in sweet sorghum should ensure rapid production of lactic acid and the pH necessary to prevent deterioration.

The objective of this project was to investigate management practices for sweet sorghum as a bioenergy crop in Iowa and its storability as an ensiled product. A management study was conducted to evaluate the effects of planting date, seeding rate, row width, and nitrogen fertility on the growth, biomass yield, and chemical composition of sweet sorghum. In addition, a silage study was conducted to determine chemical composition and fermentation potential of pressed sweet sorghum residue as well as the effects of enzymatic pretreatments.

References

- Almodares, A., R. Taheri, M.R. Hadi, and M. Fathi. 2006. The effect of nitrogen and potassium fertilizers on the growth parameters and the yield components of two sweet sorghum cultivars. *Pakistan Journal of Biological Sciences* 9:2350-2353.
- Amaducci, S., A. Monti, and G. Venturi. 2004. Non-structural carbohydrates and fibre components in sweet and fibre sorghum as affected by low and normal input techniques. *Industrial Crops and Products* 20:111-118.
- Anderson, I.C., D.R. Buxton, and A. Hallam. 1995. Performance of annual and perennial crops for forage and biomass energy production. USDA. U.S. Dairy Forage Research Center, 1995 Research Summaries.
- Bolsen, K.K. 1995. Silage: Basic principles. P. 163-176. *In* R.F. Barnes, D.A. Miller, and C.J. Nelson (eds.), *Forage Vol. II, The science of grassland agriculture*, 5th ed. Iowa State University Press, Ames, IA.
- Broadhead, D.M. 1969. Sugar production from sweet sorghum as affected by planting date, after-ripe harvesting, and storage. *Agronomy Journal* 61:811-812.
- Broadhead, D.M. 1972. Effect of planting date and maturity on juice quality of Rio sweet sorghum. *Agronomy Journal* 64:389-390.
- Broadhead, D.M., and K.C. Freeman. 1980. Stalk and sugar yield of sweet sorghum as affected by spacing. *Agronomy Journal* 72:523-524.
- Caravetta, G.J., J.H. Cherney, and K.D. Johnson. 1990. Within-row spacing influences on diverse sorghum genotypes: I. Morphology. *Agronomy Journal* 82:206-210.
- Caravetta, G.J., J.H. Cherney, and K.D. Johnson. 1990. Within-row spacing influences on diverse sorghum genotypes: II. Dry matter yield and forage quality. *Agronomy Journal* 82:210-215.
- Cox, W.J., D.R. Cherney, and J.J. Hanchar. 1998. Row spacing, hybrid, and plant density effects on corn silage yield and quality. *Journal of Production Agriculture* 11:128-134.
- Cusicanqui, J.A., and J.G. Lauer. 1999. Plant density and hybrid influence on corn forage yield and quality. *Agronomy Journal* 91:911-915.
- Darby, H.M., and J.G. Lauer. 2002. Planting date and hybrid influence on corn forage yield and quality. *Agronomy Journal* 94:281-289.

- Ferraris, R., and D.A. Charles-Edwards. 1986. A comparative analysis of the growth of sweet and forage sorghum crops. I. Dry matter production, phenology and morphology. *Australian Journal of Agricultural Research* 37:495-512.
- Freeman, K.C., D.M. Broadhead, and N. Zummo. 1973. Culture of sweet sorghum for sirup production. USDA. Agriculture Handbook No. 441.
- Gascho, G.J., R.L. Nichols, and T. Powell Gaines. 1984. Growing sweet sorghum as a source of fermentable sugars for energy. Georgia Agricultural Experiment Station. Research Bulletin No. 315.
- Graybill, J.S., W.J. Cox, and D.J. Otis. 1991. Yield and quality of forage maize as influenced by hybrid, planting date, and plant density. *Agronomy Journal* 83:559-564.
- Hipp, B.W., W.R. Cowley, C.J. Gerard, and B.A. Smith. 1970. Influence of solar radiation and date of planting on yield of sweet sorghum. *Crop Science* 10:91-92.
- Hons, F.M., R.F. Moresco, R.P. Wiedenfeld, and J.T. Cothren. 1986. Applied nitrogen and phosphorus effects on yield and nutrient uptake by high-energy sorghum produced for grain and biomass. *Agronomy Journal* 78:1069-1078.
- Jaster, E.H. 1995. Legume and grass silage preservation. P. 91-115. *In* K.J. Moore and M.A. Peterson (eds.), *Post-harvest physiology and preservation of forages*. CSSA-ASA, Madison, WI.
- Kanemasu, E.T., D.L. Bark, and E. Chin Choy. 1975. Effect of soil temperature on sorghum emergence. *Plant and Soil* 43:411-417.
- Karlen, D.L., C.R. Camp, and J.P. Zublena. 1985. Plant density, distribution, and fertilizer effects on yield and quality of irrigated corn silage. *Communications in Soil Science and Plant Analysis* 16:55-70.
- Koller, H.R., and J.M. Scholl. 1968. Effect of row spacing and seeding rate on forage production and chemical composition of two sorghum cultivars harvested at two cutting frequencies. *Agronomy Journal* 60:456-459.
- Lipinsky, E.S., D.R. Jackson, S. Kresovich, M.F. Arthur, and W.T. Lawhon. 1979. Carbohydrate crops as a renewable resource for fuels production. Volume 1: Agricultural research. Battelle Columbus Labs, Columbus, Ohio.
- Lueschen, W.E., D.H. Putnam, B.K. Kanne, and T.R. Hoverstad. 1991. Agronomic practices for production of ethanol from sweet sorghum. *Journal of Production Agriculture* 4:619-625.

- Lyons, E.S. 1957. Effect of plant spacings and fertilizers on the yield of sorgo. USDA. ARS Report 34-2.
- Martin, P.M., and F.M. Kelleher. 1984. Effects of row spacing and plant population on sweet sorghum yield. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24:386-390.
- Matherne, R.J. 1970. Sweet sorghum growing in Louisiana. USDA. Report CR 39-70.
- McBee, G.G., and F.R. Miller. 1982. Carbohydrates in sorghum culms as influenced by cultivars, spacing, and maturity over a diurnal period. *Crop Science* 22:381-385.
- McBee, G.G., and F.R. Miller. 1990. Carbohydrate and lignin partitioning in sorghum stems and blades. *Agronomy Journal* 82:687-690.
- McDonald, P., A.R. Henderson, and S.J.E. Heron. 1991. *The biochemistry of silage*, 2nd edition. Chalcombe Publications, Marlow, Buckinghamshire, UK.
- McGowan, M., H.M. Taylor, and J. Willingham. 1991. Influence of row spacing on growth, light and water use by sorghum. *Journal of Agricultural Science, Cambridge* 116:329-339.
- Rattunde, H.W.F., E. Zerbini, S. Chandra, and D.J. Flower. 2001. Stover quality of dual-purpose sorghums: genetic and environmental sources of variation. *Field Crop Research* 71:1-8.
- Ren, H. 2006. Effect of cell wall degrading enzymes and chemicals on corn stover preservation and pretreatment during ensilage processing. Ph.D. dissertation, Pennsylvania State University.
- Rutger, J.N., and L.V. Crowder. 1967. Effect of population and row width on corn silage yields. *Agronomy Journal* 59:475-476.
- Smith, G.A., and D.R. Buxton. 1993. Temperate zone sweet sorghum ethanol production potential. *Bioresource Technology* 43:71-75.
- Stickler, F.C., A.W. Pauli, H.H. Laude, H.D. Wilkins, and J.L. Mings. 1961. Row width and plant population studies with grain sorghum in Manhattan, Kansas. *Crop Science* 1:397-300.
- Stickler, F.C., and H.H. Laude. 1960. Effect of row spacing and plant population on corn, grain sorghum, and forage sorghum. *Agronomy Journal* 52:275-277.
- Vanderlip, R.L. 1993. How a sorghum plant develops. Kansas State University Agriculture Experiment Station. Contribution No. 1203.

- Widdicombe, W.D., and K.D. Thelen. 2002. Row width and planting density effect on corn forage hybrids. *Agronomy Journal* 94:326-330.
- Wiedenfeld, R.P. 1984. Nutrient requirements and use efficiency by sweet sorghum. *Energy in Agriculture* 3:49-59.
- Worker, G.F. 1973. Sudangrass and sudangrass hybrids responses to row spacing and plant maturity on yield and chemical composition. *Agronomy Journal* 65:975-977.
- Worley, J.W., J.S. Cundiff, D.H. Vaughan, and D.J. Parrish. 1991. Influence of sweet sorghum spacing on stalk pith yield. *Bioresource Technology* 36:133-139.

CHAPTER III

Management of sweet sorghum for biomass production

Abstract

In recent years, biofuels have become a national priority as demonstrated by the formation of the Renewable Fuels Standard. In response, significant research has been directed toward identifying high-yielding bioenergy crops, breeding for improved biomass yield and quality, and developing management practices specific to bioenergy crop production in order to meet the mandated production of advanced biofuels. The objective of this project was to investigate management practices for sweet sorghum (*Sorghum bicolor* (L.) Moench) as a bioenergy crop in Iowa. The effects of planting date, seeding rate, row width, and nitrogen fertility on growth, biomass yield, and chemical composition of ‘Top 76-6’ sweet sorghum were evaluated. Plots were seeded on three dates; late May, early June, and late June, at rates of 4.5, 11.2, and 17.9 kg/ha with row widths of 20, 38, and 76 cm in the years 2005 and 2006. All treatments were in factorial combination and replicated four times in a randomized complete block design (RCBD). Nitrogen fertilizer treatments of 84 and 168 kg N/ha were applied as strip-plot treatments. Harvested plots were weighed for biomass yield and a subsample was collected for chemical analysis. Our results show that dry matter yields were maximized for the second planting date in 20-cm row widths. Seeding rate and nitrogen fertility had no significant effects on biomass yield. Under these management conditions, dry matter yields of 20.9 Mg/ha and 37.1 Mg/ha were obtained in 2005 and 2006, respectively. Average dry matter yield, for this two-year period, was 29.0 Mg/ha. Given these results, sweet sorghum, when managed as a bioenergy crop in Iowa, has the potential of substantial biomass yields.

Introduction

Sweet sorghum (*Sorghum bicolor* (L.) Moench) was introduced to the United States in the early 1850s. Production of sweet sorghum has historically been practiced in the southeastern region of the United States predominately for the production of syrup. Sweet sorghum has also been utilized as a forage crop to a limited extent in the Midwest. The high carbohydrate content in the stem of sweet sorghum provides ample sugar for syrup production. Sorghum syrup was important during World War II as a substitute for granular sugar. However, land devoted to production has steadily declined since its peak the 1930s (Coleman, 1970).

During the 1970s, petroleum prices increased to unprecedented levels due to the embargo placed on shipments of oil to the United States. This motivated researchers to identify alternative sources of transportation fuels in the United States. Given its history of syrup production, sweet sorghum was immediately recognized as a possible substrate for ethanol production. Significant research was conducted on sweet sorghum during this period to evaluate management practices that would improve biomass and sugar yields.

The results of these studies show that planting date, row width, plant density, and nitrogen fertility are important factors in the accumulation of plant biomass and fermentable carbohydrates of sweet sorghum. Optimum planting date for sweet sorghum is dictated by soil conditions (Kanemasu et al., 1975; Freeman et al., 1973) with slightly earlier planting in southern latitudes (Broadhead, 1969; Lueschen et al., 1991). Regardless of production region, the results were similar; planting too early results in slight reductions while planting late results in large reductions in biomass yield (Broadhead, 1969; Broadhead, 1972; Gascho et al., 1984) and sugar yield (Broadhead, 1969; Hipp et al., 1970; Gascho et al., 1984).

Planting in narrow row widths has been shown to increase both biomass and sugar yield of sweet sorghum (Lipinsky et al., 1979; Broadhead and Freeman, 1980; Martin and Kelleher, 1984). Increasing plant density had similar results as planting in narrow rows (Martin and Kelleher, 1984; Caravetta et al., 1990b; Worley et al., 1990). Structural composition of related species does not appear to be affected by either row width (Koller and Scholl, 1968) or plant density (Koller and Scholl, 1968; Rattunde et al., 2001; Amaducci et al., 2004), however, experiments with sweet sorghum have been limited. Rates of nitrogen fertilizer application have been well evaluated. Sorghum has shown a positive response in biomass yield to nitrogen applications up to 168 kg N/ha (Hons et al., 1986; Smith and Buxton, 1993). Application rates above 168 kg N/ha reduced the concentration of soluble carbohydrates and provided no further increase in biomass production (Wiedenfeld, 1984; Anderson et al., 1995). Nitrogen fertilization showed no effects on structural composition of sorghum (Anderson et al., 1995; Amaducci et al., 2004).

These results provide insight into the management of sweet sorghum, however, further research is necessary to evaluate these management practices as they relate to the production of sweet sorghum as a feedstock for lignocellulosic ethanol production. The objective of this research was to evaluate the effects of planting date, seeding rate, row width, and nitrogen fertility on the growth, biomass yield, and chemical composition of sweet sorghum.

Materials and methods

Site description

Field plots were established in 2005 and 2006 on the Iowa State University, Sorenson Farm located 14.5 kilometers west of Ames in Boone County, Iowa (42°00' N, 93°44' W).

The experiment was located on a Webster silty clay loam (fine-loamy, mixed, superactive, mesic Typic Endoaquolls) and Clarion loam (fine-loamy, mixed, superactive, mesic Typic Hapludolls) in both years of the studies. Webster silty clay loam had a 0-2% slope and Clarion loam had a 2-5% slope.

The site utilized in 2005 has previously been used for kenaf (*Hibiscus cannabinus* L.) production on the northern half of each replication and fallow on the southern half of each replication in the year prior to establishment. The site utilized in 2006 had been used to grow conventionally managed soybeans (*Glycine max* (L.) Merr.) in the year prior to establishment.

Experiment establishment & design

Experiment locations were disked in the spring on May 5 and April 13 in 2005 and 2006, respectively. Nitrogen fertilizer was applied as ammonium nitrate (NH_4NO_3) on May 23, 2005 and as urea ($(\text{NH}_2)_2\text{CO}$) on May 10, 2006; fertilizer was incorporated with a field cultivator on the same dates. Immediately preceding planting, seedbed preparation was completed using a rotary tiller. Plots were seeded with ‘Top 76-6’ sweet sorghum (*Sorghum bicolor* (L.) Moench) that had been acquired from the Mississippi Agricultural and Forestry Experiment Station (MAFES) Foundation Seed Stocks and had been treated with the safener Concep III® (Syngenta). A preemergence herbicide application of Bicep® (Syngenta) was sprayed over plots on the date of planting at a rate of 4.7 L/ha.

Experiment plots measuring 3x12 m were seeded on three dates (Table 1): late May, early June, and late June, at rates of 4.5, 11.2, and 17.9 kg/ha with row widths of 20, 38, and 76 cm in both years of the experiment. All treatments were in factorial combination and replicated four times in a randomized complete block design (RCBD). Nitrogen fertilizer

treatments of 84 and 168 kg N/ha were applied as strip-plot treatments perpendicular to the main-plot treatments.

Climatic conditions, specifically monthly-accumulated precipitation and average monthly temperature, in 2005 were consistent with a 50-year average. Average monthly temperature in 2006 was also consistent with the 50-year average. However, monthly-accumulated precipitation in 2006 was inconsistent with the 50-year average due to limited rainfall in May and June (Table 2). Limited availability of soil moisture early in the 2006 growing season affected seed germination of the second planting date. The first killing frost ($<2.2^{\circ}\text{C}$) occurred October 28 and October 12 in 2005 and 2006, respectively.

Data collection

Plant height and stem diameter measurements were taken biweekly in 2006 starting two weeks after seedling emergence. Plant height was determined as the average height of four randomly selected plants per plot measured from the soil to the collar of last fully developed leaf. Stem diameter was also determined as the average diameter of four randomly selected plants per plot measured with vernier calipers at half-height of each plant.

Immediately prior to harvest, the outer 1.5 m of each plot was cut down thereby eliminating border effects. Plots were hand-harvested on October 7-9 and October 9-12 in 2005 and 2006, respectively. In 2005, three meters of the center two rows of each plot were harvested and weighed to calculate biomass yield per hectare. In 2006, the area harvested was reduced to 1.5 m of the center two rows. A subsample from each plot was chopped, weighed, and dried to calculate yield on a dry matter basis. The dried subsample was utilized for subsequent chemical analyses.

Chemical analysis

Dried samples were ground in a Wiley mill (Thomas Scientific, Inc.) to pass through a 1-mm screen. Dry matter (DM) of the ground sample was determined by drying 1 g of sample in a forced air oven at 103°C for 4 hours. Water soluble carbohydrates (WSC) were determined using the procedure described by Murphy et al. (2007). Total nonstructural carbohydrates (TNC) were determined using the acid extraction and phenol-sulfuric acid colorimetric procedures described by Guiragosian et al. (1977). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations were determined using the filter bag method (Vogel et al., 1999). Hemicellulose concentrations were calculated as the difference between NDF and ADF. Cellulose concentrations were calculated as the difference between ADF and ADL. Lignin is reported on an ash-free basis. Dry matter values were used to calculate water soluble carbohydrate, total nonstructural carbohydrate, and structural carbohydrate concentrations on a dry matter basis.

Statistical analysis

Field plot treatments of planting date, row width, and seeding rate were arranged in a RCBD with split-plot nitrogen treatments replicated four times in both years of the study. Effects of treatment factors and all their interactions on biomass yield and plant composition data were analyzed using the generalized linear model (GLM) of the Statistical Analysis Systems software (SAS, 2003) and the least significant difference (LSD) test. Differences were considered significant at $P \leq 0.05$. All treatment factors were considered fixed effects except for year and replication, which were random effects. Plant height and stem diameter measurements were collected from the same field plots as biomass yield and plant composition data, however, data was only collected in 2006. The same design was utilized

with the addition of measurement date as an additional split-plot treatment. Effects of treatment factors and all their interactions on plant height and stem diameter were analyzed as a split-plot in time using the generalized linear model (GLM) of the Statistical Analysis Systems software (SAS, 2003) and the least significant difference (LSD) test. Differences were considered significant at $P \leq 0.05$. All treatment factors were considered fixed effects except for replication, which was a random effect.

Results and discussion

Morphological measurements

Plant height showed an interaction between planting date, row width, seeding rate, nitrogen fertility, and day measured (Tables 3, 4, and 5). Later planting resulted in shorter plants, however, growth was more rapid in the first few weeks following emergence in the third planting date. Additionally, the effects of the other treatment factors in this interaction differed between planting dates. In the first planting date, plant height was highest when seeded in the 38-cm row width and at the 4.5 kg/ha seeding rate (Table 3). Nitrogen application of 168 kg N/ha showed an advantage over 84 kg N/ha throughout the vegetative growth period. In the second planting date, plant height was highest in row width of 20-cm and 11.2 kg/ha seeding rate (Table 4). Increased nitrogen application only showed an advantage at the lowest seeding rate. Finally, plant height in the third planting date responded similarly to the first planting date with tallest plants in the 38-cm row width and at the 4.5 kg/ha seeding rate (Table 5). Nitrogen application of 168 kg N/ha only showed a consistent advantage for plots seeded at 11.2 kg/ha and those planted in 20-cm row widths.

Stem diameter showed a response to three interactions. First, a four-way interaction existed between planting date, row width, seeding rate, and nitrogen fertility (Table 6).

Average stem diameter was increased with later planting, higher nitrogen rate, narrow row width, and low seeding rates. Additionally, average stem diameter was reduced by each increase in seeding rate across all dates measured (Figure 1). Plots seeded at 4.5 kg/ha had the thickest stems of the three seeding rates at each date. Finally, the three-way interaction between planting date, row width, and date measured showed that the response of stem diameter to row width differed between planting dates (Figure 2). Stem diameter of plots seeded on the first planting date was reduced as row width was narrowed. Plots seeded on the second and third planting date had thicker stems in 20-cm row widths than 38 and 76-cm rows. Stem diameter increased more rapidly in the third planting date when compared to the first or second. All planting dates showed reductions in stem diameter approaching the end of the growing season.

Previous research supports the effects management treatments had on plant height and stem diameter measurements in this experiment. In relation to date of planting, Freeman et al. (1973) stated that planting later would result in a faster plant growth rate, which was evident. Narrow row spacing has previously been shown to increase plant height (Martin and Kelleher, 1984) and was supported by these findings. Additionally, narrow row spacing has been shown to both increase (Martin and Kelleher, 1984) and decrease (Broadhead and Freeman, 1980) stem diameter. The results of this experiment would support the findings of Martin and Kelleher (1984). Previous studies have found that increased plant density results in taller plants (Caravetta et al., 1990a), however, the results of this experiment do not support that conclusion given the interaction involved. Lower planting density has been shown to increase plant stem diameter (McBee and Miller, 1982; Martin and Kelleher, 1984; Caravetta, et al., 1990a), which is supported by these findings.

Biomass yield

Biomass yield varied between years, planting dates, and row widths in a three-way interaction (Figure 3 and 4). Yield decreased with delayed planting across all row widths in 2005 (Figure 3). The highest biomass yield in 2005 was from plots seeded in 20-cm row widths. Biomass yield of plots seeded in 20-cm row widths at each planting date was 23.97, 20.85, and 17.29 Mg/ha, respectively. In 2006, biomass yield decreased with later planting in 38 and 76-cm row widths while biomass yield of 20-cm row widths was highest in the second planting date (Figure 4). Plots seeded in 20-cm row widths again had significantly higher biomass yield than 38 or 76-cm row widths. Biomass yield of plots seeded in 20-cm row widths at each planting date was 28.73, 37.09, and 24.56 Mg/ha, respectively. Overall, the 20-cm row width had the highest biomass yields across all planting dates in both years of the experiment. The two-year average biomass yield of plots seeded in 20-cm row widths for the three planting dates was 26.35, 28.97, and 20.93 Mg/ha, respectively. When compared to the two-year average of plots seeded in 38-cm row widths, biomass yield of 20-cm row widths was higher by 18.2%, 51.1%, and 35.1% at each of the three planting dates, respectively. Likewise, biomass yield was higher by 20.0%, 74.1%, 30.7% in 20-cm row widths compared to 76-cm at each planting date, respectively.

The effects of planting date and row width on biomass yield of sweet sorghum have been extensively documented. A review of the literature supports the results of this experiment. Freeman et al. (1973) suggested that reductions in biomass yield from later planting were due to the shortened growing season. Later planting has consistently resulted in reductions in biomass yield of sorghum across of range of environments (Broadhead, 1969; Hipp et al., 1970; Broadhead, 1972; Gascho et al., 1984; Ferraris and Charles-

Edwards, 1986). Additionally, comparison of row widths has shown that as row width is reduced biomass yield is increased (Lipinsky et al., 1979; Broadhead and Freeman, 1980; Martin and Kelleher, 1984; McGowan et al., 1991).

Water soluble and nonstructural carbohydrate concentrations

Water soluble carbohydrate (WSC) concentration showed a response to two interactions. First, the response of WSC concentration to nitrogen fertility varied between years. Water soluble carbohydrate concentration increased when 168 kg N/ha was applied compared to 84 kg N/ha in 2006 but no significant changes due to nitrogen fertility in 2005 were apparent (Figure 5). Water soluble carbohydrate concentration increased from an average of 202 to 221 g/kg with the higher nitrogen fertility in 2006 and remained around 200 g/kg in 2005 regardless of nitrogen fertility. In addition, WSC concentration responded to years, planting date, and seeding rate in a three-way interaction (Figure 6 and 7). In 2005, WSC concentration increased in the second and third planting dates when compared to the first planting date while no significant differences existed between seeding rates (Figure 6). Average WSC concentration for the three planting dates in 2005 was 185, 211, and 206 g/kg, respectively. Differences in WSC concentration between seeding rates and across planting dates were present in 2006 (Figure 7). Plots seeded at 4.5 kg/ha decreased in WSC concentration across all planting dates; average WSC concentration for each planting date was 234, 212, and 180 g/kg, respectively. Plots seeded at 17.9 kg/ha decreased in WSC concentration from 216 g/kg in the first planting date to 201 g/kg in the third. Conversely, plot seeded at 11.2 kg/ha responded as in 2005; WSC concentration increased from 194 g/kg in the first planting date to 225 g/kg in the second and third.

Total nonstructural carbohydrate (TNC) concentration decreased across planting dates (Figure 8). Two-year averages of TNC concentration for the three planting dates were 367, 341, and 321 g/kg, respectively. The decrease in TNC concentration across planting dates was likely caused by the inverse relationship between nonstructural and structural carbohydrates noted by McBee and Miller (1990).

Previous studies have found that increased nitrogen fertility has limited effects on WSC concentration of sweet sorghum. Adamucci et al. (2004) noted that an increase of nitrogen application from 60 kg N/ha to 120 kg N/ha had no effect on soluble carbohydrate concentration of sorghum. However, Wiedenfeld (1984) found that at a rate of 224 kg N/ha sugar concentration of sweet sorghum was slightly reduced. The response in 2005 supports the findings of these studies, however, the increase in WSC concentration at the 168 kg N/ha rate in 2006 is contradictory to previous findings. In relation to planting date and plant density, previous research has found that later planting (Gascho et al., 1984) and higher plant density (Martin and Kelleher, 1984; Worley et al., 1991; Adamucci et al., 2004) increased soluble carbohydrate concentration and yield. The results of this experiment were similar to Gascho et al. (1984) regarding later planting in 2005 but not 2006. Additionally, higher plant density does not increase WSC concentration as previously demonstrated in other studies.

Structural carbohydrate composition

Cellulose concentration showed a response to management treatments in two separate interactions. The response of cellulose concentration to planting date varied between years (Figure 9). In both years, cellulose concentration increased with later planting but the amount of increase was substantially higher in 2005 than 2006. Cellulose concentration increased from an average of 243 g/kg for the first planting date to 273 g/kg for the third

planting date in 2005. However, in 2006, the increase in cellulose concentration was smaller, increasing from an average of 242 g/kg for the first planting date to only 259 g/kg for the third planting date. The response of cellulose concentration to row width also varied between years (Figure 10). Average cellulose concentration decreased with increasing row width in 2005 while remaining nearly unchanged in 2006 regardless of row width. Additionally, cellulose concentration was higher in 2005 with exception of plots seeded in 76-cm row widths. Cellulose concentration in 2005 was similar for both 20 and 38-cm row widths at 263 and 260 g/kg, respectively, but decreased to 249 g/kg when planted in 76-cm row widths. As stated, cellulose concentration in 2006 remained near 251 g/kg irrespective of row width treatment.

Hemicellulose concentration also responded to management treatments in two interactions. Hemicellulose concentration varied between years in respect to row width treatments (Figure 11). Similar to cellulose concentration, hemicellulose concentration decreased with increasing row width in 2005 while remaining nearly unchanged in 2006 regardless of row width. Additionally, hemicellulose concentration was higher in 2005 across all row width treatments. Hemicellulose concentration in 2005 was similar for both 20 and 38-cm row width at 216 and 215 g/kg, respectively, but decreased to 208 g/kg when planted in 76-cm row widths. As stated, hemicellulose concentration in 2006 remained similar across row widths, ranging from 195 to 198 g/kg. In addition, hemicellulose concentration responded to years, planting date, seeding rate, and nitrogen fertility in a four-way interaction (Figure 12). Average hemicellulose concentration was higher in 2005 than 2006. Hemicellulose concentration in 2005 increased with later planting and increased seeding rates. In 2006, hemicellulose concentration increased with later planting while

seeding rate was not as influential as in 2005. The influence of nitrogen fertility treatments within this interaction was inconsistent.

Lignin concentration showed a response to three interactions. The response of lignin concentration to planting date varied between years (Figure 13). Average lignin concentration was higher in 2006 for the first two planting dates and similar for the third when compared to 2005. In general, lignin concentration in 2005 increased with later planting and decreased with later planting in 2006. The response of lignin concentration to row width also varied between years (Figure 14). Average lignin concentration decreased with increased row widths in 2005 and was unaffected by row width treatments in 2006. Lignin concentration was generally higher in 2006 than 2005. Lastly, lignin concentration responded to the treatment factors of planting date, row width, and seeding rate in a three-way (Figure 15). Lignin concentration was generally higher in the third planting date compared to the first and second planting dates. The response to an increase in row width from 20 to 38 cm varied widely among planting dates and seeding rates with no distinguishable trend. When row width was increased from 20 to 76 cm a specific response in lignin concentration was apparent. Lignin concentration for all three seeding rates decreased in the second planting date from an average of 19.4 to 17.8 g/kg when row width increased from 20 to 76 cm. The 17.9 kg/ha seeding rate showed a similar response in both the first and third planting dates, decreasing in lignin concentration from the 20 to 76 cm row width. Plots seeded at 4.5 and 11.2 kg/ha tended to follow the same trend, however, decreases in lignin concentration were not statistically significant.

Determination of sweet sorghum structural carbohydrate composition has generally been limited to variety comparison studies rather than management studies. Management

studies similar to this experiment have been conducted on related crop species. Previous research on sudangrass and sorghum-sudangrass (Koller and Scholl, 1968; Worker, 1973) and maize (Cox et al., 1998; Widdicombe and Thelen, 2002) evaluated the effect of row width on structural carbohydrate composition. These studies concluded that row width had no effect on structural carbohydrate composition of the respective species evaluated. Additionally, studies evaluating plant density on sweet sorghum (Amaducci et al., 2004), dual-purpose sorghum (Rattunde et al., 2001), and sudangrass and sorghum-sudangrass (Koller and Scholl, 1968) suggest that plant density does not affect structural carbohydrate composition. The results of this experiment conflict with these studies and suggest that management factors such as planting date, row width, and plant density may influence the structural carbohydrate composition of sweet sorghum.

Conclusions

In summary, management practices had significant effects on the growth, biomass production, and chemical composition of sweet sorghum. Earlier planting resulted in taller plants, increased nonstructural carbohydrates, decreased structural carbohydrates, and substantially increased biomass yield. Biomass yield increased significantly in narrow row widths and was highest in the 20-cm row width. Cellulose, hemicellulose, and lignin concentrations were increased in narrow rows in 2005. However, there were generally unaffected by row width treatments in 2006. Lower seeding rates tended to produce taller plants with thicker stems. Seeding rate also had minor effects on WSC, hemicellulose, and lignin concentrations of sweet sorghum. Nitrogen fertilization had limited effects on plant height, stem diameter, WSC concentration, and hemicellulose concentration. Biomass yield was not affected by seeding rate or nitrogen fertility.

The difference in climatic conditions between experiment years, specifically accumulated rainfall, was likely a contributing factor in the biomass yield response noted in this experiment. As noted, rainfall during 2006 was severely limited during the growing season after the first planting date. Sweet sorghum seeded in 20-cm rows was likely better able to utilize the available soil moisture within the plot than plants seeded in wider row widths. Additionally, relative humidity in narrow rows is generally higher than in wider rows. These conditions would suggest lower moisture stress in narrow rows, which favored the higher biomass yields in 20-cm rows compared to wider rows. Early planting was important but row width had a greater effect on biomass yield. In 2005, rainfall was similar to the 50-year average. Conversely, planting date had a greater effect on biomass yield than row width.

Given these results, planting in late May to early June in 20-cm row widths would be recommended for maximum biomass production of sweet sorghum in Iowa. Since seeding rate and nitrogen fertility had no significant effects on biomass yield and only minor effects on growth and chemical composition of sweet sorghum, lowest rates of both would be justified. Finally, the biomass yield of sweet sorghum in this study confirms its status as one of the highest yielding biomass crops considered for bioenergy production.

References

- Amaducci, S., A. Monti, and G. Venturi. 2004. Non-structural carbohydrates and fibre components in sweet and fibre sorghum as affected by low and normal input techniques. *Industrial Crops and Products* 20:111-118.
- Anderson, I.C., D.R. Buxton, and A. Hallam. 1995. Performance of annual and perennial crops for forage and biomass energy production. USDA. U.S. Dairy Forage Research Center, 1995 Research Summaries.
- Broadhead, D.M. 1969. Sugar production from sweet sorghum as affected by planting date, after-ripe harvesting, and storage. *Agronomy Journal* 61:811-812.
- Broadhead, D.M. 1972. Effect of planting date and maturity on juice quality of Rio sweet sorghum. *Agronomy Journal* 64:389-390.
- Broadhead, D.M., and K.C. Freeman. 1980. Stalk and sugar yield of sweet sorghum as affected by spacing. *Agronomy Journal* 72:523-524.
- Caravetta, G.J., J.H. Cherney, and K.D. Johnson. 1990. Within-row spacing influences on diverse sorghum genotypes: I. Morphology. *Agronomy Journal* 82:206-210.
- Caravetta, G.J., J.H. Cherney, and K.D. Johnson. 1990. Within-row spacing influences on diverse sorghum genotypes: II. Dry matter yield and forage quality. *Agronomy Journal* 82:210-215.
- Coleman, O. H. 1970. Syrup and sugar from sweet sorghum. p. 416-440. *In* J. S. Wall and W. M. Ross (eds.), *Sorghum production and utilization*. The AVI Publishing Company, Inc., Westport, CT.
- Cox, W.J., D.R. Cherney, and J.J. Hanchar. 1998. Row spacing, hybrid, and plant density effects on corn silage yield and quality. *Journal of Production Agriculture* 11:128-134.
- Ferraris, R., and D.A. Charles-Edwards. 1986. A comparative analysis of the growth of sweet and forage sorghum crops. I. Dry matter production, phenology and morphology. *Australian Journal of Agricultural Research* 37:495-512.
- Freeman, K.C., D.M. Broadhead, and N. Zummo. 1973. Culture of sweet sorghum for sirup production. USDA. *Agriculture Handbook* No. 441.
- Gascho, G.J., R.L. Nichols, and T. Powell Gaines. 1984. Growing sweet sorghum as a source of fermentable sugars for energy. Georgia Agricultural Experiment Station. *Research Bulletin* No. 315.

- Guiragossian, V.Y., S.W. Van Socyoc, and J.D. Axtell. 1977. Chemical and biological methods for grain and forage sorghum. Department of Agronomy, Purdue University, W. Lafayette, IN.
- Hipp, B.W., W.R. Cowley, C.J. Gerard, and B.A. Smith. 1970. Influence of solar radiation and date of planting on yield of sweet sorghum. *Crop Science* 10:91-92.
- Hons, F.M., R.F. Moresco, R.P. Wiedenfeld, and J.T. Cothren. 1986. Applied nitrogen and phosphorus effects on yield and nutrient uptake by high-energy sorghum produced for grain and biomass. *Agronomy Journal* 78:1069-1078.
- Kanemasu, E.T., D.L. Bark, and E. Chin Choy. 1975. Effect of soil temperature on sorghum emergence. *Plant and Soil* 43:411-417.
- Koller, H.R., and J.M. Scholl. 1968. Effect of row spacing and seeding rate on forage production and chemical composition of two sorghum cultivars harvested at two cutting frequencies. *Agronomy Journal* 60:456-459.
- Lipinsky, E.S., D.R. Jackson, S. Kresovich, M.F. Arthur, and W.T. Lawhon. 1979. Carbohydrate crops as a renewable resource for fuels production. Volume 1: Agricultural research. Battelle Columbus Labs, Columbus, Ohio.
- Lueschen, W.E., D.H. Putnam, B.K. Kanne, and T.R. Hoverstad. 1991. Agronomic practices for production of ethanol from sweet sorghum. *Journal of Production Agriculture* 4:619-625.
- Martin, P.M., and F.M. Kelleher. 1984. Effects of row spacing and plant population on sweet sorghum yield. *Australian Journal of Experimental Agriculture and Animal Husbandry* 24:386-390.
- McBee, G.G., and F.R. Miller. 1982. Carbohydrates in sorghum culms as influenced by cultivars, spacing, and maturity over a diurnal period. *Crop Science* 22:381-385.
- McBee, G.G., and F.R. Miller. 1990. Carbohydrate and lignin partitioning in sorghum stems and blades. *Agronomy Journal* 82:687-690.
- McGowan, M., H.M. Taylor, and J. Willingham. 1991. Influence of row spacing on growth, light and water use by sorghum. *Journal of Agricultural Science, Cambridge* 116:329-339.
- Murphy, P.T., K.J. Moore, T.L. Richard, and C.J. Bern. 2007. Enzyme enhanced solid-state fermentation of kenaf core fiber. *Bioresource Technology* 98:3106-3111.

- Rattunde, H.W.F., E. Zerbini, S. Chandra, and D.J. Flower. 2001. Stover quality of dual-purpose sorghums: genetic and environmental sources of variation. *Field Crop Research* 71:1-8.
- SAS. 2003. SAS User's Guide. Version 9.1. SAS Institute Inc., Cary, NC.
- Smith, G.A., and D.R. Buxton. 1993. Temperate zone sweet sorghum ethanol production potential. *Bioresource Technology* 43:71-75.
- Vogel, K.P., J.F. Pedersen, S.D. Masterson, and J.J. Troy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. *Crop Science* 39:276-279.
- Widdicombe, W.D., and K.D. Thelen. 2002. Row width and planting density effect on corn forage hybrids. *Agronomy Journal* 94:326-330.
- Wiedenfeld, R.P. 1984. Nutrient requirements and use efficiency by sweet sorghum. *Energy in Agriculture* 3:49-59.
- Worker, G.F. 1973. Sudangrass and sudangrass hybrids responses to row spacing and plant maturity on yield and chemical composition. *Agronomy Journal* 65:975-977.
- Worley, J.W., J.S. Cundiff, D.H. Vaughan, and D.J. Parrish. 1991. Influence of sweet sorghum spacing on stalk pith yield. *Bioresource Technology* 36:133-139.

Table 1. Date of planting and corresponding soil temperature at 10 cm depth in 2005 and 2006.

Year	Planting date	Soil temperature (°C)
2005	23 May	19.7
	7 June	27.1
	20 June	26.8
2006	24 May	19.8
	7 June	22.6
	23 June	23.2

Table 2. Monthly mean air temperature and accumulated precipitation for Ames, Iowa in 2005 and 2006. Mean column is the average of data from 1951-2006.

Month	Air temperature (°C)			Accumulated precipitation (cm)		
	2005	2006	Mean	2005	2006	Mean
April	12.8	13.3	10.0	8.2	10.9	8.9
May	15.6	16.7	16.1	19.4	16.4	20.3
June	23.3	22.2	21.1	31.7	18.4	32.7
July	24.4	24.4	23.3	42.1	32.6	42.9
August	22.2	22.2	22.2	59.3	48.2	53.7
September	20.6	16.1	17.8	70.4	67.3	61.9
October	12.2	10.0	11.7	71.3	73.6	67.9

Source: National Weather Service Cooperative Observer Program

Table 3. Effect of fertility, seeding rate, row width, and measurement date on plant height of sweet sorghum planted on 24 May 2006.

Nitrogen rate (kg N/ha)	Seeding rate (kg/ha)	Row width (cm)	Measurement date (DOY)						
			163	177	191	212	226	240	254
-----cm-----									
84	4.5	20	11.89	34.33	96.99	224.41	259.81	303.00	-
		38	12.23	33.43	89.09	223.33	274.81	304.94	-
		76	12.01	32.87	85.30	214.28	257.75	286.50	-
	11.2	20	12.04	35.85	105.49	229.96	270.25	302.44	-
		38	12.29	35.56	89.78	217.47	263.25	301.56	-
		76	13.64	36.98	90.58	215.74	256.50	295.25	-
	17.9	20	13.31	35.73	102.58	221.03	253.50	279.94	-
		38	13.40	37.97	102.90	216.34	255.63	292.25	-
		76	12.18	33.78	86.02	208.38	245.56	284.88	-
168	4.5	20	13.04	37.16	101.33	228.56	271.81	305.19	-
		38	12.84	35.49	90.61	221.97	267.06	302.69	-
		76	13.12	36.55	89.91	217.28	267.69	304.28	-
	11.2	20	13.08	36.93	99.44	231.50	273.94	299.94	-
		38	12.38	34.74	98.26	212.38	263.56	302.69	-
		76	13.13	34.77	92.69	211.50	263.38	290.56	-
	17.9	20	14.11	40.61	110.66	226.81	262.56	288.00	-
		38	12.26	35.83	98.56	218.38	256.00	287.56	-
		76	13.03	37.64	90.26	215.13	249.88	293.63	-

LSD_{.05} = 0.967

Table 4. Effect of fertility, seeding rate, row width, and measurement date on plant height of sweet sorghum planted on 7 June 2006.

Nitrogen rate (kg N/ha)	Seeding rate (kg/ha)	Row width (cm)	Measurement date (DOY)						
			163	177	191	212	226	240	254
-----cm-----									
84	4.5	20	-	13.09	39.52	116.72	178.70	233.97	273.81
		38	-	12.77	40.66	82.26	139.68	204.82	235.26
		76	-	12.95	42.44	94.49	150.27	219.60	253.14
	11.2	20	-	12.89	42.72	131.03	186.52	243.15	290.39
		38	-	12.32	43.36	99.74	156.80	219.38	255.19
		76	-	11.81	32.43	83.00	142.41	207.10	243.11
	17.9	20	-	12.86	49.94	139.05	165.74	233.95	274.53
		38	-	12.13	43.24	107.63	151.86	218.99	242.79
		76	-	13.16	44.73	105.81	156.75	215.92	244.03
168	4.5	20	-	11.71	40.56	116.40	171.36	240.23	286.40
		38	-	12.46	39.29	95.34	145.91	221.59	253.41
		76	-	12.14	43.31	103.45	161.89	222.04	265.11
	11.2	20	-	12.13	44.19	124.33	186.41	234.38	289.08
		38	-	12.51	43.65	95.30	148.30	211.11	253.70
		76	-	12.81	45.11	92.63	144.46	212.04	251.59
	17.9	20	-	12.33	48.36	127.51	182.63	237.35	275.95
		38	-	11.28	41.18	93.40	147.89	190.15	241.08
		76	-	13.36	46.16	98.49	149.32	215.99	240.86

LSD_{.05} = 0.967

Table 5. Effect of fertility, seeding rate, row width, and measurement date on plant height of sweet sorghum planted on 23 June 2006.

Nitrogen rate (kg N/ha)	Seeding rate (kg/ha)	Row width (cm)	Measurement date (DOY)						
			163	177	191	212	226	240	254
-----cm-----									
84	4.5	20	-	-	13.83	66.22	120.13	201.08	224.58
		38	-	-	14.86	93.75	161.06	222.96	254.71
		76	-	-	14.30	78.89	142.22	218.28	246.23
	11.2	20	-	-	16.39	85.05	154.71	217.32	244.36
		38	-	-	15.15	96.21	165.05	230.68	244.94
		76	-	-	15.20	93.47	158.54	212.35	233.66
	17.9	20	-	-	15.97	94.30	145.54	178.18	232.49
		38	-	-	15.28	95.21	154.19	213.34	234.49
		76	-	-	15.73	95.21	146.50	217.24	247.81
168	4.5	20	-	-	14.13	70.94	120.01	207.37	241.91
		38	-	-	15.65	86.01	156.68	219.91	252.54
		76	-	-	14.78	85.09	144.48	205.16	239.67
	11.2	20	-	-	15.71	90.62	152.34	218.14	245.91
		38	-	-	15.72	95.75	174.40	231.93	250.92
		76	-	-	18.27	94.77	155.81	209.51	236.48
	17.9	20	-	-	15.12	93.06	148.29	212.85	241.44
		38	-	-	14.78	102.35	155.22	211.62	229.61
		76	-	-	14.75	95.34	149.53	207.24	229.48

LSD_{.05} = 0.967

Table 6. Effect of planting date, row width, nitrogen fertility, and seeding rate on average stem diameter of sweet sorghum.

		Nitrogen rate (kg N/ha)					
		-----84-----			-----168-----		
Planting date (DOY)	Row width (cm)	Seeding rate (kg/ha)					
		4.5	11.2	17.9	4.5	11.2	17.9
		-----cm-----					
143	20	1.128	1.058	0.957	1.140	1.122	1.002
	38	1.148	1.078	1.007	1.127	1.070	0.999
	76	1.081	1.092	1.051	1.154	1.081	1.068
158	20	1.219	1.162	1.025	1.181	1.161	1.064
	38	1.022	1.106	1.027	1.130	1.083	0.934
	76	1.093	0.969	1.073	1.153	1.078	1.063
173	20	1.294	1.099	0.982	1.344	1.153	1.060
	38	1.184	1.078	1.052	1.195	1.096	1.077
	76	1.192	1.008	1.133	1.171	1.004	1.076

LSD_{.05} = 0.0128

Figure 1. Effect of seeding rate and measurement date on stem diameter of sweet sorghum.

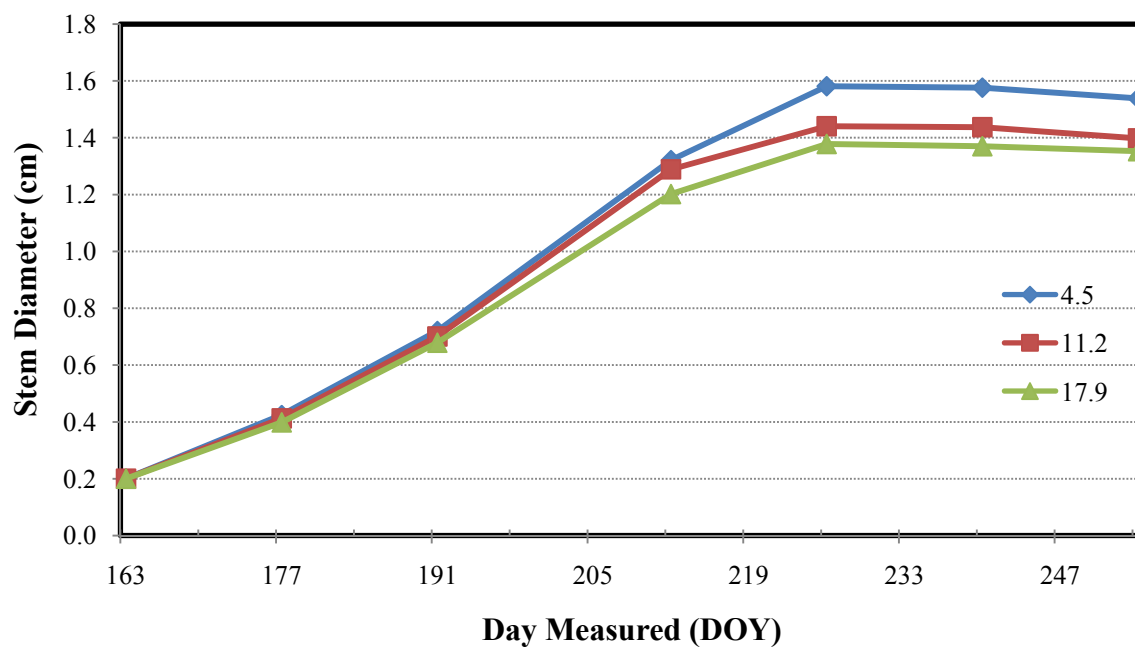


Figure 2. Effect of planting date, row width, and measurement date on stem diameter of sweet sorghum.

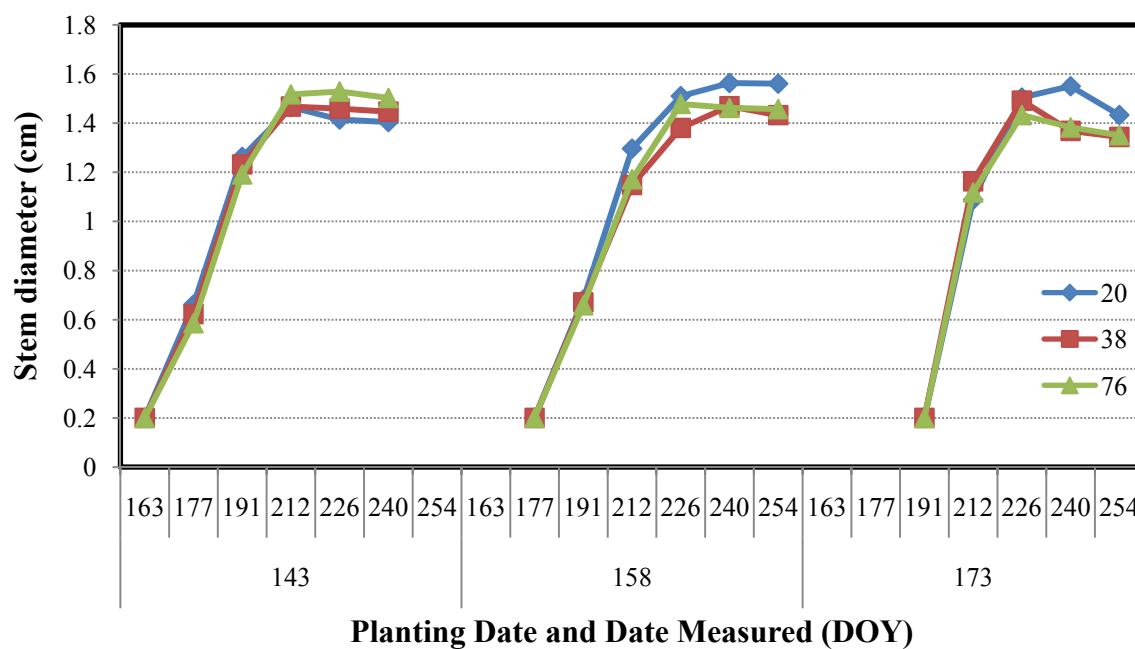


Figure 3. Effect of planting date and row width on biomass yield of sweet sorghum in 2005.

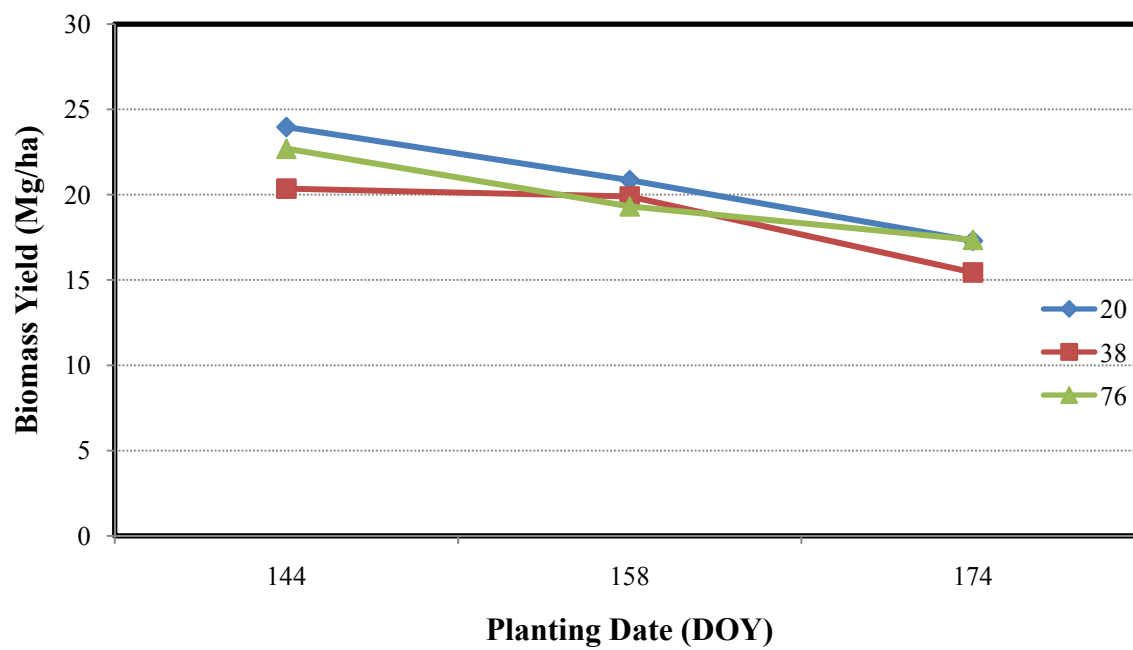


Figure 4. Effect of planting date and row width on biomass yield of sweet sorghum in 2006.

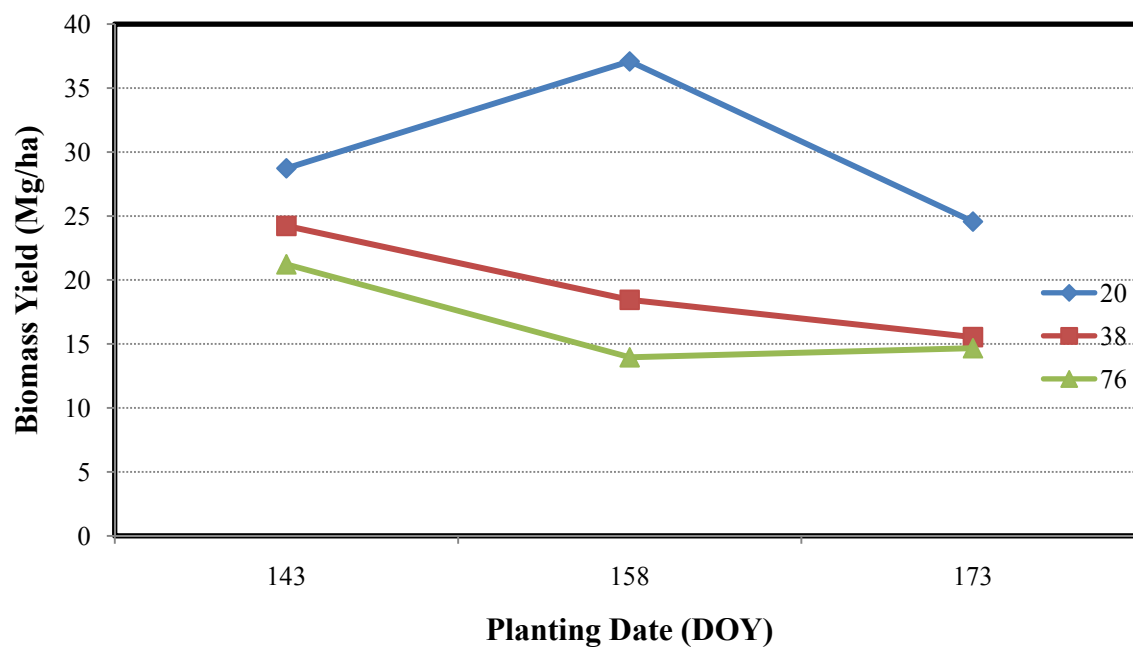


Figure 5. Effect of year and nitrogen fertility on water soluble carbohydrate concentration of sweet sorghum.

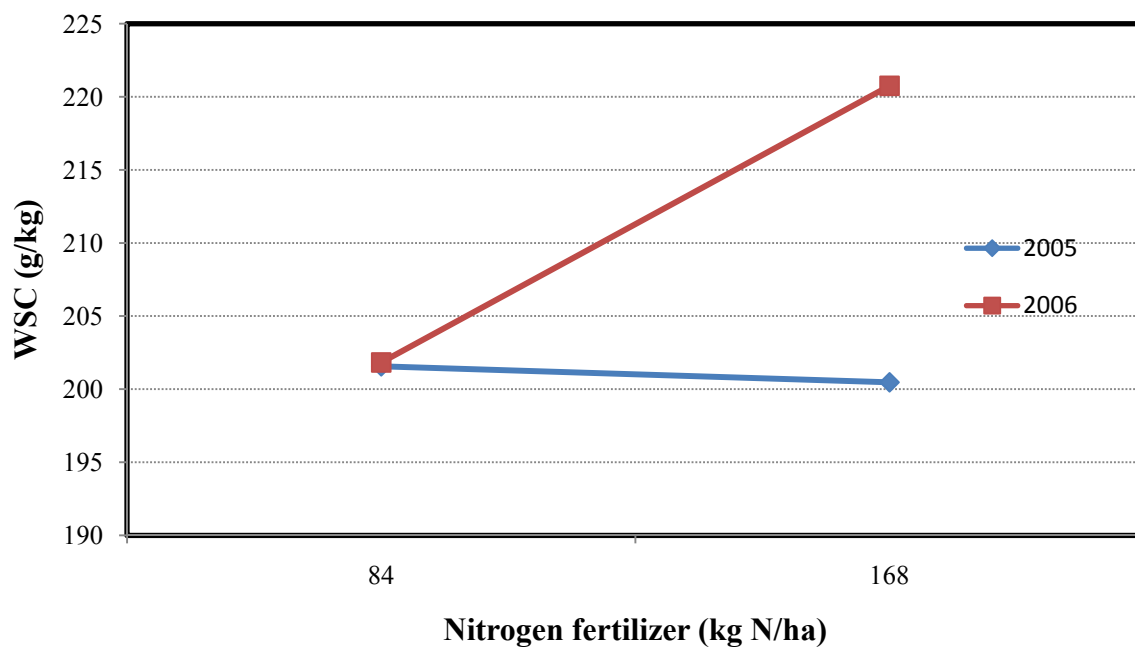


Figure 6. Effect of planting date and seeding rate on water soluble carbohydrate concentration of sweet sorghum in 2005.

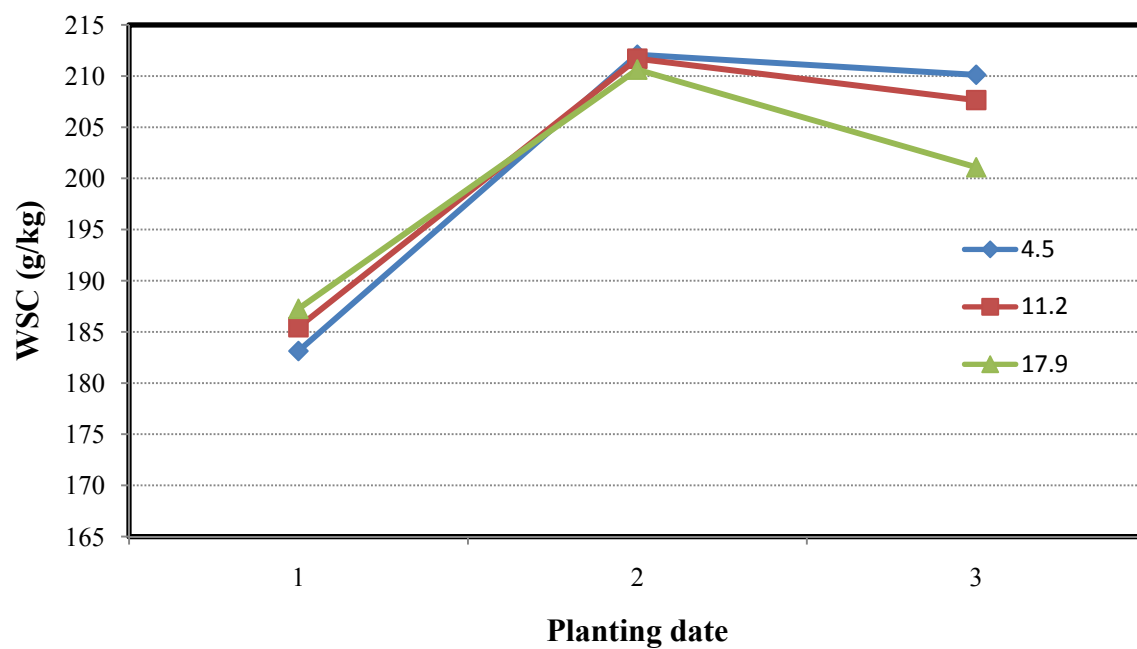


Figure 7. Effect of planting date and seeding rate on water soluble carbohydrate concentration of sweet sorghum in 2006.

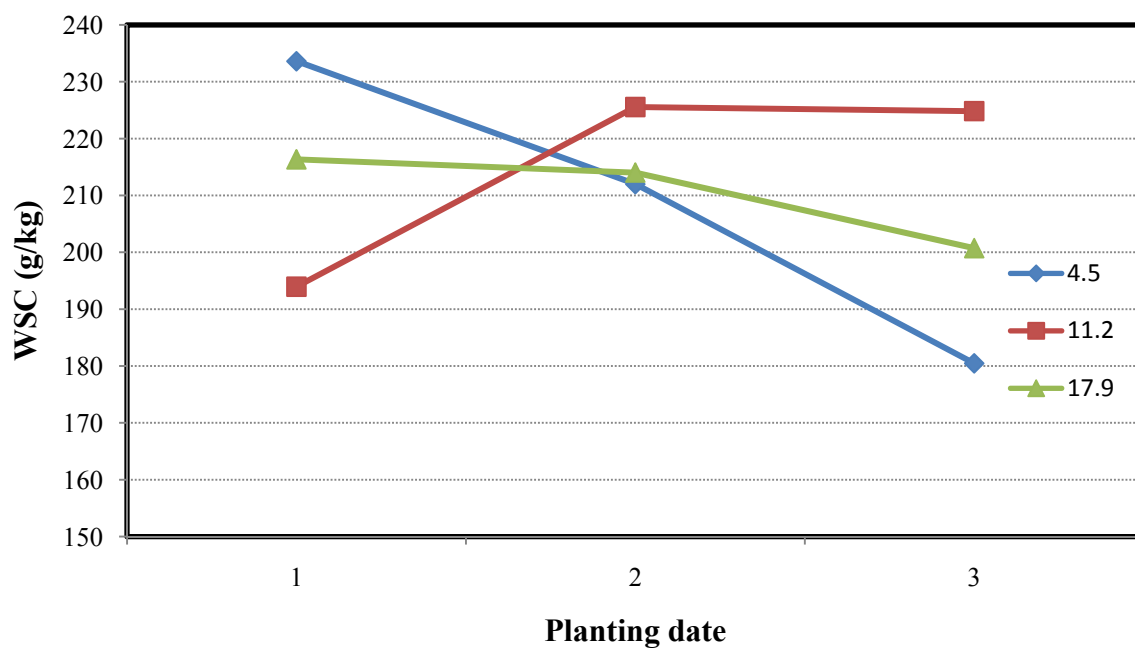


Figure 8. Effect of planting date on total nonstructural carbohydrate concentration of sweet sorghum.

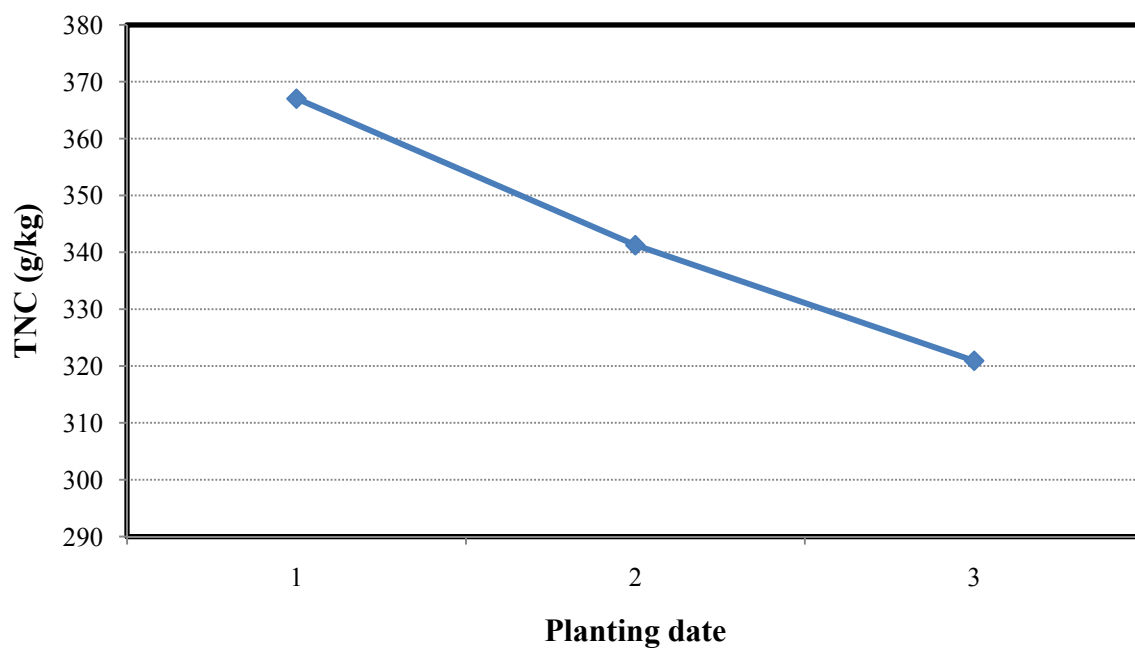


Figure 9. Effect of year and planting date on cellulose concentration of sweet sorghum.

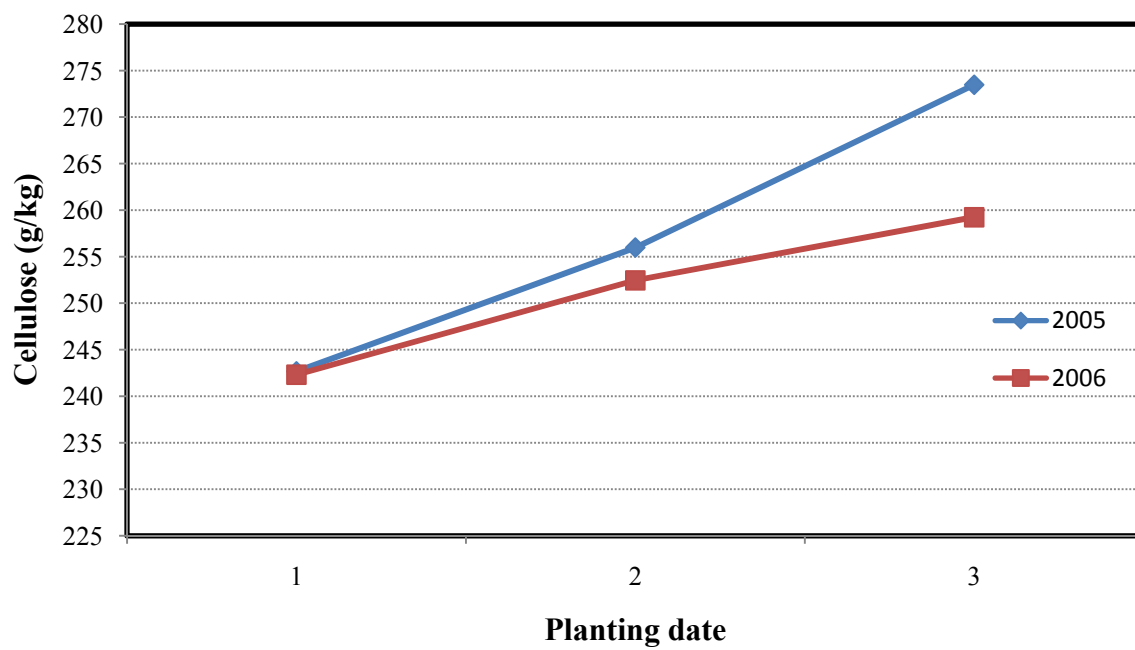


Figure 10. Effect of year and row width on cellulose concentration of sweet sorghum.

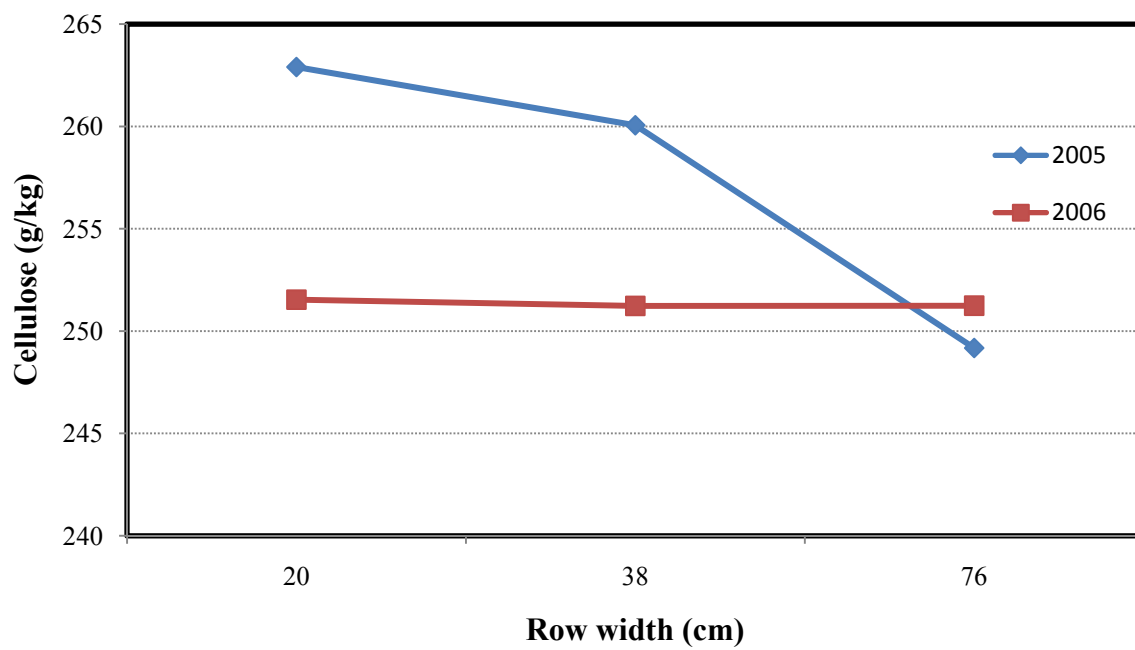


Figure 11. Effect of year and row width on hemicellulose concentration of sweet sorghum.

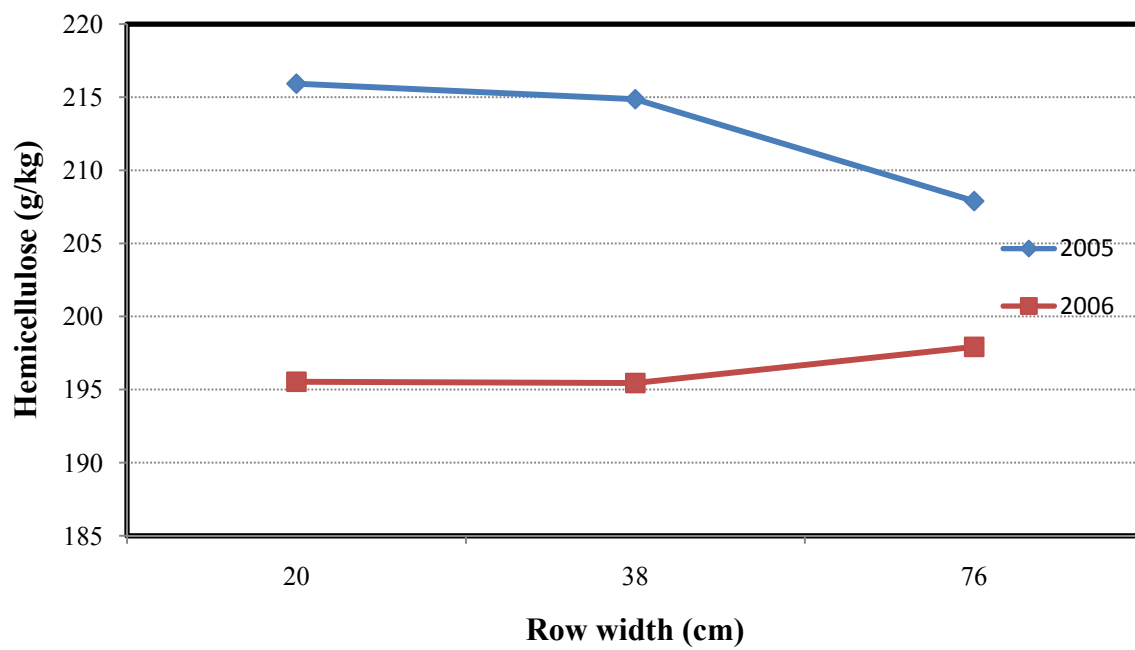


Figure 12. Effect of year, planting date, seeding rate, and nitrogen fertility on the hemicellulose concentration of sweet sorghum.

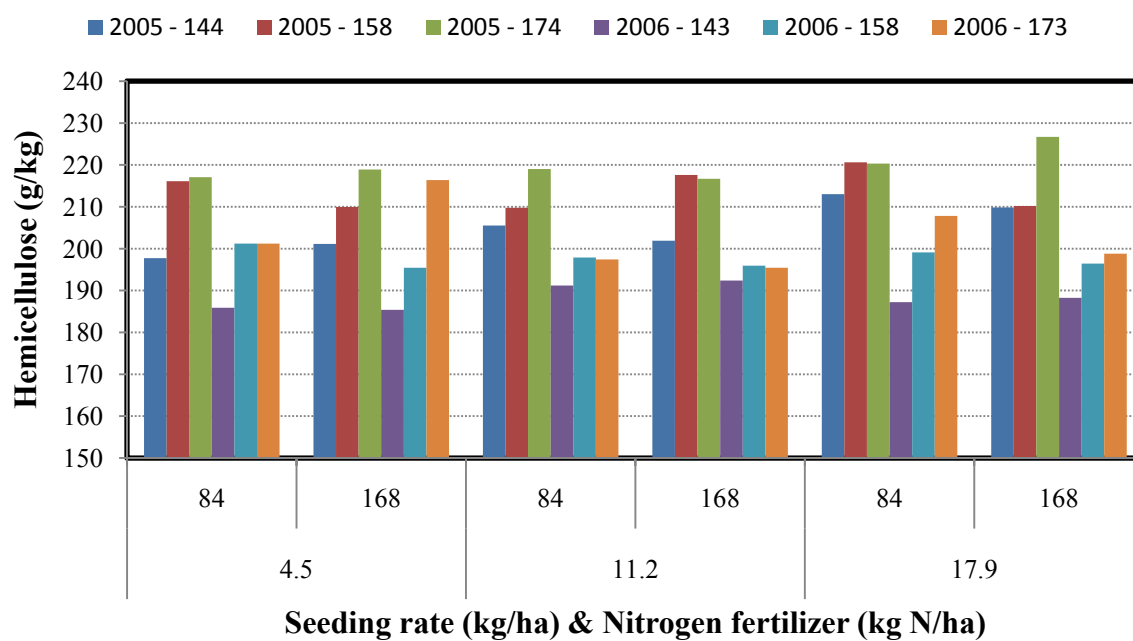


Figure 13. Effect of year and planting date on lignin concentration of sweet sorghum.

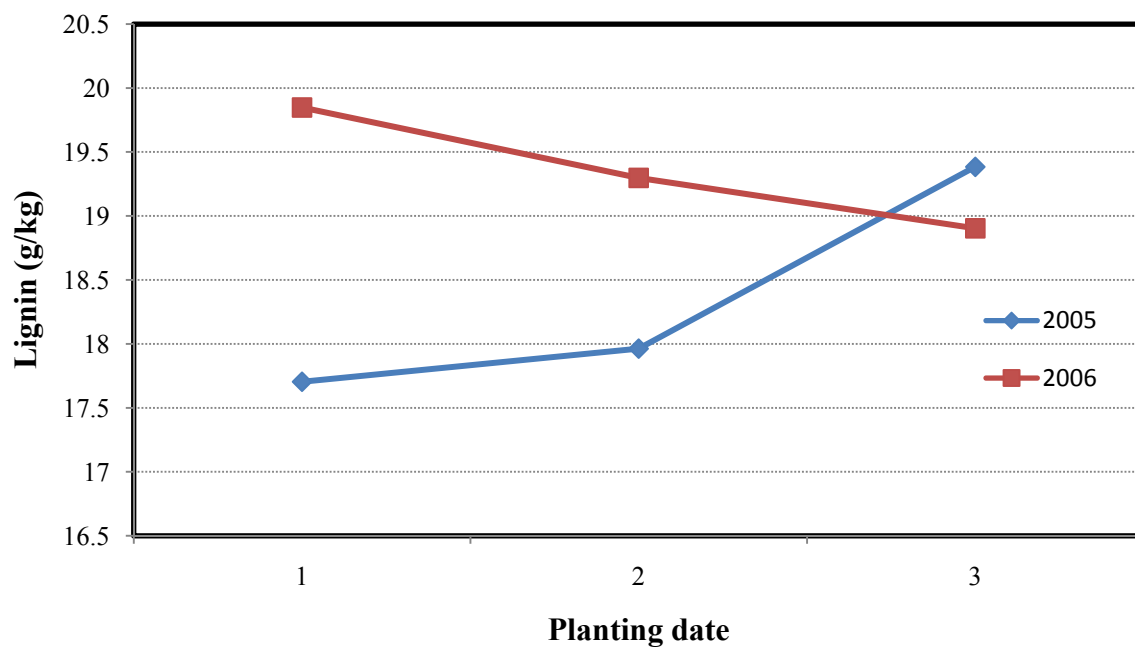


Figure 14. Effect of year and row width on lignin concentration of sweet sorghum.

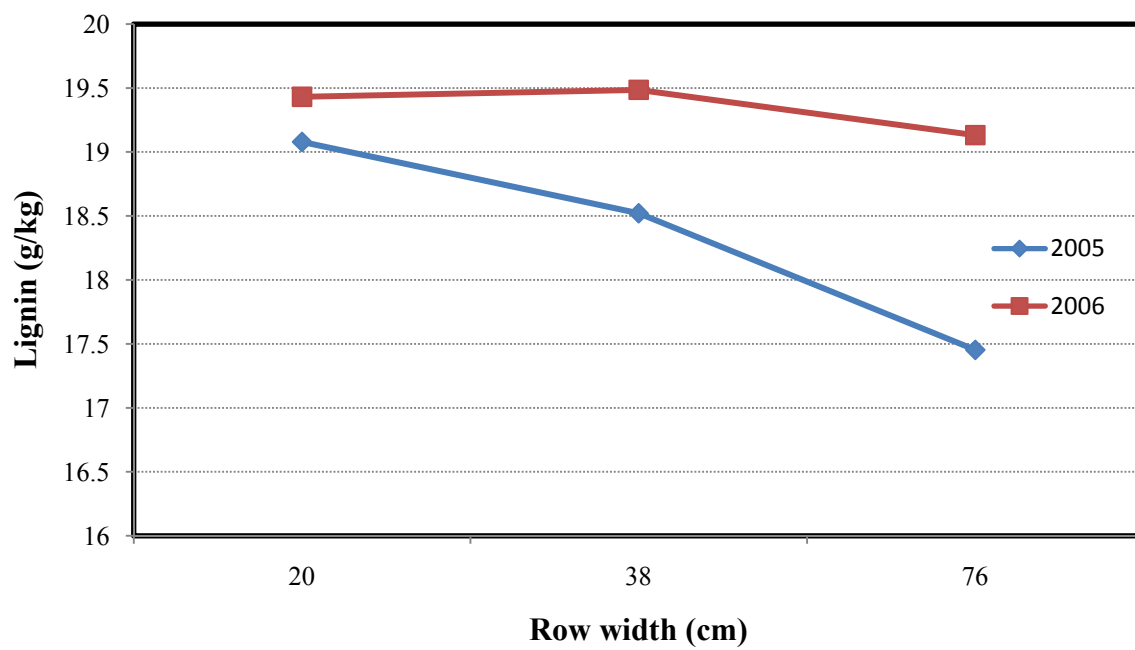
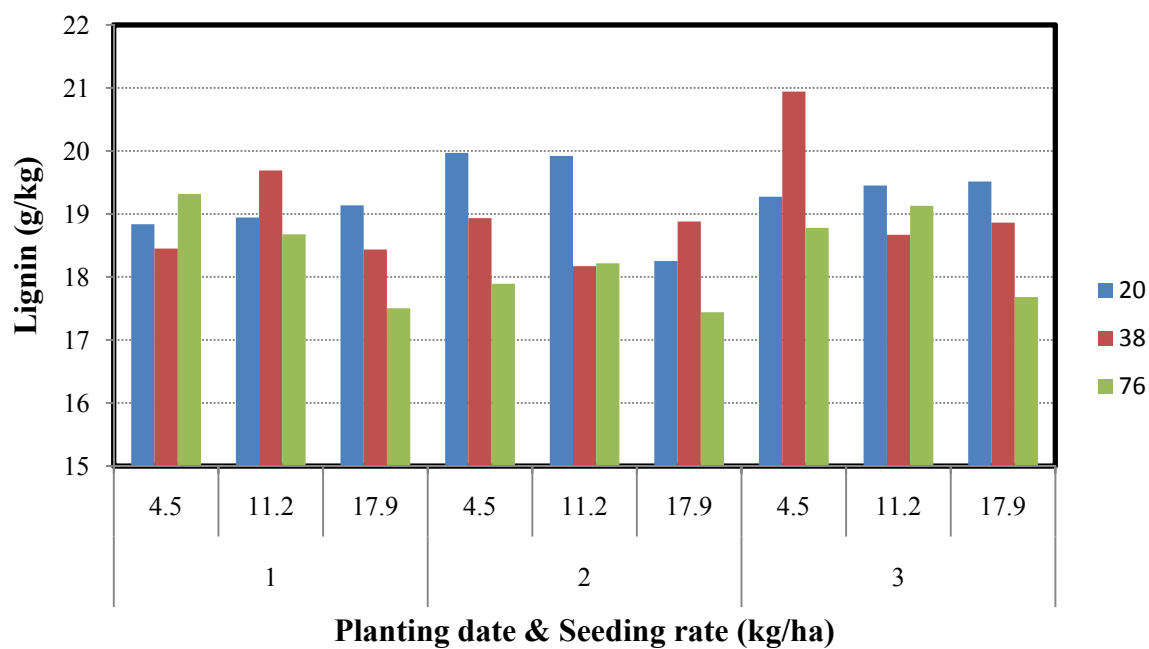


Figure 15. Effect of planting date, seeding rate, and row width on lignin concentration of sweet sorghum.



CHAPTER IV

Ensiling characteristics of pressed sweet sorghum residue

Abstract

Recent interest in biomass production systems for lignocellulosic ethanol has necessitated the evaluation of storage systems for vast quantities of feedstocks. The ensiling process has the potential to store large quantities for long periods while preserving the quality of the biomass. This study evaluated the chemical composition and fermentation characteristics of pressed sweet sorghum (*Sorghum bicolor* (L.) Moench) residue ensiled at moisture concentrations of 550, 650, and 750 g/kg for periods of 1, 7, and 21 days with additions of a fiber-degrading enzyme at concentrations of 0, 1, and 10 IU/g DM. Silage pH of all samples decline rapidly to below 4.0 within 7 days and was further reduced after 21 days. Water soluble carbohydrate concentration declined predominantly predominately during the first 7 days and were greater with increasing silage moisture concentration. Lactic acid concentration increased throughout the ensiling period reaching 31.2, 41.2, and 55.5 g/kg at moisture concentrations of 550, 650, and 750 g/kg, respectively. Acetic acid concentration increased throughout the ensiling period and was highest in samples ensiled at the 750 g/kg moisture concentration. Additions of fiber degrading enzymes provided additional carbohydrates for fermentation. At the highest enzyme concentration, cellulose and hemicellulose concentration was reduced by 13.9% and 7.8%, respectively.

Introduction

Interest in the production of ethanol from lignocellulosic sources has increased substantially in the past decade. Currently, production of ethanol from plant biomass is conducted in small-scale research facilities. When large-scale production will become an

economically viable industry still remains to be seen. Additionally, crops considered for biomass production such as sweet sorghum (*Sorghum bicolor* (L.) Moench) have limited availability during the year, which presents a problem. Large-scale production facilities will require large quantities of biomass feedstocks year-round for continual operation. Therefore, an efficient system for storage of feedstocks beyond harvest that will preserve biomass quality is necessary.

Long-term preservation of plant material for livestock feed via the ensiling process has been conducted for centuries (Bolsen, 1995). The process of ensiling (Bolsen, 1995; Jaster, 1995) and the biochemistry involved (McDonald et al., 1991) are well understood. In the earliest stage of ensiling, oxygen within the ensiled material is utilized by respiration producing the anaerobic environment necessary for fermentation and preservation (Bolsen, 1995). Once an anaerobic environment is achieved, lactic acid bacterial population increases and fermentation begins. Lactic acid bacteria convert soluble carbohydrates primarily into lactic acid, but acetic acid production is possible depending on the species of bacteria present (McDonald et al., 1991). If provided an adequate supply of soluble carbohydrates, rapid production of lactic acid will occur reducing silage pH and preventing deterioration of the silage.

Ensilage preservation of sweet sorghum has been previously studied (Caswell et al., 1983; Linden et al., 1987; Henk and Linden, 1992; Henk, 1996; Philipp et al., 2007). Philipp et al. (2007) found that varieties higher in water soluble carbohydrates produce more lactic acid and had lower pH. Additionally, adequate pH for long-term storage can be achieved within 7 days of ensiling (Philipp et al., 2007). In a study that compared the fermentation of corn, sweet sorghum, and grain sorghum it was concluded that sweet

sorghum produced the most lactic acid and achieved the lowest pH (Caswell et al., 1983). However, sweet sorghum had the highest dry matter and soluble carbohydrate losses during fermentation (Casswell et al., 1983). Linden et al. (1987) compared pressed and wilted sweet sorghum and found both reached pH below 4.0, which is considered adequate for preservation. Additionally, enzymatic hydrolysis of structural carbohydrates sufficiently replenished the sugars utilized for fermentation (Linden et al., 1987).

These studies suggest that preservation of sweet sorghum through the ensiling process is possible. The objective of this study was to determine chemical composition and fermentation potential of pressed sweet sorghum residue ensiled at three moisture concentrations for a period of 21 days with enzymatic pretreatments.

Materials and methods

Experiment Design

Sweet sorghum (*Sorghum bicolor* (L.) Moench) residue was harvested from field plots of a variety comparison study grown in 2005 to be utilized as the feedstock for ensilage. Plant material was harvested from each of the four replicated plots of the variety 'Top 76-6' and immediately pressed in the field using a sorghum press provided by the Iowa Energy Center (Ames, IA). The pressed material from each plot was chopped and immediately transported to the laboratory for silage preparation. Initial moisture concentration of the pressed material was calculated to be 550 g/kg by drying at 100°C in a forced air oven until weight stabilized.

Silage samples were prepared at 550, 650, and 750 g/kg moisture concentrations with enzyme concentrations of 0, 1, and 10 IU/g DM and incubated for periods of 1, 7, and 21 days. Deionized water was added to raise silage moisture concentration from 550 g/kg to

650 and 750 g/kg. A commercial enzyme preparation was utilized (MULTIFECT® A40, Danisco US Inc., Genencor Division, Palo Alto, CA) and had a cellulase:hemicellulase activity ratio of 2.54:1. Samples were vacuum-sealed in cryovac bags and incubated for the appropriate period at 37°C in a forced air heated chamber. All treatments of silage moisture, enzyme concentration, and incubation time were in factorial combination and replicated four times based on field replications. Control samples were prepared from nonpressed material without the addition of enzyme and ensiled for periods of 1, 7, and 21 days. Moisture concentration of the nonpressed material was 650 g/kg. Additionally, original material of both the pressed and nonpressed material was frozen on the day of preparation for later comparison with ensiled material.

Immediately following incubation subsamples were taken to measure pH and final dry matter of the ensiled material; the remaining material was frozen. The dried sample was used to determine water soluble carbohydrate concentrations and structural carbohydrate composition. Frozen material was utilized for lactic acid, volatile fatty acid, and hydrolyzed monomeric sugar concentration analyses.

Chemical Analysis

Sample pH was determined by a 10:1 (H₂O:sample) mass dilution and measured by a pH electrode after 30 minutes. Dried samples were ground in a Wiley mill (Thomas Scientific, Inc.) to pass through a 1-mm screen. Dry matter (DM) of the ground sample was determined by drying 1 g of sample in a forced air oven at 103°C for 4 hours. Water soluble carbohydrates (WSC) were determined using the procedure described by Murphy et al. (2007). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations were determined using the filter bag method (Vogel et al., 1999).

Hemicellulose concentrations were calculated as the difference between NDF and ADF.

Cellulose concentrations were calculated as the difference between ADF and ADL. Lignin is reported on an ash-free basis. Dry matter values were used to calculate water soluble carbohydrate and structural carbohydrate concentrations on a dry matter basis.

Concentrations of the hydrolyzed monomers arabinose, galactose, glucose, xylose, and mannose were quantified by high performance liquid chromatography (HPLC). Monomeric sugars were extracted in 10:1 (H₂O:sample) mass dilution agitated for a period of 30 minutes at 155 rpm. Extracts were filtered through four layers of cheese cloth. An aliquot of the filtered extract was centrifuged for 15 minutes at 9,000 rpm. A subsample of the centrifuged extract was filtered through a 0.45 µm membrane filter and subsequently diluted to a 1:200 (sample:H₂O) concentration. Monomers were separated by anion exchange chromatography in a Dionex CarboPac PA20 column (Dionex, Inc., Sunnyvale, CA) and quantified by electrochemical detection. The eluent utilized was 0.2 mmol NaOH at a flow rate of 0.45 ml/min. High purity standards of each monomer were utilized to establish a four-point calibration.

Statistical Analysis

When pressed sorghum residue was prepared for ensiling, treatments of silage moisture, enzyme concentration, and incubation time were in factorial combination and replicated four times. Effects of treatment factors and all their interactions on silage pH, organic acid concentration, water soluble carbohydrate concentration, structural carbohydrate composition, and concentration of hydrolyzed monomeric carbohydrates were analyzed using the generalized linear model (GLM) of the Statistical Analysis Systems software (SAS, 2003) and the least significant difference (LSD) test. Differences were considered significant

at $P \leq 0.05$. All treatment factors were considered fixed effects except for replication, which was a random effect. The original pressed material is utilized as an initial reference point for comparison to the pressed ensiled material but was not included in statistical analyses. Nonpressed control samples were evaluated for the effect incubation time had on all characteristics measured. Samples were replicated four times and analyzed using the generalized linear model (GLM) of the Statistical Analysis Systems software (SAS, 2003) and the least significant difference (LSD) test. Differences were considered significant at $P \leq 0.05$. Incubation time was considered a fixed effect and replication was a random effect.

Results

Composition of pressed and nonpressed control samples

The composition of control samples can be found in Table 1. The original pressed material utilized for silage preparation is included as a reference point for comparison to the pressed ensiled material. Nonpressed control samples showed a response to incubation time for most characteristics measured. As expected for ensiled plant material, pH and WSC concentration decreased while concentrations of lactic acid and acetic acid increased. Cellulose and lignin concentrations appeared to increase slightly over time but were likely an artifact of the reduction in WSC concentration. The concentration of the carbohydrate monomers arabinose, galactose, and xylose increased over the incubation period while glucose and mannose concentrations were unaffected. The increase in these specific monomers was likely due to hydrolysis of hemicellulose as pH decreased (McDonald et al., 1991).

Fermentation

Silage pH responded to silage moisture and incubation time in a two-way interaction (Figure 1). Samples with moisture concentrations of 550 and 650 g/kg decreased in pH from 1 day of incubation to 7 days of incubation and pH remained stable out to 21 days of incubation. Samples with moisture concentration of 750 g/kg responded similarly except a small increase in pH was noted at 21 days of incubation. Nonpressed control samples decreased in pH similar to samples ensiled at the 650 g/kg moisture concentration. The pH was lower in higher moisture samples. However, pH for all samples was quickly reduced from pH above 5.0 to below 4.0 within seven days.

Water soluble carbohydrate (WSC) concentration, as the substrate for organic acid production, was affected by enzyme concentration treatments. Average WSC concentration increased with each increase in enzyme concentration. When compared to the treatment without enzyme additions average WSC concentration increased 6.8% and 11.2% at enzyme concentrations of 1 and 10 IU/g DM, respectively. Water soluble carbohydrate concentration also responded to silage moisture treatments and incubation time in a two-way interaction (Figure 2). The concentration of WSC decreased more rapidly at higher silage moisture concentration over the incubation period. Samples with moisture concentrations of 550 and 650 g/kg decreased slightly in WSC concentration from 1 day of incubation to 7 days of incubation and remained stable out to 21 days of incubation. Water soluble carbohydrate concentration decreased at each day tested when ensiled at the 750 g/kg moisture concentration. When compared to the initial composition of pressed material the ensiled material preserved 81%, 72%, and 55% of the WSC concentration after 21 days of incubation at moisture concentrations of 550, 650, and 750 g/kg, respectively. Nonpressed control

samples had higher initial WSC concentration but responded similarly to pressed samples ensiled at the 650 g/kg moisture concentration with 69% of the initial WSC concentration remaining.

Lactic acid was the primary organic acid produced in the ensiled sorghum residue. Lactic acid concentration increased with increased incubation time as well as increased silage moisture in a two-way interaction (Figure 3). Lactic acid concentrations after 21 days of incubation were 31.2, 41.2, and 55.5 g/kg at silage moisture concentrations of 550, 650, and 750 g/kg, respectively. Nonpressed samples also increased in lactic acid concentration across the entire incubation period, reaching a final concentration of 41.6 g/kg. As previously noted the nonpressed samples responded most similarly to samples ensiled at the 650 g/kg moisture concentration.

Acetic acid was produced to a lesser extent than lactic acid but was present in significant concentrations suggesting heterofermentative lactic acid bacteria were present (McDonald et al., 1991). Acetic acid concentration responded to silage moisture (Figure 4) and incubation time (Figures 5). Acetic acid concentration increased with increased silage moisture. Average acetic acid concentration was 7.0, 11.1, and 15.5 g/kg at silage moisture concentrations of 550, 650, and 750 g/kg, respectively. Additionally, acetic acid concentration increased with longer incubation time. Average acetic acid concentration was 9.3, 11.4, and 12.8 g/kg at 1, 7, and 21 days of incubation, respectively.

Propionic acid was present but represented only a small fraction of the total organic acids produced. The concentration of propionic acid responded to incubation time (Figure 6) and enzyme concentration (Figures 7). Propionic acid concentration increased after 21 days of incubation and at each increase in enzyme concentration. However, propionic

concentration of an individual sample never exceeded 5.0 g/kg. Detection of small quantities of propionic acid is normal even in well-preserved silage (McDonald et al., 1991).

Chemical composition

Cellulose concentration responded to enzyme concentration and silage moisture treatments in a two-way interaction (Table 2). Cellulose concentration decreased at each higher enzyme concentration regardless of silage moisture concentration. The response in cellulose concentration to silage moisture treatments differed between enzyme concentration treatments. When no enzyme was added (0 IU/g DM), cellulose concentration was similar between all moisture concentrations tested with a range of 300 to 303 g/kg. When the enzyme was added at a rate of 1 IU/g DM cellulose concentration decreased 1.7%, 5.9%, and 3.3% at each respectively higher moisture concentration. At a concentration of 10 IU/g DM, the decrease in cellulose concentration was even higher. When applied at 10 IU/g DM the enzyme caused reductions in cellulose concentration of 6.0%, 9.9%, and 13.9% at moisture concentrations of 550, 650, and 750 g/kg, respectively.

Hemicellulose concentration responded to both silage moisture treatments and enzyme concentration treatments (Table 2). Hemicellulose concentration differed between silage moisture treatments. Samples with moisture concentrations of 550 and 750 g/kg had similar hemicellulose concentrations of 224 and 222 g/kg, respectively. Hemicellulose concentration was lower in samples with moisture concentrations of 650 g/kg at 218 g/kg. In addition, hemicellulose concentration decreased with each increase in enzyme concentration. When compared to the treatment without the enzyme hemicellulose concentration was reduced by 3.0% and 7.8% at enzyme concentrations of 1 and 10 IU/g DM, respectively.

Lignin concentration responded to incubation time and the two-way interaction of silage moisture and enzyme concentration treatments (Table 2). Average lignin concentration increased from 1 day of incubation to 7 days of incubation and remained stable out to 21 days of incubation. Increased lignin concentration was likely due to reduced WSC concentration. The response in lignin concentration to changes in enzyme concentration differed between silage moisture treatments. Lignin concentration of samples at a moisture concentration of 550 g/kg was not affected by changes in enzyme concentration. Lignin concentration of samples at moisture concentrations of 650 and 750 g/kg decreased with addition of enzyme. This interaction cannot be explained, as fiber-degrading enzymes should not affect lignin concentration.

Hydrolyzed monomeric carbohydrates

The concentrations of arabinose, galactose, glucose, xylose, and mannose responded to silage moisture, enzyme concentration, and incubation time (Table 3). Arabinose, galactose, and mannose were found in the smallest concentrations, never exceeding 5 g/kg. Xylose was found in the second highest concentration, at 11.5 g/kg after 21 days of incubation at the highest enzyme concentration averaged over silage moistures. Glucose concentration was the highest of all carbohydrates evaluated. Xylose and glucose were expected to be the present in the highest concentrations of the sugars evaluated. Xylose is the predominant carbohydrate in sorghum hemicellulose (Nandra et al., 1983) and cellulose is polymer of exclusively glucose monomers (Ren, 2006). As silage moisture increased so did the concentration of the carbohydrates evaluated with the exception of mannose, which was not affected by moisture concentration. All sugars evaluated increased in concentration as the concentration of enzymes increased. Hydrolytic activity over the incubation period

was apparent as concentrations of the sugars increased. Concentration of arabinose, galactose, and xylose increased over the incubation period due to hydrolytic activity while mannose content remained unchanged. When compared to the pressed control, glucose concentration increased from 80.9 to 88.5 g/kg after one day of incubation. Thereafter, glucose concentration decreased but remained higher than the initial concentration of the pressed control at 81.7 g/kg after 21 days of incubation. The decrease in glucose concentration later in the incubation period was likely due to inactivity of the enzyme at low pH and continued utilization of glucose in lactic acid fermentation.

Discussion

The results of this study show that rapid reduction in silage pH is achieved regardless of silage moisture concentration. The rapid reduction in silage pH was achieved due to the high water soluble carbohydrate concentration available for lactic acid production. However, ensiling at lower moisture concentration is advantageous for the preservation of initial WSC concentration. Preservation of WSC concentration decreased as silage moisture concentration increased. Samples ensiled at the 550 g/kg moisture concentration preserved the most carbohydrates, containing 81% of the initial WSC concentration after 21 days of incubation. Lactic acid production was still sufficient for rapid reduction in silage pH and acetic acid production lowest in samples ensiled at a moisture concentration of 550 g/kg. Additions of the enzyme caused increased WSC concentration through the hydrolysis of cellulose and hemicellulose. Hydrolytic activity of the enzyme ceased early in the incubation period likely due to increasingly acidic conditions.

Silage pH is an important factor in the long-term stability of ensiled plant material. A pH value below 4.0 is considered satisfactory for long-term storage of ensiled material

(Jaster, 1995). Low silage pH prohibits the deleterious activity of entobacteria and clostridia and promotes chemical hydrolysis of hemicellulose (McDonald et al., 1991). Similar reductions in silage pH to those in this experiment were noted in previous experiments that utilized ensiling for preservation of sweet sorghum (Caswell et al., 1983; Linden et al., 1987; Henk and Linden, 1992; Henk, 1996; Philipp et al., 2007).

Additionally, Philipp et al. (2007) noted similar reductions in WSC concentration as this experiment when comparing numerous varieties of sorghum over a 21-day incubation period. Caswell et al. (1983) ensiled sweet sorghum at a moisture concentration of 740 g/kg for a period of 31 days and noted that WSC concentration was reduced by 57.5%. Likewise, Linden et al. (1987) ensiled pressed sweet sorghum at a moisture concentration of 660 g/kg and after 155 days of ensiling, 65% of the initial fermentable carbohydrate concentration was preserved. The findings of Caswell et al. (1983) and Linden et al. (1987) are similar to the results of this experiment in respect to preservation of WSC concentration at higher silage moisture concentrations.

Hydrolysis of cellulose and hemicellulose in ensiled sweet sorghum was studied in an experiment conducted by Henk and Linden (1992). Similar concentrations of an enzyme with both cellulase and hemicellulase activity was utilized as was in this experiment. Similar to the findings of this experiment, hydrolysis of both cellulose and hemicellulose was increased at higher enzyme concentrations. However, the amount of cellulose hydrolyzed was significantly higher in the study by Henk and Linden (1992) and actually caused an increase in available carbohydrates over the period ensiled.

In conclusion, the process of ensiling can achieve preservation of the quality of sweet sorghum biomass for subsequent lignocellulosic ethanol production. However, moisture

concentration of ensiled sweet sorghum is important for carbohydrate preservation. Higher moisture silages fermented excessive amounts of soluble carbohydrates reducing quality. Additionally, the enzymes added were effective at hydrolyzing cellulose and hemicellulose but were not effective at maintaining soluble carbohydrate concentration over the incubation period. Therefore, moisture concentration must be low enough to preserve carbohydrates to be utilized in subsequent lignocellulosic ethanol production while still reaching the necessary acidic environment for preservation. This was demonstrated by samples ensiled at the 550 g/kg moisture concentration.

References

- Bolsen, K.K. 1995. Silage: Basic principles. P. 163-176. *In* R.F. Barnes, D.A. Miller, and C.J. Nelson (eds.), Forage Vol. II, The science of grassland agriculture, 5th ed. Iowa State University Press, Ames, IA.
- Caswell, L.F., R.S. Kalmbacher, and F.G. Martin. 1983. Yield and silage fermentation characteristics of corn, sweet sorghum, and grain sorghum. *Proceedings – Soil and crop science society of Florida* 42:139-142.
- Henk, L.L. 1996. Processing sorghum silage to fuel alcohol. Ph.D. dissertation, Colorado State University.
- Henk, L.L., and J.C. Linden. 1992. Simultaneous ensiling and enzymatic hydrolysis of structural polysaccharides. *Enzyme and Microbial Technology* 14:923-930.
- Jaster, E.H. 1995. Legume and grass silage preservation. P. 91-115. *In* K.J. Moore and M.A. Peterson (eds.), Post-harvest physiology and preservation of forages. CSSA-ASA, Madison, WI.
- Linden, J.C., L.L. Henk, V.G. Murphy, D.H. Smith, B.C. Gabrielsen, R.P. Tengerdy, and L. Czako. 1987. Preservation of potential fermentables in sweet sorghum by ensiling. *Biotechnology and Bioengineering* 30:860-867.
- McDonald, P., A.R. Henderson, and S.J.E. Heron. 1991. The biochemistry of silage, 2nd edition. Chalcombe Publications, Marlow, Buckinghamshire, UK.
- Murphy, P.T., K.J. Moore, T.L. Richard, and C.J. Bern. 2007. Enzyme enhanced solid-state fermentation of kenaf core fiber. *Bioresource Technology* 98:3106-3111.
- Nandra, K.S., B.K. Gupta, and A.K. Chopra. 1983. The effect of stage of maturity on the digestion of hemicelluloses of sorghum (*Sorghum bicolor*). *Journal of the Science of Food and Agriculture* 34:962-964.
- Philip, D., K.J. Moore, J.F. Pedersen, R.J. Grant, D.D. Redfearn, and R.B. Mitchell. 2007. Ensilage performance of sorghum hybrids varying in extractable sugars. *Biomass and Bioenergy* 31:492-496.
- Ren, H. 2006. Effect of cell wall degrading enzymes and chemicals on corn stover preservation and pretreatment during ensilage processing. Ph.D. dissertation, Pennsylvania State University.
- SAS. 2003. SAS User's Guide. Version 9.1. SAS Institute Inc., Cary, NC.

Vogel, K.P., J.F. Pedersen, S.D. Masterson, and J.J. Troy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. *Crop Science* 39:276-279.

Table 1. Chemical composition of pressed and nonpressed control samples.

Days *	Pressed	Nonpressed			
	0	0	1	7	21
pH	5.47	5.20 [‡] _a	3.96 _b	3.72 _b	3.71 _b
WSC ^{†±}	226.7	263.0 _a	226.4 _b	200.2 _{bc}	181.6 _c
Cel	275.2	251.0 _c	252.8 _{bc}	267.6 _{ab}	271.3 _a
Hemi	216.4	192.9 _a	192.1 _a	205.2 _a	205.4 _a
Lig	23.2	20.9 _b	22.3 _b	24.8 _a	25.2 _a
Lac	11.42	4.72 _c	22.82 _b	39.62 _a	41.60 _a
Ace	0.57	1.60 _b	9.66 _a	12.79 _a	14.43 _a
Prop	0.86	1.70 _a	0.00 _b	0.36 _{ab}	0.00 _b
Ara	0.52	0.56 _b	0.88 _b	0.84 _b	1.38 _a
Gal	1.80	1.69 _c	2.00 _b	2.93 _a	2.74 _a
Glu	80.86	98.52 _a	92.90 _a	95.37 _a	84.82 _a
Xyl	1.16	1.31 _b	1.52 _b	3.54 _a	3.23 _a
Man	1.37	1.30 _a	0.76 _a	1.26 _a	0.64 _a

* Number of days ensiled.

† Chemical constituents abbreviated as follows: Water soluble carbohydrates (WSC), Cellulose (Cell), Hemicellulose (Hemi), Lignin (Lig), Lactic acid (Lac), Acetic acid (Ace), Propionic acid (Prop), Arabinose (Ara), Galactose (Gal), Glucose (Glu), Xylose (Xyl), and Mannose (Man).

± Chemical constituent values are expressed in units of g/kg.

‡ Means for a particular chemical constituent with different letters are significantly different ($P < 0.05$).

Figure 1. Effect of incubation time and silage moisture on pH of sweet sorghum silage.

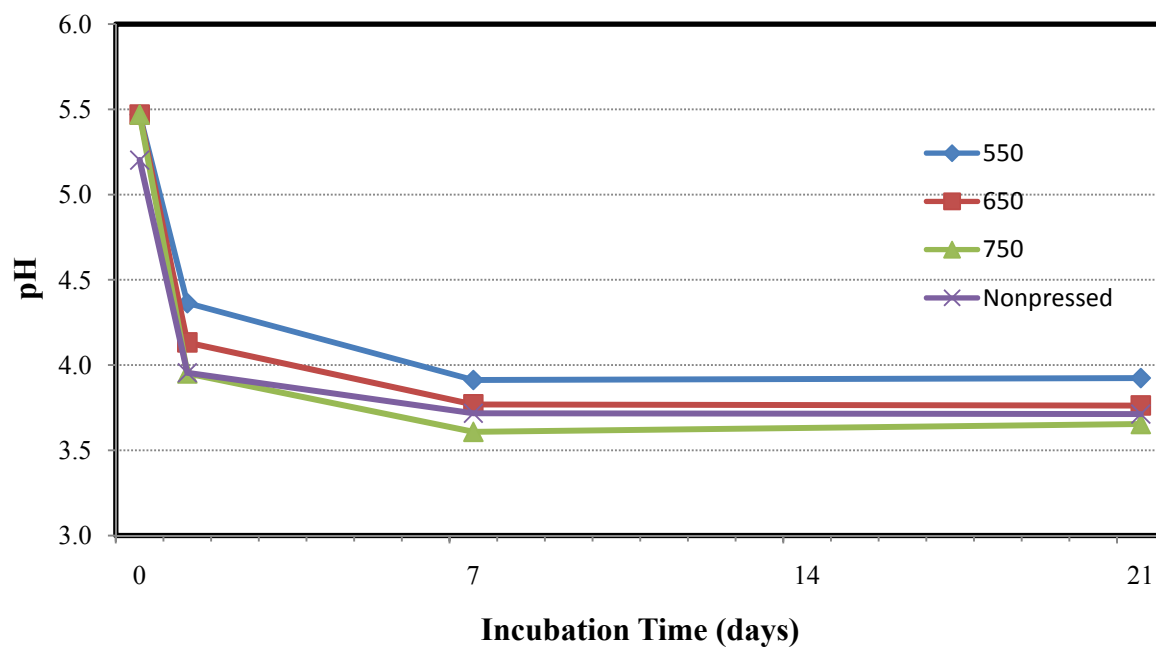


Figure 2. Effect of incubation time and silage moisture on water soluble carbohydrate concentration of sweet sorghum silage.

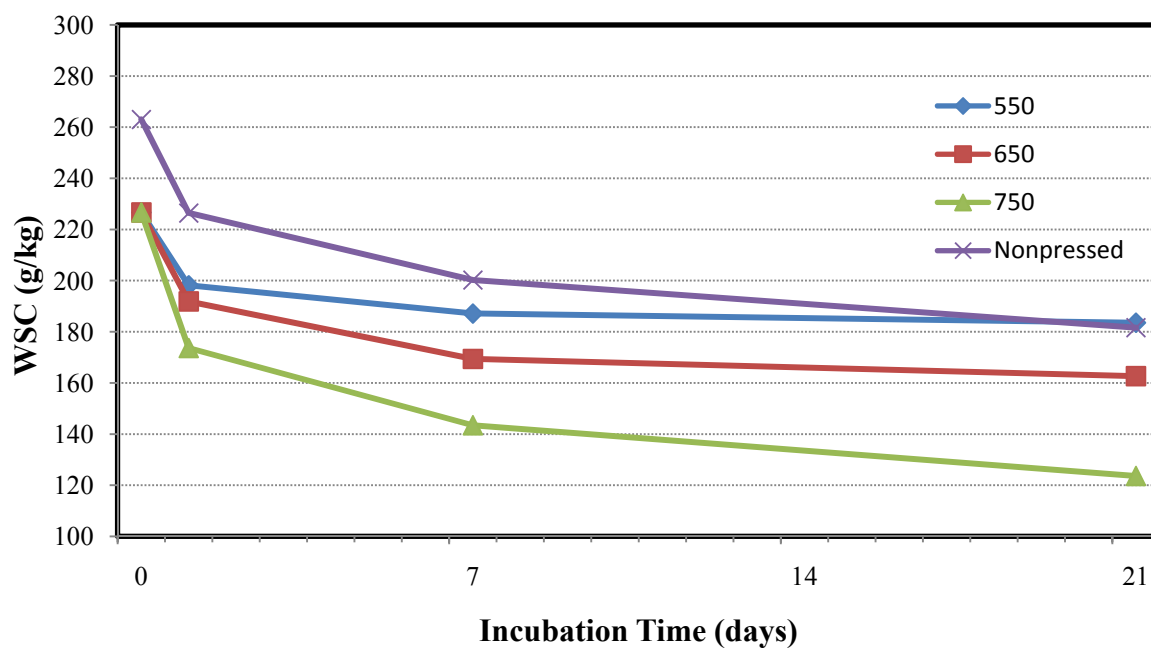


Figure 3. Effect of incubation time and silage moisture on lactic acid concentration of sweet sorghum silage.

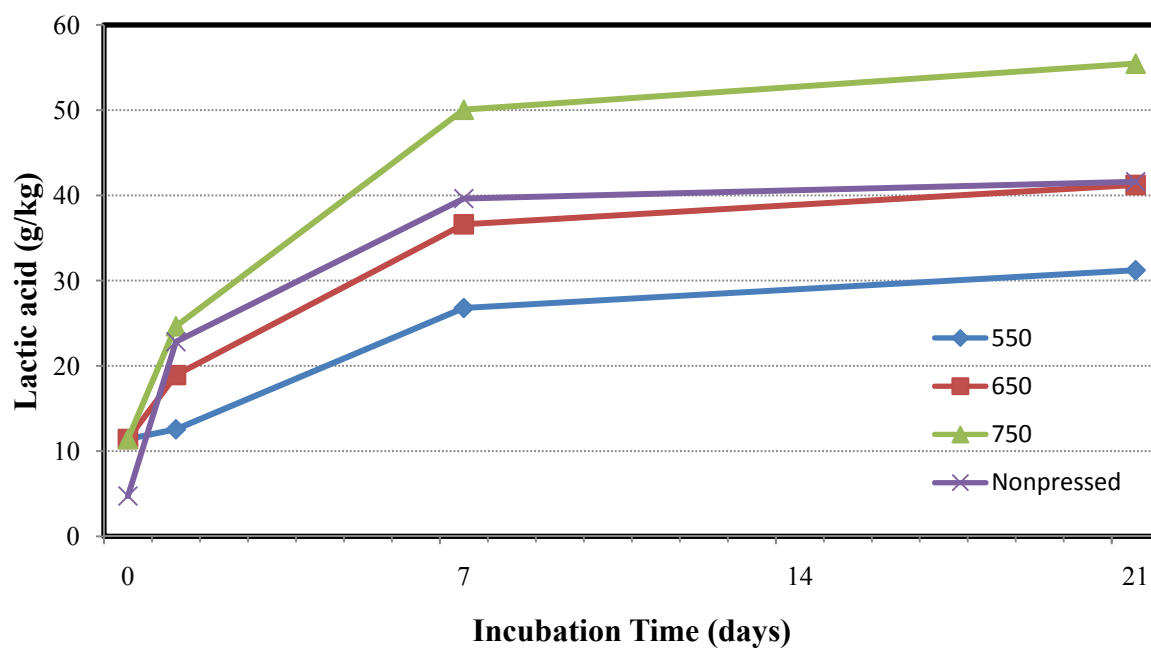


Figure 4. Effect of silage moisture on acetic acid concentration of sweet sorghum silage.

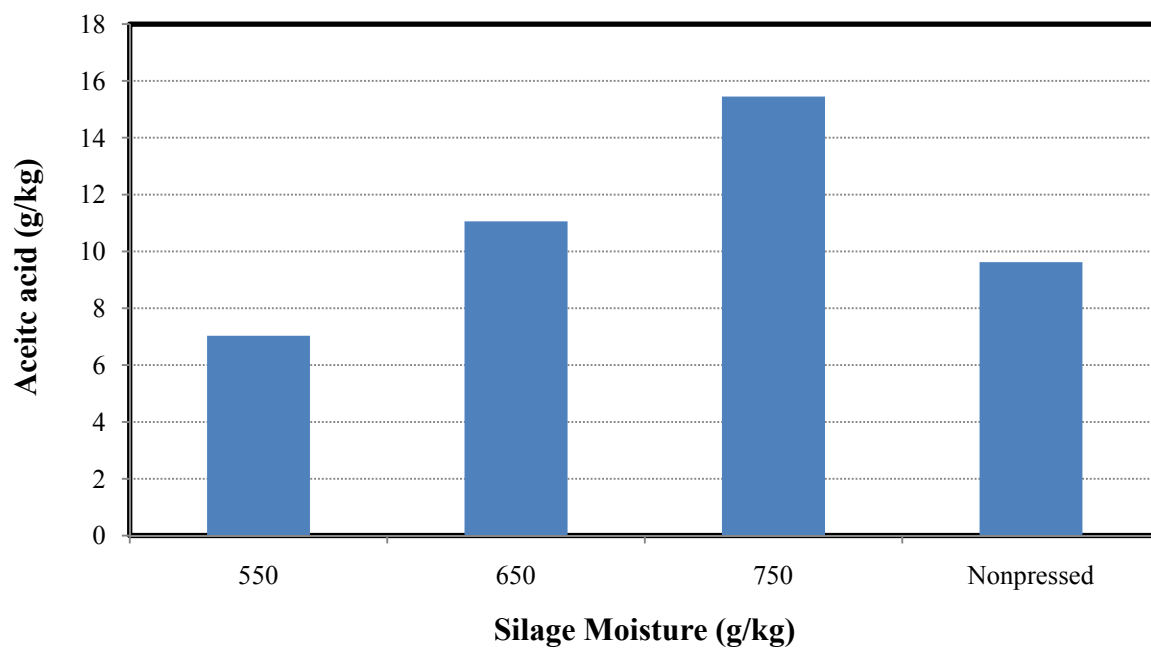


Figure 5. Effect of incubation time on acetic acid of sweet sorghum silage.

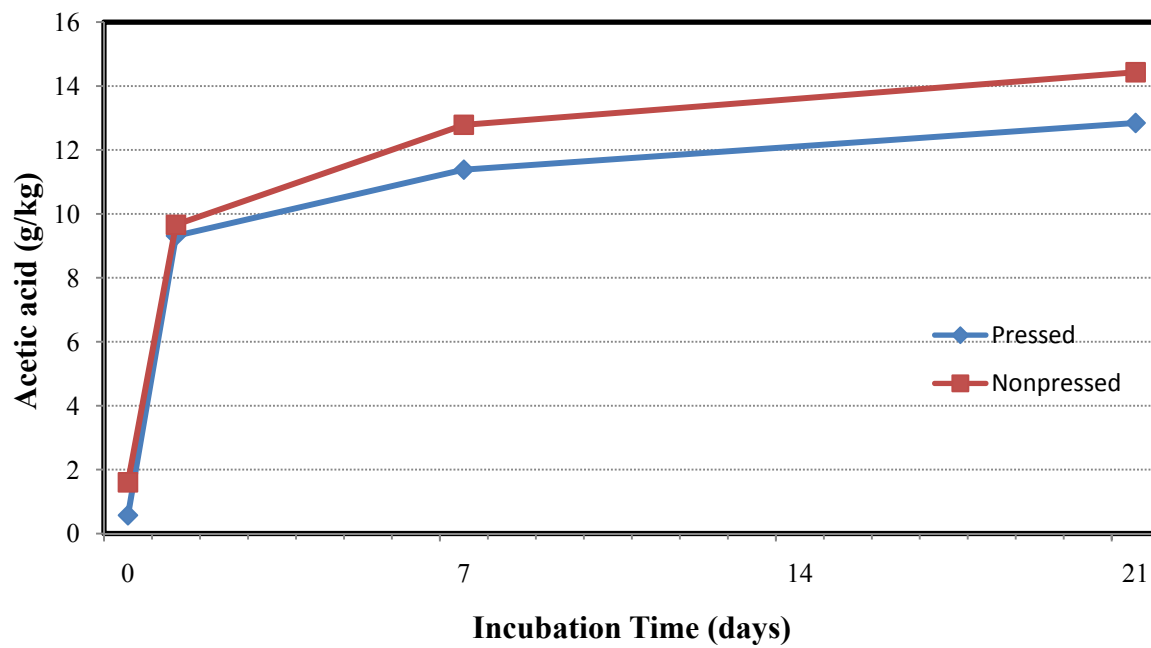


Figure 6. Effect of incubation time on propionic acid concentration of sweet sorghum silage.

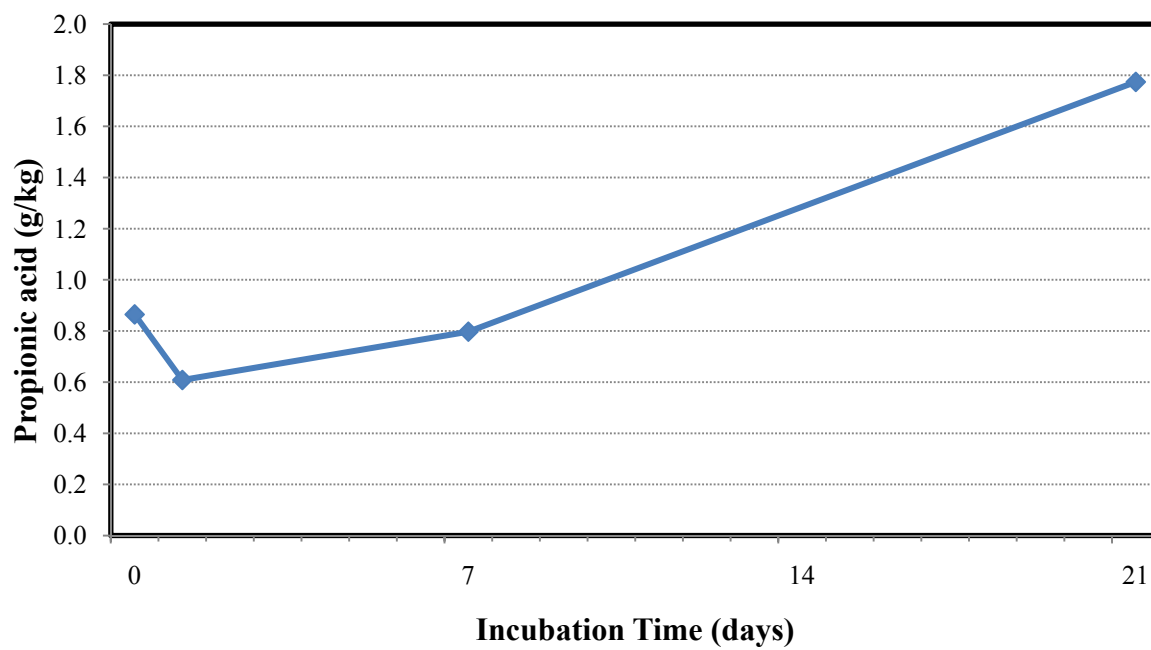


Figure 7. Effect of enzyme concentration on propionic acid concentration of sweet sorghum silage.

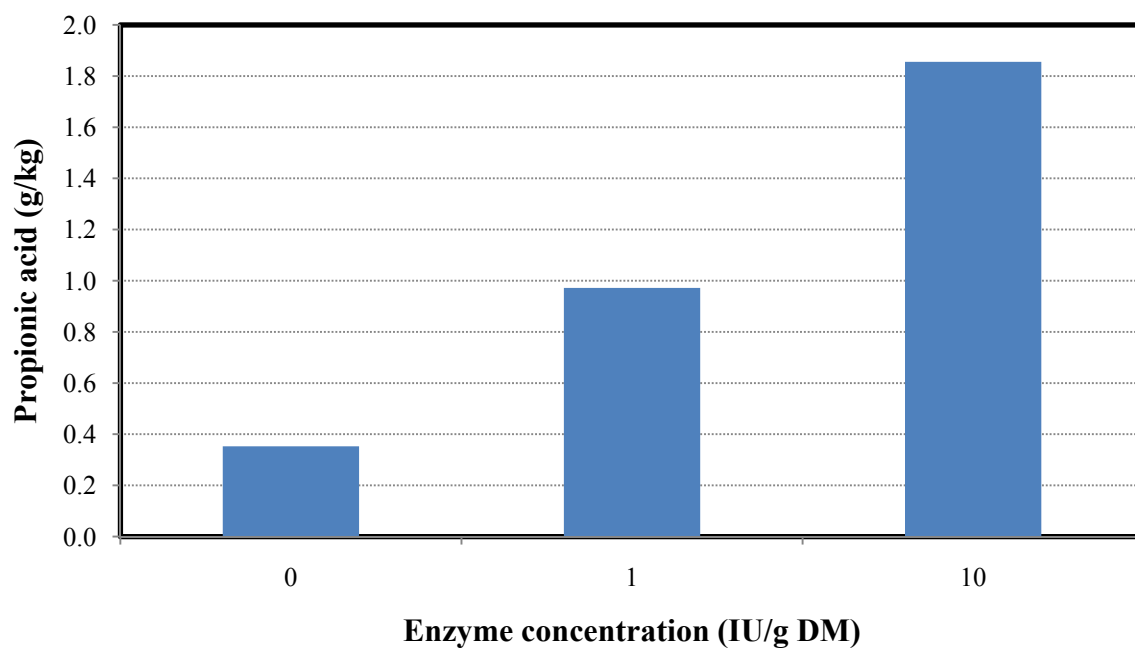


Table 2. Effect of silage moisture, enzyme level, and incubation time on the chemical constituents of pressed sweet sorghum silage.

Silage Moisture (g/kg)	Enzyme Level (IU/g DM)	Incubation Time (days)	WSC* (g/kg)	Cell (g/kg)	Hemi (g/kg)	Lig (g/kg)
550	0	1	200.5 [†] _a	293.6 _b	225.0 _b	24.8 _b
		7	186.5 _b	302.5 _a	233.3 _a	27.1 _a
		21	171.2 _c	303.5 _a	232.6 _a	26.8 _a
	1	1	197.6 _a	292.1 _a	224.7 _a	25.3 _b
		7	186.6 _b	295.8 _a	223.5 _a	27.1 _a
		21	194.9 _a	296.1 _a	224.4 _a	27.8 _a
	10	1	196.4 _a	282.0 _{ab}	213.9 _b	25.5 _b
		7	188.3 _b	286.4 _a	219.3 _a	27.6 _a
		21	184.5 _b	278.7 _b	216.7 _{ab}	27.0 _a
	650	1	178.7 _a	306.5 _a	228.6 _a	27.1 _b
		7	159.6 _b	302.2 _{ab}	227.7 _a	28.5 _a
		21	154.7 _b	300.1 _b	222.7 _b	27.3 _b
	1	1	195.9 _a	276.5 _c	214.0 _b	23.5 _b
		7	164.4 _b	292.0 _a	219.6 _a	28.1 _a
		21	157.4 _c	286.7 _b	217.9 _a	27.8 _a
	10	1	201.0 _a	277.6 _a	213.2 _b	25.6 _b
		7	184.0 _b	274.5 _a	207.1 _a	27.9 _a
		21	175.8 _c	268.0 _b	207.3 _a	26.3 _b
	750	1	164.2 _a	294.9 _c	227.0 _b	25.3 _c
		7	118.9 _b	311.4 _a	236.2 _a	28.4 _a
		21	111.0 _c	301.2 _b	234.3 _a	26.7 _b
	1	1	176.0 _a	288.7 _b	221.0 _c	25.9 _b
		7	154.7 _b	285.9 _b	224.9 _b	26.4 _b
		21	119.2 _c	303.5 _a	232.8 _a	31.0 _a
	10	1	180.7 _a	263.2 _a	206.0 _b	23.8 _b
		7	156.6 _b	257.6 _b	211.7 _a	24.3 _b
		21	140.6 _c	262.4 _{ab}	208.6 _{ab}	26.7 _a

* Chemical constituents abbreviated as follows: Water soluble carbohydrates (WSC), Cellulose (Cell), Hemicellulose (Hemi), and Lignin (Lig).

† Means in the same column within an enzyme treatment with different letters are significantly different ($P < 0.05$).

Table 3. Effect of silage moisture, enzyme level, and incubation time on the monomeric sugar composition of pressed sweet sorghum silage.

Silage Moisture (g/kg)	Enzyme Level (IU/g DM)	Incubation Time (days)	Ara*† (g/kg)	Gal (g/kg)	Glu (g/kg)	Xyl (g/kg)	Man (g/kg)
550	0	1	0.91 _{ab}	2.29 _b	78.77 _a	1.45 _b	0.48 _b
		7	0.81 _b	2.31 _b	63.52 _b	2.73 _a	1.00 _a
		21	1.27 _a	2.71 _a	67.37 _b	2.80 _a	0.42 _b
	1	1	1.53 _b	2.55 _b	74.90 _a	2.09 _c	0.92 _b
		7	1.63 _b	2.80 _b	68.92 _b	4.33 _b	1.64 _a
		21	2.47 _a	3.34 _a	74.94 _a	5.38 _a	0.54 _b
	10	1	2.79 _c	2.72 _c	82.73 _a	3.34 _c	1.39 _b
		7	3.35 _b	3.45 _b	71.90 _c	7.05 _b	1.95 _a
		21	4.65 _a	4.15 _a	78.27 _b	9.10 _a	1.21 _b
650	0	1	0.99 _b	2.77 _c	86.15 _a	2.16 _c	1.14 _a
		7	1.49 _a	3.25 _b	83.60 _{ab}	3.08 _b	0.97 _a
		21	1.81 _a	3.99 _a	80.02 _b	3.99 _a	0.68 _b
	1	1	0.98 _b	3.04 _c	86.40 _a	2.91 _c	1.25 _a
		7	3.52 _a	3.70 _b	79.41 _{ab}	5.31 _b	1.09 _a
		21	3.38 _a	5.03 _a	78.90 _b	7.59 _a	1.10 _a
	10	1	1.81 _c	2.94 _c	89.46 _a	4.07 _c	1.65 _b
		7	3.13 _b	3.94 _b	87.40 _a	8.90 _b	2.29 _a
		21	4.38 _a	4.60 _a	90.77 _a	11.59 _a	2.02 _{ab}
750	0	1	0.70 _c	2.87 _c	95.66 _a	2.50 _c	1.11 _a
		7	2.11 _b	3.66 _a	85.48 _b	3.35 _b	0.64 _b
		21	3.55 _a	3.32 _b	82.18 _b	4.05 _a	0.36 _b
	1	1	1.06 _c	2.97 _c	101.24 _a	3.39 _c	1.00 _b
		7	1.89 _b	3.60 _b	96.13 _b	5.86 _b	0.94 _b
		21	3.00 _a	4.77 _a	82.83 _c	8.40 _a	1.63 _a
	10	1	1.58 _c	3.26 _c	101.36 _b	5.17 _c	1.29 _b
		7	2.51 _b	4.23 _b	106.42 _a	9.60 _b	1.14 _b
		21	4.61 _a	5.49 _a	99.80 _b	13.79 _a	1.99 _a

* Monomeric sugars abbreviated as follows: Arabinose (Ara), Galactose (Gal), Glucose (Glu), Xylose (Xyl), and Mannose (Man).

† Means in the same column within an enzyme treatment with different letters are significantly different ($P < 0.05$).

CHAPTER V

Summary

The first experiment was a management study conducted in 2005 and 2006. It focused on determining how planting date, row width, seeding rate, and nitrogen fertility would affect the growth, biomass yield, and quality of sweet sorghum grown as a bioenergy crop in Iowa. The results of this study suggest that sweet sorghum should be planted in late May or early June at a 20-cm row width to maximize biomass production. Seeding rate and nitrogen fertility did not affect biomass production and lowest rates evaluated would be recommended. Management factors tested did have marginal effects on biomass quality but the substantial increases in total biomass yield due to early planting and narrow row width would likely dominate these effects during lignocellulosic ethanol production. The results of this study confirm that sweet sorghum managed as a bioenergy crop can produce substantial biomass yields in Iowa that are competitive with other potential bioenergy crops.

The second experiment was an ensiling study conducted in 2005. It evaluated the fermentation of pressed sweet sorghum residue. Silage moisture concentration, incubation time, and enzyme treatments were utilized in this evaluation. Sweet sorghum silage, regardless of moisture concentration, had adequate soluble carbohydrates necessary for rapid lactic acid production. Silage pH was reduced below 4.0 within 7 days of ensiling, which is considered adequate for long-term preservation. However, higher moisture silage fermented more carbohydrates to organic acids. Additions of enzymes responsible for the breakdown of structural carbohydrates were effective at that task but were not at maintaining the soluble carbohydrate concentration of silage samples. The results of this study suggested that carbohydrate preservation was best achieved at the 550 g/kg moisture concentration.

APPENDIX

Table 1. Plant height measurement data of the management experiment.

Planting date	Row width (cm)	Seeding rate (kg/ha)	Nitrogen rate (kg N/ha)	Plant height (cm)						
				Measurement date (DOY)						
				163	177	191	212	226	240	254
1	20	4.5	84	11.89	34.33	96.99	224.41	259.81	303.00	
1	20	4.5	168	13.04	37.16	101.33	228.56	271.81	305.19	
1	20	11.2	84	12.04	35.85	105.49	229.96	270.25	302.44	
1	20	11.2	168	13.08	36.93	99.44	231.50	273.94	299.94	
1	20	17.9	84	13.31	35.73	102.58	221.03	253.50	279.94	
1	20	17.9	168	14.11	40.61	110.66	226.81	262.56	288.00	
1	38	4.5	84	12.23	33.43	89.09	223.33	274.81	304.94	
1	38	4.5	168	12.84	35.49	90.61	221.97	267.06	302.69	
1	38	11.2	84	12.29	35.56	89.78	217.47	263.25	301.56	
1	38	11.2	168	12.38	34.74	98.26	212.38	263.56	302.69	
1	38	17.9	84	13.40	37.97	102.90	216.34	255.63	292.25	
1	38	17.9	168	12.26	35.83	98.56	218.38	256.00	287.56	
1	76	4.5	84	12.01	32.87	85.30	214.28	257.75	286.50	
1	76	4.5	168	13.12	36.55	89.91	217.28	267.69	304.28	
1	76	11.2	84	13.64	36.98	90.58	215.74	256.50	295.25	
1	76	11.2	168	13.13	34.77	92.69	211.50	263.38	290.56	
1	76	17.9	84	12.18	33.78	86.02	208.38	245.56	284.88	
1	76	17.9	168	13.03	37.64	90.26	215.13	249.88	293.63	
2	20	4.5	84		13.09	39.52	116.72	178.70	233.97	273.81
2	20	4.5	168		11.71	40.56	116.40	171.36	240.23	286.40
2	20	11.2	84		12.89	42.72	131.03	186.52	243.15	290.39
2	20	11.2	168		12.13	44.19	124.33	186.41	234.38	289.08
2	20	17.9	84		12.86	49.94	139.05	165.74	233.95	274.53
2	20	17.9	168		12.33	48.36	127.51	182.63	237.35	275.95
2	38	4.5	84		12.77	40.66	82.26	139.68	204.83	235.26
2	38	4.5	168		12.46	39.29	95.34	145.91	221.59	253.41
2	38	11.2	84		12.32	43.36	99.74	156.80	219.38	255.19
2	38	11.2	168		12.51	43.65	95.30	148.30	211.11	253.70
2	38	17.9	84		12.13	43.24	107.63	151.86	218.99	242.79
2	38	17.9	168		11.28	41.18	93.40	147.89	190.15	241.08
2	76	4.5	84		12.95	42.44	94.49	150.27	219.60	253.14
2	76	4.5	168		12.14	43.31	103.45	161.89	222.04	265.11
2	76	11.2	84		11.81	32.43	83.00	142.41	207.10	243.11
2	76	11.2	168		12.81	45.11	92.63	144.46	212.04	251.59
2	76	17.9	84		13.16	44.73	105.81	156.75	215.92	244.03
2	76	17.9	168		13.36	46.16	98.49	149.32	215.99	240.86

Table 1. (continued)

Planting date	Row width (cm)	Seeding rate (kg/ha)	Nitrogen rate (kg N/ha)	Plant height (cm)						
				Measurement date (DOY)						
				163	177	191	212	226	240	254
3	20	4.5	84			13.83	66.22	120.13	201.08	224.58
3	20	4.5	168			14.13	70.94	120.01	207.37	241.91
3	20	11.2	84			16.39	85.05	154.71	217.32	244.36
3	20	11.2	168			15.71	90.62	152.34	218.14	245.91
3	20	17.9	84			15.97	94.30	145.55	178.18	232.49
3	20	17.9	168			15.12	93.06	148.29	212.85	241.44
3	38	4.5	84			14.86	93.75	161.06	222.96	254.71
3	38	4.5	168			15.65	86.01	156.68	219.91	252.54
3	38	11.2	84			15.15	96.21	165.05	230.68	244.94
3	38	11.2	168			15.72	95.75	174.40	231.93	250.92
3	38	17.9	84			15.28	95.21	154.19	213.34	234.49
3	38	17.9	168			14.78	102.35	155.22	211.62	229.61
3	76	4.5	84			14.30	78.89	142.22	218.28	246.23
3	76	4.5	168			14.78	85.09	144.48	205.16	239.67
3	76	11.2	84			15.20	93.47	158.54	212.35	233.66
3	76	11.2	168			18.27	94.77	155.81	209.51	236.48
3	76	17.9	84			15.73	95.21	146.50	217.24	247.81
3	76	17.9	168			14.75	95.34	149.53	207.24	229.48

Table 2. Stem diameter measurement data of the management experiment.

Planting date	Row width (cm)	Seeding rate (kg/ha)	Nitrogen rate (kg N/ha)	Stem diameter (cm)						
				Measurement date (DOY)						
				163	177	191	212	226	240	254
1	20	4.5	84	0.20	0.68	1.33	1.57	1.52	1.48	
1	20	4.5	168	0.20	0.69	1.36	1.56	1.52	1.51	
1	20	11.2	84	0.20	0.66	1.24	1.48	1.34	1.44	
1	20	11.2	168	0.20	0.69	1.34	1.53	1.49	1.48	
1	20	17.9	84	0.20	0.59	1.14	1.36	1.29	1.17	
1	20	17.9	168	0.20	0.66	1.18	1.28	1.33	1.36	
1	38	4.5	84	0.20	0.66	1.33	1.49	1.59	1.61	
1	38	4.5	168	0.20	0.64	1.34	1.51	1.56	1.51	
1	38	11.2	84	0.20	0.63	1.17	1.58	1.49	1.41	
1	38	11.2	168	0.20	0.61	1.24	1.48	1.48	1.41	
1	38	17.9	84	0.20	0.60	1.20	1.37	1.29	1.38	
1	38	17.9	168	0.20	0.60	1.11	1.37	1.35	1.36	
1	76	4.5	84	0.20	0.59	1.16	1.54	1.52	1.48	
1	76	4.5	168	0.20	0.63	1.25	1.61	1.62	1.62	
1	76	11.2	84	0.20	0.59	1.23	1.50	1.54	1.49	
1	76	11.2	168	0.20	0.58	1.15	1.51	1.57	1.48	
1	76	17.9	84	0.20	0.53	1.17	1.51	1.45	1.44	
1	76	17.9	168	0.20	0.60	1.19	1.43	1.48	1.51	
2	20	4.5	84		0.20	0.68	1.35	1.71	1.74	1.63
2	20	4.5	168		0.20	0.68	1.37	1.58	1.65	1.61
2	20	11.2	84		0.20	0.69	1.29	1.49	1.65	1.66
2	20	11.2	168		0.20	0.70	1.39	1.46	1.58	1.65
2	20	17.9	84		0.20	0.66	1.16	1.34	1.39	1.40
2	20	17.9	168		0.20	0.68	1.22	1.48	1.39	1.42
2	38	4.5	84		0.20	0.63	0.93	1.42	1.53	1.43
2	38	4.5	168		0.20	0.68	1.23	1.49	1.59	1.60
2	38	11.2	84		0.20	0.71	1.37	1.40	1.51	1.45
2	38	11.2	168		0.20	0.73	1.17	1.45	1.51	1.45
2	38	17.9	84		0.20	0.66	1.14	1.38	1.42	1.37
2	38	17.9	168		0.20	0.63	1.05	1.16	1.27	1.30
2	76	4.5	84		0.20	0.66	1.23	1.46	1.50	1.50
2	76	4.5	168		0.20	0.67	1.28	1.67	1.56	1.55
2	76	11.2	84		0.20	0.56	1.05	1.33	1.26	1.41
2	76	11.2	168		0.20	0.68	1.08	1.54	1.59	1.39
2	76	17.9	84		0.20	0.69	1.16	1.45	1.49	1.47
2	76	17.9	168		0.20	0.70	1.24	1.43	1.38	1.44

Table 2. (continued)

Planting date	Row width (cm)	Seeding rate (kg/ha)	Nitrogen rate (kg N/ha)	Stem diameter (cm)						
				Measurement date (DOY)						
				163	177	191	212	226	240	254
3	20	4.5	84			0.20	1.14	1.70	1.87	1.56
3	20	4.5	168			0.20	1.13	1.77	1.92	1.71
3	20	11.2	84			0.20	1.15	1.41	1.39	1.34
3	20	11.2	168			0.20	1.16	1.52	1.51	1.37
3	20	17.9	84			0.20	0.95	1.23	1.25	1.28
3	20	17.9	168			0.20	1.00	1.39	1.37	1.33
3	38	4.5	84			0.20	1.18	1.61	1.48	1.46
3	38	4.5	168			0.20	1.24	1.63	1.41	1.49
3	38	11.2	84			0.20	1.16	1.46	1.29	1.28
3	38	11.2	168			0.20	1.20	1.41	1.35	1.32
3	38	17.9	84			0.20	1.06	1.44	1.28	1.29
3	38	17.9	168			0.20	1.14	1.42	1.40	1.23
3	76	4.5	84			0.20	1.19	1.55	1.53	1.49
3	76	4.5	168			0.20	1.22	1.58	1.41	1.44
3	76	11.2	84			0.20	1.06	1.26	1.25	1.26
3	76	11.2	168			0.20	1.04	1.29	1.29	1.20
3	76	17.9	84			0.20	1.09	1.50	1.41	1.47
3	76	17.9	168			0.20	1.11	1.41	1.42	1.24

Table 3. Analysis of variance for measurement data of the management experiment.

Source†	df	Mean square‡	
		Height	Diameter
Rep	3	9023.25	0.33
Row	2	4184.58*	0.20*
Srate	2	1113.74	1.41*
Date	2	816909.26*	11.56*
Row*Srate	4	844.60	0.22*
Row*Date	4	10021.58*	0.09
Srate*Date	4	1001.99	0.12
Row*Srate*Date	8	348.53	0.03
Error a	78	859.45	0.05
Nrate	1	423.63	0.07*
Row*Nrate	2	267.20	0.02
Srate*Nrate	2	207.13	0.01
Date*Nrate	2	133.12	0.00
Row*Srate*Nrate	4	217.08	0.03
Row*Date*Nrate	4	265.07	0.05*
Srate*Date*Nrate	4	305.07	0.01
Row*Srate*Date*Nrate	8	149.06	0.03*
Error b	81	221.28	0.01
Mday	6	1822434.58*	43.84*
Row*Mday	12	866.98*	0.02
Srate*Mday	12	795.23*	0.11*
Date*Mday	8	26821.93*	3.41*
Row*Srate*Mday	24	130.02	0.02
Row*Date*Mday	16	822.48*	0.06*
Srate*Date*Mday	16	310.41*	0.03
Row*Srate*Date*Mday	32	95.84	0.02
Error c	378	171.96	0.02
Nrate*Mday	6	54.80	0.01
Row*Nrate*Mday	12	76.14	0.01
Srate*Nrate*Mday	12	78.15	0.01
Date*Nrate*Mday	8	47.49	0.01
Row*Srate*Nrate*Mday	24	69.78	0.01
Row*Date*Nrate*Mday	16	91.90*	0.01
Srate*Date*Nrate*Mday	16	88.75*	0.01
Row*Srate*Date*Nrate*Mday	32	73.91*	0.01
Error d	378	49.34	0.01

† Source abbreviations are the following: Replications (Rep), Row width (Row), Seeding rate (Srate), Planting date (Date), Nitrogen fertility (Nrate), and Measurement date (Mday).

‡ Mean squares marked with * indicates significance at $P < 0.05$.

Table 4. Biomass yield and chemical composition data of the management experiment.

Year	Planting date	Row width (cm)	Seeding rate (kg/ha)	Nitrogen rate (kg N/ha)	Yield (Mg/ha)	WSC* (g/kg)	TNC (g/kg)	Cell (g/kg)	Hemi (g/kg)	Lig (g/kg)
2005	1	20	4.5	84	23.15	186.22	374.05	231.82	199.03	16.43
2005	1	20	4.5	168	23.40	178.56	348.21	252.31	211.91	19.55
2005	1	20	11.2	84	23.18	178.70	368.49	245.70	211.39	18.12
2005	1	20	11.2	168	27.49	200.34	364.58	241.30	204.48	18.16
2005	1	20	17.9	84	21.09	163.74	334.24	271.74	223.50	19.14
2005	1	20	17.9	168	25.51	168.70	392.25	249.03	213.16	18.52
2005	1	38	4.5	84	20.84	180.29	366.38	249.00	205.76	18.97
2005	1	38	4.5	168	18.32	203.38	394.44	234.69	200.47	16.91
2005	1	38	11.2	84	22.26	179.41	377.57	243.86	205.69	20.14
2005	1	38	11.2	168	21.26	173.53	395.22	237.88	199.68	16.47
2005	1	38	17.9	84	20.71	205.92	367.79	249.02	218.63	16.52
2005	1	38	17.9	168	18.75	203.34	353.61	260.01	218.76	19.07
2005	1	76	4.5	84	22.52	178.04	397.11	226.14	188.37	16.70
2005	1	76	4.5	168	22.33	172.33	381.49	229.91	190.99	17.12
2005	1	76	11.2	84	23.45	204.98	386.82	237.37	199.48	16.96
2005	1	76	11.2	168	22.97	175.84	368.52	242.97	201.50	18.07
2005	1	76	17.9	84	23.37	176.70	400.75	229.84	196.94	15.59
2005	1	76	17.9	168	21.53	205.16	388.89	235.43	197.58	16.25
2005	2	20	4.5	84	18.57	216.88	350.82	259.21	211.61	20.07
2005	2	20	4.5	168	20.40	218.27	333.77	256.40	208.75	20.28
2005	2	20	11.2	84	20.13	207.45	325.09	261.86	213.36	19.00
2005	2	20	11.2	168	22.17	221.48	353.04	256.81	218.80	18.88
2005	2	20	17.9	84	21.14	174.15	344.14	265.56	225.40	18.22
2005	2	20	17.9	168	22.71	201.72	357.02	255.48	214.39	17.04
2005	2	38	4.5	84	18.82	207.97	360.02	262.75	219.69	18.13
2005	2	38	4.5	168	18.26	211.43	357.31	253.84	216.75	18.02
2005	2	38	11.2	84	20.64	223.15	383.77	250.78	204.51	17.21
2005	2	38	11.2	168	20.35	181.74	330.73	262.49	220.33	18.14
2005	2	38	17.9	84	19.69	204.49	319.41	269.80	222.38	18.28
2005	2	38	17.9	168	21.63	219.36	359.42	265.23	212.12	18.56
2005	2	76	4.5	84	18.67	209.66	345.68	262.15	217.03	17.63
2005	2	76	4.5	168	19.41	208.37	362.09	237.39	204.33	16.24
2005	2	76	11.2	84	19.71	221.59	368.80	252.43	211.45	18.81
2005	2	76	11.2	168	20.37	214.82	344.02	244.94	213.72	16.57
2005	2	76	17.9	84	17.16	223.83	358.91	253.71	214.07	15.74
2005	2	76	17.9	168	20.55	240.28	373.10	236.88	204.03	16.51

* Chemical constituents abbreviated as follows: Water soluble carbohydrates (WSC), Total nonstructural carbohydrates (TNC), Cellulose (Cell), Hemicellulose (Hemi), and Lignin (Lig).

Table 4. (continued)

Year	Planting date	Row width (cm)	Seeding rate (kg/ha)	Nitrogen rate (kg N/ha)	Yield (Mg/ha)	WSC (g/kg)	TNC (g/kg)	Cell (g/kg)	Hemi (g/kg)	Lig (g/kg)
2005	3	20	4.5	84	16.11	196.91	318.97	274.85	222.68	19.83
2005	3	20	4.5	168	18.64	211.96	354.46	259.87	212.15	21.62
2005	3	20	11.2	84	16.39	206.18	316.46	279.07	215.94	19.57
2005	3	20	11.2	168	18.37	209.31	309.84	289.91	220.49	20.04
2005	3	20	17.9	84	15.81	191.38	305.61	293.73	227.55	20.13
2005	3	20	17.9	168	18.45	171.87	294.84	287.59	232.03	18.82
2005	3	38	4.5	84	14.84	208.33	308.35	285.81	217.19	21.77
2005	3	38	4.5	168	15.05	219.65	317.17	280.90	221.95	21.97
2005	3	38	11.2	84	13.40	195.75	306.46	276.57	226.69	18.54
2005	3	38	11.2	168	17.98	187.35	344.63	259.14	214.45	17.39
2005	3	38	17.9	84	15.06	211.07	313.95	269.11	215.33	18.32
2005	3	38	17.9	168	16.32	202.43	307.12	270.15	227.06	18.98
2005	3	76	4.5	84	17.65	230.79	331.50	257.88	211.35	19.24
2005	3	76	4.5	168	15.01	193.18	297.74	279.55	222.59	19.41
2005	3	76	11.2	84	19.60	223.38	346.35	261.83	214.42	19.89
2005	3	76	11.2	168	18.84	224.00	322.31	269.02	215.08	19.15
2005	3	76	17.9	84	17.41	235.40	347.09	252.99	218.08	16.45
2005	3	76	17.9	168	15.61	194.40	317.72	274.77	221.07	17.81
2006	1	20	4.5	84	31.23	206.89	366.09	243.86	185.49	19.36
2006	1	20	4.5	168	27.41	239.27	373.07	246.12	187.40	20.00
2006	1	20	11.2	84	26.92	180.68	345.13	242.52	186.31	18.37
2006	1	20	11.2	168	35.28	192.03	345.15	253.44	200.07	21.12
2006	1	20	17.9	84	23.08	181.55	334.58	254.76	190.84	20.38
2006	1	20	17.9	168	28.46	228.59	362.16	235.27	184.70	18.52
2006	1	38	4.5	84	25.33	236.64	374.96	235.70	180.35	19.73
2006	1	38	4.5	168	25.62	247.34	381.52	230.26	182.95	18.20
2006	1	38	11.2	84	23.16	193.49	349.30	249.88	191.17	22.46
2006	1	38	11.2	168	23.51	235.62	374.84	238.22	183.39	19.69
2006	1	38	17.9	84	24.08	237.07	371.42	238.40	182.46	19.10
2006	1	38	17.9	168	23.64	224.25	376.79	245.46	188.26	19.06
2006	1	76	4.5	84	19.34	218.83	343.73	244.48	191.86	21.11
2006	1	76	4.5	168	21.26	252.77	378.41	234.87	185.85	22.34
2006	1	76	11.2	84	21.54	171.30	335.24	247.25	196.12	19.71
2006	1	76	11.2	168	23.35	190.52	321.61	243.11	193.66	19.96
2006	1	76	17.9	84	20.66	203.23	338.42	238.79	188.32	19.89
2006	1	76	17.9	168	21.19	223.52	380.58	239.14	191.75	18.28

Table 4. (continued)

Year	Planting date	Row width (cm)	Seeding rate (kg/ha)	Nitrogen rate (kg N/ha)	Yield (Mg/ha)	WSC (g/kg)	TNC (g/kg)	Cell (g/kg)	Hemi (g/kg)	Lig (g/kg)
2006	2	20	4.5	84	32.69	215.46	331.74	252.02	196.33	19.17
2006	2	20	4.5	168	35.61	212.26	320.23	255.21	197.39	20.36
2006	2	20	11.2	84	41.15	252.41	379.19	245.40	187.85	21.01
2006	2	20	11.2	168	45.18	253.73	353.51	251.64	195.77	20.79
2006	2	20	17.9	84	40.11	194.70	318.91	256.42	200.04	19.72
2006	2	20	17.9	168	27.80	237.79	322.15	247.98	192.66	18.03
2006	2	38	4.5	84	15.22	201.63	319.19	257.97	206.80	18.86
2006	2	38	4.5	168	19.15	210.47	320.26	254.29	196.82	20.72
2006	2	38	11.2	84	23.64	214.43	329.79	247.43	195.81	19.23
2006	2	38	11.2	168	16.45	223.52	338.03	251.77	199.05	18.11
2006	2	38	17.9	84	20.49	227.16	357.08	247.54	193.18	20.16
2006	2	38	17.9	168	15.72	237.64	340.05	249.06	196.76	18.51
2006	2	76	4.5	84	12.93	213.02	354.64	246.34	200.49	19.47
2006	2	76	4.5	168	15.43	219.17	315.51	245.72	192.12	18.22
2006	2	76	11.2	84	11.31	163.21	282.96	268.09	209.94	19.80
2006	2	76	11.2	168	13.16	246.11	354.76	250.42	193.04	17.68
2006	2	76	17.9	84	17.30	187.84	316.80	260.73	204.06	18.87
2006	2	76	17.9	168	13.66	199.10	303.48	256.24	199.90	18.63
2006	3	20	4.5	84	19.38	194.96	312.88	253.87	199.54	17.28
2006	3	20	4.5	168	19.91	182.46	312.66	257.37	215.38	18.37
2006	3	20	11.2	84	23.52	190.31	294.16	260.38	201.80	19.68
2006	3	20	11.2	168	29.09	236.54	340.16	255.06	201.15	18.51
2006	3	20	17.9	84	27.89	183.65	316.68	264.58	200.35	19.26
2006	3	20	17.9	168	27.59	225.58	360.93	251.76	196.59	19.85
2006	3	38	4.5	84	17.58	202.03	319.18	253.77	198.38	18.69
2006	3	38	4.5	168	17.60	177.58	296.52	277.11	214.22	21.34
2006	3	38	11.2	84	15.13	213.51	348.00	255.72	195.31	19.14
2006	3	38	11.2	168	16.00	238.42	334.40	253.79	191.31	19.60
2006	3	38	17.9	84	12.36	152.62	275.09	273.17	217.37	19.44
2006	3	38	17.9	168	14.54	200.31	325.64	262.70	204.46	18.71
2006	3	76	4.5	84	13.63	172.90	303.47	260.26	205.74	18.16
2006	3	76	4.5	168	13.38	152.92	267.27	275.05	219.58	18.30
2006	3	76	11.2	84	14.16	226.18	342.80	256.68	195.19	19.86
2006	3	76	11.2	168	14.67	244.10	371.76	252.38	193.83	17.62
2006	3	76	17.9	84	16.58	213.59	339.37	255.50	205.74	17.91
2006	3	76	17.9	168	15.59	228.70	331.56	247.34	195.37	18.56

Table 5. Analysis of variance for biomass yield and chemical composition data of the management experiment.

Source†	df	Mean square‡					
		Yield	WSC±	TNC	Cell	Hemi	Lig
Year	1	603.61*	11386.71	15375.04	3941.09	29724.15*	107.82
Error a	6	29.37	4072.01	16702.61	2397.31	1614.13	29.69
Row	2	2257.22*	1017.79	1127.65	1946.88*	320.53	35.89*
Srate	2	108.97	452.48	889.03	289.73	409.57	21.83*
Date	2	1377.87*	7869.53*	76926.01*	20532.37*	7948.53*	10.09
Year*Row	2	1489.76*	3094.28	5025.21	1833.26*	1190.81*	15.85*
Year*Srate	2	3.31	358.31	729.57	277.92	571.25*	5.72
Year*Date	2	22.62	10738.96*	5177.77	1903.98*	3.26	65.02*
Row*Srate	4	35.05	2341.42	1206.52	513.05	133.98	4.30
Row*Date	4	249.62*	2668.39	1460.59	132.39	144.08	4.46
Srate*Date	4	17.63	5082.76*	3124.92	320.75	440.03*	2.01
Year*Row*Srate	4	57.00	1938.79	647.67	222.15	131.84	7.26
Year*Row*Date	4	275.74*	1234.63	990.08	275.20	230.56	3.42
Year*Srate*Date	4	18.49	6145.58*	3050.64	163.33	241.99	8.82
Row*Srate*Date	8	26.48	1367.28	2366.69	352.04	108.29	10.77*
Year*Row*Srate*Date	8	33.82	1815.91	2391.36	333.75	177.29	2.29
Error b	156	40.95	1922.94	2016.16	322.50	152.42	4.48
Nrate	1	33.60	8585.39*	1499.13	380.49	38.68	1.62
Year*Nrate	1	4.05	10824.83*	1529.86	0.00	1.14	1.77
Row*Nrate	2	40.43	969.63	1440.02	40.65	47.18	2.95
Srate*Nrate	2	28.85	1382.98	1803.44	174.96	172.24	11.14
Date*Nrate	2	10.85	1014.80	694.62	313.56	214.63	3.39
Year*Row*Nrate	2	10.12	576.16	1371.32	223.16	132.11	0.77
Year*Srate*Nrate	2	35.24	2182.58	1473.98	236.39	64.29	5.20
Year*Date*Nrate	2	39.87	179.83	900.79	203.62	15.28	0.55
Row*Srate*Nrate	4	20.24	467.95	499.37	445.71	112.86	9.16
Row*Date*Nrate	4	28.71	1027.74	1508.62	521.92	115.32	7.21
Srate*Date*Nrate	4	32.12	855.30	530.21	158.03	308.67	1.02
Year*Row*Srate*Nrate	4	26.29	1392.03	655.48	67.12	33.63	3.86
Year*Row*Date*Nrate	4	1.14	282.86	696.64	225.35	10.39	0.97
Year*Srate*Date*Nrate	4	37.78	1942.33	2888.60	304.97	430.85*	3.12
Row*Srate*Date*Nrate	8	20.66	1068.67	1609.77	355.99	139.33	7.56
Year*Row*Srate*Date*Nrate	8	4.80	713.59	2865.07	163.42	134.10	2.14
Error c	162	19.32	1638.78	1658.85	278.95	140.27	5.01

± Chemical constituents abbreviated as follows: Water soluble carbohydrates (WSC), Total nonstructural carbohydrates (TNC), Cellulose (Cell), Hemicellulose (Hemi), and Lignin (Lig).

† Source abbreviations are the following: Experiment years (Year), Replications (Rep), Row width (Row), Seeding rate (Srate), Planting date (Date), and Nitrogen fertility (Nrate).

‡ Mean squares marked with * indicates significance at $P < 0.05$.

Table 6. Fermentation data of the silage experiment.

Moisture (g/kg)	Enzyme (IU/g DM)	Incubation (days)	pH	WSC*	Cell	Hemi	Lig	Lac	Ace	Prop
550	0	1	4.37	200.55	293.57	224.98	24.85	12.42	5.52	0.49
		7	3.89	186.47	302.51	233.27	27.08	27.15	7.92	0.32
		21	3.90	171.24	303.46	232.65	26.76	32.99	7.47	0.42
	1	1	4.39	197.61	292.13	224.72	25.35	11.91	5.79	0.34
		7	3.90	186.64	295.83	223.50	27.12	26.12	6.94	0.00
		21	3.87	194.95	296.06	224.42	27.79	31.70	8.31	1.80
	10	1	4.33	196.39	281.95	213.89	25.54	13.37	6.16	1.52
		7	3.95	188.33	286.41	219.30	27.59	27.10	7.60	1.10
		21	4.00	184.46	278.71	216.66	27.04	28.98	7.56	2.42
650	0	1	4.13	178.68	306.49	228.64	27.09	18.47	8.54	0.79
		7	3.77	159.64	302.21	227.66	28.52	36.86	11.99	0.00
		21	3.77	154.67	300.13	222.73	27.28	42.57	12.97	0.46
	1	1	4.12	195.90	276.54	214.04	23.51	18.77	9.61	0.51
		7	3.80	164.43	291.97	219.61	28.13	36.96	11.13	1.51
		21	3.75	157.43	286.66	217.91	27.77	41.79	11.44	1.02
	10	1	4.15	201.01	277.62	213.15	25.59	19.52	9.54	1.36
		7	3.74	184.04	274.48	207.06	27.86	35.98	12.00	1.95
		21	3.77	175.82	268.02	207.30	26.30	39.32	12.31	3.40
750	0	1	3.96	164.21	294.93	226.96	25.29	24.18	13.30	0.00
		7	3.62	118.89	311.43	236.16	28.42	53.91	15.52	0.00
		21	3.63	110.98	301.17	234.30	26.73	58.21	20.15	0.69
	1	1	3.91	176.00	288.71	221.00	25.93	24.39	12.55	0.46
		7	3.58	154.75	285.85	224.88	26.43	48.00	12.96	0.54
		21	3.63	119.17	303.45	232.78	31.01	54.69	17.23	2.56
	10	1	3.99	180.73	263.19	206.01	23.83	25.36	12.81	0.00
		7	3.63	156.64	257.60	211.66	24.31	48.25	16.37	1.75
		21	3.71	140.64	262.44	208.56	26.67	53.50	18.18	3.19

* Chemical constituents abbreviated as follows: Water soluble carbohydrates (WSC), Cellulose (Cell), Hemicellulose (Hemi), Lignin (Lig), Lactic acid (Lac), Acetic acid (Ace), Propionic acid (Prop), Arabinose (Ara), Galactose (Gal), Glucose (Glu), Xylose (Xyl), and Mannose (Man).

Table 6. (continued)

Moisture (g/kg)	Enzyme (IU/g DM)	Incubation (days)	Ara (g/kg)	Gal (g/kg)	Glu (g/kg)	Xyl (g/kg)	Man (g/kg)
550	0	1	0.91	2.29	78.77	1.45	0.48
		7	0.81	2.31	63.52	2.73	1.00
		21	1.27	2.71	67.37	2.80	0.42
	1	1	1.53	2.55	74.90	2.09	0.92
		7	1.63	2.80	68.92	4.33	1.64
		21	2.47	3.34	74.94	5.38	0.54
	10	1	2.79	2.72	82.73	3.34	1.39
		7	3.35	3.45	71.90	7.05	1.95
		21	4.65	4.15	78.27	9.10	1.21
650	0	1	0.99	2.77	86.15	2.16	1.14
		7	1.49	3.25	83.60	3.08	0.97
		21	1.81	3.99	80.02	3.99	0.68
	1	1	0.98	3.04	86.40	2.91	1.25
		7	3.52	3.70	79.41	5.31	1.09
		21	3.38	5.03	78.90	7.59	1.10
	10	1	1.81	2.94	89.46	4.07	1.65
		7	3.13	3.94	87.40	8.90	2.29
		21	4.38	4.60	90.77	11.59	2.02
750	0	1	0.70	2.87	95.66	2.50	1.11
		7	2.11	3.66	85.48	3.35	0.64
		21	3.55	3.32	82.18	4.05	0.36
	1	1	1.06	2.97	101.24	3.39	1.00
		7	1.89	3.60	96.13	5.86	0.94
		21	3.00	4.77	82.83	8.40	1.63
	10	1	1.58	3.26	101.36	5.17	1.29
		7	2.51	4.23	106.42	9.60	1.14
		21	4.61	5.49	99.80	13.79	1.99

Table 7. Analysis of variance for fermentation data of the silage experiment.

Source†	df	Mean square‡							
		pH	WSC±	Cell	Hemi	Lig	Lac	Ace	Prop
Rep	3	0.01	13581.56	959.69	214.56	77.15	966.10	41.33	5.56
Moist	2	0.98*	16922.37*	460.78	379.97*	1.55	3566.96*	638.66*	0.78
Level	2	0.01	3002.77*	8002.78*	3024.31*	9.10	29.61	7.37	20.54*
Moist*Level	4	0.00	575.11	625.66*	120.44	11.02*	7.87	3.85	0.68
Error a	24	0.00	516.86	191.46	90.31	3.49	27.63	9.30	1.40
Day	2	1.71*	9194.58*	132.51	110.16	56.37*	5765.03*	113.37*	14.09*
Moist*Day	4	0.02*	968.59*	84.74	78.05	6.15	144.04*	14.20	1.89
Level*Day	4	0.01	101.12	179.59	35.95	8.30	21.43	3.73	2.94
Moist*Level*Day	8	0.00	253.55	147.95	42.29	4.03	4.07	1.64	1.08
Error b	54	0.01	194.27	111.84	54.44	3.69	36.35	5.91	1.59

± Chemical constituents abbreviated as follows: Water soluble carbohydrates (WSC), Cellulose (Cell), Hemicellulose (Hemi), Lignin (Lig), Lactic acid (Lac), Acetic acid (Ace), Propionic acid (Prop), Arabinose (Ara), Galactose (Gal), Glucose (Glu), Xylose (Xyl), and Mannose (Man).

† Source abbreviations are the following: Replications (Rep), Silage moisture concentration (Moist), Enzyme concentration (Level), and Incubation time (Day).

‡ Mean squares marked with * indicates significance at $P < 0.05$.

Table 7. (continued)

Source	df	Mean square				
		Ara	Gal	Glu	Xyl	Man
Rep	3	0.20	0.74	1602.18	13.61	0.31
Moist	2	0.54	8.24*	4006.93*	36.19*	0.86
Level	2	26.11*	6.55*	879.72*	242.79*	7.45*
Moist*Level	4	3.40	0.47	80.88	3.19	0.23
Error a	24	1.27	0.42	109.08	1.53	0.55
Day	2	31.31*	15.99*	500.37*	175.84*	0.37
Moist*Day	4	2.64*	0.51	148.81	2.47*	1.19
Level*Day	4	1.34	1.09*	72.41	24.93*	0.40
Moist*Level*Day	8	0.67	0.29	50.40	0.57	0.34
Error b	54	0.75	0.34	74.66	0.70	0.63

ACKNOWLEDGEMENTS

I would first like to thank Dr. Ken Moore for taking me on as a Master's student, serving as my major professor, and providing guidance through my graduate career. Your advice and patience throughout this process has been greatly appreciated. Next, I would like to thank my graduate committee members Dr. Rob Anex and Dr. Steve Fales for their critique of this thesis.

I would like to extend a special thank you to everyone who helped me with field and laboratory data collection especially Trish Patrick and Roger Hintz. This project would not have been possible without the time they devoted to helping me.

To my mom, you are my hero! You have always been there for me whenever I needed you and I could never thank you enough. The support and love you have given to me unconditionally throughout my life is truly a blessing. To my brothers, thank you for being my role models and always encouraging me to achieve my goals.

Finally, to my wife Sabrina, you have shown me why life is truly worth living and I want to thank you for living it with me every day. Without your patience and support over the past couple years I would not have succeeded. Thank you again for everything you are and everything you have done. I love you, My Beautiful Girl.