Double-cropping sorghum for biomass production

by

Ben Michael Goff

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Program of Study Committee:
Ken Moore, Co-Major Professor
Steven Fales, Co-Major Professor
Emily Heaton
Basil Nikolau

Iowa State University

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ABSTRACT

The production of biomass using double-cropping systems may have the advantage of producing more feedstock for refineries by extending the growing season, while also providing many environmental benefits, such as the reduction of erosion. Past research indicates that there may be a genotypic effect for the suitability of a crop for use within these systems. There has been little research conducted to explicitly examine this effect in sorghum, despite the crop's diverse genetic background. The objective of this study was to evaluate the biomass production of twelve sorghum genotypes grown as a sole crop and within a double-cropping system with triticale. It was shown that both triticale and sorghum are acceptable as potential feedstocks for ethanol conversion. Because of adverse weather conditions, the chemical composition of both crops varied over all study environments and was the result of the differences in maturity at the time of harvest. Although genotypes within the single-cropping system produced higher biomass system yields than the double-cropping systems, the difference was not significant for several genotypes. These sorghums were characterized as being earlier maturing varieties and had nearly maximized dry matter production at the earlier harvest of the double-cropping system. Thus, the additional biomass that the crop would have accrued was capable of being supplemented by the growth of the triticale. However, the theoretical ethanol yields were significantly higher within the single-cropping system for these cultivars. This indicates that although it could offset any loss in dry matter production, the triticale crop was of lower quality for conversion to ethanol compared to the sorghum biomass. The double-cropping systems were more costly to produce than the single-cropped sorghums; however, there are favorable environmental benefits associated with the double-cropped sorghum that may warrant the additional costs.

CHAPTER I: GENERAL INTRODUCTION

There has been considerable interest in recent years to replace petroleum based fuels with those derived from plant materials, particularly ethanol. In addition to reducing dependency on foreign oil, biofuels have been associated with several other advantages (Greene et al., 2004). Biofuels are among the most renewable of energy sources and may be produced from feedstocks that are considered waste products from other industries (Chen et al., 2007; McKendry, 2002). In addition to these feedstocks, dedicated energy crops have the potential to open new markets for farmers (Greene et al., 2004). Also, because they are produced from plant materials, biofuels will release approximately the same amount of CO_2 from production and combustion as the plant, and will thus aid in the reduction of greenhouse gas emission (Ragauskas et al., 2006; Farrell et al., 2006).

As of 2005, the United States transportation sector consumed approximately 180 billion gallons of fuel, of which about four billion gallons were provided by biofuels (EIA, 2007). The majority of the ethanol currently produced from within the United States is derived from the hydrolysis and fermentation of starch obtained from corn (*Zea mays*) grain. Although this is a suitable temporary solution, there are some long term issues associated with its continued use as a feedstock. It has been estimated that if the entire U.S. corn crop was used for ethanol production, it would only meet approximately 15-25% of the countries transportation fuel need (Houghton et al., 2006; Rooney et al., 2007). Also, corn is already largely used as a major source for livestock feeds and human consumption (Cassman et al., 2006) and use for ethanol production would compete with the ability to maintain adequate levels of food production, making the need for alternative fuels sources a must.

Ethanol produced from biomass has been promoted to help meet the energy needs that grain ethanol may not provide (Perlack et al., 2005; Greene et al., 2004; Wallace et al., 2005). The

structural carbohydrates found within the plant cell wall (i.e. cellulose and hemicelluloses) make up a large portion of the cell's total mass. While not as easily digestible as starch due to the presence of lignin, pretreatment of these feedstocks with various chemical and physical treatments has been shown to significantly increase enzyme accessibility to the carbohydrate polymers (McKendry, 2002; Sun and Cheng, 2002). Because of the diversity and high-cost of these methods, cellulosic ethanol is still considered an emerging technology, however, there have been and will continue to be significant breakthroughs (Sticklen, 2007; Hahn-Hägerdal et al., 2006; Sun and Cheng, 2002), which should make this a reasonable future energy source.

For cellulosic ethanol to be considered a reasonable fuel alternative, there must be an ample supply of feedstock to provide for our energy needs. It has been estimated that it will take over one billion tons of biomass (Greene et al., 2004; Perlack et al., 2005) to accomplish this, with approximately 600-700 billion tons needed within the next twenty years to meet government goals to reduce petroleum usage (Fales et al., 2007, Perlack, et al., 2005). There are several ways this may be done agronomically. First, the development of new crops, such as Miscanthus (*Miscanthus* x *giganteus*) (Heaton et al., 2008), and the selective breeding of existing crops, such as switchgrass (*Panicum virgatum*) and hybrid sorghums, that will produce considerable seasonal biomass with limited resources (Mitchell et al., 2008). Although these are essential for the long term needs, they do not to provide much aid to current production. A short-term alternative for providing biomass feedstocks may be in the modification of current management practices, such as the harvesting of crops residues previously left in the field, or the development of alternative cropping systems, such as the use of a double-cropping system.

Within a double-cropping system, two crop species of complementary growing characteristics are grown on the same land. Most commonly a winter annual crop is planted in the fall of the year and harvested in the spring, while a warm-season crop with a relatively short growing season is

planted immediately following the harvest of first crop (Karpenstein-Machan, 2001). In addition to having beneficial environmental benefits (Fisher, 1989; Kasper et al., 2007), the use of double-cropping may be well suited for biomass production because, unlike traditional double-cropping systems, harvest is not limited to the physiological maturity of either crop. In fact the use of a double-cropping system has shown some potential for the production of biomass (Heggenstaller et al., 2008; Hesel and Wedin, 1981), but is not without its short-comings (Buxton et al., 1999; Crookston et al., 1978). Some of the limitations reported with double-cropping were limited moisture for the second crop, increased costs for the producer, and excess removal of soil nutrients (Buxton et al., 1999; Heggenstaller et al., 2008; Murdock and Wells, 1978).

Triticale (x *Triticosecale* Wittmack) and Sorghum (*Sorghum bicolor*) both have shown to be productive as a sole-crop (Gibson et al., 2007; Rooney et al., 2007; Pedersen et al., 1995) and in a double-cropping system (Heggenstaller et al., 2008; Helsel and Wedin, 1981). However, most of the work done on sorghums in these systems has only consisted of a few select genotypes; even though there has been some evidence showing that success of a crop within a double-cropping system may depend on genotypic variation (Sheaffer et al., 1977; Crookston et al., 1978). There also has been evidence showing that forage quality of a crop may be altered within a double-cropping system compared to a single-crop system, due to differences in maturities at harvest (Buxton et al., 1999). The objectives of this study were to the evaluate biomass production potential, feedstock quality, and production costs of triticale and twelve sorghum genotypes grown as a season-long crop, as well as grown in a double-cropping system.

This thesis is laid out in the following format. Chapter's I-V will be focused on the use of double-cropping sorghum genotypes for biomass production, and meets the requirements of the Masters of Science requirements for the Agronomy department. Chapter I is a brief introduction of the theory behind the main project and lignocellulosic ethanol production, while Chapter II gives a

more detailed description of double-cropping and the use of triticale and sorghum as feedstocks for ethanol production. Chapter III is a detailed account of how the experiment was conducted. The data is presented and discussed in Chapter IV, and Chapter V summarizes the findings of this study. Chapter VI focuses on the work done to meet the requirements for the Biochemistry, Biophysics, and Molecular Biology (BBMB) minor. It is presented in a journal format, and details the work done on finding alternative methods for the quantification of prussic acid within sorghums. Any references, tables, or figures cited within a chapter are present at the end of the section.

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CHAPTER II: LITERATURE REVIEW

Double-Cropping System

Biomass Production Potential

The use of a double-cropping system for producing biomass has been well documented. While most of the research was done in the 1970's and is centered on forage production (Camper et al., 1972; Sheaffer et al., 1977; Murdock & Wells, 1978), interest in these systems has risen in recent years to take advantage of these systems to produce large amounts of biomass (Heggenstaller et al., 2008, Karpenstein-Machan, 2001). Although there has been a considerable amount of research done, there seems to be no conclusive evidence about the production suitability of these systems when compared to mono-cropping systems. Most of the variability seems to due to climatic and other environmental conditions, as well as the producer's goals for the system (Helsel and Wedin, 1981; Crookston et al., 1978; Singer et al., 2007).

Variability in production potential for double-cropping systems is particularly evident in research conducted in Midwest. Helsel and Wedin (1981) evaluated in ten crops produced both as a sole crop and double-cropped with winter rye (*Secale cereal* L.) and spring oats (*Avena sativa* L.). They found that although the grain and forage yield of the main crops were reduced due to later planting dates in the double-cropping systems, the total season dry matter yields of eight of the ten crops double-cropped with rye were equal or higher than when grown as a sole crop, with a maximum yield of over 22 Mg ha^{-1} being produced by a rye/sorghum x sudangrass system. The only exceptions being that corn grown for either silage or grain produced higher yields under a mono-cropping system. Similarly, Heggenstaller et al. (2008) concluded that winter triticale (x *Triticosecale* Wittmack) double-cropped with corn (*Zea mays* L.) and sorghum x sudangrass had larger total

seasonal biomass yields (22.7 and 23.0 Mg ha^{-1}, respectively) compared to mono-cropped corn (18.2 Mg ha^{-1}).

In contrast to these studies, Buxton et al. (1999) found that with the exception of the winter annual crop fertilized at the highest nitrogen application rate, the reduction in yield of the second crop could not be recovered by that of the first crop. They found that double-cropped sorghums (*Sorghum bicolor* (L.) Moench) with rye yielded 72, 84, 95, and 110% of single-cropped sorghum fertilized at the rates of 0, 70, 140, and 280 kg ha^{-1} of nitrogen, respectively. The authors concluded that although, higher yields could be gained in a double-cropping system, a partial budget analysis showed that the double-cropping system at this fertilizer rate had a cost of $147 per ha more than the when sorghum was grown as a sole crop, making it impractical to producers. In Minnesota, Crookston et al. (1978) found that although their study showed that on average corn double-cropped with rye had larger total system yields (25.9 Mg ha^{-1}) than single-cropped corn (18.8 Mg ha^{-1}), the total yield depended on climatic conditions. For example, rye had greater yields (15.3 Mg ha^{-1}) in 1974, which were more than double the yields in any other year. Also in 1976, both sites experienced a drought which further reduced the yields of the double-cropped corn more than the sole cropped corn, indicating that single-cropped corn would be more beneficial in drier years.

Outside of the Midwest, particularly in the southeastern United States, the use of double-cropping systems have been shown to be quite suitable for production, more than likely due to a longer and more favorable growing season. At two sites in Kentucky, Murdock and Wells (1978) showed that small grains double-cropped with corn grown for silage were 19% and 31% more productive than corn grown alone for silage. When averaged across years and sites the small grain/corn system averaged 58.5 Mg ha^{-1} compared to 46.9 Mg ha^{-1} with the corn silage system. The authors also showed that choice of small grain had an effect on the subsequent corn yield, as corn following barley (*Hordeum vulgare* L.) produced higher yields (50.6 Mg ha^{-1}) compared to corn

following wheat (45.8 Mg ha^{-1}) and oats (41.2 Mg ha^{-1}). In eastern Virginia, it was shown that corn and grain sorghum could be successfully grown for silage following barley harvested for forage (Camper et al., 1972) if planted by mid-late June. However, if planting was delayed any later, then the double-cropping of corn was shown to be less lucrative, while the sorghums produced equal yields under both planting dates.

Other Possible Benefits

In addition to a potential increase in dry matter production, there have been other benefits associated with the use of a double-cropping system. Because the winter annual begins growth in the early spring, there is the potential for the crop to capture mineralized soil nitrogen that would otherwise be lost from the soil. Triticale has been shown to be accumulate nearly 86 kg of soil nitrogen per hectare more than corn at the time of harvest (Heggenstaller et al., 2008), which would otherwise would have leached from the production system. Similarly, the same authors showed that by mid-April, double-cropping systems reduced soil nitrogen by 34% (17 kg ha^{-1}) compared to a single crop system. In tile drainage discharge, it has been shown that use of cover crops decreased the amount of nitrate in tile water decreases up to 61% (Kasper et al., 2007). The reduction in nitrogen loss from cropping systems is essential, as its loss means an increase in production cost for producers, and nitrogen runoff has been shown to be a large contributor to the contamination of water sources (Burkart and James, 1999).

In addition to improved nitrogen cycling, double-cropping systems may also offer other environmental benefits that should be considered. Because there is residue on the soil surface during the winter and early spring with these systems, there is less potential for soil erosion. Based on estimates using the Universal Soil Loss Equation (USLE), Buxton et al. (1999) saw an approximate 38% decrease in erosion on two different sites in Iowa. The incorporation of a vegetative cover on the soil surface will also increase the efficiency of solar radiation capture. The energy from the sun is

captured by plants and stored in the form of various chemical bonds within the plant tissue (Taiz and Zeiger, 2006). These bonds are harvested and used to provide energy in other sources, such as with digestion to maintain cellular metabolism or combusted to provide warmth. An actively growing herbaceous cover before traditional crop planting occurs will allow for a greater amount of the solar energy to be captured and will therefore increase the stored energy to be harvested from an area of land (Fisher, 1989).

Possible Limitations

There are also certain disadvantages related with growing sequential crops on a given area of land. Although there may be an increase in production associated with harvesting more biomass from a given area of land, there is also a large risk of removing an excessive amount of soil nutrients along with the biomass. Heggenstaller et al. (2008), showed that double-cropping systems removed 42, 26, 71 % more N, P, and K than mono-cropped corn. The authors estimated that sole cropped corn (grain and stover) removed on average 153 kg of N ha^{-1}, 32 kg of P ha^{-1}, and 91 kg of K ha^{-1} compared to 266 kg of N ha^{-1}, 45 kg of P ha^{-1}, and 240 kg of K ha^{-1} for corn double-cropped with triticale. These removal estimates were similar to those reported for a barley and corn system by Murdock and Wells (1978) in Kentucky, where it was estimated that double-cropping systems may remove up to 293, 66, and 312 kg of N, P, and K, respectively. Excess removal of soil nutrients may be a major limitation to the acceptance of the double-cropping system by producers. For an area of land to remain productive under these systems, the nutrients removed will need to be replaced resulting in a greater cost for the producer. It has been anticipated that double-cropping could increase P and K fertilization rates by 67 and 567 %, respectively, compared to sole-cropped corn (Heggenstaller et al., 2008).

Additional cost to producers may also come from the need for additional weed control and irrigation cost. Because an earlier planting date is essential for high yields within a double-cropping

system (Crookston et al., 1978), many producers may use a no-till system to plant the second crop. This allows for an earlier planting date with less cultivation, but may also lead to more herbicide application. Several studies have shown that weed control is an issue in no-till double-cropping systems (Sanford et al., 1973; Ndon et.al, 1982). It has also been shown that crops grown in a double-cropping system respond to irrigation (Okoli et al., 1984). Although this response may not be solely due to double-cropping alone, it seems logical that crops grown sequentially may be at a higher risk of a moisture shortage, due to a greater utilization of a soil's moisture supply. Under dry conditions, producers may have to provide supplemental moisture or risk of chance of reduced yield.

Because of some of the limitations described here, the implementation of double-cropping systems has been met with a considerable lack of interest from producers. A recent poll of northern Midwestern farms showed that only 11% had grown a cover crop in the five years prior to the survey, and only 8% planted a cover crop in the fall prior to the survey (Singer et al., 2007). The potential benefits were not lost on the survey recipients, as 80 and 96 % perceived the use of cover crops improved soil and water conditions, respectively. The major restriction to the adoption of the use of a cover crop appears to be due to financial limitations. The main reasons cited for not using cover crops included increased time, costs, and risk associated with establishing and maintaining an additional crop. These concerns were echoed in a focus group conducted in Michigan by Snapp et al. (2005). Although there is some skepticism about growing a winter crop, there appears there may be some hope for future adoption, as over half of the producers surveyed (55.7%) acknowledged that they would use of cover crops if a cost-sharing program was available and would require a minimum payment of $56.81 per hectare.

Genotypic Variation in Double-Cropping

Although past research seems to indicate that there may be a genotypic effect for the suitability of crops grown in double-cropping systems (Sheaffer et al., 1977; Crookston et al., 1978),

research specifically comparing the interaction of different genotypes has been limiting. In Maryland, Shaeffer et al. (1977) compared several sunflower (*Helianthus annuus* L.) genotypes for production of silage in a double-cropping system, and concluded that when averaged across plant population, there were significant differences in the production of the different cultivars. More importantly, some of the data in this study indicated that total dry matter yields did not vary as much between types (oil and confectionary types) of sunflowers, as much as it did between specific cultivars, regardless of type. Similarly in Minnesota, Crookston et al. (1978) showed than corn hybrids having different relative maturities produced significantly different yields when planted at traditional times. However, this trend was lost when the planting date was extended, thereby demonstrating again the importance of date of planting within a double-cropping system.

Sorghum

General Overview

Sorghum has been an economically important crop worldwide. Internationally, it ranks as the sixth most produced crop, providing 57 million MT of grain on 43 million hectares (Martin et al., 2006). It is also grown abundantly throughout the world as a seasonal forage crop for livestock production. In the U.S., it has been estimated to be grown on approximately seven million hectares of land, either as a forage or grain, annually (Rooney et al., 2007). In recent years, there has been a renewed interest in sorghum's use as a potential feedstock for cellulosic ethanol production, mainly due to the ability of some varieties to produce large quantities of biomass with minimal inputs. Sorghum is a member of the *Poaceae* family, and has been classified into the *Andropogoneae* tribe and *Sorghinae* subtribe, respectively (Pedersen and Rooney, 2004). It is believed to have originated in Africa and was first domesticated approximately 3,000 years ago (Harlan and de Wit, 1972). Since its initial cultivation, selection and distribution by humans has lead to the development of several sorghum types: grain, sweet, forage, and sorghum x sudangrass.

Grain sorghum is characterized as being of shorter stature and produces large seed yields. The kernels contain large amounts of starch, and are the main cereal produced for human consumption in tropical and semi-arid regions (Martin et al., 2006). Sweet sorghums contain stems that have a high concentration of soluble carbohydrates. These traits have led to its use as a high-quality silage for livestock (Cogdill, 2008) and production of syrup for human consumption (Bitzer, 1997). Forage sorghums are generally categorized as taller sorghums that have lower stalk sugar content than sweet sorghums, and are generally harvested as silage (Pedersen and Rooney, 2004). Sorghum x sudangrasses, like forage types, generally tend to accumulate less soluble sugars in their culms and have been described as having smaller stems and an increased tillering capacity (Pedersen and Rooney, 2004). They are normally grown throughout the southern United States as hay and silage crops, and were derived as a result of a cross between grain sorghum and sudangrass parental lines.

There are several traits associated with sorghums that make them suited for forage and biomass production. Sorghums have a C_4 metabolism that allows for a lower photorespiration rates, and therefore, higher photosynthetic rates and potentially larger dry matter yields (Kramer, 1981). Also, C_4 photosynthesis has been associated with greater water-use efficiency (WUE) and nitrogen-use efficiency (Turner and Knapp, 1996; Long, 1999). Photoperiod sensitivity is also a trait associated with some high-yielding sorghum varieties (Pederson and Rooney, 2004). These sorghums are generally adapted to tropical conditions and require a long days to become reproductive. When these sorghums are grown in temperate regions (shorter days) their day length requirement for reproductive growth is not met and the plants tend to remain in a vegetative state, leading to greater biomass yields.

Biomass Production Potential

Sweet Sorghums

Sweet sorghums are perhaps the most researched of the sorghum types, and have been shown to be highly productive over a diverse area. Smith et al. (1987) conducted a yield trial at nine sites in the United States, ranging from latitudes of 21°N to 47°N. They concluded that sorghum dry matter yields at these sites ranged from 29.3 Mg ha^{-1} to 13.3 Mg ha^{-1}. At the site with the closest proximity to Iowa (East Lansing, MI: 43°N), the yields averaged 21.1 Mg ha^{-1} over the three years the study was conducted. In central Iowa, sweet sorghums have been shown capable of produce yields approaching 19.9 Mg ha^{-1} (Buxton et al., 1999). Similarly in Minnesota, Putnam et al. (1991) showed that of the sweet sorghums evaluated, approximately 60% of the cultivars produced higher dry matter yields than a corn hybrid, with a reported maximum sorghum yield of 35.8 Mg ha^{-1} compared to 21.7 Mg ha^{-1} with corn. In temperate regions of China, sweet sorghums have been shown to have a maximum yield of 31.9 Mg ha^{-1} (Zhao et al., 2009).

Forage Sorghums

Although not as extensively researched as sweet sorghums, forage sorghums have been shown to be capable of producing similar or greater yields. In Nebraska, Pedersen et al. (1995) found that three forage sorghum genotypes produced higher dry matter yields (17.1 Mg ha^{-1}) than sweet sorghum cultivars (15.8 Mg ha^{-1}), with a maximum forage sorghum yield of 20.7 Mg ha^{-1}. However, in a study conducted in Iowa, sweet sorghums produced roughly 7% more biomass than forage sorghum, but no specific yield data was reported (Buxton et al., 1999). In central Oklahoma, Venuto and Kindiger (2008) evaluated several forage sorghum genotypes for biomass production. They showed all the cultivars within the study averaged 27.0 Mg ha^{-1}, with a range in yields of 18.2 to 40.3 Mg ha^{-1}. They also showed the potential for using photoperiod sensitive (PS) sorghums for biomass

production, as the two PS sorghums (Tentaka and WXF-113) produced yields of 40.3 and 34.5 Mg ha^{-1}. These yields were similar to the yields for the PS sorghum 'Grassl' in Texas (Powell et al., 1991), with a yield of 23.2 Mg ha^{-1} being reported.

Sorghum x Sudangrasses

Although a common forage, there has not been much research done on sorghum x sudangrasses for total season long biomass. However, what has been done shows that sorghum x sudangrasses acre capable of producing substantial amounts of biomass yields, comparable to the other sorghum types. In their comparison of genotypes, Pedersen et al. (1995) showed that sorghum x sudangrass hybrids consistently had higher yields than the other sorghum types, averaging 19.5 Mg ha^{-1} across the evaluated cultivars. This is comparable to the results reported in Venuto and Kindiger (2008) where the assessed sorghum x sudangrasses averaged 27.6 Mg ha^{-1}. As previously mentioned with the forage sorghums in this study, the photoperiod sensitive (PS) sorghums produced yields of approximately 30 Mg ha^{-1}. In Louisiana, Tew et al. (2008) showed that these PS sorghum x sudangrass hybrids produced fresh biomass yields similar to sweet sorghums at various harvest dates. The maximum yield obtained with these sorghum x sudangrass hybrids was 105.3 Mg ha^{-1} (fresh weight) compared to 90.6 Mg ha^{-1} reported for the maximum yield of a sweet sorghum ('Theis').

Forage/Feedstock Quality

Nonstructural Carbohydrates

Nonstructural carbohydrate concentration is perhaps the most studied quality parameter in sorghums, due to the ability of some sorghums to accumulate sugars in their culms. From a forage/ethanol perspective, nonstructural carbohydrates represent a substrate than is easily digested or fermented. In Iowa, Buxton et al. (1999) showed that the sweet sorghum 'M-81E' had a total nonstructural carbohydrate (TNC) concentration of 244 g kg^{-1} when grown as a sole crop, but

decreased to 218 g kg^{-1} when grown in a double-cropping system with rye. Also in this study, the authors showed that the forage sorghum 'FFR 201' had a TNC concentration of 146 g kg^{-1} which was not significantly different across cropping systems. Similarly in Italy, Dolciotti et al. (1998) showed that the sweet sorghum 'Wray' and forage sorghum 'H173' had a fermentable sugar (i.e. sucrose, glucose, and fructose) concentration of 43.58 and 19.49 % on a dry matter basis, with the total soluble sugar yield of the sorghums being 9.10 and 3.92 Mg ha^{-1}, respectively. In Minnesota, Putnam et al. (1991) showed that the total fermentable carbohydrate yield ranged from 2.28 to 7.01 Mg ha^{-1} for the 15 sweet sorghum cultivars evaluated. As with the forage sorghums, the TNC concentration of sorghum x sudangrass has been shown to be much lower than sweet sorghums, within the 142-159 g kg^{-1} range (Cherney et al., 1986).

Numerous studies have predicted ethanol yields based only on nonstructural carbohydrate fraction in sorghums. In Minnesota, the potential ethanol yields from sweet sorghums ranged from 1,300-3,985 L ha^{-1}, compared to 2,582 L ha^{-1} estimated from the grain yield of a corn hybrid used in the study (Putnam et al., 1991). The range of ethanol yield in the cultivars illustrates that selection of specific cultivars may be important for production. These estimates are similar to the ones reported in Smith et al. (1987), where ethanol yields ranged from 2182 L ha^{-1} in Fargo, ND to 6388 L ha^{-1} in Aiea, HI. In Iowa (Anderson et al., 1995) it was found that a maximum yield of ethanol of 4,714 L ha^{-1} was produced from the sweet sorghum 'Keller'. More recent studies (Zhao et al., 2009; Dolciotti, 1998) also have reported ethanol yields from sorghum within this range.

Structural Carbohydrates

In recent years, there has been considerable interest in the use of structural carbohydrates (i.e. carbohydrates located within the plant cell wall) in the production of ethanol (Perlack et al., 2005). Although not as readily digested as their nonstructural counterparts, they represent a larger fraction of the plant. One of the reasons why these are not are readily available for fermentation is that

carbohydrate matrixes also contain lignin, which inhibits their contact with enzymes and degrading organisms (Jung and Deetz, 1993). Thus lignin concentration is one of the most limiting factors of a material as a feedstock for livestock production. Dolciotti et al. (1998) found that the forage sorghums contained a larger concentration of cellulose, hemicelluloses, and lignin (41.85 %, 27.21%, and 7.82 %, respectively) than did the sweet sorghum (25.41 % C, 22.34 % HC, 3.84 % L). They estimated that this translated to total structural carbohydrate yields of 13.74 and 10.07 Mg ha^{-1} for the forage and sweet sorghums, respectively. In contrast, Buxton et al. (1999) found in their study that sweet sorghums had greater levels of cell wall polysaccharides than did the forage sorghum evaluated (55% vs. 51%). The authors reasoned, as this was unexpected, that the since the whole plant was used for analysis, the grain produced from the forage sorghum could have diluted the cell wall concentrations in the sample. Reported concentrations of cellulose and hemicellulose for sorghum x sudangrasses also are approximately within this range of values (Beck et al., 2007; Fritz et al., 1990).

Because lignocellulosic ethanol is still a developing technology and the conversion of structural carbohydrate to ethanol is more difficult than nonstructural carbohydrates, the estimates of ethanol yields reported from biomass in literature may be considered somewhat ambiguous. In the double-cropping system reported by Heggenstaller et al. (2008), the authors reported the sorghum x sudangrass was capable of producing approximately 5,500 L of ethanol per hectare of the total 8,000 L ha^{-1} produced by the system. Their estimates were based on the conversion factor of 501 L Mg^{-1} biomass reported in a National Renewable Energy (NRE) report (Wallace et al., 2005). Similarly in Louisiana, Tew et al. (2008) estimated 3,030 to 8,860 L of ethanol per hectare being produced by sorghum x sudangrasses, depending on harvest date, by using another conversion factor based on the amounts of biomass produced. The issues associated with using these methods for estimating ethanol yields via are that they are based on the assumption that the composition and conversion efficiencies of all biomass are consistent across materials and environmental conditions, while considerable research has shown that there may be sizeable variation among feedstocks (Buxton and Fales, 1994;

Buxton and Casler, 1993). In contrast, Zhao et al. (2009) reported ethanol yields ranging 709 to 5414 L ha^{-1} for sorghums grown as a sole-crop. These estimations were derived from conversion efficiencies that take into account the various fractions of cell wall carbohydrates. Regardless of the approximation method, it seems evident that sorghums are currently capable of producing roughly 4,000 to 5,000 L of ethanol per hectare, with this number likely to increase with the continued development of new conversion technologies.

Use in Double-cropping

There have been numerous studies that incorporated sorghums (Sanford et al., 1973; Camper et al., 1972; Nelson et al., 1977) into double-cropping systems, but only a few have shown the potential for their use for biomass production. Helsel and Wedin (1981) showed that of the ten double-cropping systems evaluated, the ones that incorporated forage sorghums and sorghum x sudangrasses were among the most productive systems, producing equal or greater yields than the single-cropped system. They hypothesized that because these crops were adapted to hot dry climates and had a higher tillering capacity, they were able to overcome some of the issues associated with double-cropping, such as limited moisture. Interestingly in this study, the sorghum yields in the double-cropping system were significantly higher than the yields of the sorghums grown as a sole crop. The authors attributed this to the fact that the sole cropped sorghums were planted early and possibly had slower germination and growth of the crop due to cooler soil conditions. Similarly, Heggenstaller et al. (2008) showed that sorghum x sudangrass double-cropped with triticale had system yields of 23 Mg ha^{-1}, which is comparable to the yields reported for sorghum x sudangrass as a sole crop (Venuto and Kendiger, 2008). However in contrast to these studies, Buxton et al. (1999) reported that sweet and forage sorghums double-cropped with rye only had higher yields than the sole-cropped sorghums when the winter annual crop was fertilized at the high nitrogen rates. Overall, they concluded that although slightly higher yields were obtained, the increased cost would limit use

of such systems and that future use of double-cropping systems would be due solely to the system's positive environmental effects.

Triticale

General Overview

Triticale has been shown to be a productive crop, both as a grain or forage. Originally developed in the 1870's by plant breeders who were trying to develop a crop with the respective agronomic traits of both wheat (*Triticum aestivum*) and rye, triticale did not become extensively bred until the 1970's by the University of Manitoba (Oelke et al., 1989). As of 2007, triticale was produced on approximately 3.7 million hectares worldwide (FOA, 2007). While a majority of this crop is grown for grain, triticale has been shown to be an adequate forage. Winter varieties have been shown in the Midwest to yield up to as much as 4.1 and 12.1 Mg ha^{-1} as grain and forge crops, respectively (Gibson et al., 2007; Harmoney and Thompson, 2005). In addition to having high yield potential, triticale has been of considerable interest as a cover crop. Schwarte et al. (2005) found that triticale is an effective crop for capturing mineralized spring nitrogen, accumulating up to 20 g kg^{-1} of nitrogen. In addition to accumulating nutrients, triticale may provide also valuable residue cover in the winter, which could considerably reduce erosion potential (Gibson et al., 2007).

Biomass Production Potential

There has been considerable research done to compare the forage yield of triticale to other cereal crops. In western Idaho, Brown (2006) showed that winter triticale produced considerably higher forage yields than other winter cereal crops, with an average yield of 6.61 Mg ha^{-1} compared to 5.44 and 5.43 Mg ha^{-1} for wheat and barley, respectively. Similarly in northern Alabama, it was shown that triticale cultivars '6TA 131' and '6TA 298' harvested in the boot stage yielded approximately 8.40 Mg ha^{-1} compared to 7.38, 7.07, 6.66, and 6.99 Mg ha^{-1} for barley, oat, wheat, and

rye forages, respectively (Bishnoi et al., 1978). In contrast to these studies, there are some instances where rye has been shown to produce a higher yield than triticale. In Alberta, Canada, Juskiw et al. (2000) reported that 'Prima' rye had higher yields than 'Pika' triticale at standard seeding rates (10.75 vs. 12.07 Mg ha^{-1}). It was not until seeding rates were increased to three times the standard rates did the triticale produce yields equal to that of rye (11.4 and 11.34 Mg ha^{-1}, respectively). These results are similar to the ones found from the comparison of rye and triticale in Ohio and Georgia (McCormick et al., 2006; Brown and Almodares, 1976).

Triticale has shown great potential for biomass production in the Midwest. In Iowa, Gibson et al. (2007) showed that winter triticale was able to produce total dry matter yields of 9.2 and 10.3 Mg ha^{-1}, when succeeding corn silage and soybeans, respectively. Also in this study, the authors concluded that maximum yields could be obtained with the application of only 33 kg ha^{-1} of nitrogen. Similar forage triticale yields were reported in Lekgari et al. (2008), where 29 triticale varieties were evaluated at two locations in Nebraska. In Kansas at similar fertilization rates, triticale yields harvested at boot stage had yields ranging from 1.7-9.9 Mg ha^{-1} (Harmoney and Thompson, 2005). The wide range in yields over the years of this study was conducted may indicate that triticale is susceptible to environmental conditions, as the large increase in yield was attributed to a large increase in rainfall when the forage was beginning to break its winter dormancy.

Forage/Feedstock Quality

Based on reported forage quality parameters, triticale may be a high-quality feedstock for lignocellulosic ethanol production. Brown and Almodare (1976) showed that triticale forage had a lower cell wall content (46.9%) than did rye (55.8%) and wheat (48.5%), but had a larger fraction than oat forages (40.7%). On the contrary, Juskiw et al. (2000) estimated the neutral detergent fiber (NDF) and acid detergent (ADF) components to be higher in triticale (NDF: 583 g kg^{-1}, ADF: 358 g kg^{-1}) than in rye (NDF: 523 g kg^{-1}, ADF: 316 g kg^{-1}). These estimates of NDF and ADF are lower

than those reported in Harmoney and Thompson (2005), which gave estimates of 63.4 and 39.5 % for these respective portions. Lekgari et al. (2008) reported ranges in NDF, ADF, and acid detergent lignin (ADL) of 594-635, 321-348, and 38-43 g kg^{-1}, respectively. Based on these reported estimates, the yield of structural carbohydrates produced from triticale biomass would be approximately 2.30-2.90 Mg ha^{-1} of hemicellulose and 2.38-3.01 Mg ha^{-1} of cellulose.

It has been estimated that triticale is capable of producing approximately 2600 L of ethanol per hectare based on its structural carbohydrate concentration alone (Heggenstaller et al., 2008). One area that is usually underappreciated is the ability of triticale produced as a forage to still be able to produce generous amounts of grain (Lekgari et al., 2008). The added starch supplied by the grain in whole plant biomass will likely increase the amount of ethanol produced as it adds additional amounts of nonstructural carbohydrates that are more readily fermented to ethanol by microbes. The total nonstructural carbohydrate (TNC) concentration of triticale forage has been shown to increase from 208 g kg^{-1} at the onset of flowering to 296 g kg^{-1} at soft dough stage (Guedes et al., 2006). As these concentrations make up a considerable portion of the material, is would be reasonable to assume that the estimates of ethanol produced from triticale could significantly increase when TNC is taken in account.

Use in Double-Cropping

Although triticale has become popular as grain and popular and has been estimated to be an effective cover crop (Gibson et al., 2007; Nance et al., 2007), there has been few accounts of its use in double-cropping systems. In central Iowa, Heggenstaller et al. (2008) evaluated triticale in three different cropping systems (triticale/corn, triticale/sorghum x sudangrass, and triticale/sunn hemp [*Crotalaria juncea* L.). Between systems, the triticale dry matter yields did not vary significantly and averaged 7.83 Mg ha^{-1}, when harvested in early June. The total yields of the systems were 23.0, 22.7, 15.1 Mg ha^{-1} for the triticale/sorghum x sudangrass, triticale/corn, triticale/sunn hemp systems,

respectively, compared to 18.2 Mg ha^{-1} being produced by corn grown as a sole crop. The triticale component of the system yields varied some, as the crop accounted for approximately 34% of the total biomass produced by the triticale/corn and triticale/sorghum systems and increased to 53% of the total production in the triticale/sunn hemp system.

Although the use of triticale in a double-cropping system is limited, its productivity may be better estimated by looking at the previous use of other cereal grains into these types of systems. Murdock and Wells (1978) reported more total silage from harvesting corn and a small grain silage (barley or oats) from the same acreage than only harvesting mono-cropped corn for silage (58.45 vs. 46.95 Mg ha^{-1}). Helsel and Wedin (1981) showed that oats had higher forage yields than rye (5.5 vs. 4.75 Mg ha^{-1}) when produced in a double-cropping system, however, this translated to lower yields of the subsequent crop and lower total system yields for the oat cropping system. However, these authors reported the rye double-cropping system produced equal or greater yields than the sole cropping system for many of the crops evaluated; suggesting that effectiveness of a double-cropping system may depend on the selection of the winter crop. However, Buxton et al. (1999) illustrated that incorporation of winter cereals into a cropping system may not always be beneficial, as they found that with except at the highest nitrogen rate, rye double-cropped with sorghum were less productive than sorghum grown as a sole crop.

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CHAPTER III: MATERIALS AND METHODS

Experimental Design

Location and Layout

This experiment was conducted at the Iowa State University Sorenson Research Farm (42° 01' N, 93° 46' W) for the 2007-08 and 2008-09 growing seasons. An additional site was included for the 2008-09 growing season at the Iowa State University Northwest Research Farm (42° 55'N, 95° 32'W). The predominate soil types for these sites were a Nicollet loam soil (fine-loamy, mixed, superactive, mesic Aquic Hapludoll) and a Primghar silty clay loam (fine-silty, mixed, superactive, mesic Aquic Hapludoll) at the Sorenson and Northwest locations, respectively. Initial soil test showed that the Nicollet soil had a pH of 6.75 and available P and K concentrations of 16 and 148 ppm in 2007-08. For this soil during the 2008-09 growing season this soil had a pH of 6.68 and P and K concentrations of 15 and 131 ppm. At the NW farm, the Primghar soil had a pH of 6.6 and P and K concentrations of 15 and 191 ppm.

The experimental layout was randomized complete block split-plot design that was replicated four times. The whole plots consisted of sorghum (*Sorghum bicolor* (L.) Moench) grown as a season long crop and in a double-cropping system with triticale (x *Triticosecale* Wittmack). The subplots consisted of 12 sorghum genotypes representative of three sorghum types (Table 1) and were chose based on their relatively high production potential. Within the double-cropping plots, an additional subplot was included to represent triticale grown as a sole forage crop, with no sorghum subsequently planted into these plots. The whole plots had dimensions of 39.6 by 7.6 meters and 36.6 by 7.6 meters for the double-cropping and sole-cropping systems, respectively, while the subplots were 3.1 by 7.6 meters.

Cultural Practices

Triticale was planted on September 12th, 2007 with a grain drill at the Sorenson location into a prepared seedbed. For the 2008-09 growing season, triticale was seeded on September 16th and 18th at the Sorenson and Northwest farms, respectively. At the Northwest farm, the crop was planted into a prepared seedbed, while at the Sorenson site it was no-tilled into sorghum stubble. All plots were seeded at 112 kg of pure live seed (PLS) per hectare. Each spring the triticale received with 34 kg of N/ha (urea) at the beginning of April each year. The target harvest date for the triticale was approximately the first week of June. This was accomplished during the 2008-09 (Sorenson: June 1st; Northwest: June 3rd) growing season; however, harvest was delayed until mid-June for the 2007-08 (June 16th) growing season due to flooded field conditions. Because the plots were already in the mid-reproductive stages at this time, the single-cropped triticale plots were harvested with the double-cropped triticale plots.

The sorghums were planted within eight inch rows at 13.4 kg of PLS/ha, in the spring of the year. Because of the previously mentioned wet conditions in 2008, both the single and double cropped sorghums were planted in mid-June (June 18th). For the 2009 growing season, the single cropped sorghums were planted in mid-May (Sorenson: May 18th; Northwest: May 20th), while the double-cropped sorghums were planted early June (Sorenson: June 4th; Northwest: June 11th). All of the sorghum plots received 123 kg of N/ha (urea) at the start of the growing season. The double-cropped sorghums were harvested on September 11th for the 2007-08 growing season and on September 14th and 16th for the Sorenson and Northwest locations during the 2008-09 growing season. This was done to allow adequate time for establishment of the winter annual crop. The sole-cropped sorghums were harvested immediately after frost, which occurred on October 27th for the 2007-08 season and on October 5th and 7th for the Sorenson and Northwest farms during the 2008-09 season.

Several herbicides were used to control weeds throughout the growing season. Glyphosphate (Round-up Weather-max®) applied at a rate of 1.61 liters of active ingredient (a.i) per hectare before planting of each crop. Throughout the growing season, atrazine (Aatrex-90df at 2.24 kg of a.i. /ha) and 2, 4-D (1.75 L/ ha) were sprayed as needed to control cool-season grasses and broadleaves weeds in the sorghums. No additional weed control was needed for the triticale.

Data Collection

Triticale yields were estimated by harvesting the plant material from two square meters within each subplot. Sorghum yields were estimated by harvesting the middle 5.6 meters of two rows from each plot. All materials were harvested to an approximate height of 5 cm. Subsamples were taken for moisture determination and chemical analyses. Sorghum plant populations were estimated by counting the number of stems removed from the rows at harvest. Sorghum heights were measured from ground level to the upper most portion of the canopy, while stem diameter was measured approximately between the third and fourth palpable node. Plant maturities were estimated using the Nebraska system (Moore et al., 1991). The amount of lodged sorghum and weeds were also estimated for each plot. This was done via visual inspection using a scale of 0-5, with 0 representing no reported incidents and 5 representing total occurrence.

Chemical Analysis

All samples were dried at 60°C for three days in a forced-air dryer. The samples were then ground with a Thomas-Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a one millimeter screen.

Dry Matter and Ash

All quality analyses of the biomass were estimated on a dry matter basis. To accomplish this, weighed samples were placed in a 105°F oven for four hours. The approximate moisture of each sample was estimated from measuring the difference in weights of the dried sample. To conserve material and determine the ash content, these samples were then placed overnight into a muffle furnace at 600°C. The ash content was determined by measuring the amount of sample left after ignition within the furnace.

Structural Carbohydrates

The amount of structural carbohydrates of each sample was calculated using the fiber analysis procedure described in Vogel et al. (1999). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations were estimated sequentially using an ANKOM 200/220 fiber analyzer (ANKOM Technologies, Macedon, NY). The concentration of hemicelluloses was approximated from subtraction of the ADF from the NDF, while as the concentration of cellulose of each sample was estimated by subtracting the ADL from the ADF. All lignin values reported are on the ash-free basis. Standards of similar and known chemical composition were run with samples groups to identify any potential errors within a specific run. Smooth brome (*Bromus inermis* Lyess.) of late vegetative to mid-reproductive stage was used as a standard for the winter annuals. For the sorghums, a mid-reproductive stage sweet sorghum was used.

Total Nonstructural Carbohydrates

The concentration of total nonstructural carbohydrates (TNC) was estimated using the colorimetric method described by Guiragosian et al. (1977). Samples were digested in 25 ml of 0.2 N H_2SO_4 at 135°F for 75 minutes. Samples were then scanned at the 490 nm wavelength using a

Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments; Columbia, MD). D-glucose was used as a standard to develop a TNC concentration curve.

Total Nitrogen

The concentration of total nitrogen for each sample were estimated by the Iowa State University Plant and Soil Analysis Laboratory (Ames, IA) using a LECO analyzer (Model CHN-2000, LECO Co., St. Joseph, MI). The crude protein concentration of each sample was approximated by multiplying total nitrogen by 6.25%.

Theoretical Ethanol

The potential ethanol yields of cellulose and hemicellulose were estimated by the equation described in Kirkpatrick et al. (2008). The amount of cellulose was estimated via subtraction of the values obtained for ADL from the concentration of ADF. The amounts of hemicelluloses were estimated via subtraction of the ADF from the NDF values obtained for each sample. The total yield of TNC was obtained by multiplication of the percent TNC of each sample by its respective plot yield. The ethanol yields from TNC were estimated using the same method as the structural carbohydrates and were assumed to consist entirely of hexoses. The ethanol yields from each carbohydrate source were then added to obtain the total theoretical ethanol potential of each plot.

Cost Analysis

The production cost of each system was estimated using the 2009 crop production budgets from Iowa State University (Duff and Smith, 2009). It was assumed that the triticale was removed as a hay crop, while the sorghum was removed for silage. The predicted equipment usage for each system, as well as the estimated fixed and variable cost for the machinery, is summarized in Table 2. Fertilizer prices were estimated at $0.68, $0.90, and $0.72 for each pound of nitrogen, phosphate, and

potash applied, respectively. Lime was applied yearly at a cost of $10 per acre. The herbicide prices used were $80/gal, $16/gal, and $2.27/lb for round-up®, 2,4-D, and atrazine, respectively. The total phosphate and potash costs were based on Iowa State fertilizer recommendations of optimum soil test ratings (ISU, 2002) for sorghum silage and tall cool-season grass hay. The total seed, nitrogen, and herbicide costs were estimated from amounts used in the study. It was assumed that the producer had an interest rate on preharvest variable cost of 6.25% for eight months, and had rent cost of $205/acre. The assumed labor cost was $11 per hour and that five and two hours of labor were required for each acre of sorghum and triticale, respectively. The producer was also expected to have approximately $9 per acre of miscellaneous cost for each crop.

Statistical Analysis

The experiment was analyzed as a traditional split plot design. The year and location factors were combined and analyzed as environments due to the unbalance of years at each location. Significance of all treatments was determined by using the generalized linear model (GLM) of the Statistical Analysis Software (SAS, 2003). Comparisons between sorghum cultivars were done using a least significant differences (LSD) test and orthogonal contrasts. LSMEANS was used to compare variables within significant interactions. Differences were considered significant at $P \leq 0.05$ level. Cropping system and sorghum varieties were considered fixed factors for the study, while blocks were considered random. Because of diverse climatic conditions during the study, growing environments were considered fixed factors in order to accurately detail their effect on the measured parameters of the study.

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Table 1. Summary of Study Sorghum Cultivars

Cultivar	Source
Sweet Sorghums	
M-81E	Mississippi State University
Sucrosorgo 405	Sorghum Partners, Inc.
Sugar T	Texas A&M University
Topper 76-6	Mississippi State University
Forage Sorghums	
1990	Sorghum Partners, Inc.
FS-5	Monsanto
Pacesetter BMR	Richardson Seeds, Ltd.
Silo Master D	Richardson Seeds, Ltd.
Sorghum x Sudangrass	
Maxi Gain	Coffey Forage Seed, Inc.
Mega Green	Walter Moss Seed Company, Ltd.
Pacesetter	Richardson Seeds, Ltd.
Sugar Graze Ultra	Coffey Forage Seed, Inc.

Table 2. Summary of Machinery Costs

Operation	Assumed Hours of Use	Fixed Cost per Acre	Variable Cost per Acre
Triticale			
Bulk Fertilizer Spreader	60	$1.60	$1.20
Grain Drill	100	$4.80	$4.00
Sprayer	150	$1.20	$1.00
Mower-conditioner	120	$3.80	$2.90
Rake	120	$1.90	$1.40
Round Baler	120	$7.60	$4.60
Haul Large Round Bales	120	$1.00	$1.35
Sorghum			
Bulk Fertilizer Spreader	60	$1.60	$1.20
Grain Drill	100	$4.80	$4.00
Sprayer	150	$1.20	$1.00
Silage Harvester	200	$27.00	$12.60
Haul Silage	140	$3.70/ ton	$3.30/ ton

CHAPTER IV: RESULTS AND DISCUSSION

Weather

The weather conditions observed at the locations during the study are summarized in Figures 1-4. Figures 1 and 2 show the monthly growing degree days (GDD) for the growing season at the Sorenson and Northwest (NW) farm, respectively, along with the averages for each site. The Sorenson and NW farms had a seasonal accumulation of 3,850 and 3,700 GDD, respectively. The fall of 2007 was slightly warmer than average (Fig. 1) with 751 GDD occurring in September and October, compared with the average for this time period being 670 GDD. The following spring was slightly cooler than typical conditions. At the end of May only 432 GDD had amassed, which was noticeably lower than the amount that normally occurs during this time (620 GDD). During June through August of 2008, there were approximately 1,816 GDD, roughly equal to the average for this period (1,891 GDD) Overall, the 2007-08 growing season accumulated a total of 3,633 GDD, slightly less than the average growing conditions.

The fall and spring of the following growing season (2008-09) at the Sorenson location were slightly colder than the previous season (Fig. 1). Approximately 634 GDD accumulated during September and October of 2008 and was followed by 562 GDD in March through May of 2009. The NW farm accrued 616 and 566 GDD for the fall of 2008 and spring of 2009 (Fig. 2), respectively. These temperatures were fairly close to the average, being within roughly 20 GDD. By the end of the spring, both sites had accumulated nearly the same GDD (Sorenson: 1,196; NW: 1,182 GDD), and each was slightly below the average for their respective site. The summer months of 2009 were among of the coolest on record. This resulted in there being nearly 200 GDD less occurring from June to August at both sites, than normally occurs (Sorenson: 1,670; NW: 1,638). The fall of 2009 had an earlier killing frost at both sites, which resulted in there being much less GDD accumulated for

October (GDD < 90). Overall both sites had accrued nearly the same GDD (Sorenson: 3,384; NW: 3,309), and were considerably less than the site averages.

The monthly precipitation for both growing seasons may be seen in Figures 3 and 4. The 2007-08 growing season was typified by very wet conditions. October of 2007 (Fig. 3) had more than double the average rainfall (139.7 mm vs. 63.5 mm). This was followed by large amounts of rain during the spring and summer of 2008. The months of May, June, and July each had over 241.3 mm of rainfall, more than double the average amounts. By the beginning August, the site collected 934.2 mm of precipitation. This was more than the total seasonal average of precipitation for the Sorenson site (979.9 mm). This additional rain caused massive flooding, and made field work difficult. The total precipitation for this entire growing season was 1,385.6 mm.

Precipitation during the 2008-09 growing season was the opposite of the preceding season and was characterized as being drier than average. The Sorenson and NW farms (Figs. 3 and 4), as with the previous season, had higher than average rainfall during the fall of 2008, with 172.2 and 220.0 mm for each site, respectively. The Sorenson site had greater than average during the early spring (March and April), but visibly less than average amounts for the late spring (May) and summer months. However, this did not have an effect on the total seasonal precipitation for this site (1,002.5 mm), which was approximately identical to the average for the site. The NW farm followed similar trends during the summer, but the magnitude was more severe. There was no additional rainfall in the early spring at this site, and by the end of June there was an annual rainfall deficit of 128.3 mm. There was above average precipitation in July, before the onset of another drought period during August and September of 2009. Overall, the NW farm had 697.0 mm of precipitation for the growing season, which was much lower than the site average of 811.0 mm.

Triticale

The statistical analysis of the triticale grown is summarized in Table 1. The growing environment had the largest effect on the yield and quality of triticale, as it had a large effect on nearly all measurements. The lack of a cropping system (i.e. single- or double-cropped) influence is most likely the result of the timing of harvest. The initial goal of the study was to harvest the single-cropped triticale when the crop was at a maturity stage that would be typical for harvest as a forage, and to harvest the double-cropped triticale when conditions were ideal for the establishment of the succeeding sorghum crop. For all years and locations of this study, the timing of two these harvests overlapped, and both cropping systems were harvested at approximately the same maturity. The trends between the two cropping systems were consistent for all of the site environments, and because of this, the triticale data reported is the average of the two systems at each site.

Yield

Average triticale yields at each site are shown in Figure 5. When averaged across all environments, the triticale had an approximate yield of 4.54 Mg ha^{-1}. Yields were significantly different between each environment. The crop grown at the Sorenson location during 2007-08 growing season had the highest yield (6.63 Mg ha^{-1}), followed by the NW and Sorenson farms during the 2008-09 season, with 4.56 and 2.44 Mg ha^{-1}, respectively. One of the reasons for the higher yield of 2007-08 was the large amount of rainfall during the spring (Fig. 3) that caused flooding in the area, delaying triticale harvest approximately two weeks. This caused the triticale during this year to be harvested in the early stages of grain development, compared to early reproductive stages in the following years, and allowed the crop to accumulate more dry matter and consequently higher yields. The fall of 2007 also experienced warmer than normal temperatures and accumulated more growing degree days (GDD) during this period. GDD have been implicated to have an effect on the amount of leaves that are produced by cereal crops (Cao and Moss, 1989; Siddique et al., 1989). A greater

amount of leaves would have a direct effect on the amount of carbohydrate reserves of the plant (Grueb and Wedin, 1971; White, 1973), and could have resulted in earlier and more rapid spring growth during this growing season.

It is not immediately known why the triticale at the Sorenson site experienced lower yields than the other site locations. It is possible that the reduced growth could be due to the cropping system of the previous year. At this location, the triticale was planted directly into to the sorghum residue of the previous year. Sorghum has been shown to be capable of producing alleopathic compounds that are capable of diminished growth in sequentially planted crops (Ben-Hammouda et al., 1995). While this seems plausible, the triticale that was grown as a sole crop also experienced a noticeably lower yield (2.74 Mg ha^{-1}) than other locations and was comparable to the yields of its double-cropped counterpart. A more likely cause for this yield reduction could be due to the climatic conditions during this growing season. During the spring of 2009 at this site, there was a considerably higher precipitation (Fig. 3) and lower temperatures (Fig. 1) than the average for the site. These cool, wet conditions have been shown to stunt the development of grasses due to a climate induced phosphorus deficiency. Although no quantitative measurements were taken to verify this, the triticale at this site was visibly shorter and there was no need for phosphorus application based on the soil test for the site.

The triticale yields of this study are predominately lower than the yields produced in other studies. In central Iowa, Gibson et al. (2007) reported yields of 9.2 and 10.3 Mg ha^{-1} for winter triticale grown as a forage following corn and soybeans, respectively. Similarly in Idaho, Brown (2006) showed that triticale was capable of producing yields of 6.61 Mg ha^{-1}, which was comparable to the triticale during the first year of the study. The reasons for these discrepancies are likely a combination of differences in cultural practices (i.e. fertilizer use, time of harvest) and unfavorable weather conditions for this study. Other researchers have described similar differences among

triticale yields due to variation in climatic conditions. Harmoney and Thompson (2005) reported a range in yields of 1.69 to 9.91 Mg ha^{-1} for triticale fertilized at similar rates as this study, and attributed much of this variation to differences in amounts and timing of rainfall.

Chemical Composition

The quality of triticale for use as a forage/feedstock are summarized in Table 2, and also varied with the site environments. Most of the variance between environments may be described by the differences in maturity of the crop at the times of harvest. The neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (ADL) fractions of the triticale were all higher for the Sorenson site during the 2007-08 year, but were not different between the two sites during the following year. This is due to the triticale being harvested at a more immature stage during the last year of the study. It has been shown that the concentrations of these components increase with physiological maturity (Hatfield, 1993). Similarly, it has been well documented that the protein level of a forage decreases with age, which would explain the reduced crude protein (CP) concentration of the 2007-08 Sorenson site (100.6 g kg^{-1}) when compared to the 2008-09 Sorenson and NW farms (147.8 and 137.3 g kg^{-1}, respectively).

The total nonstructural carbohydrate (TNC) concentration of the samples varied slightly between environments, but there were still some apparent differences. At the Sorenson site, there were significant differences in TNC concentration between the different years (2007-08: 178.5 g kg^{-1}; 2008-09: 205.2 g kg^{-1}), however, the NW farm did vary from either of these sites (190.7 g kg^{-1}). The concentrations of TNC for the 2008-09 growing seasons were equivalent to what has been reported for triticale forage at similar stages of growth (208 g kg^{-1}) (Guedes et al., 2006). However, during the 2007-08 growing season they were much lower than the reported concentration for its relative maturity (238 g kg^{-1}), and is likely due to the triticale reported in the Guesdes paper having a lower cell wall concentration (NDF: 571 g kg^{-1}). As the cell wall fraction of a plant increases, it has

been shown that the content of the protoplasm (i.e. cell solubles), decreases proportionally (Van Soest, 1994). Since the TNC fraction is located within this portion, its reasonable that its concentration may concomitantly decrease.

The concentration of ash concentration varied with location rather than to a specific growing environment (i.e. year and location). The concentration of ash of the triticale was lower for the Sorenson sites (2007-08: 73.0 g kg^{-1}; 2008-09: 70.0 g kg^{-1}) than at the NW location (80.0 g kg^{-1}). This trend is reflective of the initial mineral fertility of the sites (see Materials and Methods). Since most nutrients enter the plant via mass flow into the roots, higher concentrations within the soil increase the amount of minerals that enter and accumulate within the plant. From an animal nutrition standpoint, this is rather insignificant as excess mineral nutrients may be excreted by the animal, however, for cellulosic ethanol production it may be prove to a problem. An increase in the ash concentration of a feedstock represents a comparative decrease in its available energy (McKendry, 2002). The ash concentration of the triticale is much greater than other crops that are considered ideal for thermo-chemical conversion (McKendry, 2002).

Ethanol

The amount of ethanol that may be potentially derived per Mg of triticale follows similar trends as their respective carbohydrate fractions (Table 2). The Sorenson site during 2008-09 had the largest potential ethanol from nonstructural carbohydrate (NSTE) with 148 L Mg^{-1}, followed the NW farm (137 Mg ha^{-1}) and 2007-08 Sorenson site (129 L Mg^{-1}), respectively. Similarly, the triticale at the 2007-08 Sorenson site was capable of producing the most ethanol from structural carbohydrates (450 L Mg^{-1}), compared to 396 and 401 L Mg^{-1} for the 2008-09 Sorenson and NW sites, respectively. These ethanol estimates were determined empirically, and because of this, the differences between the growing environments are the same as described for their respective carbohydrate fractions. Overall, the Sorenson plots during 2007-08 tended to have the highest total theoretical ethanol potential (TTE)

with 579 L Mg^{-1}, followed by the two sites during the 2008-09 growing seasons (543 and 538 L Mg^{-1} for the Sorenson and NW farms, respectively). When averaged across the different site environments, an estimated 553 L of ethanol may be produced from each Mg of triticale.

The total potential ethanol yields from triticale at all sites are shown in Figure 6, and are reflective of the total biomass yields for the environments. The triticale at the 2007-08 Sorenson site was capable of producing at total 3,834 L of ethanol per ha, with 852 and 2,982 L derived from nonstructural (NSC) and structural (SC) carbohydrate respectively. The other sites produced a total of 1,337 L ha^{-1} (NSC: 352 L ha^{-1}; SC: 985 L ha^{-1}) and 2,456 L ha^{-1} (NSC: 613 L ha^{-1}; SC: 1,843 L ha^{-1}) for the 2008-09 Sorenson and NW farms. On average triticale was triticale produced total ethanol yields of 2,542 L ha^{-1}, with 605 and 1,937 L ha^{-1} from the NSC and SC fractions.

The majority of the total potential ethanol was consistently derived from structural carbohydrates (SC), accounting for approximately 75% of the total yields at each location. Although this may seem to indicate that the amount of structural carbohydrates has the largest effect on the total ethanol potential of a feedstock, it should be noted that these estimates are based on complete conversion of these carbohydrates into ethanol. It has been well documented conversion of lignocellosic feedstocks into ethanol is less efficient than nonstructural carbohydrates (Sticklen, 2007). Conversion efficiencies for starch and sucrose into ethanol approach total conversion, while cellulosic materials are approximately 60-80%, depending on the conversion process and feedstock (Chen et al., 2007; Sun and Cheng, 2002). The NSC, however, provides a substrate that may be readily and almost entirely converted to fuel, and thus, its importance within a feedstock should not be underestimated.

Sorghum

A statistical summary of the sorghum genotypes within a double-cropping system with triticale and a single-cropping system is shown in Table 3. As with the triticale, the growing environment had a large influence on the study parameters, however, there was also a cropping system and variety effect. If there was a significant interaction between factors, the data of its interaction is reported in place of the main effects.

Yield

The average yield of sorghums grown at each environment is shown in Figure 7. Among the double-cropping systems, 2008-09 Sorenson site produced the highest yields (12.3 Mg ha^{-1}), while the NW farm during the same growing year produced the lowest (8.9 Mg ha^{-1}). The yields of the 2007-08 Sorenson site (10.0 Mg ha^{-1}) did not vary considerably from either of these sites. The lower yields of the double-cropped sorghums at the NW farm are likely the result of a moisture limitation brought on by the double-cropping system. The spring at this site was considerably drier than typical conditions (Fig. 4), and at the time of harvest these sorghums were less mature than those at the Sorenson site for the same year (Table 4). The additional moisture limitation caused by incorporation of another crop into the system more than likely caused to sorghums to become dormant until adequate soil moisture was attained. The single-cropped sorghums yields at the NW site yielded the highest (26.0 Mg ha^{-1}), indicating that the amount of precipitation from the site was not enough to delay growth in the sorghums. The single-cropped sorghums at the Sorenson yielded slightly different between years (2007-08: 17.5Mg ha^{-1}; 2008-09: 15.5 Mg ha^{-1}), but was not significantly different and was most likely due to the drier conditions of the 2008-09 growing season. However, both years were substantially lower than the NW farm. It is not immediately clear why the single-cropped sorghums at the NW farm had substantially higher yields than the other sites. It is plausible that, although slightly drier, the moisture level still was adequate for this system, and when combined with

lower than normal summer temperatures may have created a growing environment that was more conducive for plant growth.

Figure 8 shows the yields of each sorghum variety within the two cropping systems, with their relative rank for each system. Among the double-cropping system, the cultivars FS-5, M-81E, and Sugar T had the highest yields with 12.7, 12.3, and 11.4 Mg ha^{-1}, respectively. Sugar T was also the highest producing variety in the single-cropping system (23.5 Mg ha-1), followed by Mega Green (Mg ha^{-1}) and Pacesetter (22.6 Mg ha^{-1}). The lower yields of the double-cropping system for varieties was expected, as under ideal conditions these sorghums were planted two weeks later harvested approximately two weeks earlier than in the single-cropping system. There appeared to be no difference between a specific sorghum type and cropping system, as all types were represented in the top producing cultivars for a cropping system. There was no interaction between the growing environment and sorghum varieties, indicating that performance of each sorghum was consistent across all of the locations.

There seems to be some noticeable differences for the suitability of sorghum genotypes within a cropping system. The genotypes which did the best within the double-cropping system (i.e. FS-5, M81-E, and Silo Master D) were among the lower half of rankings within the single-cropping system. And with the exception of Sugar T, the same was true for the top ranking genotypes (i.e. Mega Green, Pacesetter, and Sugar Graze Ultra) within the sole cropped sorghums. This may be explained by looking at the growth pattern of each cultivar. The top ranking sorghums in the double-cropping system were earlier maturing varieties and entered reproductive growth sooner. Conversely, the highest producing varieties within the single-crop system were photoperiod sensitive and remained vegetative throughout the growing season. At the time of harvest for the double-cropping system the earlier maturing types have already maximized dry matter production, while the photoperiod sensitive sorghums were still accumulating biomass, and this additional biomass of these

cultivars was lost. This necessitates that future selections of sorghums, both by producers and breeders, should be based on the goals of the production system.

Chemical Composition

The chemical composition of the sorghum varieties is shown in Tables 5-7. The amount of nonstructural carbohydrate (TNC) of the sorghums varied the most across the factors studied. Within the double-cropping system (Table 5), the TNC of the sorghums was much lower at the Sorenson farm for 2007-08 (221.9 g kg^{-1}) than during the other environments (2008-09 Sorenson: 269.8 g kg^{-1}; 2008-09 NW: 262.5 g kg^{-1}). Because wet conditions of the spring of 2008 (Fig. 3), the planting of these sorghums was delayed two weeks, and both cropping systems being planted at the same time in mid-June. This resulted in the double-cropped sorghums being less mature at harvest compared to the other growing environments (Table 4), which may account for these lower values for the 2007-08 growing season. Soluble sugars have been shown to accumulate within the culms of sweet sorghums after the late reproductive stages (McBee and Miller, 1993). Many of the sorghum varieties, particularly the sweet sorghums, had failed to reach these stages at this growing environment, but did for others (Table 4). This, along with visibly less grain production, would have decreased the TNC concentration of these sorghums. The delay in planting for this growing environment did not have an effect on the TNC concentration of the sorghums in the single-cropping system, however, as there was no significant difference between (Table 5). A difference in the maturity at the time of harvest is also the primary reason why the single-cropped sorghums had greater TNC concentrations.

As expected, there were substantial differences in the TNC concentration of the sorghum varieties, which may be attributed to the sorghum type. The sweet sorghums tended to have the largest TNC concentration within each cropping system (Table 6). The cultivars Topper 76-6 (390.2 g kg^{-1}) and Sugar T (369.2 g kg^{-1}), both sweet sorghums, had the greatest concentrations of nonstructural carbohydrates within the single-cropping system. While the cultivars 1990, a forage

sorghum, and Sugar Graze Ultra, a sorghum x sudangrass, had the lowest amount of soluble sugars in this cropping system, with 245.6 and 269.9 g kg^{-1}, respectively. The varieties within the double-cropping system followed similar trends in cultivars (Table 6). Surprisingly, two forage sorghums cultivars, FS-5 and Silo Master D, and a sorghum x sudangrass, Pacesetter BMR, had TNC concentrations similar to the sweet sorghum cultivars within the single cropping system. The high values of the forage sorghums are likely due to these cultivars reaching physiological maturity and producing considerable amount of grain within most of the cropping systems (Table 4).

It is not clear why the cultivar Pacesetter BMR obtained such high TNC values, but several explanations may be deduced. Since the amount of photoassimilate supply is directly proportional to the leaf area, it is possible that the greater leaf area of this vegetative cultivar, particularly at later growth stages may have lead to a greater TNC concentration. In fact, the TNC fraction for this cultivar increased approximately 21% from the time of harvest of the double-cropping system to the single-cropping system (Table 6), while maintaining the same maturity. However, the only other cultivar that stayed vegetative throughout the study (1990) only increased roughly 12% (Table 6), and had the lowest TNC concentration in both cropping systems. It also seems possible that the elevated TNC fraction within the cultivar Pacesetter BMR may be a result of its diverse genetic heritage. Brown mid-rib (BMR) mutants have been shown to have an altered lignin metabolism, and a smaller cell wall fraction, compared to their normal counterparts (Casler et al., 2003; Fritz et al., 1990). Because of this lower cell wall concentration, it may be possible that a greater amount of cell solubles could lead to higher TNC concentration in photoperiod sensitive sorghums, although no relationship like this has been shown in normal genotypes (Cherney et al., 1986).

The magnitude of the increase in TNC between cropping systems varied greatly between cultivars and types (Table 6). Between the sorghum types, the sweet sorghums had the largest increase in TNC from the double-cropping system to the single-cropping systems (~32%). This

compares to increases of nearly 20 and 24% for the forage sorghums and sorghum x sudangrasses, respectively. One of the reasons for the lower increase for the forage sorghums is likely due to the small increase (~12%) for the cultivar 1990, as was previously mentioned. The sweet sorghum Topper 76-6 had the largest increase, with approximately 40%. These differences in the extent of the TNC increase may be attributed the diverse genetic background the cultivars, as their genetic potential is ultimately what drives carbohydrate production and accumulation.

The degree of TNC concentration of the sorghum varieties also varied across study environments (Table 7), particularly for the sweet sorghums, and is probably due to the previously mentioned differences between maturities at harvest. The double-cropped sorghums of 2007-08 were considerably less mature at harvest (Table 4), which would lead to lower TNC concentration for the cropping systems and site average. Several sorghum cultivars (i.e. Sugar Graze Ultra and Mega Green) had higher concentrations at the Sorenson site during the 2008-09 growing season, and this is believed to be due to these double-cropped cultivars becoming dormant during the dry conditions at the NW site (Table 4). It had been demonstrated in this study that the growth stage at the time of harvest has a large impact the sorghum's TNC production, as both cropping system and environmental effects have been shown to significantly affect the rate a sorghum may mature. It is likely that these effects, along with the intrinsic genetic potential of the sorghums, culminated in the formation of a significant three-way interaction that is most likely an artifact the underlying conditions affecting the onset of maturity within the other factors.

There were some slight differences within the cell wall composition of the sorghums between the different growing environments. The NDF and ADF concentration did not vary among the study environment for two cropping systems (Table 5), despite the single-cropping system being considerably more mature (3.83 vs. 2.88). In fact, the amount of cell wall (NDF) was slightly lower for single-cropping system. However, the ADF concentration were significantly different at each site

(2007-08 Sorenson: 352.0 g kg^{-1}; 2008-09 Sorenson: 335.7 g kg^{-1}; 2008-09 NW: 326.9 g kg^{-1}) when averaged across cropping systems. In contrast to the NDF and ADF concentrations, the lignin concentrations of these sorghums differed greatly between sites (Table 5). Despite previous research showing that these concentrations increase with development (Van Soest, 1994), no consistent relationship could be derived between these parameters and the average maturities of the sorghums. It is possible that the differences between these components may have resulted due to dilution by the higher concentrations of TNC (Table 5). Because the samples were derived from whole plant material, the addition of these carbohydrates would have lowered the overall proportion of cell wall within the composite material. Similar examples of this type of dilution have been previously seen with sorghums (Buxton et al., 1999; Zhao et al., 2009).

The sorghum varieties varied considerably between the concentrations of their cell wall components. On average, the forage sorghums and sorghum x sudangrasses had higher amounts of NDF and ADF compared to the sweet sorghums (Tables 6 and 7). The lower fiber component of the sweet sorghums may be due to dilution by the high TNC fractions, although the fiber concentration is not given in much of the sweet sorghum literature. The levels of NDF and ADF for the sorghum cultivars appears to be approximately inversely proportional to their respective TNC concentration, and this is potentially further evidence that TNC has a diluting effect on the measured cell wall constituents of the plant.

Nearly all the varieties exhibited no difference between the NDF and ADF concentrations between the two cropping systems (Table 6), even though the single-cropped sorghums were more mature (Table 4). It is likely that the increase in these components that typically occurs was "masked" by the larger TNC concentrations with this cropping system. Although the lignin was higher within the single-cropping systems for each cultivar, it would be expected that their concentration should have been much greater (Van Soest, 1994). Similarly, many sorghums

experienced this decrease in its cell wall constituents due to high TNC concentration at different environments, particularly the sweet sorghums and the high TNC forage sorghums (FS-5 and Silo Master D) (Table 7). Among these sorghums, the 2007-08 Sorenson sites were typically lower in TNC and higher in fiber (NDF, ADF, and ADL) than the other sites because they were less mature (Table 4). Several of the sorghums (Maxi Gain, Mega Green, 1990, and Pacesetter) showed trends that have been traditionally thought of as typical. These sorghums had greater NDF, ADF, and ADL concentrations (Table 7) at the 2008-09 sites, likely due to the greater maturity at harvest for these environments. It is possible that the significant three-way interaction observed for TNC directly caused the perceived three-way interactions of these parameters, as TNC production noticeably diluted these components. The factors that interacted to cause an increase in TNC concentration also caused a decrease in the cell wall fractions.

The variation in the crude protein (CP) and ash concentration of the sorghums (Tables 5-7) followed similar trends as the other quality parameters, and may be explained by differences in crop maturity between environments and cropping systems, as were previously mentioned. It has been well documented that the protein concentration of forage crops decreases with maturity (Farhoomand and Wedin, 1968). Similarly, the minerals that make up the ash of the plant are largely contained within the protoplasm of the plant. This fraction, usually termed cell solubles, usually decreases with maturity of the crop and is inversely related to the cell wall (Van Soest, 1994).

Ethanol

As with their yields, the sorghums had theoretical ethanol potentials that varied considerably across both environments and cropping systems. For the double-cropping systems, the sorghums grown during the 2007-08 growing season were lower in TTE (563 L Mg^{-1}) than the other locations (2008-09 Sorenson: 615 L Mg^{-1}; 2008-09 NW: 613 L Mg^{-1}). This is reflective of this site also having lower NSTE and STE, which was a result of these sorghums being harvested at more

immature growth stages (Table 4). Within the single-cropping systems, the sorghums at Sorenson (2007-08: 642 L Mg^{-1}; 2008-09: 644.79 L Mg^{-1}) sites were capable of producing considerably more ethanol than the NW location (612 L Mg^{-1}). This is likely due to the lower STE for the sorghums at this location (389 L Mg^{-1}) compared to Sorenson sites (2007-08: 416 L Mg^{-1}; 2008-09: 411 L Mg^{-1}). The sorghum at this site had a noticeably less cell wall fraction (NDF: 567.3 g kg^{-1}) than the other environments (2007-08 Sorenson: 593.9 g kg^{-1}; 2008-09 NW: 602.7 g kg^{-1}). It is possible that these sorghums could have possible been more leafy, which would have lowered the overall average. However, there is no way to definitively prove this. There was no differences in NSTE for the sorghums (2007-08 Sorenson: 226 L Mg^{-1}; 2008-09 Sorenson: 234 L Mg^{-1}; 2008-09 NW: 223 L Mg^{-1}) within the single-cropping system.

The growing environment and cropping system had significant effects on the theoretical ethanol potentials for the sorghum cultivars (Tables 8 and 9), and were the results of the previously described effects on the carbohydrate fractions (nonstructural and structural). Compared to the double-cropping systems, the NSTE of the single-cropping systems was higher for all varieties. In some cases, the varieties also experienced a decrease in the STE concentration, which was likely due to dilution of the structural carbohydrates by the high TNC concentration. In spite of this decrease in STE, the sorghum varieties had greater TTE within the single-cropping system. Several of the varieties also had noticeable divergence in ethanol potential among the study's growing environments. The NSTE, STE, and TTE tended to be lower at the 2007-08 Sorenson and/or the 2008-09 NW sites, and was due to the double-cropping systems at these sites being less mature at harvest (due to later planting and drought-induced dormancy, respectively). This would have decreased the amount of carbohydrate, as was discussed in the chemical composition section, within the plant would have lead to a lower overall site average.

The total ethanol yields followed similar trends as the biomass yields (Fig. 7). Not surprisingly, the single-cropped sorghums produced more ethanol than the double-cropping systems. The contribution of ethanol from nonstructural carbohydrates increased from 30 to 35% between the double- and single-cropping systems. The double-cropping systems of the 2007-08 Sorenson (5,618 L ha^{-1}) and 2008-09 NW (5,472 L ha^{-1}) sites were lower in total ethanol yield than the 2008-09 Sorenson site (7,553 L ha^{-1}), and were related to later planting and drought-induced dormancy, respectively. As with their biomass yield (Fig. 8), the single-cropped sorghums was highest at 2008-09 NW location (15,881 L ha^{-1}), but there was no difference between the two Sorenson sites (2007-08: 11,222 L ha^{-1}; 2008-09: 9,982 L ha^{-1}).

Within the single-cropping systems, the cultivars Sugar T (15,121 L ha^{-1}), Mega Green (14,423 L ha^{-1}), and Pacesetter (13,950 L ha^{-1}) produced the highest ethanol yields (Fig. 10). In the double-cropping system (Fig. 9), the varieties FS-5 (7,572 L ha^{-1}) and M-81E (7,443 L ha^{-1}) had the highest yields. These cultivars were also among the highest within the biomass (Fig. 8). Among the lowest for both cropping systems were the cultivars Topper 76-6 (SC: 9,534 L ha^{-1}; DC: 4,993 L ha^{-1}) and Pacesetter BMR (SC: 10,890 L ha^{-1}; DC: 4,789 L ha^{-1}), which were also among the highest in theoretical potential (Table 9 and 10). The ranking of the sorghum cultivars for total ethanol yield within each cropping system (Figs. 9 and 10), were approximately the same as the ranks for the total biomass yield (Fig. 8). This further reiterates that the selection of genotypes should be based on production goals, as the highest producing cultivars within one system were often the lowest producers within the other (Figs. 9 and 10).

System Analysis

A summarization of the significance of the study parameters for total production of the two cropping systems is shown in Table 10. As with the sorghums, the growing environment, cropping system and cultivar had large effects on productivity of the systems. The purpose of this section was

to compare to the production of the systems, and because of this, the discussion is focused on how the study factors affect the cropping system. Unless otherwise stated values reported for the double-cropping system represents total production (i.e. triticale plus sorghum yields).

Yield

The total production of the two cropping systems in the different study environments is shown in Figure 11. As mentioned previously in the sorghum section, the yields varied considerably among environments, with 26.0, 17.5, and 15.5 Mg ha^{-1} for the single-cropping systems at the NW and 2007-08 and 2008-09 Sorenson sites, respectively. The total production of the double-cropped system was slightly higher for the 2007-08 Sorenson site (16.6 Mg ha^{-1}), but was not significantly different between the sites during the 2008-09 growing season (Sorenson: 14.4 Mg ha^{-1}; NW: 13.1 Mg ha^{-1}). Interestingly, the dry matter yields of the double- and single- cropping systems were not statistically different from each other during of the growing years at Sorenson locations. However, there was a large difference in production of these two systems at the NW site, and this was likely the product of the reduced growth of the double-cropped sorghums and the extensive growth of the single-cropped sorghums. As previously mentioned, it is believed that the use of an additional crop caused several of the double-cropped sorghums to go dormant during the summer months at this site. It is worth noting that the 2008-09 double-cropping system at the Sorenson site produced significantly less triticale forage yields (Fig. 5) than has been reported in the literature (Gibson et al., 2007; Brown, 2006; Harmoney and Thompson, 2005). It is reasonable to assume that if this yield was increased due to more favorable conditions, then the production of the double-cropping system would have equaled, if not succeeded, the yields of single-cropped sorghums.

There is a clear difference in the suitability of the sorghum varieties for their use within the double-cropping system (Fig. 12). Since there was no difference in triticale yield (Average: 4.3 Mg ha^{-1}) among the subplots, it may be assumed that the variation in total dry matter production within

the system is due to the sorghum growth. Although slightly less, the cultivars FS-5, M-81E, Silo Master D, and Topper 76-6 had total yields within the double-cropping system that were not significantly different than when grown as a sole crop. These sorghums had total system yields of 16.80, 16.61, 15.38, and 12.3 Mg ha^{-1}, respectively. As was previously mentioned, this is because these varieties have the ability to mature sooner, and at the time of harvest for the double-cropping system dry matter production had nearly maximized. Within this system, the production of the triticale in the spring was enough to supplement the loss of what additional biomass that the sorghum may have incurred. The highest producing sorghums within the single-cropping system (Fig. 12) were either photoperiod sensitive or reached physiological maturity later in the growing season, and were still accumulating dry matter at a high rate during this earlier harvest. Thus, the yield of the triticale was not enough to supplement the sorghum biomass that would occurred by the end of the growing season.

Past research has indicated similar differences in the suitability of various cultivars for use in double-cropping systems. In Maryland, Sheaffer et al. (1977) showed that there was a clear difference in the suitability between sunflower (*Helianthus annus* L.) types grown for silage within a double-cropping system. Cultivars of sunflower used for confectionary purposes produced roughly 12 % more dry matter than oil types. In central Iowa, Helsel and Wedin (1981) reported higher or equal dry matter yields for forage sorghums, pearl millet, and sorghum x sudangrass when double-cropped with rye than when grown as a sole-crop. However, grain sorghum and corn grown for silage produced significantly less yield in a double-cropping system. The authors attributed this lack of production to a shortened growing season and reduced stand establishment. In Minnesota, Crookston et al. (1978) reported similar trends in cultivars of corn grown in a double-cropping system. They reported that earlier maturing varieties produced greater total system biomass when double-cropped with rye than when single-cropped.

Ethanol

At the Sorenson sites, the production of each system was not different between years for either the double-cropping system (2007-08: 9,441 L ha^{-1}; 2008-09: 8,714 L ha^{-1}) or the single-cropped sorghum (2007-08: 9,982 L ha^{-1}). The production of the NW location was substantially larger for the single-cropping system (15,881 L ha^{-1}) and lower for the double-cropping system (7,641 L ha^{-1}) than the other locations and was described in the previous section. Despite there being no significant differences in dry matter yields among several sites, the total ethanol production of the single-cropping system was greater at all of the study locations.

The total ethanol production of the sorghum varieties also was varied compared to their dry matter production (Fig. 14). The single-cropped sorghums were consistently higher for each cultivar, with the highest ethanol yields being produced by the varieties Sugar T (15,121 L ha^{-1}), Mega Green (14,436 L ha^{-1}), and Pacesetter (13,950 L ha^{-1}). Of the cultivars that produced similar biomass yields between cropping systems (Fig. 12), none produced equivalent ethanol yields, although they were still among the most productive varieties within the double-cropping system (FS-5: 9,896 L ha^{-1}; M-81E: 9,846L ha^{-1}; Silo Master D: 9,076 L ha^{-1}).

These estimates are similar to what has been reported from previous research, despite a diversity in methods used to estimate ethanol yields. Heggenstaller et al. 2008 reported predicted ethanol yields of 8,948 and 7,659 L ha^{-1} for double-cropping systems of triticale with corn and sorghum x sudangrass, respectively. These estimates were less than the sorghum x sudangrasses within this study, however, their approximation was based on universal conversion factors for biomass (Wallace et al., 2005), instead of being based on actual composition of the feedstocks in this study. The ethanol yields predicted from the triticale in this study were similar to the yields reported here (Fig. 6). The ethanol yields of the single-cropped sorghums are similar to other reported

estimates (Tew et al., 2008), however, these yields were also calculated from generic conversion factors, and therefore, are potentially less reliable.

The development of a significant difference in ethanol yields, despite similar dry matter yields, for several cultivars demonstrates the different in chemical compositions of the two crops. The sorghums derived a greater amount of ethanol from nonstructural carbohydrate (227 L Mg^{-1} vs. 137 L Mg^{-1}) due their greater amounts of TNC. The sorghums also provided more theoretical ethanol from structural carbohydrates than the triticale (615 L Mg^{-1} vs. 553 L Mg^{-1}). Thus, although capable of supplying the additional biomass, the triticale harvested at this stage produced a feedstock that was slightly less efficient for ethanol conversion. This illustrates the importance that selection of both cropping system and sorghum cultivar should be based on their respective purpose. Although they produced appreciably less ethanol, the cultivars that produced equivalent dry matter between systems still are advantageous for other purposes. The relative feeding values (RFV) of the crops (Rohweder et al., 1978) were approximately the same (Triticale: 97.0; Sorghum: 100). The sorghums cultivars with equivalent production between systems themselves had a RFV range of 98.8 to 108.50. Although higher in RFV, the sorghums were considerably lower in crude protein (Tables 5-8), and animals consuming these forages may require protein supplementation. Both of the crops fell within approximate hay grades of 3 or 4 (Rohweder et al., 1978), which are still adequate as a feed source for ruminant production. Thus, it may be possible for producers to take advantage of the environmental benefits of double-cropping systems without a noticeable difference in the yield or quality of the forage.

Cost Analysis

A summarization of the cost analysis of the different production systems is shown in Table 11. The total cost per hectare to produce triticale as a hay crop was considerably less ($977.00 ha^{-1}) than the production cost of the sorghums, regardless of cropping system (SC: $2,064.03 ha^{-1}; DC:

$2,342.92 ha^{-1}). A majority of the cost differences between the two crops could be attributed to the greater variable costs of producing the sorghums. The cultivation of this crop requires higher levels of inputs (i.e. fertilizer, herbicide, and seed) and more intensive management (i.e. more expensive equipment). The total fixed cost between crops was not as large as the total variable cost, but was still considerable. This difference is primarily due to the crop's requirement for different harvest machinery between the crops.

The double-cropping system had highest cost of production of the cropping systems, however, its cost was not additive of that for the two other cropping systems. This is because some of the estimated costs were credited to the section of land (i.e. rent, lime), and not with the crops themselves. Despite being more costly to produce per land area, both of the sorghum cropping systems had noticeably lower cost per unit of output than the triticale (Table 11). This was due to the sorghum's ability to produce considerably larger yield, which helped offset the additional production costs.

Within a given cropping system, the total cost of production seemed to vary slightly among sorghum varieties (Tables 12 & 13). For the single-cropped sorghums (Table 12), these costs ranged from the cultivar Topper 76-6 ($1,996.65 ha^{-1}) being the least expensive, to Sugar T ($2,105.80 ha^{-1}) being the most. Within the double-cropping systems (Table 13), the cultivars seemed to vary even less, with Pacesetter BMR ($2,311.82 ha^{-1}) and FS-5 ($2,370.27 ha^{-1}) being the least and most costly, respectively. The deviations among cultivars within a cropping system are the result of the cost of production being the same among varieties. The variable and fixed costs for the transportation of the silage were the only way these estimates varied, and were based on the average yield of the cultivars. The yields among the sorghum cultivars varied less within the double-cropping system than in the single-cropping system (Fig. 8), which would account for the narrower range of cost for the double-cropping systems.

From the above cost analysis, the growing sorghums using a single-cropping system appears to be the most cost efficient for producers. However, this analysis did not take into account any of the environmental benefits of the double-cropping systems. Soil erosion was estimated to be reduced by the larger ground cover that the fall crop provides over the winter and early spring months (Buxton et al., 1999). This crop was also shown to be valuable for sequestering mineralized nitrogen in the spring, with the amount of nitrate in tile drainage being decreased by 61% (Kasper et al., 2007). While there is no reliable method to estimate these effects economically, it is logical to assume that they will be advantageous to society and would potentially make the double-cropping system more beneficial in the long term.

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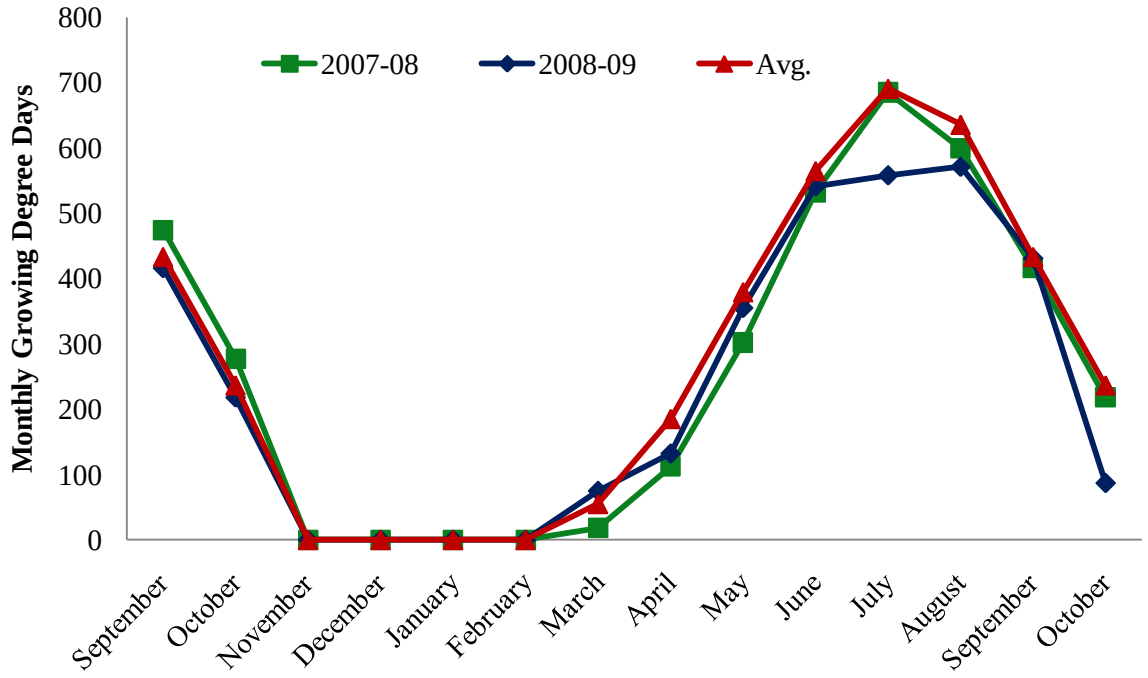

Figure 1. Monthly growing degree days (Base 50°F) at the Sorenson Farm for the 2007-08 and 2008-09 growing seasons, along with the seasonal average.

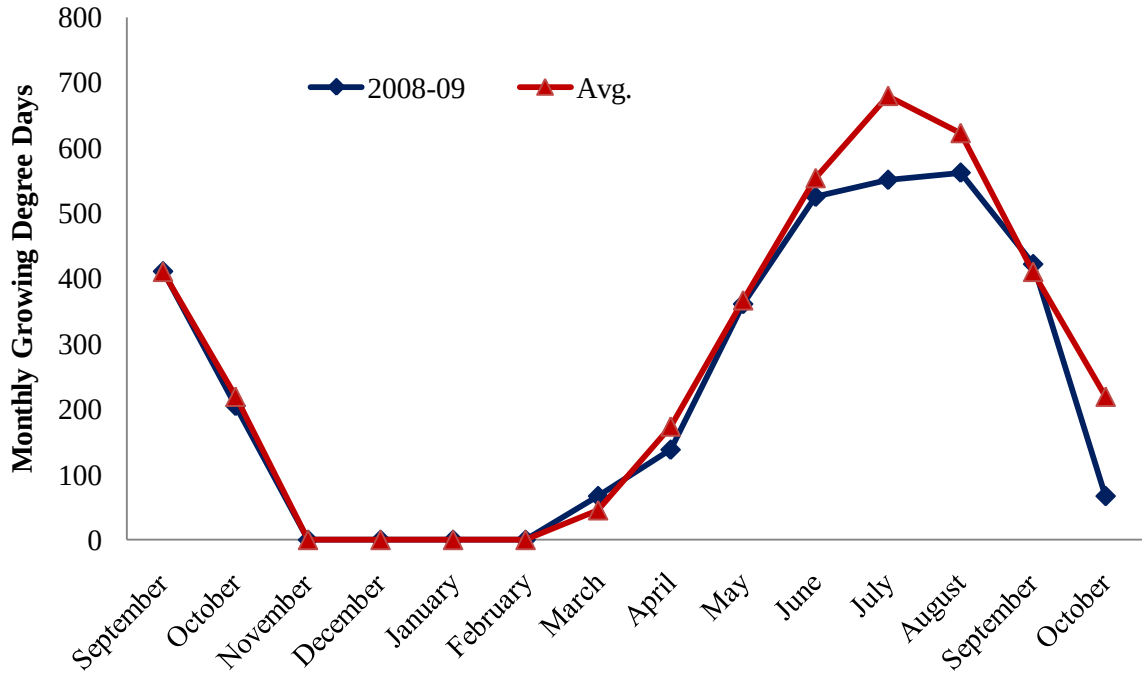

Figure 2. Monthly growing degree days (Base 50°F) at the Northwest Farm for the 2008-09 growing season and the seasonal average.

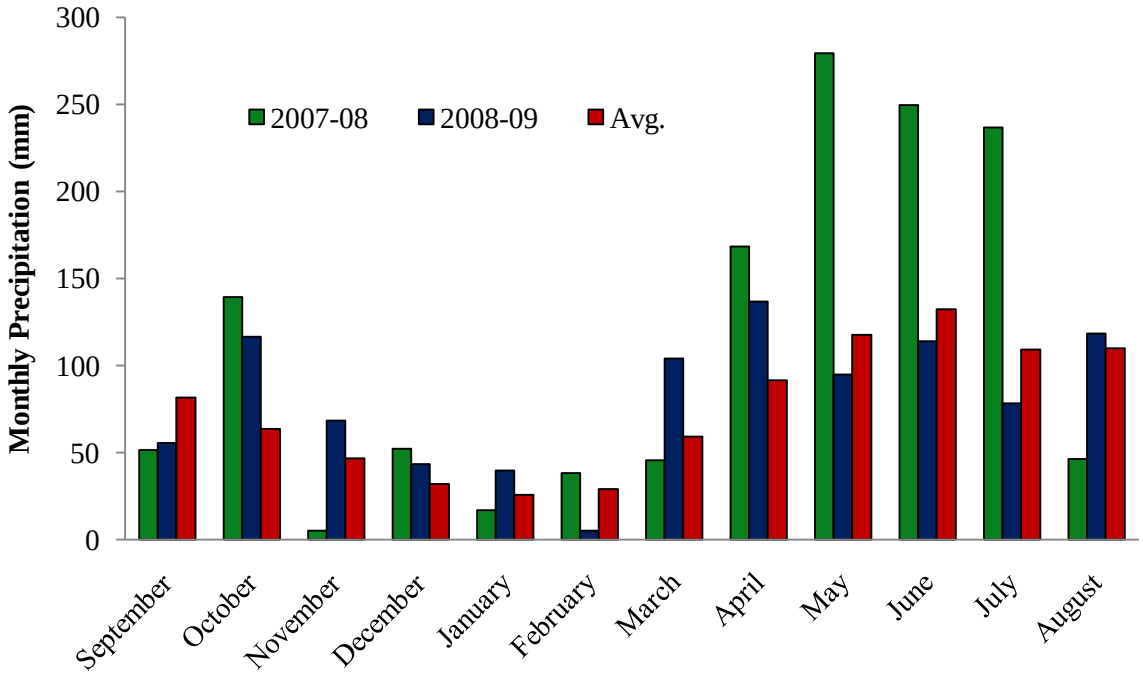

Figure 3. Monthly precipitation at the Sorenson Farm for the 2007-08 and 2008-09 growing seasons, along with the seasonal average.

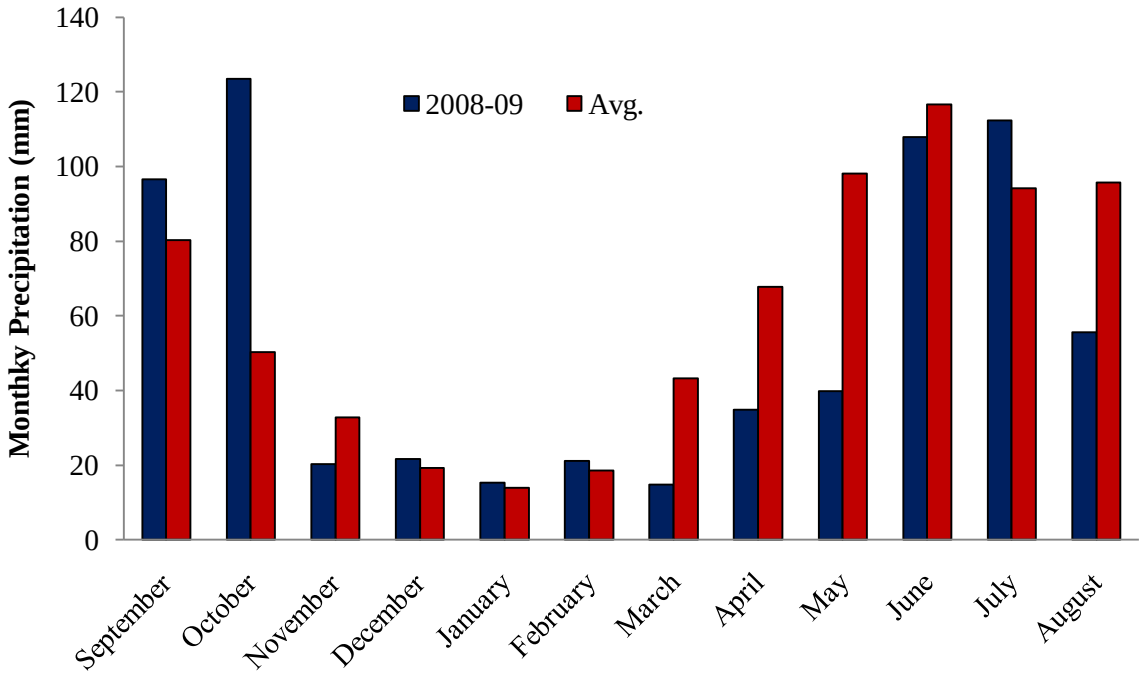

Figure 4. Monthly precipitation at the Northwest Farm for the 2008-09 growing season and the seasonal average.

Table 2. Summary of the significance of triticale variables across study factors.

	Environment	Cropping System	Env*CS
Yield	**	NS	NS
Ash	*	NS	NS
Total Nonstructural Carbohydrates	NS	NS	NS
Neutral Detergent Fiber	**	NS	NS
Acid Detergent Fiber	**	NS	NS
Lignin	**	NS	NS
Total Carbon	**	NS	NS
Crude Protein	**	NS	NS
Nonstructural Carbohydrate Theoretical Ethanol	NS	NS	NS
Nonstructural Carbohydrate Total Ethanol Yield	**	NS	NS
Structural Carbohydrate Theoretical Ethanol	**	NS	NS
Structural Carbohydrate Total Ethanol Yield	**	NS	NS
Total Theoretical Ethanol	**	NS	NS
Total Ethanol Yield	**	NS	NS

NS: Not Significant

** Significance at $p < 0.01$ Level

* Significance at $0.05 < p < 0.01$ Level

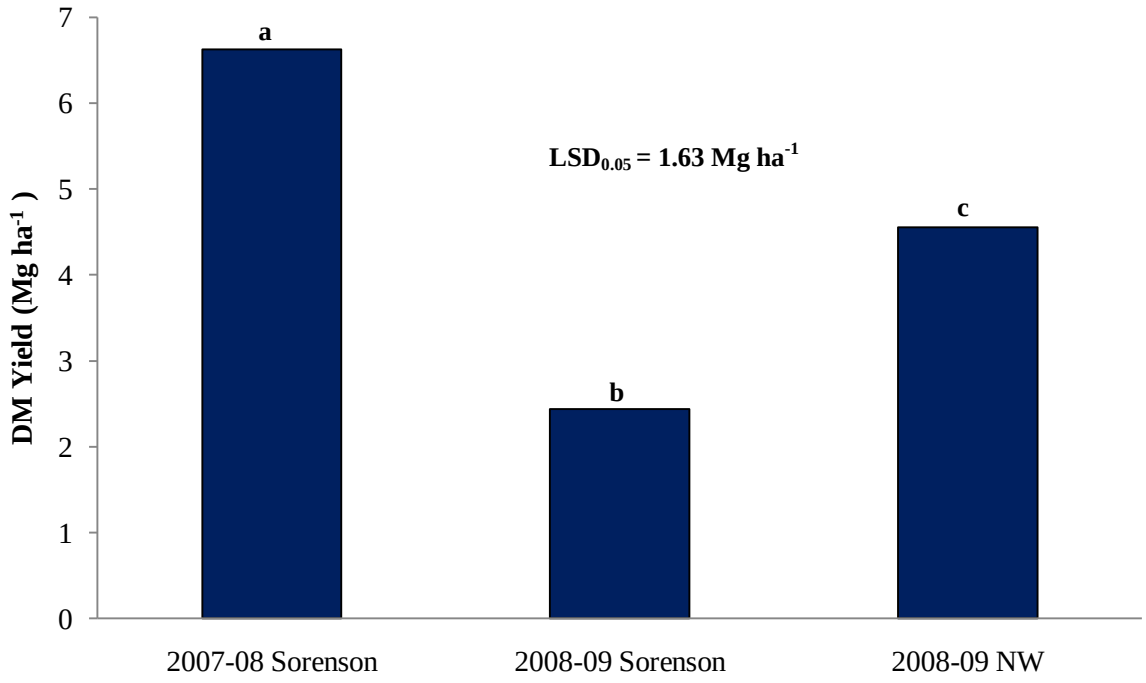

Figure 5. Average triticale yields (Mg ha^{-1}) at study locations. Letters refer to significant differences at the p < 0.05 level.

Table 3. Summarization of triticale chemical composition across environments. Letters refer to significant differences at the p < 0.05 level.

	Ash	TNC	NDF	ADF	ADL	CP	NSTE	STE	TTE
	---------- *g kg^{-1}* ----------						*L Mg^{-1}*	*L Mg^{-1}*	*L Mg^{-1}*
2007-08 Sorensen	73.0^{a}	178.5^{a}	653.6^{a}	378.9^{a}	28.9^{a}	100.2^{a}	128.6^{a}	450.3^{a}	578.9^{a}
2008-09 Sorensen	70.0^{a}	205.2^{b}	571.6^{b}	320.1^{b}	20.2^{b}	149.5^{b}	147.8^{b}	395.6^{b}	543.4^{b}
2008-09 NW	80.0^{b}	190.7^{ab}	576.8^{b}	331.9^{b}	21.3^{b}	147.0^{b}	137.3^{ab}	400.8^{b}	538.1^{b}
$LSD_{0.05}$	6.9	26.2	38.7	30.7	3.7	24.6	18.8	26.2	18.7

TNC: Total Nonstructural Carbohydrate
NDF: Neutral Detergent Fiber
ADF: Acid Detergent Fiber
ADL: Acid Detergent Lignin
CP: Crude Protein
NSTE: Theoretical Ethanol from Nonstructural Carbohydrate
STE: Theoretical Ethanol from Structural Carbohydrate
TTE: Total Theoretical Ethanol

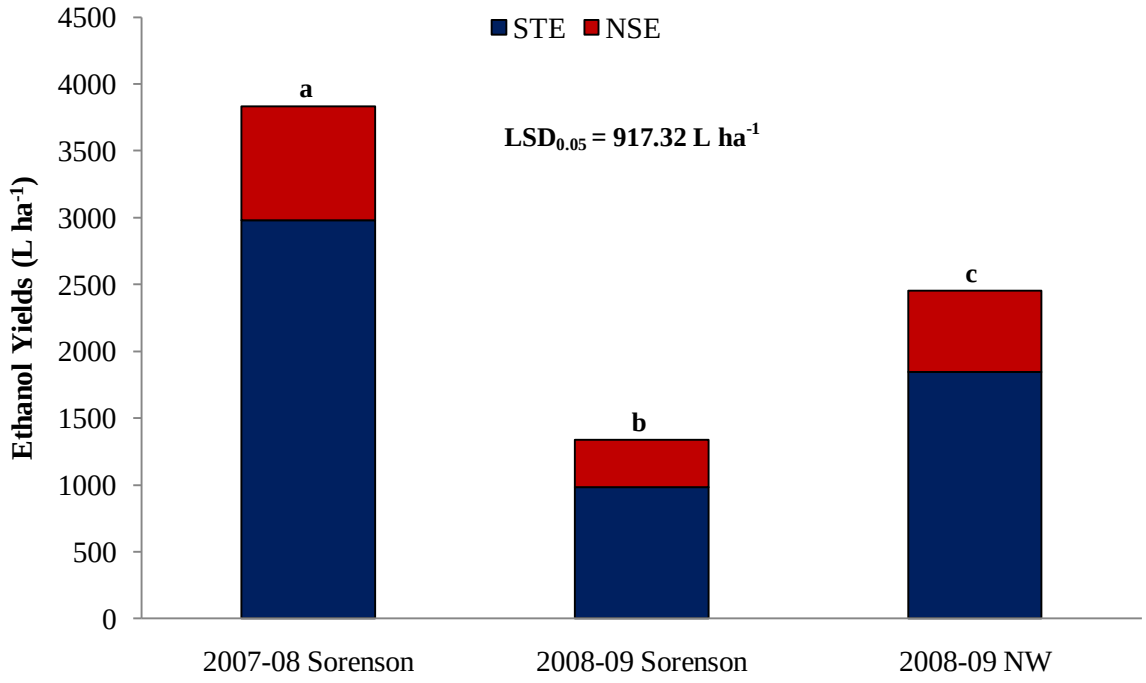

Figure 6. Total ethanol yields of triticale from nonstructural carbohydrate (NSC), structural carbohydrate (SC) at study locations. Letters refer to significant differences at the $p < 0.05$ level.

Table 3. Summary of the significance of sorghum variables across study factors.

	Environment	Cropping System	Env*CS	Variety	Env*Var	CS*Var	Env*CS*Var
Yield	**	**	**	**	NS	**	NS
Ash	**	**	NS	**	*	**	NS
Total Nonstructural Carbohydrates	*	**	*	**	**	**	**
Neutral Detergent Fiber	NS	NS	NS	**	**	**	**
Acid Detergent Fiber	**	NS	NS	**	**	NS	**
Lignin	NS	**	**	**	NS	**	**
Total Carbon	**	**	**	**	*	**	NS
Crude Protein	**	**	NS	**	NS	**	NS
Nonstructural Carbohydrate Theoretical Ethanol	*	**	*	**	**	**	**
Nonstructural Carbohydrate Total Ethanol Yield	**	**	**	**	NS	*	NS
Structural Carbohydrate Theoretical Ethanol	NS	NS	NS	**	**	**	**
Structural Carbohydrate Total Ethanol Yield	**	**	NS	**	NS	**	NS
Total Theoretical Ethanol	*	*	**	**	**	NS	NS
Total Ethanol Yield	**	**	**	**	NS	**	NS

NS: Not Significant

** Significance at $p < 0.01$ Level

* Significance at $0.05 < p < 0.01$ Level

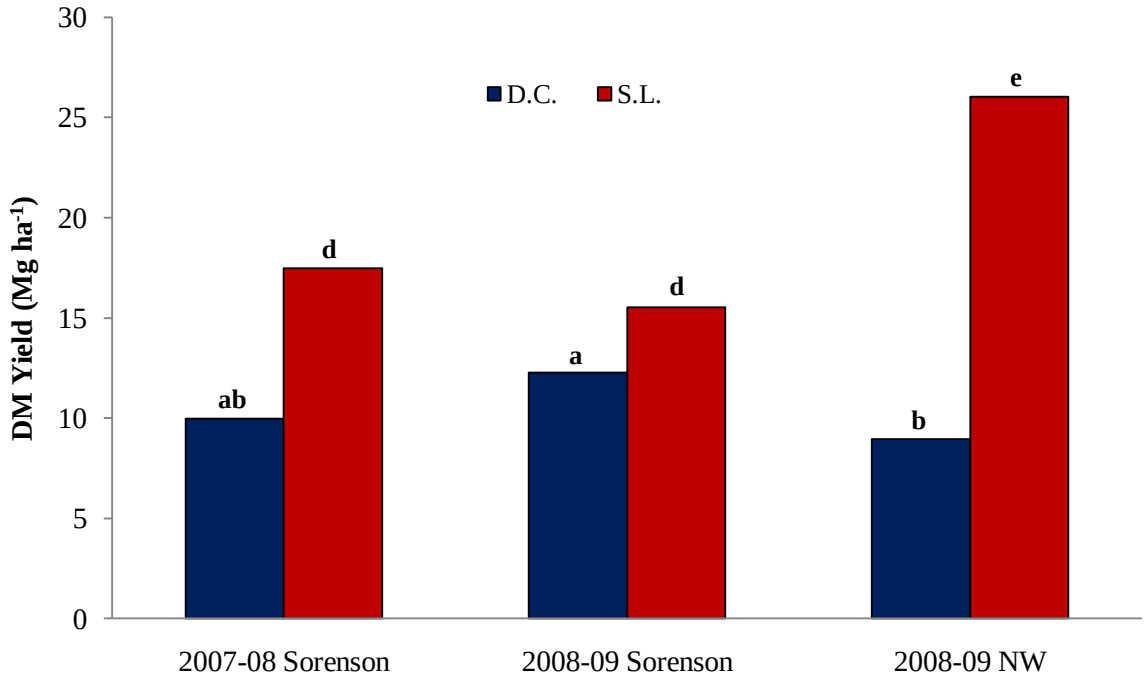

Figure 7. Yields (Mg ha^{-1}) of sorghum grown as a season long (S.L.) crop and within a double-cropping (D.C.) system. Letters refer to significant differences within a cropping system at the $p < 0.05$ level.

Table 4. Relative maturity for sorghums at each cropping system at study locations.

	2007-08 Sorenson		2008-09 Sorenson		2008-09 NW	
	D.C.	*S.C.*	*D.C.*	*S.C.*	*D.C.*	*S.C.*
1990	2.50	2.50	2.50	2.50	2.50	2.50
FS-5	3.16	4.70	3.63	4.50	3.33	4.78
Pacesetter	2.50	3.25	2.79	3.45	2.56	3.40
Silo Master D	2.72	4.50	3.90	4.85	3.53	4.63
Maxi Gain	2.50	3.30	3.15	3.48	2.50	3.43
Mega Green	2.50	3.23	3.04	3.43	2.50	3.43
Pacesetter BMR	2.50	2.50	2.50	2.50	2.50	2.50
Sugar Graze Ultra	2.50	3.13	2.56	3.45	2.50	3.35
M-81E	2.50	4.50	3.14	4.25	2.75	4.43
Sucrosorgo 405	2.50	4.60	3.73	4.63	3.21	4.60
Sugar T	2.84	4.50	3.63	4.80	3.53	4.75
Topper 76-6	2.50	4.50	3.54	4.53	2.89	4.55

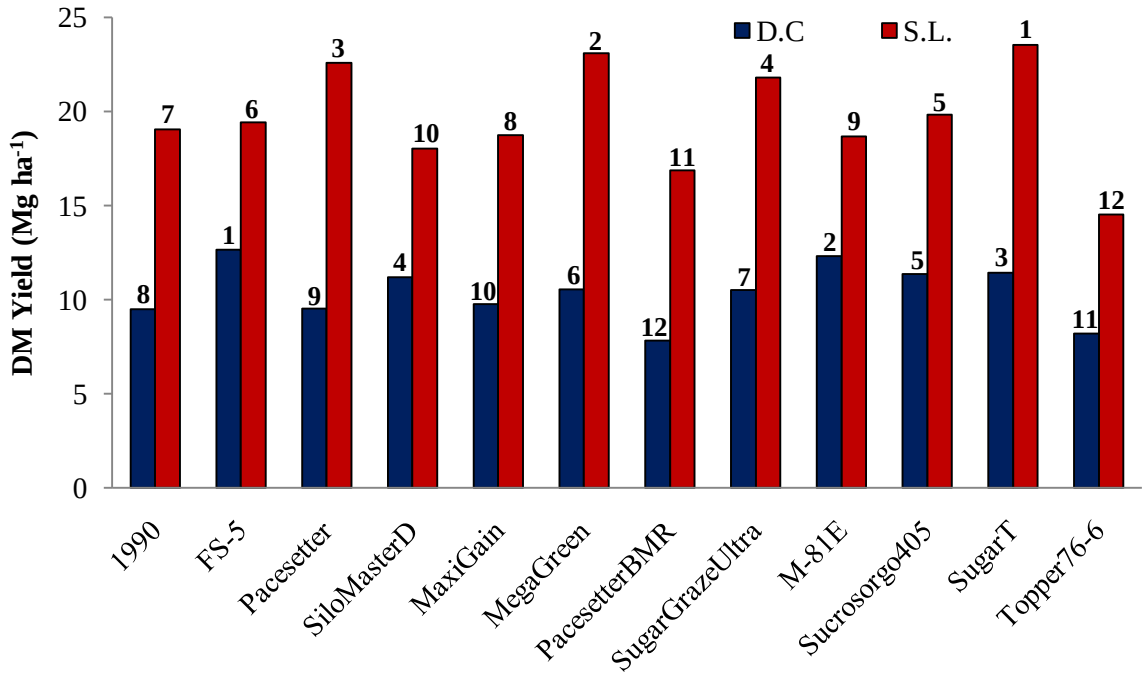

Figure 8. Yields (Mg ha^{-1}) of each sorghum variety grown as a season long crop (S.L.), and within a double-cropping (D.C.) system.

Table 5. Chemical composition of sorghums with cropping systems at each environment. Letters refer to significant differences at the p < 0.05 level within a cropping system.

	Ash	TNC	NDF	ADF	ADL	CP
	---------- *g kg^{-1}* ----------					
Double-cropping System						
2007-08 Sorensen	63.4[a]	221.9[a]	585.8[a]	348.5[a]	24.1[a]	70.4[a]
2008-09 Sorensen	50.8[b]	269.8[b]	606.3[a]	338.1[a]	22.5[b]	59.9[b]
2008-09 NW	55.1[b]	262.5[b]	605.6[a]	321.9[a]	18.2[c]	83.5[c]
Single-cropping System						
2007-08 Sorensen	57.7[d]	313.0[d]	602.7[d]	355.5[d]	26.3[d]	41.7[d]
2008-09 Sorensen	48.9[e]	324.5[d]	593.9[d]	333.3[d]	24.3[e]	30.5[e]
2008-09 NW	48.6[e]	309.7[d]	567.3[d]	332.2[d]	28.5[f]	55.9[f]

TNC: Total Nonstructural Carbohydrate
CP: Crude Protein
NDF: Neutral Detergent Fiber
ADF: Acid Detergent Fiber
ADL: Acid Detergent Lignin

Table 6. Chemical composition of sorghum varieties by cropping system. Letters refer to significant differences with the cropping system for each variety at the p < 0.05 level.

System	Ash	TNC	NDF	ADF	ADL	CP
	---------- *g kg^{-1}* ----------					
1990						
Double-cropped	58.4[a]	219.7[a]	642.3[a]	361.5[a]	23.6[a]	67.3[a]
Single-cropped	49.3[b]	245.6[b]	646.7[a]	375.6[a]	32.8[b]	43.1[b]
FS-5						
Double-cropped	51.9[a]	259.2[a]	598.1[a]	338.5[a]	21.7[a]	74.3[a]
Single-cropped	53.2[a]	318.8[b]	582.4[a]	336.1[a]	24.5[b]	42.6[b]
Pacesetter						
Double-cropped	60.0[a]	234.3[a]	601.1[a]	335.8[a]	23.5[a]	72.0[a]
Single-cropped	51.1[a]	289.6[b]	600.5[a]	345.0[a]	30.8[b]	42.6[b]
Silo Master D						
Double-cropped	57.5[a]	276.5[a]	580.5[a]	327.0[a]	21.5[a]	66.9[a]
Single-cropped	56.3[a]	337.5[b]	560.4[a]	324.1[a]	25.7[b]	46.9[b]
Maxi Gain						
Double-cropped	58.7[a]	232.9[a]	590.0[a]	324.4[a]	23.1[a]	74.5[a]
Single-cropped	49.3[b]	308.7[b]	584.6[a]	333.5[a]	27.8[b]	41.6[b]
Mega Green						
Double-cropped	57.9[a]	235.9[a]	599.0[a]	335.1[a]	23.0[a]	68.8[a]
Single-cropped	53.3[a]	293.0[b]	608.7[a]	355.3[b]	31.6[b]	40.2[b]
Pacesetter BMR						
Double-cropped	61.0[a]	256.0[a]	606.5[a]	340.4[a]	13.8[a]	78.6[a]
Single-cropped	53.9[b]	309.7[b]	609.6[a]	349.7[a]	13.6[a]	48.2[b]

Table 6 (cont.) Chemical composition of sorghum varieties by cropping system.

System	Ash	TNC	NDF	ADF	ADL	CP
	---------- *g kg^{-1}* ----------					
Sugar Graze Ultra						
Double-cropped	61.5[a]	227.0[a]	613.9[a]	347.4[a]	24.3[a]	69.6[a]
Single-cropped	46.7[b]	269.9[b]	606.3[a]	356.4[a]	31.6[b]	46.8[b]
M-81E						
Double-cropped	51.1[a]	251.1[a]	612.5[a]	345.3[a]	24.0[a]	75.1[a]
Single-cropped	51.6[a]	344.8[b]	570.6[b]	335.2[a]	26.7[b]	39.3[b]
Sucrosorgo 405						
Double-cropped	55.0[a]	261.1[a]	588.3[a]	336.3[a]	23.3[a]	65.9[a]
Single-cropped	55.4[a]	312.2[b]	589.5[a]	347.9[a]	30.0[b]	43.3[b]
Sugar T						
Double-cropped	47.8[a]	285.1[a]	577.7[a]	324.3[a]	22.1[a]	66.5[a]
Single-cropped	51.9[a]	369.2[b]	552.5[b]	318.1[a]	26.1[b]	35.8[b]
Topper 76-6						
Double-cropped	56.9[a]	277.6[a]	580.1[a]	316.7[a]	14.9[a]	76.9[a]
Single-cropped	48.5[b]	390.2[b]	543.9[b]	307.0[a]	15.1[a]	42.5[b]

TNC: Total Nonstructural Carbohydrate
CP: Crude Protein
NDF: Neutral Detergent Fiber
ADF: Acid Detergent Fiber
ADL: Acid Detergent Lignin

Table 7. Chemical composition of sorghum varieties by environment. Letters refer to significant differences with the cropping system for each variety at the p < 0.05 level.

Environment	Ash	TNC	NDF	ADF	ADL	CP
			g kg^{-1}			
1990						
2007-08 Sorenson	62.6^{a}	233.6^{a}	606.8^{a}	358.1^{a}	30.0^{a}	52.5^{a}
2008-09 Sorenson	48.6^{b}	232.4^{a}	672.2^{b}	378.7^{b}	27.1^{b}	44.7^{a}
2008-09 NW	50.3^{b}	232.0^{a}	654.4^{b}	368.9^{ab}	27.5^{ab}	68.4^{b}
FS-5						
2007-08 Sorenson	58.6^{a}	281.8^{a}	599.7^{a}	353.9^{a}	24.9^{a}	55.7^{a}
2008-09 Sorenson	51.5^{b}	293.5^{a}	596.2^{a}	337.5^{ab}	22.2^{b}	45.7^{b}
2008-09 NW	47.7^{b}	291.8^{a}	574.9^{a}	320.5^{b}	22.1^{b}	74.0^{c}
Pacesetter						
2007-08 Sorenson	62.5^{a}	243.9^{a}	585.2^{a}	343.1^{a}	28.7^{a}	53.9^{a}
2008-09 Sorenson	50.0^{b}	283.0^{b}	611.4^{b}	337.5^{a}	25.3^{b}	48.7^{a}
2008-09 NW	54.2^{b}	259.1^{a}	605.8^{ab}	340.7^{a}	27.4^{ab}	67.9^{b}
Silo Master D						
2007-08 Sorenson	64.2^{a}	282.8^{a}	603.6^{a}	359.3^{a}	24.4^{a}	56.1^{a}
2008-09 Sorenson	55.9^{b}	319.7^{b}	563.9^{b}	316.3^{b}	24.2^{a}	46.8^{b}
2008-09 NW	50.5^{b}	318.5^{b}	543.9^{b}	301.0^{b}	22.2^{a}	67.8^{c}
Maxi Gain						
2007-08 Sorenson	62.7^{a}	247.5^{a}	561.9^{a}	324.9^{a}	26.6^{a}	57.1^{a}
2008-09 Sorenson	47.3^{b}	296.9^{b}	601.0^{b}	331.4^{a}	25.1^{a}	45.3^{b}
2008-09 NW	52.0^{b}	268.0^{a}	598.9^{b}	330.6^{a}	24.7^{a}	71.8^{c}
Mega Green						
2007-08 Sorenson	59.8^{a}	251.0^{a}	581.1^{a}	340.1^{a}	27.4^{a}	54.0^{a}
2008-09 Sorenson	48.4^{b}	287.0^{b}	614.3^{b}	344.1^{a}	26.4^{a}	45.0^{a}
2008-09 NW	58.4^{a}	255.3^{a}	616.2^{b}	351.5^{a}	28.9^{a}	64.5^{b}

Table 7 (cont.). Chemical composition of sorghum varieties by environment.

Environment	Ash	TNC	NDF	ADF	ADL	CP
	---------- *g kg^{-1}* ----------					
Pacesetter BMR						
2007-08 Sorenson	60.9^{a}	288.8^{a}	599.6^{a}	357.4^{a}	12.5^{a}	61.9^{a}
2008-09 Sorenson	53.3^{b}	277.3^{a}	629.4^{b}	351.0^{a}	14.7^{a}	52.0^{b}
2008-09 NW	58.1^{ab}	282.4^{a}	595.2^{a}	326.8^{b}	13.8^{a}	76.3^{c}
Sugar Graze Ultra						
2007-08 Sorenson	63.0^{a}	234.9^{a}	595.1^{a}	352.7^{a}	30.6^{a}	55.6^{a}
2008-09 Sorenson	45.8^{b}	268.4^{b}	627.1^{b}	359.7^{a}	27.1^{b}	40.1^{b}
2008-09 NW	53.5^{c}	242.0^{a}	608.0^{ab}	343.3^{a}	25.9^{b}	78.8^{c}
M-81E						
2007-08 Sorenson	55.4^{a}	281.2^{a}	608.9^{a}	366.4^{a}	28.8^{a}	54.1^{a}
2008-09 Sorenson	46.4^{b}	309.5^{b}	590.6^{ab}	333.8^{b}	24.5^{b}	45.6^{a}
2008-09 NW	52.4^{ab}	303.0^{ab}	575.2^{b}	320.5^{b}	22.8^{b}	71.9^{b}
Sucrosorgo 405						
2007-08 Sorenson	64.5^{a}	249.6^{a}	594.1^{a}	360.4^{a}	27.7^{a}	57.9^{a}
2008-09 Sorenson	51.9^{b}	313.2^{b}	587.3^{a}	332.8^{b}	26.3^{a}	41.8^{b}
2008-09 NW	49.3^{b}	297.2^{b}	585.3^{a}	333.0^{b}	25.8^{a}	64.1^{a}
Sugar T						
2007-08 Sorenson	53.0^{a}	305.7^{a}	590.4^{a}	348.9^{a}	25.1^{a}	52.9^{a}
2008-09 Sorenson	48.0^{a}	347.2^{b}	551.9^{b}	307.7^{b}	22.7^{a}	39.0^{b}
2008-09 NW	48.6^{a}	328.6^{ab}	553.0^{b}	307.1^{b}	24.5^{a}	61.6^{a}
Topper 76-6						
2007-08 Sorenson	59.0^{a}	309.0^{a}	604.8^{a}	358.8^{a}	15.5^{a}	60.7^{a}
2008-09 Sorenson	51.1^{b}	337.9^{b}	555.6^{b}	297.8^{b}	14.6^{a}	47.9^{b}
2008-09 NW	50.4^{b}	354.8^{b}	525.6^{c}	278.8^{c}	14.9^{a}	70.5^{c}

TNC: Total Nonstructural Carbohydrate
CP: Crude Protein
NDF: Neutral Detergent Fiber
ADF: Acid Detergent Fiber
ADL: Acid Detergent Lignin

Table 8. Theoretical ethanol potentials of sorghum varieties by cropping system. Letters refer to significant differences within the cropping system for each variety at the p < 0.05 level

	NSTE	STE	TTE		NSTE	STE	TTE
	L Mg^{-1}				*L Mg^{-1}*		
	1990				**Pacesetter BMR**		
Double-cropped	158.25[a]	445.59[a]	603.84[a]	*Double-cropped*	184.38[a]	425.34[a]	609.72[a]
Single-cropped	176.87[b]	445.27[a]	622.14[b]	*Single-cropped*	223.03[b]	425.92[a]	648.95[b]
	FS-5				**Sugar Graze Ultra**		
Double-cropped	186.70[a]	414.86[a]	601.56[a]	*Double-cropped*	163.52[a]	425.21[a]	588.73[a]
Single-cropped	229.62[b]	402.22[a]	631.84[b]	*Single-cropped*	194.38[b]	417.36[a]	611.74[b]
	Pacesetter				**M-81E**		
Double-cropped	168.78[a]	416.77[a]	585.55[a]	*Double-cropped*	180.84[a]	424.29[a]	605.13[a]
Single-cropped	208.58[b]	413.02[a]	621.60[b]	*Single-cropped*	248.31[b]	392.38[b]	640.70[b]
	Silo Master D				**Sucrosorgo 405**		
Double-cropped	199.14[a]	402.44[a]	601.58[a]	*Double-cropped*	188.04[a]	407.54[a]	595.58[a]
Single-cropped	243.07[b]	386.05[b]	629.12[b]	*Single-cropped*	224.88[b]	403.29[a]	628.18[b]
	Maxi Gain				**Sugar T**		
Double-cropped	166.31[a]	410.14[a]	576.45[a]	*Double-cropped*	205.33[a]	399.96[a]	605.29[a]
Single-cropped	222.32[b]	402.93[a]	625.24[b]	*Single-cropped*	265.94[b]	380.43[b]	646.37[b]
	Mega Green				**Topper 76-6**		
Double-cropped	169.92[a]	415.50[a]	585.42[a]	*Double-cropped*	199.91[a]	405.42[a]	605.34[a]
Single-cropped	211.03[b]	417.91[a]	628.94[b]	*Single-cropped*	281.07[b]	378.70[b]	659.77[b]

NSTE: Theoretical Ethanol from Nonstructural Carbohydrates STE: Theoretical Ethanol from Structural Carbohydrate

TTE: Total Theoretical Ethanol

Table 9. Theoretical ethanol potentials of sorghum varieties by growing enviroments. Letters refer to significant differences within the environments for each variety at the p < 0.05 level

	NSTE	STE	TTE		NSTE	STE	TTE
	L Mg^{-1}				*L Mg^{-1}*		
	1990				**Pacesetter BMR**		
2007-08 Sorenson	168.24[a]	417.86[a]	586.10[a]	*2007-08 Sorenson*	207.99[a]	412.72[a]	620.71[a]
2008-09 Sorenson	167.38[a]	465.57[b]	632.95[b]	*2008-09 Sorenson*	199.75[a]	443.94[b]	643.69[b]
2008-09 NW	167.07[a]	452.85[b]	619.91[b]	*2008-09 NW*	203.37[a]	420.23[a]	623.60[a]
	FS-5				**Sugar Graze Ultra**		
2007-08 Sorenson	202.98[a]	412.64[a]	615.62[a]	*2007-08 Sorenson*	169.19[a]	410.93[a]	580.12[a]
2008-09 Sorenson	211.37[a]	413.78[a]	625.15[a]	*2008-09 Sorenson*	193.34[b]	432.93[b]	626.27[b]
2008-09 NW	210.13[a]	399.20[a]	609.33[a]	*2008-09 NW*	174.32[a]	419.99[b]	594.31[a]
	Pacesetter				**M-81E**		
2007-08 Sorenson	175.65[a]	404.73[a]	580.38[a]	*2007-08 Sorenson*	202.54[a]	417.39[a]	619.94[a]
2008-09 Sorenson	203.81[b]	422.52[b]	626.33[b]	*2008-09 Sorenson*	222.93[b]	408.76[ab]	631.69[a]
2008-09 NW	186.58[a]	417.43[ab]	604.01[c]	*2008-09 NW*	218.25[b]	398.86[b]	617.11[a]
	Silo Master D				**Sucrosorgo 405**		
2007-08 Sorenson	203.65[a]	416.83[a]	620.48[a]	*2007-08 Sorenson*	179.77[a]	408.51[a]	588.28[a]
2008-09 Sorenson	230.26[b]	389.40[b]	619.66[a]	*2008-09 Sorenson*	225.59[b]	404.44[a]	630.02[b]
2008-09 NW	229.42[b]	376.51[b]	605.92[a]	*2008-09 NW*	214.03[b]	403.30[a]	617.33[b]
	Maxi Gain				**Sugar T**		
2007-08 Sorenson	178.27[a]	390.41[a]	568.68[a]	*2007-08 Sorenson*	220.16[a]	407.35[a]	627.51[a]
2008-09 Sorenson	213.85[b]	415.71[b]	629.56[b]	*2008-09 Sorenson*	250.08[b]	381.53[b]	631.61[a]
2008-09 NW	194.32[a]	413.96[b]	608.28[c]	*2008-09 NW*	236.66[ab]	381.70[b]	618.36[a]
	Mega Green				**Topper 76-6**		
2007-08 Sorenson	180.81[a]	401.47[a]	582.28[a]	*2007-08 Sorenson*	222.58[a]	416.37[a]	638.95[a]
2008-09 Sorenson	206.70[b]	423.89[b]	630.60[b]	*2008-09 Sorenson*	243.38[b]	390.64[b]	634.02[a]
2008-09 NW	183.91[a]	424.74[b]	608.65[c]	*2008-09 NW*	255.52[b]	369.17[c]	624.69[a]

NSTE: Theoretical Ethanol from Nonstructural Carbohydrates STE: Theoretical Ethanol from Structural Carbohydrate

TTE: Total Theoretical Ethanol

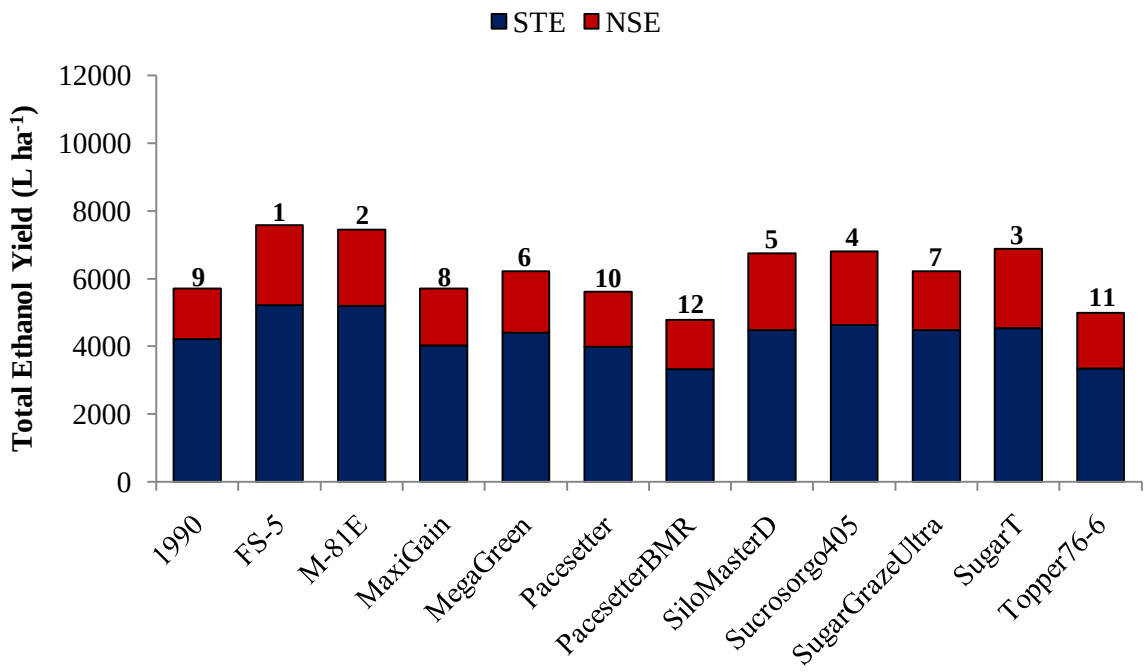

Figure 9. Ethanol yield and ranking of sorghum varieties within double-cropping system.

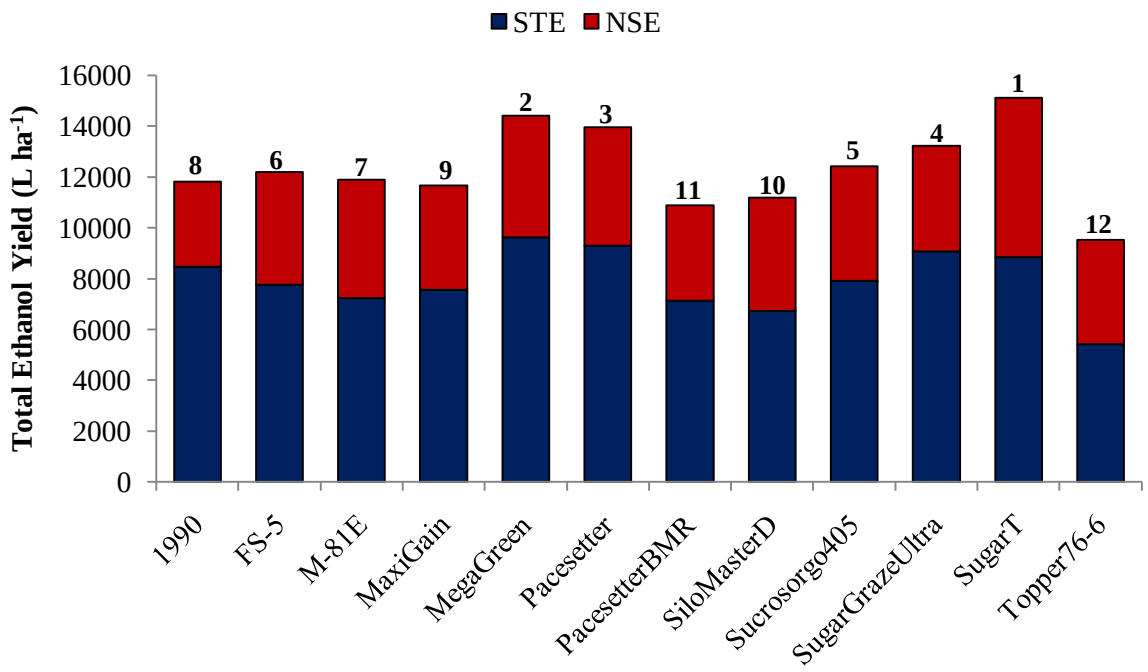

Figure 10. Ethanol yield and ranking of sorghum varieties within single-cropping system.

Table 10.Summary of the significance of system analysis across study factors.

	Environment	Cropping System	Env*CS	Variety	Env*Var	CS*Var	Env*CS*Var
Yield	**	**	**	**	NS	**	NS
Nonstructural Carbohydrate Total Ethanol Yield	**	**	**	**	NS	*	NS
Structural Carbohydrate Total Ethanol Yield	**	**	**	**	NS	*	NS
Total Ethanol Yield	**	**	**	**	NS	*	NS

NS: Not Significant

** Significance at p <0.01Level

* Significance at 0.05 < p < 0.01 Level

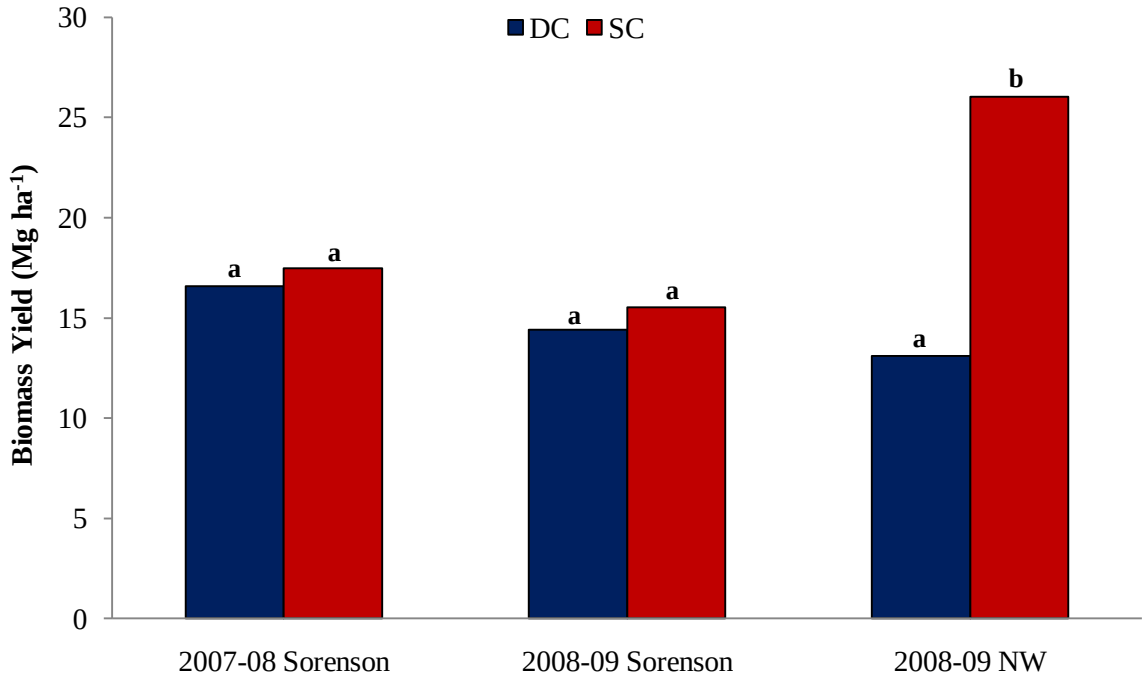

Figure 11. Total biomass yields of cropping systems at each environment. Letters refer to significant differences with a site at the $p < 0.05$ level.

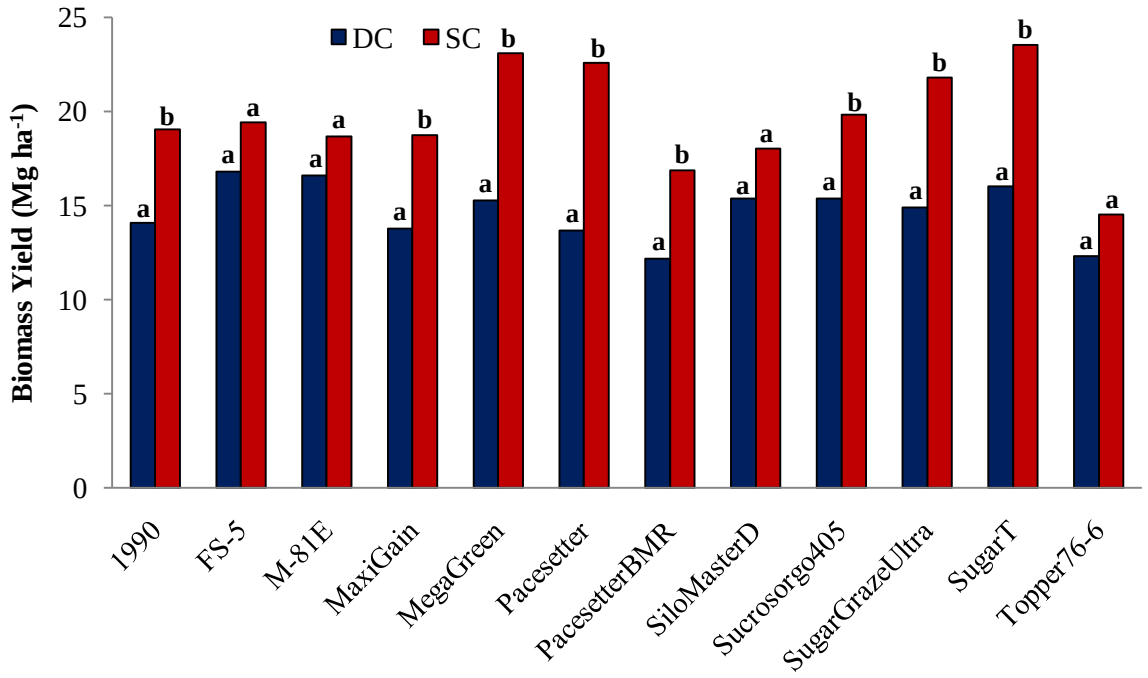

Figure 12. Total biomass yield of cropping systems broke down by sorghum variety. Letters refer to significant differences within a sorghum variety at the p < 0.05 level.

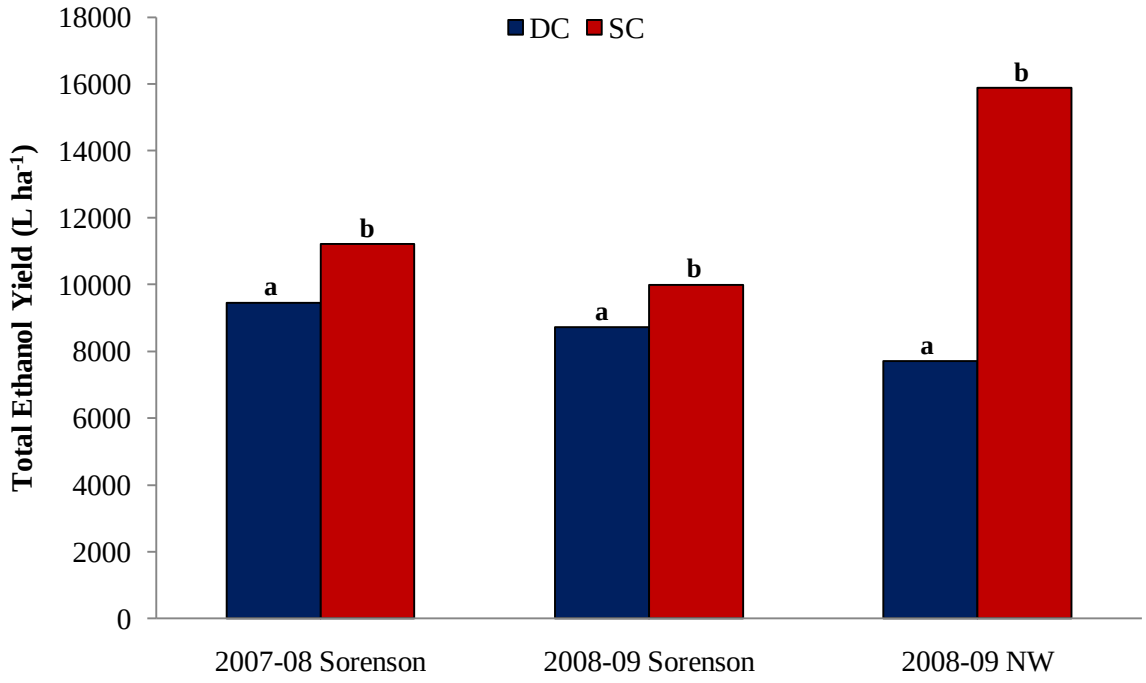

Figure 13. Total ethanol yields of cropping systems by environment. Letters refer to significant differences within an environment at the p < 0.05 level.

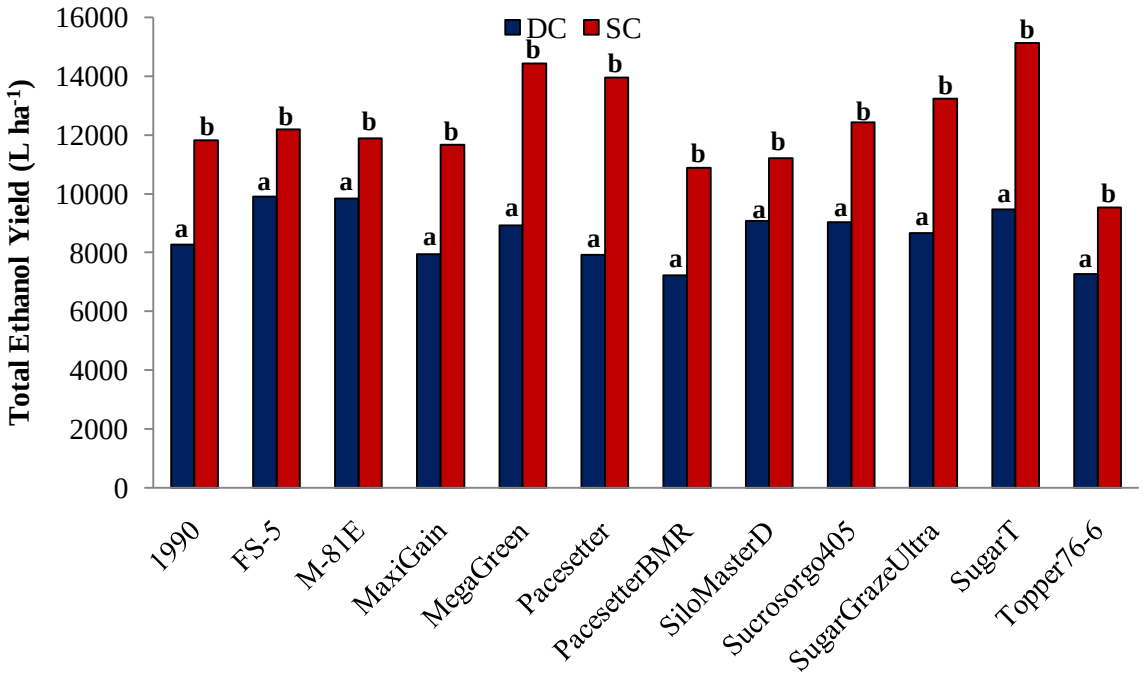

Figure 14. Total ethanol yields of cropping systems by sorghum variety. Letters refer to significant differences within a sorghum variety at the p < 0.05 level.

Table 11. Summary of cost analysis for different cropping systems.

Cost	SC Triticale	SC Sorghum	DC Sorg. /Trit.
	Cost per Area ($ ha^{-1})		
Variable	$358.02	$1,206.25	$1,433.35
Fixed	$618.98	$857.77	$909.57
Total	$977.00	$2,064.02	$2,342.92
	Cost per Biomass Yield ($ Mg^{-1})		
Variable	$78.86	$61.32	$97.51
Fixed	$136.34	$43.61	$61.88
Total	$215.20	$104.93	$159.38
	Cost per Ethanol Yield ($ L^{-1})		
Variable	$0.14	$0.10	$0.17
Fixed	$0.24	$0.07	$0.11
Total	$0.38	$0.17	$0.27

Table 12. Summary of cost analysis for sorghum varieties within single-cropping system.

	1990	FS-5	Pacesetter	Silo Master D	Maxi Gain	Mega Green
			Cost per Area ($ ha^{-1})			
Variable	$1,200.00	$1,202.16	$1,220.64	$1,194.06	$1,198.26	$1,223.55
Fixed	$851.34	$853.66	$873.55	$844.94	$849.46	$876.69
Total	$2,051.35	$2,055.82	$2,094.19	$2,039.00	$2,047.72	$2,100.24
			Cost per Biomass Yield ($ Mg^{-1})			
Variable	$63.03	$61.94	$54.06	$66.26	$63.94	$53.01
Fixed	$44.71	$43.98	$38.69	$46.89	$45.33	$37.98
Total	$107.74	$105.92	$92.75	$113.15	$109.27	$91.00
			Cost per Ethanol Yield ($ L^{-1})			
Variable	$0.10	$0.10	$0.09	$0.11	$0.10	$0.08
Fixed	$0.07	$0.07	$0.06	$0.08	$0.07	$0.06
Total	$0.17	$0.17	$0.15	$0.18	$0.18	$0.15

	Pacsesetter BMR	Sugar Graze Ultra	M-81E	Sucrosorgo 405	Sugar T	Topper 76-6
			Cost per Area ($ ha^{-1})			
Variable	$1,187.30	$1,216.15	$1,197.79	$1,204.67	$1,226.23	$1,173.66
Fixed	$837.67	$868.72	$848.96	$856.36	$879.57	$822.99
Total	$2,024.97	$2,084.87	$2,046.75	$2,061.03	$2,105.80	$1,996.65
			Cost per Biomass Yield ($ Mg^{-1})			
Variable	$70.42	$55.76	$64.19	$60.72	$52.09	$80.83
Fixed	$49.68	$39.83	$45.50	$43.16	$37.36	$56.68
Total	$120.10	$95.59	$109.69	$103.88	$89.46	$137.51
			Cost per Ethanol Yield ($ L^{-1})			
Variable	$0.11	$0.09	$0.10	$0.10	$0.08	$0.13
Fixed	$0.08	$0.07	$0.07	$0.07	$0.06	$0.09
Total	$0.19	$0.16	$0.17	$0.17	$0.14	$0.21

Table 13. Summary of cost analysis for sorghum varieties within double-cropping system.

	1990	FS-5	Pacesetter	Silo Master D	Maxi Gain	Mega Green
			Cost per Area ($ ha^{-1})			
Variable	$1,427.99	$1,446.53	$1,428.17	$1,437.84	$1,429.62	$1,434.11
Fixed	$903.80	$923.75	$903.99	$914.40	$905.56	$910.39
Total	$2,331.79	$2,370.27	$2,332.15	$2,352.24	$2,335.18	$2,344.50
			Cost per Biomass Yield ($ Mg^{-1})			
Variable	$101.56	$86.10	$104.55	$93.49	$103.82	$93.86
Fixed	$64.28	$54.99	$66.18	$59.45	$65.76	$59.58
Total	$165.85	$141.09	$170.73	$152.94	$169.58	$153.44
			Cost per Ethanol Yield ($ L^{-1})			
Variable	$0.17	$0.15	$0.18	$0.16	$0.18	$0.16
Fixed	$0.11	$0.09	$0.11	$0.10	$0.11	$0.10
Total	$0.28	$0.24	$0.29	$0.26	$0.29	$0.26

	Pacsesetter BMR	Sugar Graze Ultra	M-81E	Sucrosorgo 405	Sugar T	Topper 76-6
			Cost per Area ($ ha^{-1})			
Variable	$1,418.38	$1,434.00	$1,444.54	$1,439.07	$1,439.42	$1,420.59
Fixed	$893.45	$910.26	$921.62	$915.72	$916.09	$895.83
Total	$2,311.82	$2,344.26	$2,366.16	$2,354.78	$2,355.51	$2,316.42
			Cost per Biomass Yield ($ Mg^{-1})			
Variable	$116.45	$96.24	$86.97	$93.57	$89.80	$115.50
Fixed	$73.35	$61.09	$55.49	$59.54	$57.15	$72.83
Total	$189.80	$157.33	$142.45	$153.11	$146.94	$188.33
			Cost per Ethanol Yield ($ L^{-1})			
Variable	$0.20	$0.17	$0.15	$0.16	$0.15	$0.20
Fixed	$0.12	$0.10	$0.09	$0.10	$0.10	$0.12
Total	$0.32	$0.27	$0.24	$0.26	$0.25	$0.32

CHAPTER V: CONCLUSION

The yield of triticale in this study was characterized by large variation between study locations. The average yield and feedstock quality, however, did not vary greatly from what has been previously reported in the literature. The triticale had a theoretical ethanol potential of 553 L Mg^{-1} of DM, with approximately 415 and 137 L Mg^{-1} coming from structural and nonstructural carbohydrates, respectively. This translated to an average ethanol yield for the triticale grown as a hay crop of 2,542 L ha^{-1}.

Similarly, dry matter yields varied considerably across the growing environments of the study. The yields of the single-cropping systems averaged 19.7 Mg ha^{-1}, compared to 10.4 Mg ha^{-1} with the double-cropping systems. This difference is the result of earlier sorghum harvest for the later system. There was no statistical difference in the yields of the sorghum types (sweet, forage, and sorghum x sudangrass) within the cropping systems. Within the single-cropping system, the top producing cultivars were either photoperiod sensitive or late maturing varieties. This was in contrast to the double-cropping systems, in which early maturing varieties had the highest sorghum yields. This seems to indicate that selection of varieties for use within double-cropping systems should be based on the cultivar's ability to maximize dry matter production before the earlier harvest date, and is not necessarily associated with a particular sorghum type.

The average forage/feedstock quality of the sorghums changed with the growing environment, cropping system, and cultivar. Most of this alteration may be attributed to the sorghum maturity at harvest, which had a large influence on the total nonstructural carbohydrate (TNC) fraction. The TNC increased drastically when sorghums were grown within the single-cropping system and at the sites during the 2008-09 growing season. This was due to these sorghums being more mature. The TNC was also shown to have a dilution effect on the measured cell wall components of the sorghum, as many varieties did not experience a difference in neutral detergent

fiber (NDF) or acid detergent fiber (ADF) between the cropping systems despite the sorghums being more mature within the single-cropping systems. This lead to variations within the theoretical ethanol fractions (nonstructural and structural carbohydrates), however, the single-cropped sorghums had the greater ethanol potential.

The total biomass production of the double-cropping system was not significantly different than the single-cropping for several of the sorghums varieties. These varieties were also the early maturing varieties that produced the highest sorghum yields, and further support the selection of sorghums with these maturities for use in double-cropping systems. However, when total ethanol yields were compared, the single-cropping cropping system produced higher yields. This was due to differences within the theoretical ethanol potential of the triticale and sorghums. While the triticale was capable of supplementing the additional biomass that would otherwise be lost by earlier sorghum harvest, the quality of the feedstock for ethanol conversion was lesser to that of the sorghum. This may limit the use of double-cropping system for the production of ethanol. However, these systems may be profitable for forage production, as the triticale and sorghum had relatively equivalent ruminant feeding values without a decreased yield. Thus, forage producers may receive the additional environmental benefits of the double-cropping system without a change in the availability of a feed source.

The incorporation of sorghums into the cropping system seems to make production more cost effective than growing the triticale as a sole crop. This was due to the higher yields of the sorghum compensating for some of the additional overhead costs. Within a cropping system, the production of the sorghum varieties did not vary greatly, and this was due to the similar cultivation practices of the cultivars. The single-cropping system was the more cost efficient for producers than the double-cropping system, and was due to the greater yields without the extra cost of growing an additional crop. However, the environmental benefits of the double-cropping system were not considered in this

cost analysis, and the greater sustainability of these systems would be of greater importance than the higher costs.

CHAPTER VI. ALTERNATIVE METHODS FOR THE QUANTIFICATION OF PRUSSIC ACID POTENTIAL IN FORAGES

A paper to be submitted for publication in *Crop Science*

Ben M. Goff, Kenneth J. Moore, Steven L. Fales, Basil J. Nikolau, and Jeffery F. Pedersen

Abstract

Sorghum [*Sorghum bicolor* (L.) Moench] has been shown to contain the cyanogenic glycoside dhurrin, which is responsible for the disorder known as prussic acid poisoning in livestock. When the forage is consumed, mastication releases enzymes that lead to the hydrolysis of hydrogen cyanide (HCN) from dhurrin. HCN is a potent inhibitor of cellular respiration, and may potentially lead to death of the animal. There is potential to breed for varieties containing low levels of HCN. However to be able to select for this trait, the potential for HCN must accurately quantified. The current standard method for estimating HCN uses spectrophotometery to measure the aglycone of the dhurrin, p-hydroxybenzaldehyde (p-HB). Errors may occur due to the inability of this method to solely estimate the absorbance of p-HB at a given wavelength. This study compared the use a gas chromatography (GC) and Near-Infrared Spectroscopy (NIRS) methods to estimate the potential for prussic acid (HCNp) of sorghum and sudangrasses over three maturities. It was shown that the GC method yielded more accurate estimates of HCNp than did the spectrophotometery method. Both methods yielded robust equations with the NIRS method, however, using GC as the calibration method resulted in more accurate and repeatable estimates.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] has long been an important and valuable crop for livestock producers. It is capable of producing large quantities of forage under a shortened growing

season, which has lead to its use as an emergency crop during summer months, particularly in the Southeast (Tew et al., 2008; Venuto and Kinger, 2008). Because it is a warm-season grass (C4), sorghum has more efficient photosynthetic (Kramer, 1981) and nutrient use efficiency (Saeed and El-Nadi, 1998; Gardner et al., 1994). Sweet sorghums have also been shown to produce a high-quality, palatable silage that is capable of fermenting to a stable pH within a short time (Bolsen et al., 2003; Cogdill, 2008). While it is a valuable crop, one of the potential issues associated with use of sorghum as a forage is its potential to cause prussic acid poisoning in livestock (Gorz et al., 1983).

Sorghum has been shown to contain the cyanogenic glycoside dhurrin [(S)-p-hydroxymandelonitrile β-D-glucopyranoside], which when hydrolyzed produces hydrogen cyanide (HCN), and evolutionary adaptation to inhibit herbivory (Dunstan and Henry, 1902; Gorz et al., 1979). Dhurrin is stored within the vacuole of epidermal cells of the plant's leaves, while the enzymes (dhurrinase and α-hydroxynitrile lyase) responsible for its degradation are stored with in the leaf mesophyll (Kojima et al., 1979). This selective localization prevents the premature release of HCN and subsequent death of the tissue. Damage to the plant tissue, via mastication by grazing animals, brings the enzymes into contact with the dhurrin, which is immediately metabolized. HCN has been shown to be a potent inhibitor of cytochrome *c* oxidase (Jones et al., 1984), which leads to hindrance of cellular respiration and asphyxiation of the animal. The time of death ranges between cases, with most occurring anywhere from a few minutes up to several hours, depending on the amount of forage consumed (Hoveland, 1998; Stanton and Whittier, 1992; Thiex, 2002).

While prussic acid poisoning is a lethal disorder, it has been readily controlled with proper management. The prussic acid potential (HCNp) has been shown to decrease as the forage matures (Akazawa et al., 1977; Loyd and Gray, 1970), and most recommendations call for delaying grazing until the sward is between 18 - 24 inches tall (Stanton and Whittier, 1992; Thiex, 2002). Preserving forages via the production of hay or silage has also been shown to reduce the HCNp, most likely due

to inadequate moisture for hydrolysis or the denaturation of the degrading enzymes (Moser, 1995). However, even with proper management the HCNp of a forage has been shown to be susceptible to various environmental factors such as frost (Wattenberger et al., 1968) and moisture stress (Nelson, 1953). Because of the unpredictability of these conditions and the supplementary costs required by additional management, a more practical and direct choice for reducing the risk of toxicities would be to breed for sorghum and sudangrass lines with low HCNp. Previous research has shown that the production of dhurrin is a heritable trait (Gorz et al., 1986; Lamb et al., 1987). However, to be able to do this efficiently, one must be able to accurately quantify the amount of dhurrin being produced.

The hydrolysis of dhurrin results in the production of D-glucose, HCN, and p-hydroxybenzaldehyde (p-HB) (Figure 1) (Akazawa et al., 1960). Because these components are produced in equimolar ratios, it is likely that the dhurrin content of the original sample may be deduced from the quantification of any one of these compounds. A majority of the research developed for estimating the HCNp of foods and feeds has been aimed directly measuring the amounts of HCN produced (Hogg and Ahlgren, 1942; Gyorgy et al, 1969). Unfortunately, most of the techniques have been demonstrated to be characterized by low and unreliable estimates of HCNp, largely due to the partial recovery of the volatile HCN (Gorz et al., 1977). The first, and so far only, method that estimates dhurrin via the quantification of p-HB was developed by Gorz et al. (1977), who reasoned that p-HB was more suitable for measurement because of its stability under normal laboratory conditions, as well as having been shown to absorb ultraviolet light at 330 nm when dissolved in NaOH (Akazawa et al., 1960).

While this method is simple and has been shown to be precise (Haskins et al., 1979), there may be issues with its overall accuracy. With a spectrophotometer, compounds do not absorb light at a specific wavelength of light; rather they absorb light over a range with the maximum absorption occurring at the measured wavelength. In addition to measuring the maximum absorption of p-HB, it

is probable that this method is also measuring the residual absorbance of other plant constituents, such as amino acids and nucleic acids. There have been methods derived to quantify the amount of p-HB produced by the degradation of lignin using gas chromatography (Hartley, 1971; Fritz and Moore, 1987). These methods have been shown to estimate p-HB with greater resolution and sensitivity than would be possible with spectrophotometery. The objective of this study was to determine whether the HCNp of sorghums could be accurately quantified using gas chromatography, as well as, to determine how this method would compare to the traditional spectrophotometery method. Also, as the determination of the HCNp for forages usually is done on vegetative, and therefore a small quantity of samples, an additional aim was to evaluate the effectiveness of these two methods for a nondestructive, empirical method such as near-infrared reflectance spectroscopy (NIRS).

Materials and Methods

Field samples were grown at the Iowa State University Sorenson Research Farm (42° 01' N, 93° 46' W) in Boone, IA. Four cultivars of grain sorghum (B Redlan, BCK 60, BN 102, and BN 103) and two cultivars of sudangrass (BN 113, BN 114) were planted in 4.6 m foot rows with approximately 30 inch spacing between rows. Rows were arranged as a randomized complete block with four replications. Weeds were controlled using atrazine (Aatrex-90df at 2.24 kg of a.i. /ha). Whole plant samples were taken at random within each row. Samples were collected at approximately the first, third, and fifth leaf stage of growth, which corresponds to roughly the V1, V2 and V3 maturity stages of grain sorghum (Vanderlip, 1993).

All samples were dried at 60°C for three days in a forced-air dryer. The samples were then ground with a Wiley mill to pass through a one millimeter screen. Dhurrin and its degradation products were extracted according to Gorz et al. (1977). Approximately 0.5 g of sample were suspended in 50 ml of H_20 and placed on a shaker for two hours. Samples were filtered through Whatman 42 paper filter (Whatman International Ltd., Maidstone, England). Approximately 9 ml of

the resulting supernatant was autoclaved for 60 minutes at 121°C. The sample was then allowed to cool to room temperature before 1 ml of internal standard (1 mg/ml p-Chlorobenzaldyde) was added.

Oasis HLB 3cc solid-phase extraction (SPE) cartridges (Waters Corporation, Milford, MA) were used to prepare the sample with an SUPLECO Visiprep 24™ (SUPLECO Analytical, Bellefonte, PA) manifold system according to the method described it Matějíček et al. (2003). SPE cartridges were conditioned with 2 ml of 0.1 N HCl followed by 2 ml of water. Approximately 2 ml of each sample was added to each cartridge, and was subsequently washed with 2 ml of 0.1 N HCl. Columns were eluted with two washings of 2.5 ml acetic acid. Acetic acid was used as an alternative to methanol, which was used in the original method. It offers approximately the same polarity, but has a higher boiling temperature. This was done to allow an increase in the initial oven temperature of the gas chromatograph, and therefore, would reduce the timing of the run while avoiding potential issues from solvent effects with the column (Rood, 2007). The approximate recoveries for each cartridge were estimated from the concentration of p-cholorbenzaldyde that was recovered, as determined by gas chromatography (GC).

After elution, all samples were mixed thoroughly before two aliquots were removed for quantification. One aliquot was diluted ten-fold with 0.1 N NaOH and was scanned at 330 nm using a Shimadzu UV-1601 spectrophotometer (Shimadzu Scientific Instruments; Columbia, MD). Quantification of the HCNp was done for this sample via the equation originally published in Gorz et al. (1977) that accounted for such factors as dilution and extinction coefficient. A separate aliquot was quantified using an Agilent 7890A GC system (Agilent Technologies, Santa Clara, CA) with a 5% phenyl methyl-fused silica capillary column (0.33μm stationary phase thickness, 25m x 0.2mm). The GC operating parameters were modified from the ones listed in Fritz and Moore (1987). The oven temperature was held at 100°C for one minute then increased at a rate of 7°C per min to 150°C. The temperature was held at this level for five minutes before being increased at the same rate to the

final temperature of 210°C. A splitless inlet was used with a temperature of 220°C and a septum purge flow rate of 3 ml/min. The flame ionization detector (FID) was also used with a temperature of 240°C. The flow rates for the flame of the detector were 40 and 400 ml/min for H_2 and air, respectively. Nitrogen gas was used as a carrier with a flow rate of 0.33 ml/min. The HCNp values reported are on a dry matter basis, and both methods were corrected for recovery rate of each SPE cartridge.

Chemical analysis data was analyzed using a split-plot design. Whole plots consisted of each variety and stage, with the method of determination being the sub-plot. Significance was determined at $p \leq 0.05$ level by using the mixed model approach (PROC MIXED) of the Statistical Analysis Software (SAS, 2003). Least significant differences and orthogonal contrasts were used to describe differences between treatments. The limit of detection was determined for the GC method from the standard curve data using the procedures described in Long and Winefordner (1983). A constant of K=2 (corresponding to a 97.7% confidence level), was used because it gave the most precise estimate while still being conservative.

Samples were scanned at 1100-2500 nm using a NIRSystems 6500 scanning monochromator (NIRSystems, Silver Spring, MD) with Infrasoft International (Port Matilda, PA) software. Both the GC and spectrophotometery data were regressed against the spectral data using modified partial least squares regression (Shenk and Westerhaus, 1991). A 3,5,5,1 math treatment with SNV scatter correction was used for the development of each equation. Equations were validated internally with every sixth sample reserved for validation.

Results and Discussion

One of the most vital observations of this study was that the method of determination had a substantial effect on the HCNp of the sample. When the method of determination was considered, all study factors had substantial effect on the prussic acid content of the forage, including the variety and

maturity stage interaction that was previously insignificant. A summary of the statistical analysis of the study parameters is shown in Table 1. Both the variety and maturity stage had a significant effect on the measured prussic acid potential (HCNp) of the samples. This was to be expected, as these data points of the parameters were selected to allow for a broad range in cyanide (HCN) concentrations. However, these variables did not interact at any significant level, which would seem to indicate that the HCNp decreased at approximately the same rate as the variety matured. This is similar to the data reported in Gorz et al. (1977) that showed that the HCNp of forage sorghums and sudangrasses decreased at similar rates.

The amount of recovery of each sample from solid phase extraction (SPE) was approximately 89.8 %, which was not significantly different than the values reported for the original method (Matějíček et al., 2003). The gas chromatography (GC) method tended to have greater values of HCNp than the spectrophotometery method. When averaged across varieties and stages, the GC method had an average HCNp of 759 ± 29 ppm HCN compared to 487 ± 10 ppm with the spectrophotometery method. The GC had slightly higher standard errors than with the spectrophotometer, which was more than likely due to the method of reporting HCNp values within a larger range (Figure 2). It was determined that the limit of detection (LOD) for the GC method was around 209 ppm of HCN. While this is an acceptable estimate, it should be noted that this could easily be lowered by concentrating the sample more, either by lowering the initial extraction volume or evaporating a portion of the solvent after elution from the solid phase extraction (SPE) cartridge. Caution should be taken if the volume of the extraction is to be reduced, as there were issues with incomplete removal of dhurrin from the plant tissue (data not shown).

There was a significant difference in the HCNp for the sorghums and sudangrasses that was independent of the method used. However, what did vary was the magnitude of the difference (Figure 2). For the sorghums (B Redlan, BCK 60, BN 102, BN 103), the GC method produced

higher values than the spectrophotometery method (919 ± 94 ppm vs. 466 ± 48 ppm), however, for the sudangrasses (BN 113 and BN 114) the GC method averaged 426 ± 63 ppm in contrast to 531 ± 78 ppm with the spectrophotometery method. The spectrophotometery method did not detect any significant variation ($p > 0.19$) in HCNp when averaged across the forage varieties after the first growth stage, while the GC method found large differences ($p < 0.0001$) at all of the tested maturities. The interaction between these two variables is illustrated in Figure 2. With the exception of BN 102, the GC was able to describe a considerable change with each maturity for the sorghums, while the spectrophotometery method was only able to describe changes of lower magnitude. The HCNp of the sudangrasses did not show a decrease with either of the methods used. One possible reason for this could be due to the samples having lower initial values than the sorghums. However, even with these low values, the two methods did show notably different values for these materials.

There was considerable variance between the two methods, and because of this, there is a question of which is correct. To be able to answer this, one must look at the analytical processes that each method incorporates. Spectral analysis operates by measuring the transmittance of a light of known intensity through a given sample. The amount of light absorbed by the sample may then be translated to a concentration of a compound via a known relationship between the two, generally done with a standard curve. Issues may arise when these types of methods are used to measure single constituents within a solution. Even if the samples are believed to be relatively pure, there still could be possible interfering compounds that could contain chromophores (i.e. functional groups) that absorb light at the wavelength of interest. The absorbance of the compound of interest is most usually sensitive to specific chemical environment, thus making the presence of additional compounds all that more troublesome.

An alternative to using spectral analysis is the use of a type of chromatography, the most common being gas and high-pressure liquid chromatography (HPLC). With chromatography, the

sample is passed through a column that contains a silica phase containing phenolic subunits. As the sample moves through the column, the constituents react with the immobile phase depending on their affinity to be attracted to it, and thus move through the column at various rates. This difference in elution time allows for adequate separation of the compounds within the sample so that they may be quantified individually and less susceptible to interfering compounds. Because of this, it is probable that the HCNp values reported by the GC represent the true HCNp of the material. The true cause of the discrepancies with the spectrophotometery method is not immediately known. The most probable explanation is that the samples contained an interfering compound that absorbed light within the same absorption spectrum as p-hydroxybenzaldehyde (p-HB). All of the values reported by the spectrophotometery method fell within a narrow range (187-850 ppm) compared to the GC data (135-1709 ppm). This seems to indicate that the p-HB could have been "masked" by one or several compounds that were present at a higher and more constant concentration.

The regression statistics of the two HCNp methods and the spectral data measured via near-infrared reflectance spectroscopy (NIRS) are shown in Table 2. The standard error of calibration (SEC) and the standard error of cross validation (SECV) for both methods were within the acceptable range reported in Marten et al. (1989). These errors were slightly elevated when the GC data was used as the reference data, but as mentioned previously, this is due to the method having larger estimates. The R^2 of both methods were satisfactory. The GC method had greater 1-VR (roughly equal to the coefficient of determination for cross validation) compared to the spectrophotometery method. This indicates that GC values are more closely correlated with the observed spectral analysis of the sample, providing further evidence that GC is the more accurate method. NIRS equations have been developed with similar 1-VR values as the spectrophotometery derived equation, and were considered acceptable because the calibrations assays measured constituents at very low concentrations as is this study (Roberts et al., 1991; Gray et al., 2001). However, the GC method still

yielded a superior NIRS equation because of its greater correlation of predicted values and capability to predict samples with a wider range of values (Figure 3).

When the practical application of this method is considered, its value is even more evident. According to several extension publications (Stanton and Whittier, 1992; Thiex, 2002), the threshold at which a feed is no longer safe is approximately between 1,000 to 1,800 ppm of HCN (dry matter basis). Between 500-1,000 ppm the forage is considered potentially toxic and should not be consumed as the sole source of feed. The GC method routinely measured concentrations within these ranges, while a majority of the spectrophotometery estimates were not within the potential dangerous range. The spectrophotometery also failed to detect samples that were within the lethal range. This type of underestimation may prove costly for producers, as this will result in the loss of livestock. Also, the tendency to overestimate HCNp could be just as detrimental, as this could cause producers to delay grazing which will result in consumption of forages with lower nutritive value and reduced animal gain. Overall, it may be concluded that the HCNp of a forage may be accurately quantified using the GC, and that this method is well-suited as a wet-chemistry calibration method for the development of empirical NIRS equations.

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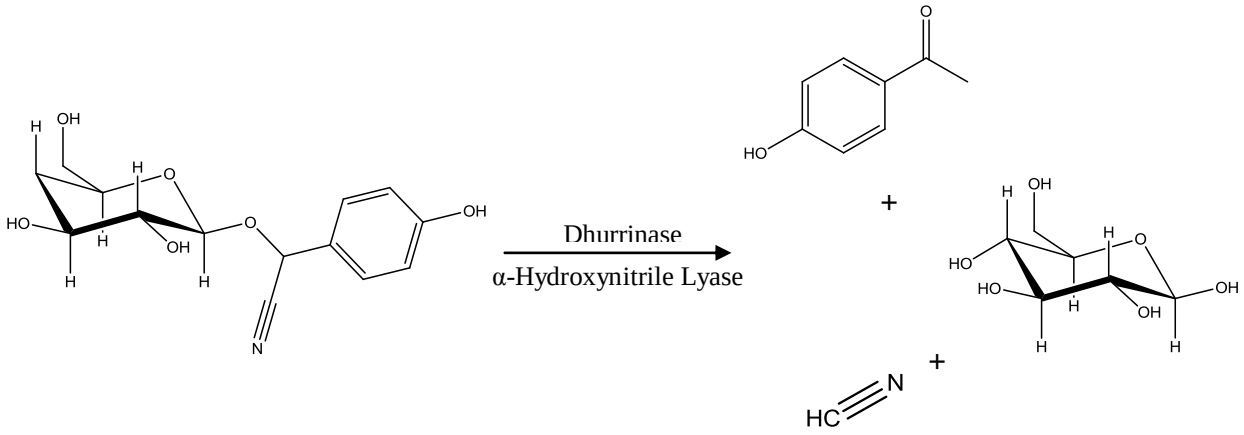

Figure 1. Diagram of the hydrolysis of the cyanogenic glycoside dhurrin into D-glucose, hydrogen cyanide (HCN), and p-hydroxybenzaldehyde (p-HB).

Table 4. Summary of statistical analysis.

Source	d.f.	Significance
Variety	5	<0.0001
Stage	2	0.0001
Variety*Stage	10	0.7778
Method	1	<0.0001
Variety*Method	5	<0.0001
Stage*Method	2	<0.0001
Variety*Stage*Method	10	0.0005

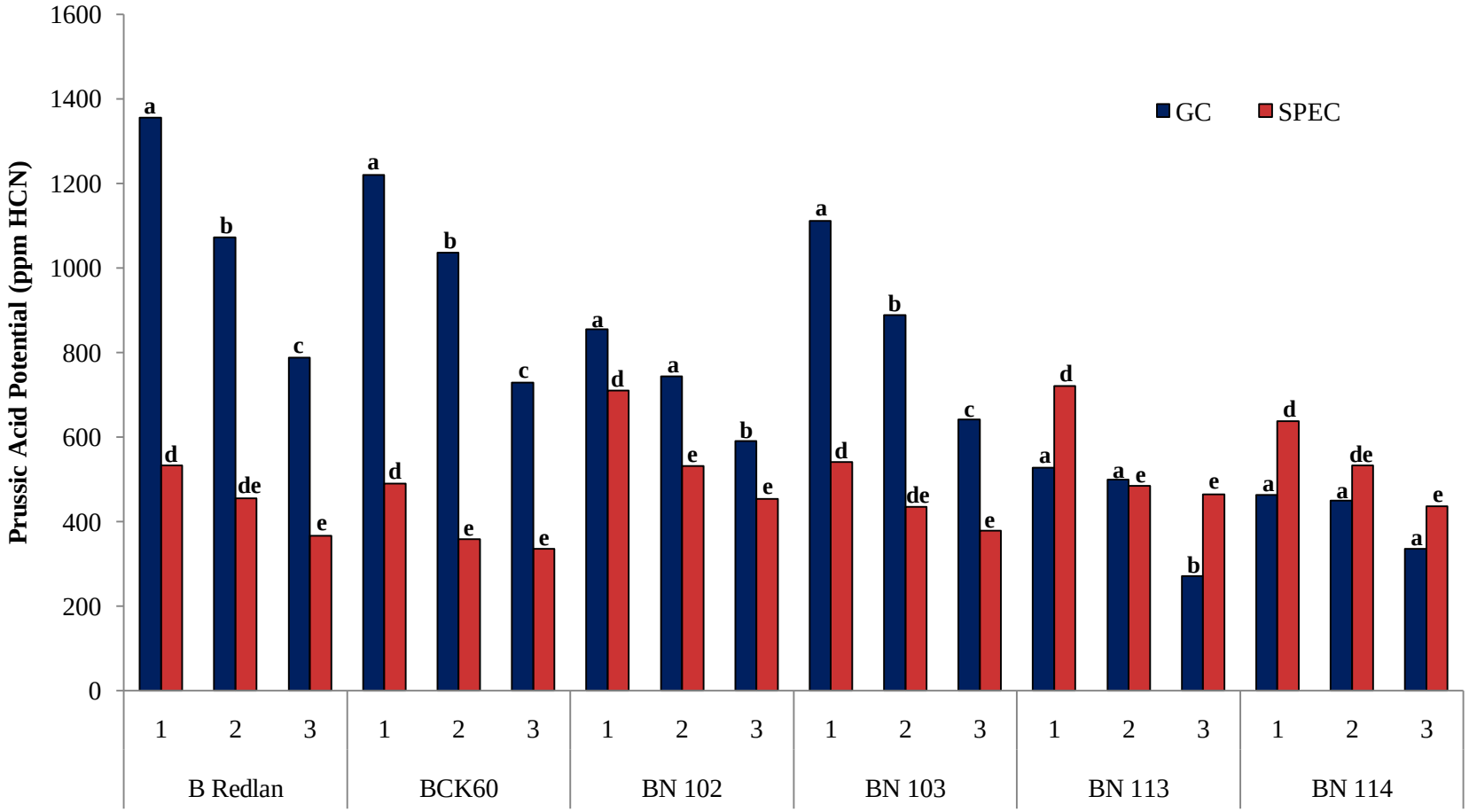

Figure 2. Comparison of the measured prussic acid potential (HCNp) of sorghum and sudangrasses using gas chromatography (GC) and spectrophotometery (SPEC) methods. Letters refer to significance ($p < 0.05$) within a cultivar and method.

Table 5. Summary of Near-Infrared Reflectance Spectroscopy (NIRS) regression statistics with gas chromatography (GC) and spectrophotometery (SPEC) used as the calibration data.

Method	n	F	Mean	SEC*	SECV**	R^2	1-VR†
			---------- *ppm* ----------				
GC	71	16.29	746.45	67.57	91.78	.96	.93
SPEC	71	17.46	483.97	30.07	49.91	.93	.81

*SEC: Standard Error of Calibration
**SECV: Standard Error of Cross Validation
†1-VR: 1 Minus the Variance Ratio

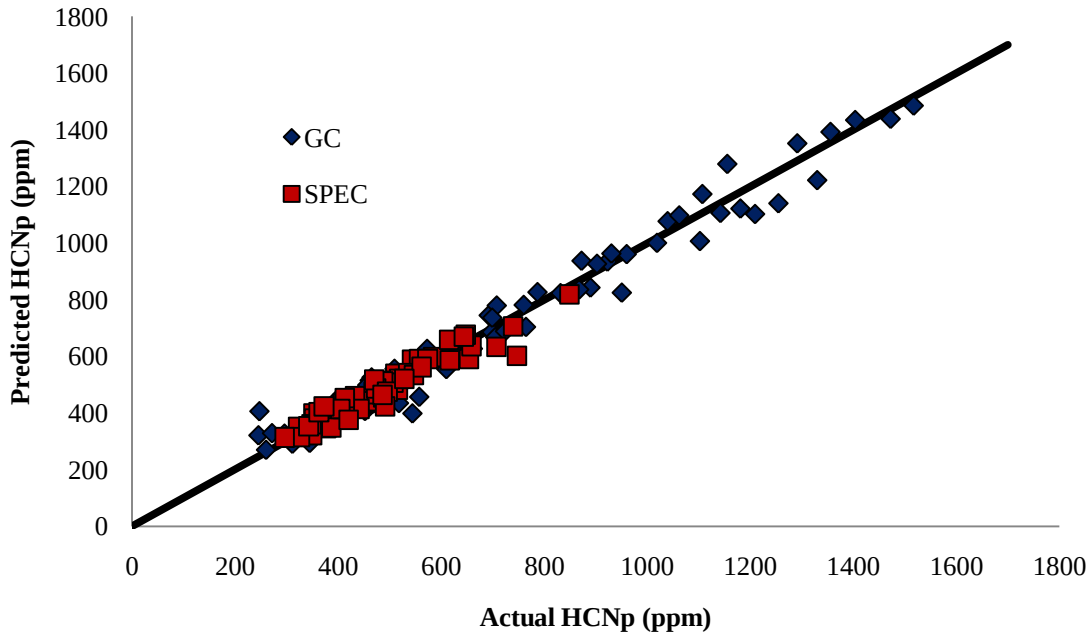

Figure 3. Actual vs. predicted prussic acid potential (HCNp) using near infrared reflectance spectroscopy (NIRS) using gas chromatography (GC) and spectroscopy (SPEC) as using reference data. Solid line represents a perfect correlation.

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