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The evaluation of maize genotypes for potential use in cellulosic ethanol production

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The evaluation of maize genotypes for potential use in cellulosic ethanol production

by

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CHAPTER 1. GENERAL INTRODUCTION

Introduction

The primary goal of this research is to investigate corn stover quality and agronomic traits that may benefit the emerging biofuels industry. Since corn stover is a widely available, low cost, renewable crop residue, it is an attractive feedstock option. However, since challenges have been posed to efficient stover harvest, transportation, and storage, as well as concerns with soil quality following stover collection, it is essential to characterize stover qualities in order to maximize the ethanol potential on a per land unit basis. Variation for agronomic and quality corn stover traits has been shown to exist (Allen et al., 2003; Shinners and Binversie, 2007). However, little breeding effort has been put forth for the improvement of corn stover traits, per se, as most breeding in corn has been done with the inclusion of grain (Lauer et al., 2001). In order to develop a breeding program to improve corn stover traits for biofuel production it is necessary to quantify agronomic and quality characteristics of specific corn stover fractions for a diverse set of corn germplasm.

In this study, 50 maize genotypes, ranging from population crosses to commercial hybrids, were evaluated for agronomic characteristics and chemical composition. An array of germplasm was included in order to assess the amount of variation that may exist for these traits. The stover residue was divided into separate fractions for evaluation, including stover (stalks and leaves), cobs, and husks. Each fraction was evaluated for yield and agronomic characteristics as well as chemical composition. Chemical composition of cell wall material was determined through a Near Infrared Reflectance Spectroscopy (NIRS) calibration developed specifically for corn stover by the National Renewable Energy Lab (NREL). Pentose and hexose sugars predicted by the NREL calibration were used to calculate theoretical ethanol potential (TEP) (www1.eere.energy.gov/biomass/ethanol_yield_calculator.html). Not all samples could be predicted by the NREL equation; therefore, detergent fiber methods (Goering and Van Soest, 1970) were utilized to determine chemical composition. From detergent fiber analysis, estimates of cellulose and hemicellulose for all fractions could be calculated. These values were then used to predict TEP based on the NREL equation. Hemicellulose and cellulose values were substituted in place of component pentose and hexose sugar values in the TEP equation. Proportions of pentose and hexose sugars accounted for in the hemicellulose fraction were adjusted for in the TEP equation and validated by HPLC methods. Theoretical ethanol yields were then calculated on a per acre basis by multiplying TEP and dry matter yield for each fraction.

This research is in conjunction with a grant sponsored by the U.S. Department of Agriculture and U.S. Department of Energy Biomass Research and Development Initiative entitled “Integrated Feedstock Supply Systems for Corn Stover Biomass.” Institutions included on this grant include Iowa State University, University of Wisconsin-Madison, Pennsylvania State University, USDA Dairy Forage Research Center, USDA Corn Insects and Crop Genetics, and the World Resources institute. The overall project is divided in to a number of tasks, ranging from harvest and storage technologies, breeding strategies, economic impacts, and life cycle assessments. The research presented in this thesis focuses on the identification and characterization of beneficial corn stover traits and the implementation of a breeding program to enhance them.

Thesis Organization

The results of the objectives described above are presented among four manuscripts. Each manuscript is followed by relevant tables and figures cited in the text. Followed by this chapter is a literature review, intended to familiarize the reader with the potential that corn stover may have as a feedstock for cellulosic ethanol production and the methods that can be used to characterize this potential. Following the manuscripts, a general conclusions chapter has been prepared to summarize the results of this study and provide suggestions for further research on this topic.

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CHAPTER 2. LITERATURE REVIEW

This literature review intends to summarize the importance and possibility of utilizing corn stover, a lignocellulosic biomass feedstock, for the production of cellulosic ethanol. Since the literature and methodologies for evaluating corn stover for ethanol production are not highly evolved, emphasis is placed on the characteristics of corn stover, how it can be utilized, and possible methods for quality evaluation based on ruminant digestion.

Lignocellulosic Feedstocks

In the 2006 State of the Union Address, President Bush proposed the Advanced Energy Initiative as a step toward overcoming the United States' dependence on foreign energy sources (Houghton et al., 2005). In order to overcome this dependence it was proposed that advancements needed to be made in the development of domestic and renewable alternatives to current transportation fuels. The United States is currently facing a number of challenges associated with energy, including economic security and growth, energy security and growth, and environmental and climate protection. As the United States consumes an average of 388.6 million gallons of gasoline per day and imports well over half of the petroleum it consumes, disruptions in oil supplies can have severe domestic impacts (DOE, 2007). According to Dhugga (2007), oil reserves throughout the world have the potential to be depleted by 2050. Environmental and climate concerns have also been raised due to the rise in greenhouse gas emissions from burning current transportation fuels, which could be reduced by using alternative energy sources. As a result of these concerns, challenges, and proposals it is necessary to investigate alternative sources to fulfill future energy needs.

Biofuels offer a promising alternative to current petroleum based energy sources. Biofuels are fuels made from biomass, or plant material, and they include ethanol, biodiesel and butanol (Dhugga, 2007). The concept of biofuels dates back to the late 1800's, as Henry Ford designed the first vehicles to run on farm ethanol. Biofuels are an attractive alternative to petroleum based products due to their abundant and renewable sources. They can be produced from crops such as sugar cane or corn grain which contain sucrose and starches that can be converted into component sugars followed by fermentation (Zaldivar et al., 2001). Biofuels can also be produced from lignocellulosic materials. Lignocellulosic biomass represents a group of low cost and abundant feedstocks that is found in agricultural residues (corn stover), industrial waste, forestry residues, or grown as dedicated energy crops (switchgrass, miscanthus). It is estimated that lignocellulose accounts for approximately 50% of the biomass in the world. Lignocellulose is a composite of three components: cellulose, hemicellulose, and lignin. These three components make up plant cell walls and form the structural materials that plants utilize to form fibrous portions of biomass, including leaves, stems, and stalks. The cellulose and hemicellulose components of biomass are structural carbohydrates, and like starches, they can be broken down into fermentable monosaccharides.

In 2005, the USDA and DOE conducted the billion ton annual supply study. The purpose of their study was to determine whether or not the United States could sustainably supply itself with enough harvestable biomass to displace 30% of the country's petroleum usage by 2030 by relying on its current land resources (Perlack et al., 2005). To meet this goal, it was estimated that one billion dry tons of harvestable biomass and 60 billion gallons of ethanol would need to be supplied each year. This study evaluated agricultural and forest lands in the United States and found that over 1.3 billion dry tons of biomass could be sustainably supplied on a per year basis. Therefore the goal of displacing 30% of petroleum usage by 2030 could be accomplished. Of the 1.3 billion dry tons of available biomass, forest lands accounted for about 368 million dry tons and agricultural lands accounted for 998 million dry tons. Perennial grasses accounted for the majority available biomass, and corn stover was the second largest source of available biomass. Nearly 20% of the harvestable biomass in the study was accounted for by corn stover. Therefore, we can conclude that corn stover is a very abundant and important source to consider for potential use as a lignocellulosic feedstock.

Lignocellulosic Biomass Composition

Lignocellulosic biomass refers to the woody and fibrous portions of plants, or the cell wall material (Houghton et al., 2005). The plant cell wall is made up of three main components: cellulose, hemicellulose, and lignin. Cellulose and hemicellulose represent structural carbohydrates, which if accessible, could be converted to other products including transportation fuels. Lignin, however poses a barrier to the accessibility of these compounds. Cellulose is the most abundant naturally occurring biological compound on earth. It is composed of six carbon glucose units. Glucose units in cellulose, unlike in starch, are connected by β 1-4 linkages (Van Soest, 1994). This linkage results in adjacent glucose molecules being rotated 180º, resulting in a disaccharide, cellobiose, as the repeating unit in cellulose chains. Due to this molecular arrangement, glucose molecules form long unbranched chains that may hydrogen bond to one another, resulting in a crystalline structure that is resistant to biological degradability. Surrounding the cellulose fibrils exists another structural polysaccharide, hemicellulose (Houghton et al., 2005). Hemicellulose is composed of five and six carbon sugars. Five carbon sugars include xylan and arabinan, and six carbon sugars include glucan, galactan, and mannan. Hemicellulose components form cross linkages to the glucose units that make up cellulose. Hemicellulose also forms cross linkages with lignin. Lignin is a polymer composed of phenylpropane units. The primary function of lignin is providing strength for cell walls and plant resistance to moisture and biological attack. Lignin may sometimes be referred to as the biologically resistant “glue” that holds the cell wall structure together. The three main components in the cell wall: cellulose, hemicellulose, and lignin, form a complex matrix which is resistant to being broken down into component sugars.

Breakdown of Lignocellulosic Biomass

Two main platforms have been proposed for the breakdown of lignocellulosic biomass to its component parts, which can then be used to produce biobased products (Houghton et al., 2005). These platforms are the thermochemical platform and the sugar platform. The thermochemical platform involves heating biomass in the presence of limited oxygen to produce a gas or liquid, which can then be burned efficiently for energy or converted to other biobased products. The sugar platform involves breaking cell wall polysaccharides that comprise biomass into component monosaccharides which can then be fermented. This process consists of pretreatment, hydrolysis, and fermentation. Pretreatment involves the adding heat, enzymes or acid to the ground feedstock in order to break apart the cellulose, hemicellulose, and lignin matrix of the cell wall. As this matrix begins to break apart, the polysaccharides become more accessible to hydrolyzing enzymes in the hydrolysis step. Following pretreatment, hydrolysis involves the breakdown of the newly accessible polysaccharides into component sugars. Once component sugars are available they can be fermented by microbes and the resulting alcohol can be distilled and used for biofuels. One process modification to the sugar platform is Simultaneous Saccharification and Fermentation (SSF). During the hydrolysis step, a feedback inhibition has been observed that is associated with hydrolyzing enzymes. As cellulose is being broken down during hydrolysis, the build up of glucose units causes a feedback inhibition associated with the enzymes, resulting in the cessation of cellulose hydrolysis. Therefore SSF involves combining of hydrolyzing enzymes and fermenting microbes into the same step to overcome the feedback inhibition. In SSF, as polysaccharides are broken down by hydrolyzing enzymes, they are simultaneously fermented by microbes to prevent the buildup of glucose.

Corn Stover as a Feedstock

Corn stover represents an attractive feedstock option for lignocellulosic biomass because it is widely abundant and inexpensive. Corn stover includes all above ground plant material excepting the grain portion of the corn plant. The possibility of corn being grown as a dedicated lignocellulosic feedstock is not promising, because the grain portion of the corn plant is a highly valued commodity. Therefore, corn stover would be considered an agricultural residue of the corn grain crop that could be collected when harvesting grain or baled after grain harvest to be used as a lignocellulosic feedstock.

Traditionally there has been little breeding effort put forth to improve maize forage yields and quality in the United States (Frey et al., 2004). Emphasis has been put on breeding corn for grain yield and agronomic characteristics that enhance yield. Corn that is grown for whole plant harvest is traditionally used in the form of silage, including the grain portion. Grain represents approximately 50% of the total above ground dry matter and is highly digestible. Therefore, increased grain yields have led to higher total dry matter yields and increased nutritional quality of silage. This is a result of traditional breeding for increased grain yield rather than for stover characteristics. Lauer et al. (2001) conducted a study to evaluate changes in silage yield and quality over the past 70 years of breeding for grain yields. They evaluated a number of corn hybrids and open pollinated varieties that have been grown since 1930 and found that total dry matter and stover yields have increased significantly, and that neutral detergent fiber (NDF) has decreased slightly. According to Allen et al. (2003) much variation exists for forage quality and forage yield among corn breeding populations in the United States. More variation exists across inbreds than hybrids for quality traits, and ranges for stover composition have been found to be greater than those for whole plant composition, including grain. Since little breeding effort has been put forth solely on corn stover and significant variation for stover quality may exist, it may be possible to make improvements to benefit the emerging biofuels industry.

Along with increasing stover quality it will be necessary to increase stover yields. In order to increase stover yields, it would be beneficial to have an understanding of what different plant fractions yield and what percentage of the plant they represent. Pordesimo et al. (2004) determined the distribution of above ground biomass for corn plants. Two plants per plot were harvested at a 15 cm height and fractioned into leaves, stalks (including tassels and sheaths), husks (including shanks), and ears. When harvested at grain physiological maturity, they found that grain, stalks, leaves, cobs, and husks accounted for 45.9, 27.5, 11.4, 8.2, and 7.0% of total plant biomass respectively. Excluding grain, dry matter proportions for stalks, leaves, cobs, and husks were 50.9, 21.0, 15.2, and 12.9%, respectively. Shinners and Binversie (2007) reported stalks, leaves, cobs, and husks to account for 56, 21, 15, and 8% of the nongrain above ground dry matter and harvest indices ranging from 41 to 62%.

Harvest index measures the ratio of grain to total dry matter. Therefore, the non-grain portion of the corn plant accounts for approximately half of the above ground biomass and each non-grain fraction is a significant contribution, but there is some variation for these proportions. Based on these observations it may be possible to breed for higher stover yields or to select specific stover fractions, such as cobs or husks to utilize as a feedstock. However, if higher stover yields are attainable, we would want to be certain not to sacrifice grain yields.

Digestibility of forages is an important characteristic, as it affects animal growth, intake, and production (Lundvall et al., 1994). Forage improvement programs have focused on increasing digestibility in order to improve forage quality. Digestibility of corn stover is influenced by cell wall composition and digestibility (Argillier et al., 2000). Therefore, forage quality is improved by selecting for increased cell wall digestibility. The cell wall characteristics of forage crops that impact feed value are traditionally measured by the Van Soest detergent fiber method (Goering and Van Soest, 1970). In this method, percentages of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) are measured. From these measurements, corresponding cell wall constituents can be calculated (Van Soest, 1994). Cellular contents are soluble in the NDF procedure, and cellulose, hemicellulose, and lignin are recovered. Hemicellulose is soluble in the ADF solution, and cellulose and lignin are recovered. Therefore, hemicellulose can be calculated as the difference between NDF and ADF. Cellulose is soluble in the ADL procedure, and lignin is recovered. Cellulose can therefore be calculated as the difference between ADF and ADL. An ashing procedure is performed after the ADL step, and lignin can then be calculated as the difference between ADL and ash.

Important mutations have been discovered and utilized in forage programs that favor quality and yield traits of corn stover. Brown midrib mutations reduce lignin content and are some of the earliest mutations described in maize (Cherney et al., 1991). Brown midrib (*bm*) is a recessive independently segregating gene that can potentially be used as a means for genetically modifying the compositional characteristics of corn stover (Coors and Lauer, 2001). Phenotypically, these mutations result in a reddish-brown pigment observed in the leaf midribs. Four brown midrib mutants have been discovered (*bm1, bm2, bm3, bm4*), with *bm1* first being discovered in 1924 at the University of Minnesota. They are located on maize chromosomes 5, 1, 4, and 9, respectively, and are thought to effect enzymes in the phenylpropanoid biosynthetic pathway leading to lignin formation (Neuffer et al., 1997).

The most extensively studied mutation is *bm3*. It has been noted that lignin, NDF, and ADF concentrations are usually lower in *bm3* hybrids (Coors and Lauer, 2001). Digestibility of corn stover, in turn, has been reported to be higher in *bm3* hybrids. However on a whole plant basis, digestibility has not been shown to increase due to reduced grain yields of *bm3* hybrids. Also, agronomic performance has been shown to be poorer for hybrids containing brown midrib mutations than for their counterparts. These characteristics include lower growth rates, poorer early season vigor, delayed flowering, increased lodging, smaller ear percentages, and poor grain and stover yields. Allen et al. (1970) compared agronomic performance of *bm3* hybrids to their near isogenic counterparts. They found that the *bm3* hybrids had 13% lower forage yields than their counterparts, 16.0 versus 18.3 Mg ha^{-1}. Gentinetta et al. (1990) reported brown midrib hybrids to have higher stalk lodging and lower forage yields than normal hybrids. Measures for stalk lodging were reported at 12.5 and 3.8%, and forage yields were 14.4 and 17.9 Mg ha^{-1} for brown midrib and normal hybrids, respectively. While the corn genotypes possessing brown midrib mutations offer a way to improve the digestibility of corn stover by having reduced lignin content, they may not offer a solution to improving biomass for cellulosic ethanol production due to their poor agronomic characteristics.

Another mutation associated with higher stover yields has also been utilized in forage programs. The dominant *Lfy1* allele is known for increasing the number of leaves above the ear on the corn plant (Coors and Lauer, 2001). These extra leaves are thought to be associated with increased photosynthate production during grain fill leading to increased yields for both grain and stover. The existence of mutations that alter yield and quality of corn stover gives promise for improvement of these important stover characteristics.

Corn stover is not typically harvested by U.S. Corn Belt grain farmers. The harvest, transportation, and storage of corn stover are considered to be major obstacles to the successful use of corn stover for biofuel production (Atchison and Hettenhaus, 2003). The removal of corn stover may also lead to a reduction in soil organic matter and an increase in soil erosion (Wilhelm et al., 2004). Hoskinson et al. (2007) have investigated the engineering challenges associated with corn stover harvest. In current grain harvest systems approximately 30% of the stover fraction passes through the combine and falls to the ground. The remaining stover, mainly stalk material, does not pass through the combine. Efficient collection of corn stover will require the development of new harvesting methods. Currently economically feasible corn stover transportation is limited to a 50 mi radius of a biofuel plant, due to its low bulk density, creating challenges to the use of corn stover (Atchison and Hettenhaus, 2003). Graham et al. (2007) investigated the effect of differing amounts corn crop residue removal on crop production. In the first year of the study, corn stover was harvested, and 0, 50, 100, and 150% of the residue was returned to the evaluation plots. In the following growing season, they found that for each Mg ha^{-1} of residue removed, grain yield was reduced by 0.13 Mg ha^{-1} and biomass yields were reduced by 0.29 Mg ha^{-1}. These data indicate the need to investigate ways to make the utilization of corn stover for biobased industries a sustainable and efficient system through maximizing agronomic and quality performance of corn stover fractions.

Quality Analysis

The detergent fiber system represents a widely used method of rapidly determining insoluble cell wall components, and further calculating estimates of hemicellulose, cellulose, and lignin (Van Soest, 1994). Near Infrared Reflectance Spectroscopy (NIRS) has also been used widely by forage breeders and physiologists to predict forage quality (Marten, 1989). The first use of NIRS to predict forage quality was reportedly in 1979. Correlations between laboratory data and NIRS predictions were $r = 0.90$ for NDF and ADF measurements and $r = 0.73$ for lignin, with standard errors of calibration less than 1 percent. Applying the methods and techniques of forage quality analysis to analyzing compositional and quality traits of biomass for conversion properties represents a valuable tool for current research in biofuel production. When using NIRS methods, quality spectroscopy should first be performed. This involves collecting spectroscopic data on the samples in wavelengths from 400 to 2500 nm. In order to obtain quality spectroscopic data, samples must be uniformly ground to a very fine particle size (1-2 mm) and mixed well. Next, a set of calibration samples should be selected from NIRS data. These samples should represent the range of compositional variance accounted for in the entire sample set. Laboratory methods should be performed on the calibration samples to obtain actual chemical values. Finally, a multivariate analysis is performed in order to relate compositional data to spectroscopic data. Partial least squares (PLS) analysis regresses compositional information against the NIRS spectra. Linear equations are then formed from this relationship and used to translate spectral data for the entire sample set to compositional data. Hames et al. (2003) evaluated 47 corn stover samples over four years in order to determine NIRS prediction equations for the chemical composition of corn stover. Near infrared reflectance spectroscopy predictions were highly correlated with wet chemical values, so it was concluded that the NIRS equations provide a complete compositional analysis that is precise and accurate for a wide range of corn stover samples.

As cell wall composition is related to animal intake and digestibility, it is likely that it is also correlated to convertibility of lignocellulosic biomass to biofuels. It is thought that factors limiting digestibility of plant cell walls in ruminant animals are the same factors limiting ethanol production from biomass, as both systems are dealing with the accessibility of fermentable polysaccharides, which are limited by lignin. The National Renewable Energy Lab (NREL) in Golden, Colorado, has derived a theoretical ethanol yield calculator (www1.eere.energy.gov/biomass/ethanol_yield_calculator.html) to predict the ethanol potential in gallons per ton of biomass. The yield calculator relates sugar content of corn residue to theoretical ethanol potential. Component hexose sugar concentrations are multiplied by a constant and added to pentose sugar proportions that are multiplied by a constant. These constants are based on monosaccharide density when in polysaccharide form. The two values are then added to determine theoretical ethanol potential measured in gallons of ethanol per dry ton of biomass. The formula for calculating theoretical ethanol yield (TEP) is:

H (hexose sugars)= (%Glucan + %Galactan + %Mannan) *172.82

P (pentose sugars)= (%Xylan + %Arabinan) *176.87

TEP (gallons/dry ton = H + P

Hames et al. (2003) reported a TEP range of 105 to 119 gal/dry ton for the stover samples that were evaluated. The TEP calculator assumes that all sugars can be calculated based on monosaccharide predictions and that all sugars can be converted into ethanol. Theoretical ethanol yield on a per unit of land area basis can be calculated by multiplying biomass yields by TEP.

Corn stover represents a valuable resource that could largely benefit the emerging biofuels industry. Since little breeding effort has been devoted to the improvement of corn stover per se, genetic variability may be present for quality and agronomic characteristics. Mutations that have been shown to improve quality and agronomic characteristics of corn stover exist, and may also offer promise to improve corn stover characteristics for biobased industries. Since many challenges still exist for efficiently utilizing corn stover for a lignocellulosic feedstock, such as efficient harvest and soil quality maintenance, maximizing agronomic and quality potential is essential. Based on the well developed methods of NIRS, detergent fiber analyses, and the stover equation developed by NREL, it may be possible to evaluate and improve the chemical characteristics and TEP of corn stover. These chemical properties could then be utilized to predict ethanol production from corn stover on a per land unit basis.

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CHAPTER 3. EVALUATION OF CORN COB CHARACTERISTICS BENEFICIAL TO THE PRODUCTION OF CELLULOSIC ETHANOL

A paper to be submitted for publication in *Agronomy Journal*

Krystal M. Kirkpatrick, Kendall R. Lamkey, and Kenneth J. Moore

Abstract

Corn cobs are a promising feedstock for cellulosic ethanol production, but little is known about dry matter and ethanol yields of corn cobs. This study was conducted to determine yield and quality characteristics of corn cobs and to make predictions of potential ethanol yields. Fifty diverse maize genotypes were evaluated for cob yield and analyzed for cob quality traits in three replications of a randomized complete block experiment, over two years and two central Iowa locations. Quality traits included cellulose (ADF-ADL), hemicellulose (NDF-ADF) and lignin (ADL). From these quality traits, estimates of pentose and hexose sugars were calculated, which were used to estimate theoretical ethanol potential (l t^{-1}) (TEP). On average, the hybrids yielded 1.3 t ha^{-1} of cob dry matter, ranging from 0.8 t ha^{-1} to 1.8 t ha^{-1} of cob dry matter. Cobs accounted for 14%, 17%, and 8% of the total ear, total nongrain above ground biomass, and total dry matter portions of the plant, respectively. Significant differences among hybrids were found for agronomic and compositional traits. Average TEP was 609 l t^{-1}, ranging from 588 to 627 l t^{-1}. Theoretical ethanol yields (TEY) were calculated by multiplying cob yields (t ha^{-1}) and TEP (l t^{-1}). Average TEY from cobs was calculated at 789 l ha^{-1}, ranging from 469 to 1103 l ha^{-1} over all genotypes. Theoretical ethanol yield and grain yield were highly correlated with cob yield ($r = 0.996$ and $r = 0.662$). Therefore, selecting for higher cob yields, through selection of higher grain yielding genotypes should also result in the best hybrids for cellulosic ethanol production.

Introduction

Improving and investigating methods of domestic, renewable fuel production is critical to achieving energy independence in the United States. The large amount of petroleum fuel used and imported in the United States on a day to day basis, creates challenges for economic security and growth, energy security, and environmental and climate protection (Dhugga, 2007). Biofuels, such as ethanol, offer a possible solution to these concerns. Currently, the majority of ethanol production is from starch derived from corn grain. Ethanol production from corn grain alone, however, is not projected to meet transportation needs (Houghton et al., 2005). Therefore, production of ethanol from cellulosic sources, such as corn stalks, leaves, and cobs will be essential to meeting the demand.

In 2005, the USDA and DOE conducted the billion ton annual supply study, which determined that the land resources of the United States were capable of sustainably supplying enough harvestable biomass to displace 30% of the country's petroleum usage by the year 2030 (Perlack et al., 2005). Of this available biomass, nearly 20% was accounted for by corn stover, or the nongrain, above-ground portions of the corn plant. Concerns with removal of soil organic matter and soil erosion, ease of harvest, and transportation have brought attention to the cob portion of the plant. As reported by Shinners and Binversie (2007) cobs account for 15% of the aboveground corn stover biomass, and would therefore result in less removal of soil organic matter than harvesting all above ground stover. The harvest and transportation of cobs also represents an attractive alternative to the harvest and transportation corn stover. Currently economically viable corn stover transportation is limited to a 50 mi radius of a biofuel plant, due to its low bulk density. Cobs have a higher bulk density than corn stover and therefore may be more economical for transportation. Cobs may also have a higher potential for ease of harvest than corn stover, as they represent a plant fraction already passing through the combine with regular grain harvest (Hoskinson et al., 2007). Therefore, collection technologies and transportation systems may be simpler and more economical with cob harvest as opposed to stover harvest. One ethanol production company, Poet LLC, has already initiated plans to harvest cobs as the main feedstock for a new cellulosic ethanol production facility in Emmetsburg, IA (Hoskins, 2007). As these ideas develop, it becomes increasingly important to agronomically and qualitatively characterize cobs to determine the profitability, productivity, and environmental impact of the emerging industry.

Two platforms have been proposed for the conversion of lignocellulosic material into energy: the biochemical or sugar platform and the thermochemical platform (Houghton et al., 2005). The thermochemical platform involves the use of heat to break biomass into gases or liquids that can be converted into other products, while the sugar platform involves the use of enzymes and acids to hydrolyze biomass into component sugars which can then be fermented. Our research focuses on the sugar platform as a means for converting biomass into energy. It is assumed that higher component sugar yields and more accessible monosaccharides will result in higher ethanol production.

Two main processes have been developed to investigate ways to estimate ethanol potential based on the above hypothesis. One process uses the stover calibration equation developed by the National Renewable Energy Lab (NREL) in Golden, CO (Hames et al., 2003). This equation uses Near Infrared Reflectance Spectroscopy (NIRS) to predict the chemical composition of corn stover. The equation is capable of predicting each component hexose and pentose sugar in the sample, including glucose, mannose, galactose, xylose, and arabinose. These component sugar values can then be used to calculate theoretical ethanol potential (TEP). The second method for estimating ethanol potential is based on the theory of ruminant digestion. Using this method, detergent fiber values are estimated by the procedures of Goering and Van Soest (1970). From the detergent fiber analysis, values of cellulose and hemicellulose can be calculated and used to predict TEP (Kirkpatrick et al., 2008).

When considering the ruminant theory, digestibility of biomass is influenced by cellular composition and cell wall digestibility, which is traditionally evaluated by analysis of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Argillier et al., 2000). Cellulose, hemicelluloses, and lignin are the three main components making up cell wall material (Van Soest, 1994). Cellulose is the most abundant naturally occurring biological compound on earth and is composed of six carbon glucose units. Surrounding the cellulose fibrils exists another structural carbohydrate, hemicellulose. Hemicellulose is composed of five carbon sugars including xylan and arabinan, and six carbon sugars including glucan, galactan, and mannan. Hemicellulose components form cross-linkages to the glucose units which comprise cellulose, and also to lignin. Lignin is a polymer composed of phenylpropane units that provides strength for cell walls and plant resistance to moisture loss and biological attack, therefore creating a hindrance to digestibility and sugar accessibility.

Neutral detergent fiber measures the cellulose, hemicellulose, and lignin content in the plant material, ADF measures the cellulose and lignin content in plant material, and ADL measures the lignin content of plant material (Van Soest, 1994). These three measures can then be used to estimate the structural carbohydrate content of plant material. For example, subtracting ADF from NDF gives an estimate of hemicelluose, and subtracting ADL from ADF gives an estimate of cellulose. Hames et al. (2003) estimated the theoretical ethanol potential of corn stover by determining the content of individual five and six carbon sugars. Hames et al. (2003) estimated the theoretical ethanol potential of corn stover to range from 105-119 gal t^{-1} (438-496 l t^{-1}).

The objective of our study was to evaluate the suitability of cobs as a lignocellulosic feedstock for the sugar platform and to assess the potential for modifying cob characteristics via plant breeding. We evaluated a group of 50 maize hybrids, populations, and other cultivar types to answer the following questions: What is the proportion of above ground corn biomass that cobs account for, and how much variation exists among a diverse germplam array for cob yields? How much variation is there for cob quality traits among different hybrids and germplam sources? What is the theoretical ethanol yield potential of cobs? What conclusions can we draw from this data regarding the development of selection criteria for breeders to develop maize hybrids with cobs best suited for ethanol production?

Materials and Methods

Germplasm

Fifty maize genotypes were selected to represent a range of germplasm in order to evaluate the amount of variation that may exist for agronomic and quality traits. The genotypes selected included commercial hybrids, open-pedigreed F1 hybrids, populations, population x population hybrids, and inbred x population hybrids (Table 1). Some of the genotypes were developed specifically for forage quality, while most of the genotypes were developed for grain production. The F1 experimental hybrids included public x public line crosses and public x private foundation line crosses. Lines beginning with “W” were developed at the University of Wisconsin. Wisconsin Quality Synthetic (WQS) lines were developed by the University of Wisconsin corn breeding project for high stover quality and forage yields (Frey et al., 2004). Inbred lines W601S, W602S, W603S, andW604S were developed from WQS CO and WQS C1, respectively. The inbred line A619 was developed at the University of Minnesota (Gerdes et al., 2003). Lines beginning with “LH” and “HC” were developed by Holden’s Foundation Seed. Lines beginning with “TR” were developed by Thurston Genetics. Lines beginning with “SGI” were developed by Seed Genetics Inc. Lines B73 (Russell, 1972), B116 (Hallauer et al., 2004), B126, and B129 were developed by Iowa State University. The BSSS population is a stiff stalk synthetic and the BSCB1 is a corn borer synthetic population (Gerdes et al., 2003). Six commercial hybrids were also evaluated, including DeKalb DKC51-43, Pioneer 34M93, Renk RK232, Mycogen F697 (*bm3*), Novartis N48V8 (*Lfy1*), and Holden’s LH244/LH295, an open pedigreed F1 hybrid.

The hybrid Mycogen F697 was a brown midrib (*bm3*) hybrid and the hybrid Novartis N48V8 is a hybrid that contains the leafy trait (*Lfy1*). Both of these hybrids are marketed as silage hybrids. Brown midrib is a recessive trait that is controlled by a series of single genes that results in lower lignin production in the plant (Cherney et al., 1991). The lower lignin production is associated with increased digestibility and forage quality. The leafy mutation results in an increase in the number of leaves above the ear on the plant (Coors and Lauer, 2001). The increased leaf production is associated with increased grain and stover yields. An isogenic series of brown midrib hybrids were evaluated in the study but are not included in this analysis because they were unadapted to Iowa and not representative of performance that would be expected in Iowa.

Field evaluation

Genotypes were evaluated in 3 replications of a randomized complete block design at 2 locations in each of 2 years. In 2005, hybrids were evaluated at Ames and Ankeny, IA, and in 2006, hybrids were evaluated at Ames and Belmond, IA. Experimental plots consisted of two rows, 5.49 m long with 0.76 m between rows, including alley ways. Data collected on plots included silking date (days after planting when 50% of the plants within a plot showed visible silks), ear height (calculated from ground level to node of the highest ear on the plant), plant height (calculated from ground level to node of the flag leaf), root lodging (percentage of plants leaning greater than 30° from vertical), stalk lodging (percentage of plants with stalks broken below the highest ear), grain yield, cob yield, stover yield, total above ground dry matter yield (TDM, including grain), ear moisture at harvest (obtained from wet and dry weights of ears), and stover moisture at harvest (obtained from wet and dry weights of stover samples).

Plots were harvested at physiological maturity. In 2005, the Ames and Ankeny locations were planted on May 5 and May 6, respectively and harvested on October 9. In 2006, plots were planted on May 3 and May 9 and harvested on September 15 and September 20 for Ames and Belmond locations, respectively. Grain and stover fractions were harvested separately by hand harvesting ears from each plot. In 2005, ears (grain and cob) were harvested from all plants in each plot. In 2006, ears were harvested from 20 plants per plot and the ear husk was harvested with the ear. A pruning shear was used to harvest ears in the husk by clipping the shank of the ear where it meets the plant. To ensure a random sample of ears, 10 ears were randomly harvested from each row of the two-row plot. Remaining ears were gleaned in the husk and discarded before stover harvest. In both years, ears from each plot were weighed at harvest (wet weight), dried at 37.8˚C for three days, and weighed again for a dry weight. After drying, ears were shelled to determine total plot grain weight and cob weight. In 2006, husks were separated at the shelling stage and weighed for a total plot husk weight. Subsamples of grain, cobs, and husks (2006 only) were kept from each plot for compositional analysis.

Stover was harvested immediately after ear harvest with a commercial silage chopper modified for agronomic research, courtesy of Mycogen Seeds, Belmond, IA. Stover was chopped at a height of approximately 6 cm. In 2005, harvested stover consisted of stalks, leaves, and husks. In 2006, harvested stover consisted of stalks and leaves only, because the husk was harvested with the ears. Total plot stover weight was obtained from the silage chopper. Subsamples of stover were collected from each plot, weighed, dried at 37.8˚C for four days, weighed again, and then kept for compositional analysis.

Lab evaluation

Cob samples were ground by first passing them through a wood chipper to reduce particle size to approximately 3 cm. These samples were ground in a Wiley Mill to pass through a 2 mm mesh screen. Ground samples were scanned with a NIRSystems 6500 near infrared reflectance spectrophotometer (NIRS) (FOSS NIRSystems Inc., Silverspring, MD). Standard NIRS procedures were used (Marten et al., 1989). The CENTER program was used to compute standardized *H* statistics for each sample's spectra, and the SELECT program was used to select calibration samples for wet-lab analysis using a standardized *H* of 1.5 for all cob samples.

Calibration samples were then analyzed to determine detergent fiber composition. A modified procedure of Goering and Van Soest (1970) was used for sequential analysis of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL). Modifications included the use of ANKOM^{200} fiber analyzer (Ankom Technologies Corp., Fairport, NY). Estimations of NDF, ADF, and ADL (g kg^{-1}) were obtained for the calibration samples from wet chemistry methods. Values for cell solubles, hemicellulose, cellulose, and lignin were calculated from the detergent fiber data. The results of the calibration set were used to develop prediction equations, relating the NIRS spectral data to chemical composition values. Calibration equations were developed for NDF, ADF, ADL, cell solubles, hemicellulose, cellulose, and lignin. Selection of prediction equations for each constituent was based on high R^2 values and low standard errors of calibration (SEC) and cross validation (SECV) (Marten et al., 1989). The final equations were then used to make predictions for all cob samples for all chemical constituents. Calibration statistics for compositional cob traits are shown in Table 2. Hemicellulose was the most difficult quality trait to predict ($R^2 = 0.85$), most likely due to the small range of variation expressed by this trait (SD= 1.2).

Hemicellulose and cellulose values were used to calculate theoretical ethanol potential (TEP) for cobs (Kirkpatrick et al., 2008). The TEP equation used was a modification of the theoretical yield calculator developed by the National Renewable Energy Lab (NREL), Golden, CO (USDOE, 2006). TEP was calculated as follows:

H (hexose sugars) = (Cellulose + Hemicellulose * 0.07) *172.82

P (pentose sugars) = (Hemicellulose*0.93) *176.87

TEP (gallons/dry ton) = H + P

Theoretical ethanol yields (TEY) (l ha^{-1}) were calculated by multiplying TEP (l t^{-1}) by cob yield (t ha^{-1}).

Data analysis

The data for the individual environments were analyzed as a randomized complete block design and then combined over environments using the MIXED procedure (SAS Institute, 2003). A residual analysis was performed on the individual environment analyses to detect outliers (Anscombe and Tukey, 1963). Entries were considered fixed effects in the analysis and all other effects were considered random. Entry means were used to calculate Pearson correlation coefficients between traits. An LSD (0.05) was calculated using the genotype x environment error mean square to compare genotype means. All tests of significance were made at the $\alpha=0.05$ p level unless otherwise noted.

Results and Discussion

Cob yield

Cob yield averaged over all genotypes and environments was 1.3 t ha^{-1} and ranged from 0.8 to 1.8 t ha^{-1} (Table 1). Cob yields were greater in 2006 (1.4 t ha^{-1}, ranging from 0.9 to 1.9 t ha^{-1}) compared to 2005 (1.2 t ha^{-1}, ranging from 0.6 to 1.8 t ha^{-1}) (Figure 1). Overall, the distribution of cob yields was similar in 2005 and 2006. Over the two years combined, the lowest yielding hybrid for cobs was WQS C3 Syn2 (0.8 t ha^{-1}), while the highest cob yielding hybrid was BS31(R)C0-246-1-01-01-01-01-B-B/TR7322 (1.8 t ha^{-1}). As might be expected, cob yield is significantly correlated with grain yield (r=0.66, Table 3, Figure 2). The WQS C3 Syn2 entry also had the lowest grain yield (5.4 t ha^{-1}), while the hybrid BS31(R)C0-246-1-01-01-01-01-B-B/B116 had the highest grain yield, (10.0 t ha^{-1}). Cob yields for the six commercial hybrids averaged 1.25 t ha^{-1} and ranged from 1.0 to 1.6 t ha^{-1}.

The cob yields we have reported are lower than those reported in previous literature. Pordesimo et al. (2005) reported average cob dry matter yields of 2.4 t ha^{-1}, while Sawyer and Mallarnio (2007), reported cob yields of 1.36 t ha^{-1}. Possible reasons for these differences in yields include maturity at time of harvest, type of germplasm, and environment and growing season. Shinners and Binversie (2007) observed a decrease in cob dry matter yield with later harvest dates and increasing plant maturity. The decrease in yield was most likely caused by respiration and microbial degradation. Our results support these findings, as cob yields were higher in 2006 and were harvested earlier and at higher moisture content than in 2005. Lower cob yields reported in 2005 could possibly be due to later time of harvest and maturity, therefore, increasing senescence. Pordesimo et al. (2005) and Sawyer and Mallarnio (2007) evaluated commercial hybrids developed specifically for grain production. We evaluated several different genotypes, including noncommercial hybrids which may have resulted in lower average dry matter yields. However, when considering our commercial entries alone, average cob yields were still lower than those reported in the literature. These differences may also be accounted for by environment or year effects. For example, Shinners and Binversie (2007) reported a significant decrease in stover and grain dry matter yields during years of below average rainfall. Therefore, growing conditions of particular environments may account for some of the differences seen between our data and data reported in the literature. From our results, it seems the best predictor of genotypes with high cob yields may be genotypes with high grain yields.

In general, high yielding grain genotypes are expected to have high cob yields, so in the absence of data, the best way to select a genotype for high cob yield is to select a high grain yielding genotype. The estimation of cob yields from grain yields, however, requires the assumption that the proportion of the total ear weight that is grain is constant from genotype to genotype. Cob yield was calculated as a percentage of the total ear weight, total nongrain aboveground biomass, and total dry matter (TDM). On the average, cobs accounted for 14% of the total ear weight with a range from 10 to 16% (Figure 3 and Table 1). This range in cob to ear proportions is evidence that slight variation for cob proportions exists among hybrids. However, when comparing average cob to total ear percentages with data from the literature, there is some consistency. Tetio-Kagho and Gardner (1988) reported cobs to account for 10 to 12% of the total ear dry matter. Hicks et al. (1977) calculated shelling percentage (the ratio of grain to total ear dry matter) to be 85.7 and 85.5% for two commercial grain hybrids. This is consistent with the data reported in our study. If the average cob to total ear percentage is 14%, the average shelling percentage would be approximately 86%. Because shelling percentage is relatively consistent from genotype to genotype, average cob yields can be calculated by multiplying grain yields by the constant 0.1628.

Cobs accounted for nearly 17% of the nongrain above ground biomass, with a range of 14 to 24%. Cobs accounted for 8% of the total dry matter (TDM), ranging from 6 to 10%. These proportions were similar to proportions reported in previous studies. Pordesimo et al. (2007) reported similar findings for cob percentages of above ground TDM and nongrain above ground biomass of 8.2 and 15.2%, respectively. Shinners and Binversie (2007) reported that cobs accounted for 15% of nongrain above ground biomass. Sawyer and Mallarino (2007) reported that cobs accounted for 7.5% of the total above ground dry matter.

The proportions calculated in our research may be slightly higher than those reported in the literature, because our calculations were made on a dry matter basis.

The amount of genetic variation that exists for cob yield will be important in determining if cob yield can be improved through breeding. If there is little variation for cob yield or if the correlation between cob yield and grain yield can not be broken, there may be little potential to improve cob yield by breeding. If there is significant variation for cob yield, however, it may be possible to implement a breeding program to increase cob yields. Our results show that significant variation does exist among genotypes for cob yield. Therefore, it may be possible to select genotypes with higher cob yields. However, selecting for higher cob yielding genotypes based on cob yield data is neither practical nor feasible. Rather, it is most likely that higher cob yielding genotypes would be selected by selecting for higher grain yielding genotypes. The variation seen in cob yield may be a result of cob diameter, kernel depth, or kernel row number; however we do not have the data to support these possible explanations.

The amount of environmental variation for cob yield is also important in order to understand what can be expected when cobs are harvested. It may be useful to have an idea of the stability of genotypes across environments for cob yield, or the significance of the genotype by environment interaction. The genotype by environment interaction for cob yield over the four environments tested in this experiment was significant. When considering the six commercial hybrids, there is evidence of change in rank across all four environments (Figure 4a.). The top three commercial hybrids, however, remained constant across all four environments. Cob yield showed significant, positive correlations with stover, grain, and TDM (Table 3.) The same trend for the genotype by environment interaction is seen for grain yield (Figure 4b). For example, hybrids performed best for grain and cob yield in the 2006 Belmond environment. The Novartis hybrid was the highest yielding for both grain and cob in the 2006 Belmond environment. The best grain and cob yielding hybrids for the 2005 Ames and 2006 Ankeny environments were Pioneer 34M93 and Novartis N48V8. Although hybrids did not always rank the same for cob and grain yield in each environment, the overall environmental performance showed the same trend. As previously mentioned the variation could be accounted for by cob diameter, kernel depth, or kernel row number.

Cob quality

The chemical composition of cobs is important, because it will determine the upper limit for ethanol production. Variation for cob composition will be important for breeding purposes if we want to improve the ethanol potential of cobs. There were significant differences among genotypes for NDF, ADF, and ADL. Genotype by environment interactions were also significant for NDF, ADF, and ADL. Therefore, there is variation among genotypes for cell wall composition that may be important for cellulosic ethanol production. The significant genotype x environment interactions indicate that environment and growing season play a role in determining cell wall composition.

We observed very little variation on average between years for chemical constituent proportions (Fig. 5). Proportions of hemicellulose, cellulose, lignin, and cell solubles were nearly constant between years. Averaged over years, the values for NDF, ADF, and ADL on a dry matter basis were 880, 469, and 44.5 g kg^{-1}, respectively (Table 1). Neutral detergent fiber and ADF values were slightly higher in 2005 than in 2006, which may reflect higher lignin content observed 2005 than in 2006 (Fig. 6). Kuehn et al. (1999) investigated the feed values of different plant fractions of grain, leafy, and blended hybrids. They reported cob

NDF values on a percent dry matter basis ranging from 80.5 to 85.7% (805 to 857 g kg^{-1}) and ADF values ranging from 38.7 to 44.7% (387 to 447 g kg^{-1}). They also reported cobs to be the least digestible fraction of the corn plant compared to leaves, stalks, and grain. Kuehn et al. (1999) estimates of chemical constituents were slightly lower than the averages reported in our study. Plant maturity and moisture content at harvest may be one explanation for our higher values of cell wall components. Variation in germplasm may also account for some differences. Our study included mostly experimental grain genotypes with a few silage genotypes, while the Kuehn et al. (1999) study included two commercial grain hybrids and a commercial silage hybrid. The greater variety of germplasm in our study may have accounted for some of the discrepancy of reported values. The difference in germplasm is the most likely explanation as we found little variation from year to year in chemical composition of cobs.

Theoretical ethanol potential and ethanol yield

Theoretical ethanol potential (TEP) is a measure of the ethanol yield potential of a ton of cobs assuming that all of the sugars can be converted to ethanol. Ethanol yield is the product of TEP and cob yield and gives ethanol yield on a per unit of land area basis. Differences among hybrids for TEP were found to be significant. Theoretical ethanol potential averaged 609 1 t^{-1} over the entire experiment. The hybrid with the lowest TEP was Novartis N48V8 (588 1 t^{-1}) and the hybrid with the highest TEP was B126/W601S (627 1 t^{-1}). Genotype by environment effects were also significant for TEP. A larger range in TEP was observed in 2005 than in 2006, most likely due to a larger range in 2005 cellulose and hemicellulose values than in 2006 (Figure 7). Average TEP was slightly greater in 2006 than in 2005, 607 1 $t^{-1}$and 605 1 t^{-1}, respectively. The distribution of TEP data is similar between

2005 and 2006, however, 2005 accounted for a greater number of low TEP values than 2006. In 2005, plots were harvested at a later harvest date and lower moisture content than in 2006. Cobs harvested in 2005 also had higher ADL and lignin values than cobs in 2006, which could be a result of the later harvest date. Later harvest date may have resulted in greater cob lignification, which in turn could lead to lower structural carbohydrate content and TEP. Theoretical ethanol potential showed a positive and significant correlation with NDF (r = 0.59) and a significant, negative correlation with cell solubles (r = -0.69) (Table 3). Hames et al. (2003) reported stover samples to have TEP ranging from 438 to 496 l t^{-1}. Because of the higher concentration of cellulose and hemicellulose, cobs have a higher TEP than stover.

Average TEY for the experiment was 789 l ha^{-1} (Table 1). The genotype with the lowest TEY was WQS C3 Syn2 (469 l ha^{-1}), while the genotype with the highest TEY was BS31(R)C0-246-1-01-01-01-01-B-B/TR7322 (1103 l ha^{-1}). The genotypes with the highest and lowest TEY values were the same genotypes that had the highest and lowest cob yields, respectively. Theoretical ethanol yield had a significant correlation of r = 0.99 with cob yield, but the correlation of TEY with TEP was not significant (r =0.11). From the data it can be concluded that cob yields have the most influence on theoretical ethanol yield as opposed to differences in cob quality.

Conclusions

On average, cobs yielded 1.3 t ha^{-1} and accounted for 7.6% of total plant above ground dry matter (TDM). Significant differences for cob yields and cob proportions were found among genotypes. However, not a lot of variation was present for cob proportions and cob yields were positively correlated to grain yields, indicating that cob yields can be estimated from grain yields. Significant differences were also found among genotypes for compositional characteristics. This would suggest that it may be possible to select for traits such as higher yielding cobs and higher cellulose and hemicellulose content in the cob fraction of the corn plant. Genotype by environment interactions were present for yield and compositional traits. Therefore, cob characteristics for specific genotypes may be affected by different environments and growing conditions. Significant differences were also found among genotypes for TEP. Theoretical ethanol yields, which were based on TEP, were most greatly influenced by cob yields. Based on these data, selection for higher cob yields as opposed to compositional traits would have the greatest effect on cob ethanol yields, and the most practical approach to selecting for higher cob yields is by selecting genotypes with higher grain yields.

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Table 1. Table of means for cob quality and agronomic traits.

	Cob composition†							Yield‡		Cob ratios§			Cob ethanol¶	
Hybrid	NDF	ADF	ADL	Sol	Hemi	Cell	Lig	Cob	Grain	Cob: ear	Cob: TDM	Cob: nongrain biomass	TEP	TEY
	----------------------------g kg^{-1}------------------------							------t ha^{-1}------		--------------Percent------------			l t^{-1}	l ha^{-1}
F1 Experimental hybrids														
W64A X A619	893	495	44.6	108	394	449	42.1	1.3	6.4	16.1	8.8	19.1	614	783
WQS C3 X HC33	873	457	45.5	127	414	417	42.9	1.3	8.1	13.2	7.5	17.0	606	776
WQS C3 X LH198	874	459	48.7	124	416	411	45.9	1.3	8.0	13.4	7.3	15.1	602	782
WQS C3 X LH332	882	469	49.2	117	414	421	46.3	1.3	7.7	14.0	8.1	19.1	608	792
WQS C3 X TR7245	884	470	45.8	112	415	422	43.1	1.3	8.7	12.3	6.9	15.3	610	771
TR7245/W601S	889	471	54.4	109	417	417	51.2	1.3	8.5	12.7	7.2	15.6	608	778
SGI912/W601S	883	465	55.8	119	414	408	52.6	1.3	8.5	13.2	7.7	17.4	599	801
B126/W601S	892	486	37.9	108	414	447	35.6	1.1	6.7	14.0	8.1	20.6	627	675
B129/W601S	894	484	50.6	107	413	434	47.8	1.5	7.9	15.4	9.2	20.9	617	927
TR7245/W602S	887	468	50.4	112	411	426	47.5	1.1	7.9	11.7	6.8	15.9	609	672
SGI912/W602S	873	465	54.3	129	407	408	51.3	1.2	8.1	12.8	7.0	17.1	594	703
B126/W602S	874	477	38.3	128	404	433	36.0	1.0	6.8	11.9	6.8	15.6	610	586
B129/W602S	880	471	46.2	123	407	423	43.5	1.3	7.9	13.3	7.6	17.4	605	756
TR7245/W603S	889	470	42.0	107	423	424	39.4	1.3	8.8	12.6	7.1	15.4	617	809
SGI912/W603S	883	464	41.5	117	419	414	39.0	1.3	7.8	13.6	7.3	15.7	607	778
B126/W603S	874	469	28.1	122	420	426	26.2	1.2	6.8	14.5	8.2	19.2	617	726
B129/W603S	882	469	34.3	115	421	426	32.1	1.4	7.9	14.4	8.5	19.5	617	839
TR7245/W604S	888	472	46.0	110	410	428	43.3	1.5	9.0	13.5	7.7	17.1	611	885
SGI912/W604S	884	468	46.3	120	405	421	43.6	1.3	7.7	13.5	7.3	15.0	602	763
B129/W604S	887	467	39.9	116	418	427	37.6	1.2	6.5	15.3	9.0	21.6	616	735
B129/TR7322	881	485	47.1	116	403	435	44.7	1.7	8.7	15.6	8.7	18.3	610	1034
B73/Mo17	887	476	44.6	113	410	436	42.1	1.4	8.9	13.1	7.0	15.0	617	846
TR7245/BS32(R)C0-249-1-02-01-01-01-B-B	881	461	51.3	117	426	412	48.3	1.3	8.8	12.5	6.9	15.1	610	796
BS31(R)C0-246-1-01-01-01-01-B-B/B116	880	465	52.3	122	411	414	49.3	1.7	10.0	14.0	8.0	17.5	601	1009
BS31(R)C0-246-1-01-01-01-01-B-B/TR7322	899	488	61.1	97	406	432	58.0	1.8	9.3	15.8	9.3	20.9	610	1103
Populations and population crosses														
WQS C3 Syn2	859	456	29.2	137	415	415	27.1	0.8	5.4	12.2	6.7	14.8	605	469
BS28(R)C4	872	468	50.1	130	420	396	47.1	1.1	5.8	14.9	7.8	14.7	595	663
BS29(R)C4	859	455	40.9	142	419	402	38.4	1.2	6.0	15.4	7.6	14.1	598	695

Table 1. (continued)

	Cob composition†							Yield‡		Cob ratios§			Cob ethanol¶	
Hybrid	NDF	ADF	ADL	Sol	Hemi	Cell	Lig	Cob	Grain	Cob: ear	Cob: TDM	Cob:nongrain biomass	TEP	TEY
	g kg^{-1}							t ha^{-1}		Percent			l t^{-1}	l ha^{-1}
Populations and population crosses														
BSSS(R)C15/BS13(S)C10	895	478	47.6	105	412	436	44.8	1.5	8.4	14.8	8.3	17.7	617	947
BSSS(R)C15/BSCB1(R)C15	886	474	44.7	110	413	433	42.1	1.3	7.3	14.7	7.7	15.7	616	807
Nokomis Gold/BS21(R)C7	884	475	38.8	113	413	433	36.5	1.1	6.6	14.0	8.2	17.9	617	692
TEPR-EC6/BS33(S)C5	873	459	37.1	127	414	423	34.8	1.1	7.1	12.5	7.3	17.3	610	653
BS33(S)C5/BS22(R)C7	859	448	32.1	142	414	416	29.9	1.0	6.4	12.6	7.1	16.0	605	584
Inbred x population crosses														
BS13(S)C10/B116	882	471	50.3	119	409	423	47.6	1.6	8.8	15.0	7.9	16.6	606	980
BSSS(R)C16/B116	887	469	46.1	113	410	426	43.3	1.3	8.4	12.8	6.9	15.4	610	778
BS32(R)C2/B129	883	479	46.4	112	416	431	43.9	1.6	8.1	15.7	8.1	16.1	617	978
BS32(R)C2/B126	887	481	47.3	109	416	434	44.8	1.3	7.2	14.4	7.2	14.0	619	799
BS31(R)C2/B114	881	472	45.9	116	410	427	43.3	1.4	7.7	15.3	8.1	16.3	610	873
BS31(R)C2/B116	883	467	49.6	118	413	419	46.7	1.5	8.8	14.0	7.1	13.8	607	904
Commercial hybrids														
LH244/LH295	865	452	42.5	132	415	416	40.0	1.1	8.5	10.9	6.1	13.5	605	637
Renk 232	890	477	48.8	109	416	430	46.2	1.4	6.9	16.1	10.2	24.7	616	844
N48V8 (leafy)	858	454	46.3	144	397	410	43.5	1.6	8.9	14.4	7.7	15.6	588	915
Pioneer 34M93	887	491	42.6	112	409	433	40.1	1.4	9.4	12.7	7.4	17.3	614	885
Mycogen F697	859	448	23.4	142	407	434	21.9	1.1	7.9	11.6	6.3	14.5	612	651
DKC51-43	867	457	35.0	132	411	424	33.0	1.0	8.8	9.9	5.9	14.5	608	605
Experiment mean	880	469	44.5	119	412	424	41.9	1.3	7.9	13.7	7.6	16.8	609	789
Minimum mean	858	448	23.4	97	394	396	21.9	0.8	5.4	9.9	5.9	13.5	588	469
Maximum mean	899	495	61.1	144	426	449	58.0	1.8	10.0	16.1	10.2	24.7	627	1103
LSD(0.05)	10	9	3.8	11	6	8	3.7	0.2	1.1	1.0	0.7	3.4	6	102
Effective error MS	72.9	62.1	11.1	92.9	23.3	49.3	10.3	0.02	0.92	0.68	0.38	8.63	30.8	7776.1

† NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; Sol, cell solubles; Hemi, hemicellulose; Cell, cellulose; Lig, lignin

‡Grain yield adjusted to a dry matter basis

§Percent of cob material accounted for in the ear, TDM, and nongrain biomass fractions

¶TEP, theoretical ethanol potential; TEY, theoretical ethanol yield

Table 2. NIRS calibration equation statistics

Trait†	Mean	SD‡	N§	R^2	SEC¶	SECV#
NDF	87.2	1.7	53	0.97	0.27	0.74
ADF	46.3	2.0	53	0.86	0.75	1.01
ADL	3.9	1.4	56	0.88	0.47	0.60
Cellulose	42.4	1.6	55	0.97	0.26	0.62
Hemicellulose	40.7	1.2	56	0.85	0.45	0.70
Lignin	3.6	1.3	56	0.88	0.45	0.57
Solubles	12.8	1.7	56	0.97	0.28	0.81

† NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.
‡SD = standard deviation.
§N = final number of data points used in NIRS calibration equation.
¶SEC = standard error of calibration.
#SECV = standard error of cross validation.

Table 3. Table of Pearson correlation coefficients for quality and agronomic cob characteristics

	NDF	ADF	ADL	SOL	CELL	LIG	HEMI	TEP	TEY	COB†	STOVER‡	GRAIN§	TDM¶
NDF	1.00												
ADF	0.81***	1.00											
ADL	0.52***	0.356*	1.00										
SOL	-0.98***	-0.81***	-0.48***	1.00									
CELL	0.56***	0.695***	-0.19	-0.58***	1.00								
LIG	0.53***	0.362*	1.00	-0.48***	-0.18	1.00							
HEMI	0.01	-0.27	-0.12	-0.08	-0.36*	-0.12	1.00						
TEP	0.59***	0.56***	-0.27	-0.65***	0.83***	-0.27	0.23	1.00					
TEY	0.56***	0.51***	0.61***	-0.56***	0.22	0.61***	-0.20	0.11	1.00				
COB	0.52***	0.47**	0.64***	-0.52***	0.16	0.65***	-0.21	0.04	0.99***	1.00			
STOVER	0.13	0.04	0.45**	-0.13	-0.13	0.45**	-0.07	-0.18	0.60***	0.62***	1.00		
GRAIN	0.28	0.09	0.47***	-0.28	0.02	0.47***	-0.18	-0.08	0.65***	0.66***	0.64***	1.00	
TDM	0.27	0.13	0.54***	-0.26	-0.04	0.55***	-0.15	-0.13	0.75***	0.76***	0.90***	0.99***	1.00

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† Cob dry matter yield

‡ Stover dry matter yield

§ Grain yield

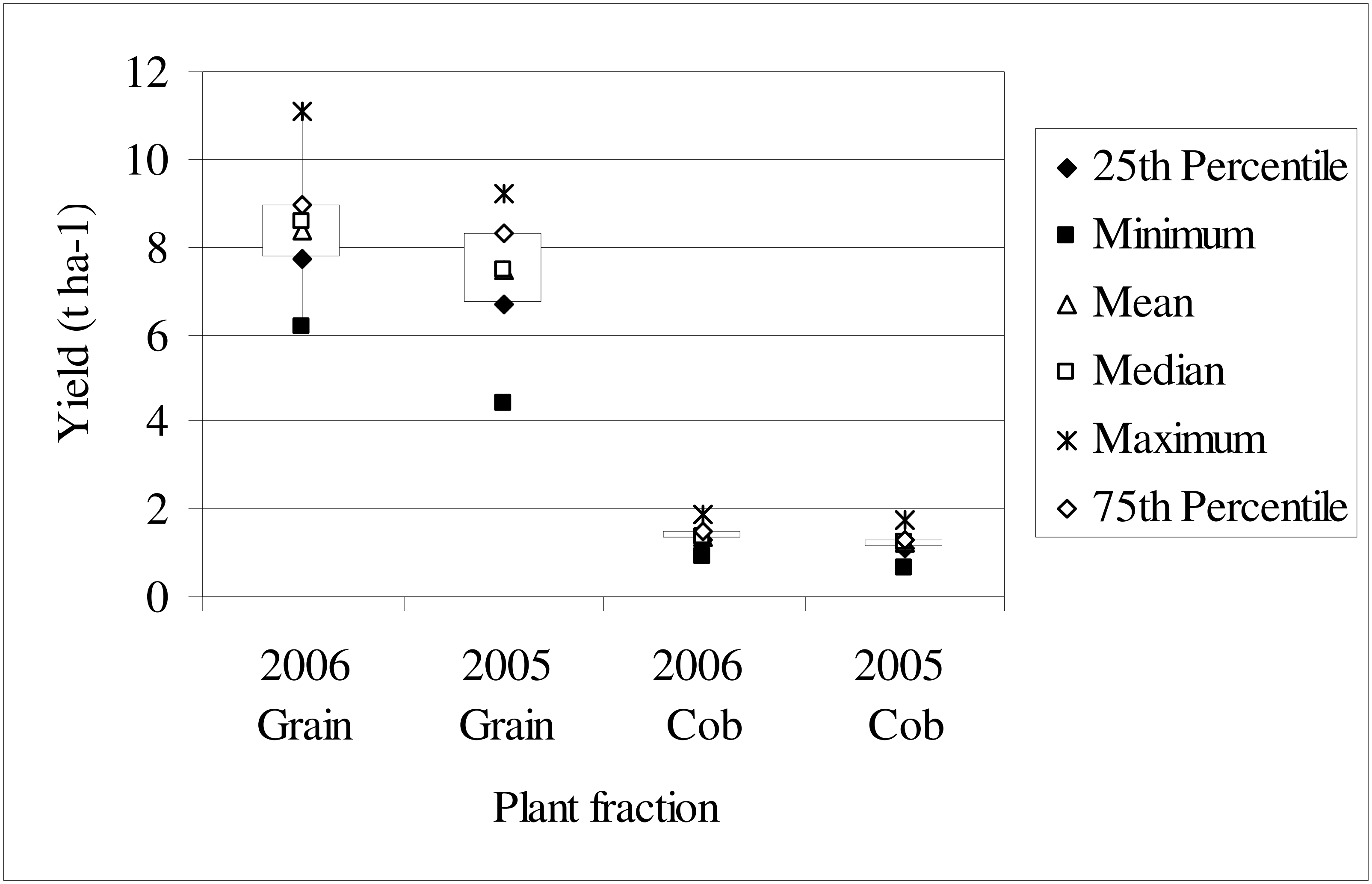


Figure 1. Distribution of grain yield and cob yield for 2006 versus 2005 growing seasons, respectively. Distribution of data is based on entry means for each year and plant fraction combination. Grain yield is adjusted to zero percent moisture.

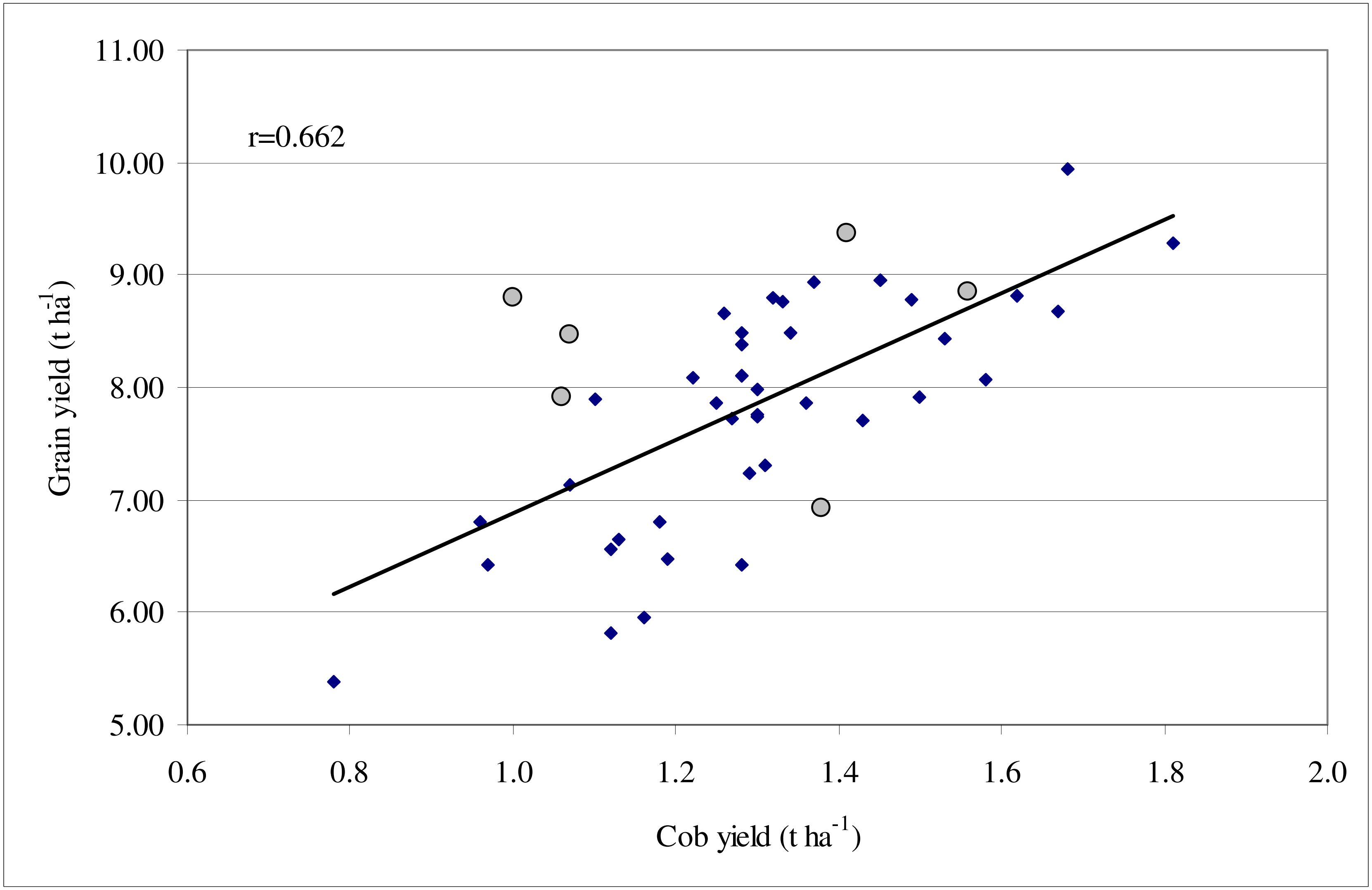


Figure 2. Scatter plot of grain yield versus cob yield. Grain yield is adjusted to zero percent moisture. Highlighted data points represent the six commercial hybrids in the study.

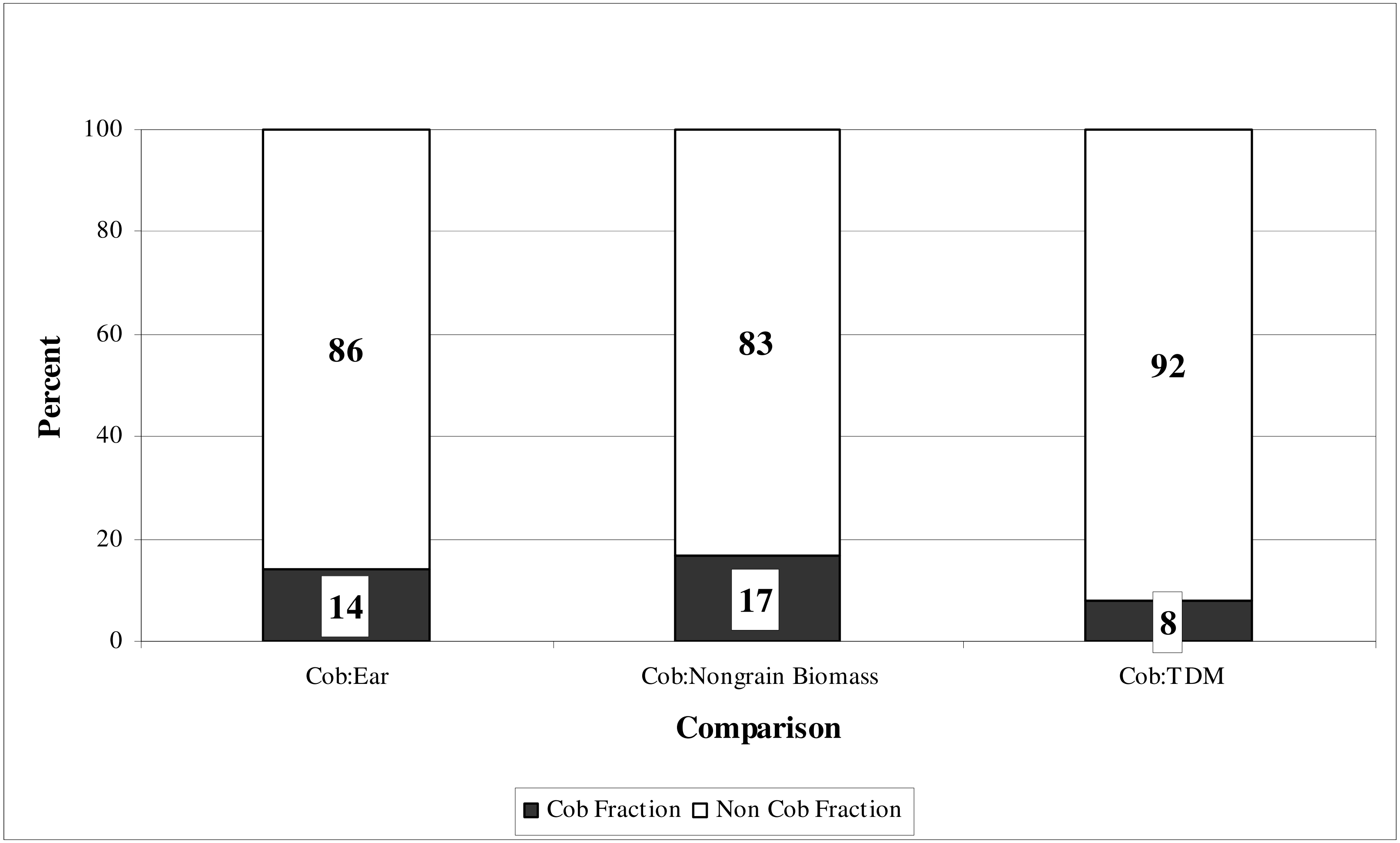
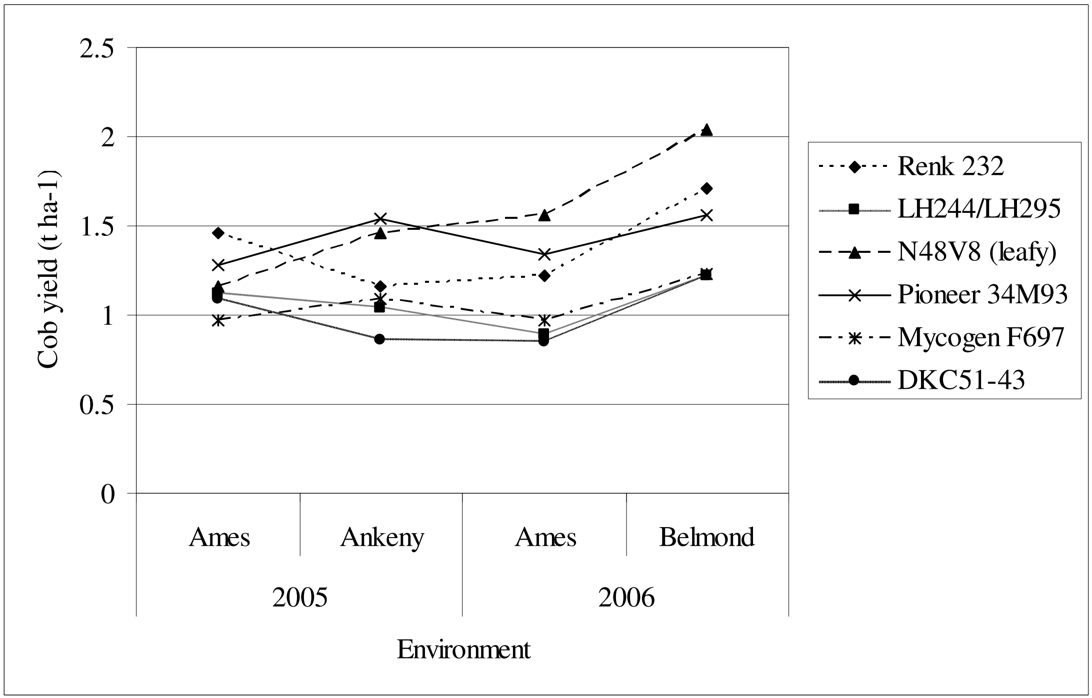


Figure 3. Proportion of cob dry matter accounted for in the ear, nongrain biomass, and total dry matter (TDM) plant fractions, respectively.

(a)



(b)

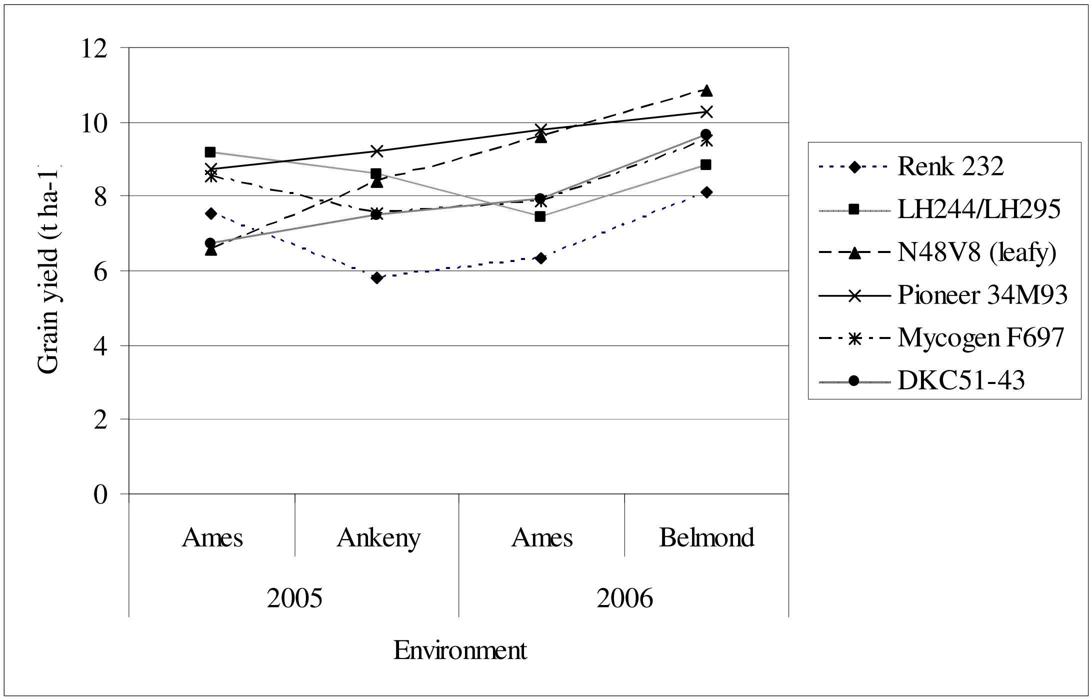


Figure 4. Genotype by environment interactions for six commercial hybrids evaluated at four environments for cob yield (a) and grain yield (b). Corresponding data points represent the entry mean yield at each environment. Grain yield is adjusted to zero percent moisture.

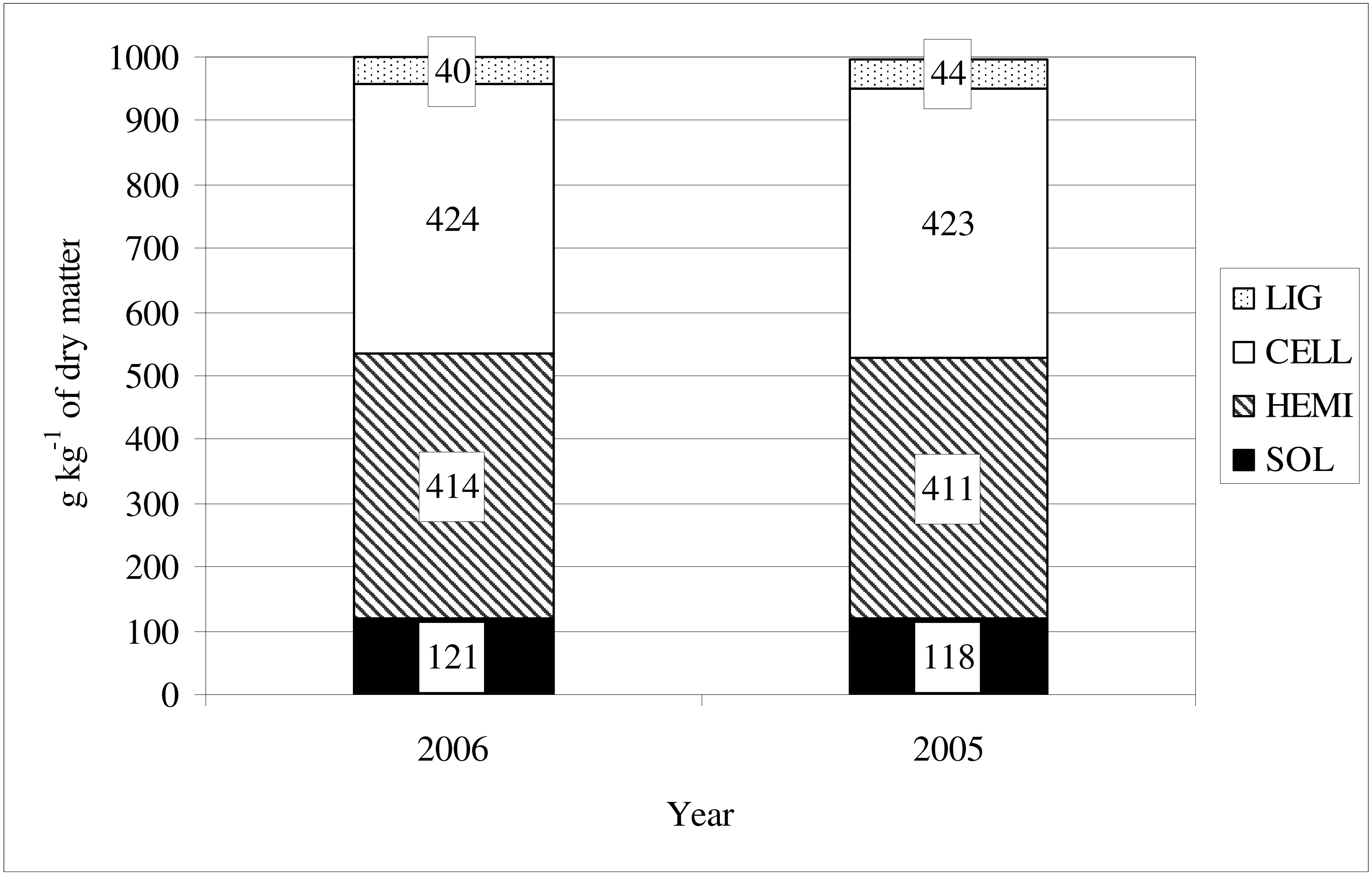
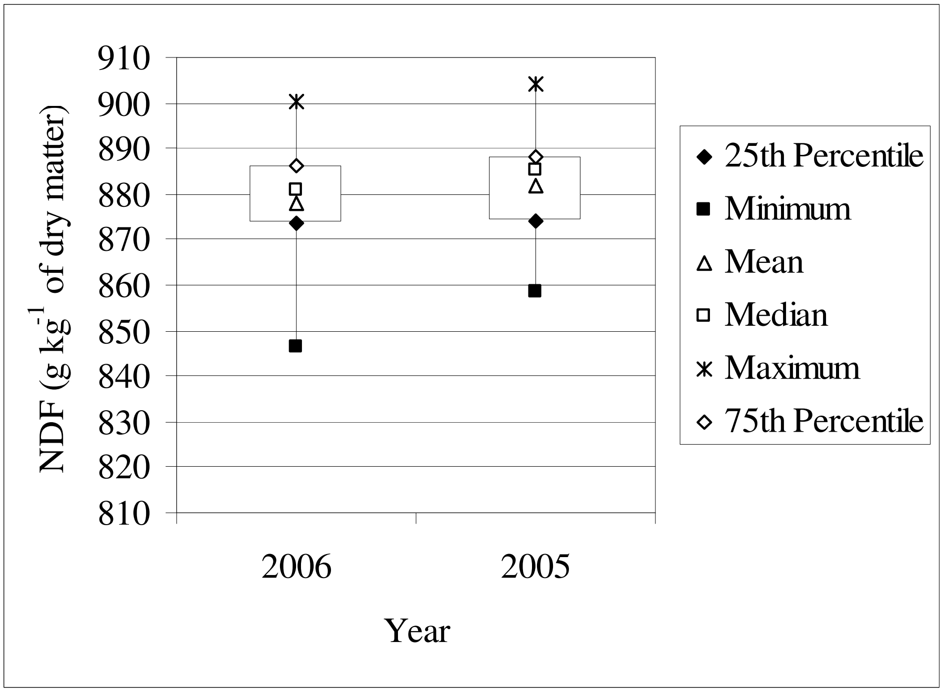
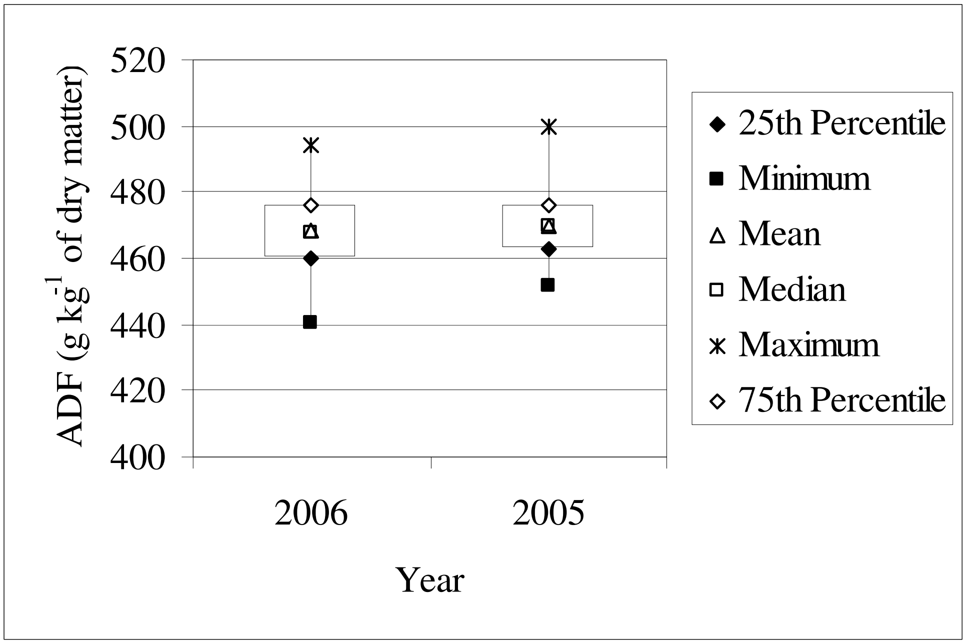


Figure 5. Comparison of average cob composition between the 2006 and 2005 growing seasons, respectively. Lig, lignin; Cell, cellulose; Hemi, hemicellulose; Sol, cell solubles.

(a)


910
900
890
880
870
860
850
840
830
820
810
NDF (g kg^{-1} of dry matter)
2006
2005
Year
◆ 25th Percentile
■ Minimum
△ Mean
□ Median
✳ Maximum
◇ 75th Percentile

(b)


520
500
480
460
440
420
400
ADF (g kg^{-1} of dry matter)
2006
2005
Year
◆ 25th Percentile
■ Minimum
△ Mean
□ Median
✳ Maximum
◇ 75th Percentile

(c)

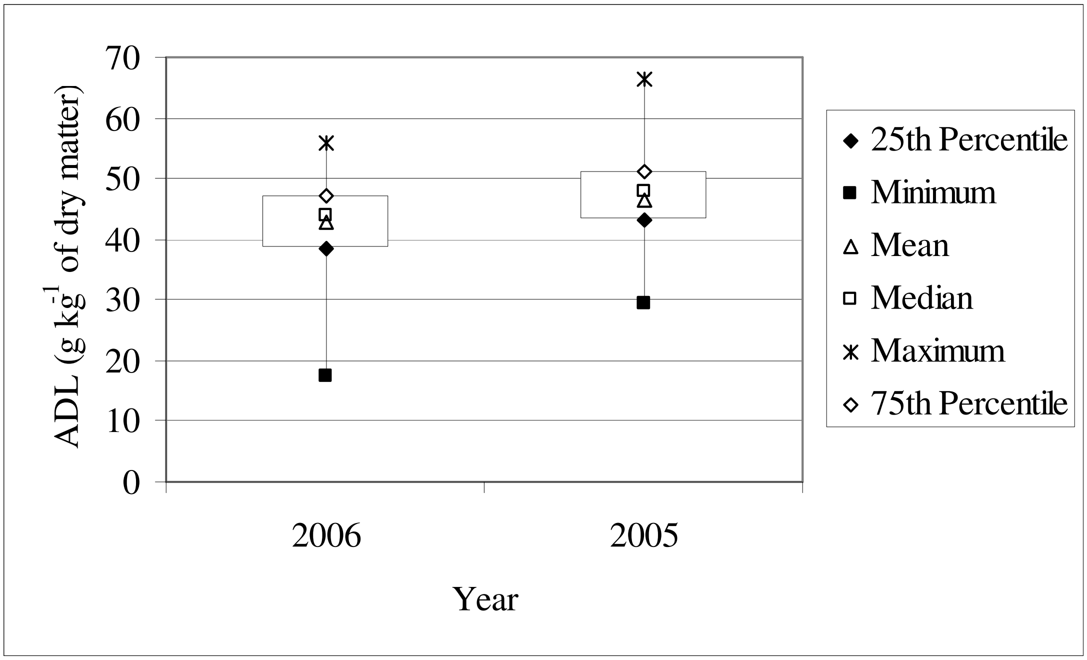


Figure 6. Distribution of cob chemical constituents for 2006 versus 2005 growing seasons, respectively. Distribution of data is based on entry means for each year and chemical trait combination. NDF, neutral detergent fiber (a); ADF, acid detergent fiber (b); ADL, acid detergent lignin (c).

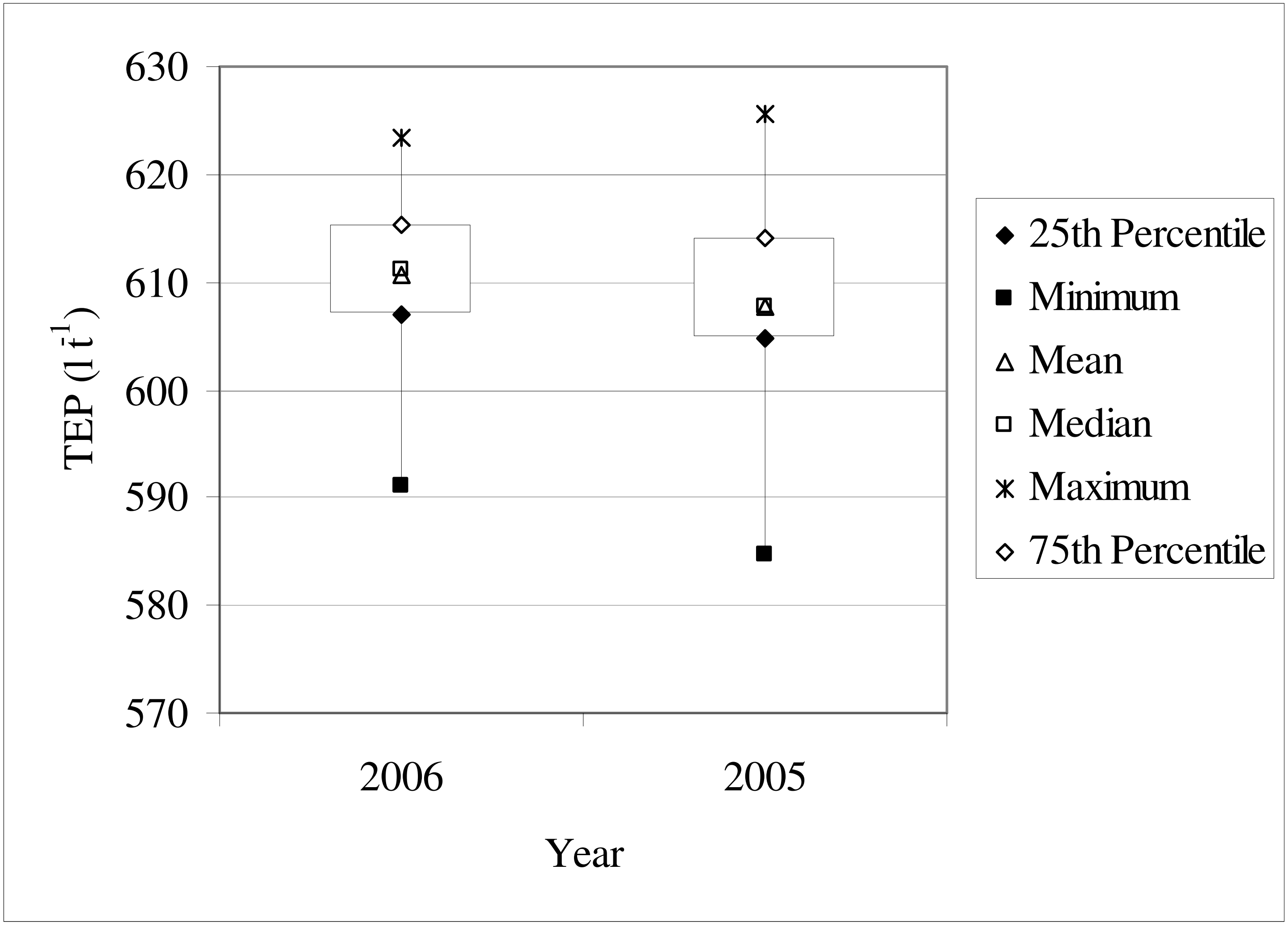


Figure 7. Distribution of cob TEP for 2006 versus 2005 growing seasons, respectively. Distribution of data is based on entry means for each year. TEP, theoretical ethanol potential.

CHAPTER 4. EVALUATION OF CORN STOVER CHARACTERISTICS BENEFICIAL TO THE PRODUCTION OF CELLULOSIC ETHANOL

A paper to be submitted for publication in *Agronomy Journal*

Krystal M. Kirkpatrick, Kendall R. Lamkey, and Kenneth J. Moore

Abstract

The low cost and abundance of corn (*Zea mays* L.) stover make it an attractive feedstock option for cellulosic ethanol production. However, little is known about the breeding potential of agronomic and compositional characteristics of corn stover and how these characteristics relate to potential stover ethanol yields. This study was conducted to evaluate yield and chemical composition of corn stover and make predictions of theoretical ethanol yields. Fifty different corn genotypes were evaluated for stover yield and analyzed for stover quality traits over two years and two central Iowa locations. Quality traits included cellulose (ADF-ADL), hemicellulose (NDF-ADF) and lignin (ADL). Theoretical ethanol potential ($l\ t^{-1}$) (TEP) was estimated based on calculations of pentose and hexose sugars. Stover dry matter yield averaged 7.3 and 5.9 $t\ ha^{-1}$ for 2006 and 2005 growing seasons, respectively. Significant differences among hybrids were found for both agronomic and compositional traits. Average TEP was 441 and 485 $l\ t^{-1}$ for 2006 and 2005. Theoretical ethanol yields (TEY), calculated by multiplying stover yield ($t\ ha^{-1}$) and TEP ($l\ t^{-1}$), averaged 3226 and 2824 $l\ ha^{-1}$ for 2006 and 2005. Theoretical ethanol yield was highly correlated with stover yield ($r = 0.98$ and $r = 0.98$ for 2006 and 2005). Stover yield showed significant correlations with grain yield for 2006 and 2005 ($r = 0.66$, $r = 0.50$) and with plant height in 2006 ($r = 0.78$). Harvest index averaged 0.49 and 0.51 for the respective growing seasons. The best ethanol producing genotypes may be the highest stover yielding genotypes, which in

most cases have the highest grain yields. Harvest index may be the best predictor of stover dry matter yields.

Introduction

With rising concerns of energy security and environmental stability, as well as the ever increasing demand for imported petroleum fuel in the United States, evaluating and implementing sources for of domestic, renewable fuel production has become a critical task (Dhugga, 2007). Biofuels have the potential to offer some resolution to this energy crisis. Currently, much of the biofuel production in the United States is in the form of ethanol. However, the ethanol industry uses starch derived from corn grain as a main feedstock for this process. Since corn grain is highly utilized in feed and food products, concern with the issue of food versus fuel has risen, and corn grain alone is not projected to meet transportation needs (Houghton et al., 2005). Therefore, production of ethanol from cellulosic sources, such as corn stalks, leaves, and cobs may be a valuable option to investigate for meeting the fuel demand.

The billion ton annual supply study, conducted by the USDA and DOE, determined that the United States was capable of sustainably supplying enough harvestable biomass to displace 30% of the country's petroleum usage by the year 2030 (Perlack et al., 2005). Corn stover accounted for a large proportion of the available biomass in this study, nearly 20%, deeming it a significant feedstock for this process. However, little breeding effort has been put forth to improve maize forage yields and quality in the United States (Frey et al., 2004). Emphasis has been put on breeding corn for grain yield and agronomic characteristics that enhance yield. Corn that is grown for whole plant harvest has been used in the form of silage, which includes the stover as well as the grain portions of the plant. Since grain represents approximately 50% of the total above ground dry matter and is highly digestible, increased grain yields have led to higher total dry matter yields and increased nutritional quality of silage. According to Allen et al. (2003), much variation exists for forage quality and forage yield among corn breeding populations in the United States. Since corn stover represents a low cost and abundant feedstock, and little breeding effort has been put forth to enhance yield and compositional characteristics that may exist, it is important to evaluate these traits in order to investigate the potential of corn stover as a feedstock for cellulosic ethanol production.

Two platforms have been proposed for the conversion of lignocellulosic material into energy: the biochemical, or sugar platform, and the thermochemical platform (Houghton et al., 2005). Thermochemically, biomass is heated to a gasesous or liquid form which can be then converted into other products. The sugar platform, which is the focus of this research, involves the use of enzymes and acids to hydrolyze biomass into component sugars which can then be fermented and used for energy. Based on the concepts of the sugar platform, it is hypothesized that higher sugar content, as well as more accessible monosaccharides, will yield higher ethanol production.

One method of estimating ethanol potential based on the sugar platform is by means of the ruminant animal digestion model. Like digestion by ruminant animals, conversion efficiency of cellulosic material to ethanol is maximized by high sugar content and accessible sugars (Van Soest, 1994). Evaluation of this model uses detergent fiber values estimated by the procedures of Goering and Van Soest (1970). For the purposes of this research, detergent fiber analyses can be used to estimate the amount of available cellulose and hemicellulose in corn stover. From these estimates, predictions of theoretical ethanol potential (TEP) can be made (Kirkpatrick et al., 2008a).

Another method that has been utilized for evaluating ethanol potential of corn stover feedstocks is by means of the stover calibration developed by the National Renewable Energy Lab (NREL) in Golden, CO (Hames et al., 2003). This equation uses Near Infrared Reflectance Spectroscopy (NIRS) to predict component hexose and pentose sugars in corn stover samples, including glucose, mannose, galactose, xylose, and arabinose. These component sugar values can then be used to calculate theoretical ethanol potential (TEP).

The objective of our study was to evaluate the suitability of corn stover as a lignocellulosic feedstock for the sugar platform and to assess the potential for modifying stover characteristics via plant breeding. A group of 50 hybrids, populations, and other germplasm types was evaluated to answer the following questions: What is the proportion of above ground total dry matter that stover accounts for, and how much variation exists among a diverse germplam array for stover yields? How much variation is there for corn stover quality traits among different hybrids and germplam sources? What is the theoretical ethanol yield potential of corn stover? What conclusions can we draw from this data regarding the development of selection criteria for breeders to develop maize genotypes with stover best suited for ethanol production? This study is a follow-up to a study involving the agronomic and quality characteristics of corn cobs (Kirkpatrick et al., 2008b).

Materials and Methods

Germplasm

Fifty maize genotypes were selected to represent a range of germplasm in order to evaluate the amount of variation that may exist for agronomic and quality traits. The genotypes selected included commercial hybrids, open-pedigreed F1 hybrids, populations, population x population hybrids, and inbred x population hybrids (Table 1, Table 2). Some of the genotypes were developed specifically for forage quality, while most of the genotypes were developed for grain production. Six commercial hybrids were included in the study: DeKalb DKC51-43, Pioneer34M93, Renk RK232, Mycogen F697 (*bm3*), Novartis N48V8 (*Lfy1*), and Holden's LH244/LH295, an open pedigreed F1 hybrid. For a complete description of the germplasm utilized in this study, see Kirkpatrick et al. (2008b).

Field evaluation

Genotypes were evaluated in 3 replications of a randomized complete block design at 2 locations in each of 2 years. In 2005, genotypes were evaluated at Ames and Ankeny, IA, and in 2006, genotypes were evaluated at Ames and Belmond, IA. Experimental plots consisted of two rows, 5.49 m long with 0.76 m between rows, including alley ways. Data collected on plots included silking date, ear height, plant height, root lodging, stalk lodging, grain yield, cob yield, stover yield, stover yield, total above ground dry matter yield, ear moisture at harvest, and stover moisture at harvest. See Kirkpatrick et al. (2008b) for a detailed description of these measurements.

Plots were harvested at physiological maturity. In 2005, the Ames and Ankeny locations were planted on May 5 and May 6, respectively and harvested on October 9. In 2006, plots were planted on May 3 and May 9 and harvested on September 15 and September 20 for Ames and Belmond locations, respectively. Grain and stover fractions were harvested separately by hand harvesting ears from each plot. In 2005, ears (grain and cob) were harvested from all plants in each plot. In 2006, ears were harvested from 20 plants per plot and the ear husk was harvested with the ear. A pruning shear was used to harvest ears in the husk by clipping the shank of the ear where it meets the plant. To ensure a random sample of ears, 10 ears were randomly harvested from each row of the two-row plot. Remaining ears were gleaned in the husk and discarded before stover harvest. In both years, ears from each plot were weighed at harvest (wet weight), dried at 37.8˚C for three days, and weighed again for a dry weight. After drying, ears were shelled to determine total plot grain weight and cob weight. In 2006, husks were separated at the shelling stage and weighed for a total plot husk weight. Subsamples of grain, cobs, and husks (2006 only) were kept from each plot for compositional analysis.

Stover was harvested immediately after ear harvest with a commercial silage chopper modified for agronomic research, courtesy of Mycogen Seeds, Belmond, IA. Stover was chopped at a height of approximately 6 cm. In 2005, harvested stover consisted of stalks, leaves, and husks. In 2006, harvested stover consisted of stalks and leaves only, because the husk was harvested with the ears. Total plot stover weight was obtained from the silage chopper. Subsamples of stover were collected from each plot, weighed, dried at 37.8˚C for four days, weighed again, and then kept for compositional analysis.

Lab evaluation

Stover samples were ground at the University of Wisconsin in a hammer mill to pass through a 1mm mesh screen. Ground samples were scanned with a NIRSystems 6500 near infrared reflectance spectrophotometer (NIRS) (FOSS NIRSystems Inc., Silverspring, MD). Standard NIRS procedures were used (Marten et al., 1989). A set of calibration samples were selected from 2005 and 2006 due to the difference in plant fractions between the two years. The CENTER program was used to compute standardized *H* statistics for each sample's spectra, and the SELECT program was used to select calibration samples for wet-lab analysis using a standardized *H* of 1.5 for all stover samples.

Calibration samples were then analyzed to determine detergent fiber composition. A modified procedure of Goering and Van Soest (1970) was used for sequential analysis of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL). Modifications included the use of ANKOM^{200} fiber analyzer (Ankom Technologies Corp., Fairport, NY). Estimations of NDF, ADF, and ADL (g kg^{-1}) were obtained for the calibration samples from wet chemistry methods. Values for cell solubles, hemicellulose, cellulose, and lignin were calculated from the detergent fiber data. The results of the calibration set were used to develop prediction equations, relating the NIRS spectral data to chemical composition values. Calibration equations were developed for NDF, ADF, ADL, cell solubles, hemicellulose, cellulose, and lignin. Selection of prediction equations for each constituent was based on high R^2 values and low standard errors of calibration (SEC) and cross validation (SECV) (Marten et al., 1989). The final equations were then used to make predictions for all stover samples for all chemical constituents.

Calibration statistics for 2005 and 2006 compositional stover traits are shown in Tables 3-4. Hemicellulose and cellulose values were used to calculate theoretical ethanol potential (TEP) for stover, (Kirkpatrick et al., 2008b):

H (hexose sugars) = (Cellulose + Hemicellulose*0.07) *172.82

P (pentose sugars) = (Hemicellulose *0.93) *176.87

TEP (gallons/dry ton) = H + P

Theoretical ethanol yields (TEY) (l ha^{-1}) were calculated by multiplying TEP (l t^{-1}) by stover yield (t ha^{-1}).

Data analysis

The data for the individual environments were analyzed as a randomized complete block design and then combined for each year using the MIXED procedure (SAS Institute, 2003). A combined analysis over the two years was not performed due to differences in stover fractions and calibration equations between the two years. A residual analysis was performed on the individual environment analyses to detect outliers, (Anscombe and Tukey, 1963). Entries were considered fixed effects in the analysis and all other effects were considered random. Entry means were used to calculate Pearson correlation coefficients between traits. An LSD (0.05) was calculated using the genotype by environment error mean square to compare hybrid means. All tests of significance were made at the $\alpha=0.05$ p level unless otherwise noted.

Results and Discussion

Stover yield

Ideal lignocellulosic feedstocks need to be high yielding and widely available in order to be cost effective. Corn stover is low cost, widely available, and potentially a large contributor of available lignocellulosic feedstocks. Determining corn stover yields and how much variation we can expect among corn genotypes and growing environments will enable predictions to be made about the economic feasibility of harvesting corn stover as a lignocellulosic feedstock for biofuel production.

There were significant differences among genotypes for stover yield and stover moisture for both growing seasons (Table 5, 6). In 2005, average ear moisture at harvest was 18.2%. Average stover yield for this year was 5.9 t ha^{-1}, ranging from 4.1 to 8.4 t ha^{-1} (Table 1, Fig.1). Grain yields, on a dry matter basis, averaged 7.4 t ha^{-1}, ranging from 4.4 to 9.2 t ha^{-1}. The lowest yielding stover genotype was also the lowest yielding grain genotype in 2005, WQS C3 Syn2. There was a positive and significant correlation between grain and stover yield (r = 0.66) (Table 7). Harvest occurred closer to physiological maturity in 2006 than in 2005, resulting in higher average ear moisture at harvest (26.7 versus 18.2%). Stover and grain yields for 2006 were also higher than in 2005. Stover yield averaged 7.3 t ha^{-1}, ranging from 4.1 to 10.6 t ha^{-1}, and grain yields averaged 8.4 t ha^{-1}, ranging from 6.2 to11.1 t ha^{-1} (Table 2, Fig. 1). The correlation between stover and grain yield in 2006 was lower than the correlation reported in 2005, but still significant (r = 0.50) (Table 8). Stover yield was also shown to have a positive and significant correlation with plant height in 2006 (r = 0.78) (plant height data was not collected in 2005). Stover moisture in 2005 averaged 35.2%, ranging from 22.7 to 47.4%, however no significant correlation between stover yield and stover moisture was found. Stover moisture in 2006 averaged 50.5%, ranging from 34.3 to 60.5%. Stover moisture showed a significant and positive correlation to stover yield (r = 0.74) in 2006.

In 2005, corn stover (stalk, leaf, and husk material) accounted for 38% of the total above ground dry matter (TDM), with genotypes ranging from 32 to 46%. Average harvest index (HI), the proportion of grain to total dry matter, was 49%, ranging from 42 to 55%. In 2006, corn stover (stalk and leaf material) accounted for 38% of the TDM, with genotypes ranging from 28 to 46%. Average harvest index was 51%, ranging from 42 to 60%, which is larger than the reported harvest indices in 2005.

Average stover yields are comparable to those reported in the literature. Hoskinson et al. (2006) reported stover yields at 5.1 and 6.7 t ha^{-1} for normal and low cut stover harvesting scenarios, respectively. The normal and low cut harvest scenarios were chopped at 40 cm and 10 cm heights. As our stover cutting height was approximately 6 cm, we would expect stover yields slightly higher than the low cut harvest scenario. The stover yields we reported in 2006 were similar to those reported by Hoskinson et al. (2006), while our 2005 stover yields were lower, reflecting year to year variation for yield. Sawyer and Mallarino (2007) reported a stover dry matter yield of 6.7 t ha^{-1} for combined weights of stalk, leaf, and sheath material, and 7.5 t ha^{-1} when husks were included. Pordesimo et al. (2004) reported total stover dry matter to average 11.2 t ha^{-1}, with 7.9 and 3.3 t ha^{-1} attributed to the stalk and leaf portions, respectively. This reported stover yield is much higher than the dry matter yield we have reported, as well as those reported by Hoskinson and Sawyer and Mallarino. Differences among stover yield reports are most likely due to variation among germplasm and different growing environments. For example most of the data reported in the literature is based on the use of commercial grain and silage hybrids, while most of the germplasm included in our study, consisted of publicly developed experimental hybrids. The Pordesimo et al. (2004) study was conducted in Tennessee under irrigated growing conditions, which may have led to substantially higher stover yields.

The observation that stover and grain yields were higher in 2006 than in 2005 may be explained by several factors. One factor may be time the time of harvest. In 2006, plots were harvested closer to physiological maturity than in 2005, which is reflected by the higher stover moisture in 2006. As a result, in 2005 plants were allowed to senesce and dry for a longer period of time. This may have resulted in more dry matter loss from dropped leaves and lodging in 2005 than in 2006. For example, Shinners and Binversie (2007) reported dry matter yield of stalks and leaves combined to be 8.53 t ha^{-1} when harvested at an early harvest date, but lower stover yields of 7.12 t ha^{-1} were reported when harvested at a later date within the same year. This difference was attributed to increased dry matter loss due to senescence and abscission during field dry down. Pordesimo et al. (2004) also reported decreases in dry matter yields of stalk and leaf fractions when harvest was delayed. Senescence and leaf droppage may account for much of the dry matter loss. Leaves contribute to a large portion of the nongrain biomass (approximately 20%), and are highly susceptible dry matter loss during field dry down (Sawyer and Mallarino, 2007). Growing season may also account for the differences between stover yield for 2005 and 2006. Conditions in 2006 may have simply been more favorable for corn production than in 2005. Shinners and Binversie (2007) show evidence of higher stover yields in years of above average precipitation for stalks and leaves.

Stover yield showed a positive and significant correlation with grain yield for both growing seasons (Table 7, Table 8). This is important from a breeding aspect, because breeders have already been selecting for high grain yield and will continue to do so. Selection for high grain yields results in high stover yields, which explains the relatively consistent 50% harvest index in corn. On average this correlation may hold true, however, there are individual exceptions. For example, high grain yielding genotypes may have a high harvest index, resulting in a large amount of grain being produced on small plant (Fig. 3). Stover yield also showed a significant and positive correlation with plant height. Therefore, genotypes that produce taller plants generally produce higher stover dry matter yields.

In the past, plant breeders have used a variety of selection methods to improve grain yields among corn germplasm sources, and a wide range of data is available for grain yields of modern corn hybrids. One possible way to estimate stover yields may be to use harvest index. Harvest index (HI) is the ratio of grain to total dry matter. On average, the harvest index of U.S. Corn Belt germplasm is 50% (Hallauer et al., 1988). Therefore, if grain yield averages 8.4 t ha^{-1}, then remaining dry matter yield, including stover, cobs, and husks together, could be estimated at 8.4 t ha^{-1}. Stover yield per se could further be estimated by using the proportion of stover to TDM ratio calculated in this study. If there is consistency in harvest index and stover to TDM ratios over time and genotypes, then grain yield may be used to estimate stover yield.

Kuehn et al., (1999) reported average HI for three commercial hybrids of 43.6%. Shinners and Binversie (2007) reported an average HI of 52% over three years. They also showed a range in HI over the three years of 41 to 62%, depending on the hybrid evaluated and the growing season. The mean and range they reported was similar to the mean and range we reported over the two years of the experiment. While HI was not always consistent among hybrids, average HI over time was near the expected 50%. Sawyer and Mallarino (2007) reported stalks, leaves, husks, and sheaths to account for 42.2% of the TDM, which was similar to the 38% we have reported. Kuehn et al. (1999), however, reported stalks, leaves, and husks to account for 47.1% of TDM. The higher percentage may be attributed to an earlier maturity at harvest, as they harvested at the half milkline stage while we harvested plots at grain physiological maturity. Like HI, stover to TDM proportions were not consistent among hybrids, but did show consistency when averaged over time. Therefore, grain yield is a good predictor of stover yields.

Stover quality

The chemical composition of stover can be utilized to predict the ethanol potential of corn stover and may also affect its conversion efficiency. Variation for corn stover composition is also important for improving the stover ethanol potential of corn. In 2005, significant differences were not observed among genotypes for NDF, but were present for lignin and ADF (Table 5). In 2006, significant differences among genotypes were observed for NDF, ADF, and lignin content (Table 6). Genotype by environment effects were not significant for any of the compositional traits evaluated at the two locations in 2005, but were significant for NDF and ADF in 2006. Neutral detergent fiber, ADF, and lignin content averaged 697, 402, and 27.1 g kg^{-1} in 2005, respectively (Table 1). In 2006, NDF, ADF, and lignin averaged 645, 376, and 23.4 g kg^{-1}, respectively (Table 2). The greatest range in chemical constituents was observed in lignin for both growing seasons (20.4 to 31.1 g kg^{-1} in 2005 and 13.7 to 26.7 g kg^{-1} in 2006).

Overall corn stover harvested in 2006 had lower percentages of cellulose, hemicellulose, and lignin, and higher values for cell solubles than 2005 stover samples (Figure 4). Differences among the chemical constituents between the two years are most likely attributed to differences in growing conditions. The separation of the husk fraction in 2006 may also account for some of the compositional differences among years. Differences could also be attributed to the later time of harvest in 2005 as compared to 2006. Lignin and NDF of stover increases and cell solubles decrease as time to harvest increases (Russell 1986). Pordesimo et al. (2004) reported increases in xylan and lignin content in corn stover shortly after grain physiological maturity. Kuehn et al. (1999) reported NDF and ADF values for stalks and leaves at 67 and 40%, respectively, which is comparable to the values we have reported for corn stover. Lee et al. (2007) reported average cellulose values of corn stover to be 37.5% (375 g kg^{-1}), ranging from 31.3 to 41.0%, and average hemicellulose values at 26.1%, ranging from 20.0 to 34.4%, which are also similar to the averages and ranges for cellulose and hemicellulose values we have reported (Table 1, Table 2).

The variability observed among genotypes and among environments for chemical composition is important as it may relate to the conversion efficiency of corn stover. Conversion efficiency refers to the ease of converting biomass to fermentable sugars. The least economical step in this process is pretreatment, which involves the use of acid or enzymes to interfere with the matrix of lignin, cellulose, and hemicellulose, making structural carbohydrates more accessible for hydrolysis (Houghton et al., 2005). To be economical, a cellulosic ethanol facility would need to have a conversion efficiency of near 100%. In other words, availability of sugars would need to be maximized so that energy inputs could be reduced. Based on our data, we could select for genotypes with higher cellulose and hemicellulose yields. However, if conversion efficiency of the ethanol facility was not 100%, these structural carbohydrates would not all be converted to fermentable sugars.

Theoretical ethanol potential and ethanol yield

Theoretical ethanol potential (TEP) is a measure of the ethanol yield potential of a ton of corn stover assuming that all of the sugars can be converted to ethanol. Ethanol yield is the product of TEP and stover yield and gives ethanol yield on a per unit of land area basis. Theoretical ethanol potential averaged 441 and 485 1 t^{-1}, ranging from 394 to 481 1 t^{-1} and 463 to 500 1 t^{-1} for the 2006 and 2005 growing seasons, respectively (Table 1, Table 2). The genotype with the lowest TEP was B129/W601S for 2006 and 2005. Genotypes with the highest TEP values were Renk 232 and TR7245/BS32(R)C0-249-1-02-01-01-01-B-B for 2006 and 2005, respectively. Theoretical ethanol potential showed positive correlations with NDF, ADF, and lignin for both growing seasons, which was expected (Table 7, Table 8). Significant negative correlations were seen between TEP and stover moisture for both years, suggesting that harvesting at a lower moisture (associated with later harvest date) may result in higher TEP values. This is also evident by observing differences between the two years of data, as 2005 had higher TEP values and was harvested at lower moisture content than 2006. Differences among hybrids for TEP were significant for 2006 but not for 2005 (Table 5, Table 6). Larger ranges in NDF and ADF values were also observed in 2006 as opposed to 2005, reflecting the larger range in TEP for 2006 (Fig. 5). Genotype by environment interactions were not found to be significant for TEP for either growing season. Stover harvested in 2005 had higher lignin values than stover in 2006, which may be related to the later harvest date in 2005. Increased senescence time may have resulted in greater stover lignification, which in turn could have lead to increrased structural carbohydrate content and TEP. Hames et al. (2003) reported corn stover samples to have TEP values ranging from 438 to 496 l t^{-1}, which is similar to what we have reported.

Theoretical ethanol yield averaged 3226 and 2824 1 ha^{-1}, ranging from 1966 to 4422 1 ha^{-1} and 1897 to 2824 1 ha^{-1} for 2006 and 2005 growing seasons, respectively (Table1, Table 2). The genotype with the highest TEY was BS31(R)C2/B116 for both years, while the lowest TEY yielding genotypes were WQS C3 Syn2 and Renk RK232 for 2005 and 2006, respectively. The genotypes with the highest and lowest TEY values were the same genotypes that had the highest and lowest stover yields, respectively. Theoretical ethanol yield had a positive and significant correlation with stover yield (r = 0.98, r = 0.98 for 2006 and 2005) (Table 8, Table 7). Theoretical ethanol yield showed a significant correlation with TEP in 2005 but no correlation in 2006. Therefore, stover dry matter yields, rather than chemical composition, have the most influence on theoretical ethanol yield, reflecting the greater variation observed for dry matter yield as opposed to chemical composition.

Conclusions

Corn stover yield has a significant and positive relationship with plant height and grain yield. Therefore, taller plants and higher grain yielding genotypes generally give rise to higher stover yields. This is important, as breeders have been selecting for high grain yields, and in turn, higher stover yields. Harvest indices are shown to vary across genotypes, however, when averaged among environments they are consistent. As a result, grain yield data can be effectively used to estimate stover yields. Significant variation was observed for compositional traits and theoretical ethanol potential of corn stover. Theoretical ethanol potentials, however, may not be attainable without 100% conversion efficiency during ethanol production. In addition, theoretical ethanol potential was not highly correlated with TEY. Theoretical ethanol yield was most greatly influenced by stover yield. Therefore, the best way to improve genotypes for cellulosic ethanol production may be by increasing stover yields, which can be estimated from grain yields.

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Table 1. Table of means for corn stover agronomic and quality traits for the 2005 growing season, averaged over two environments.

	Stover composition†						Yield								Stover ethanol	
Hybrid	NDF	ADF	SOL	HEM	CEL	LIG	Stover yield	Grain yield‡	Stover moist	Ear moist	Stover: TDM§	HI¶	SILK#	TEP††	TEY‡‡
	g kg^{-1}						t ha^{-1}		Percent				DOP	l t^{-1}	l ha^{-1}
F1 experimental hybrids															
W64A X A619	712	415	288	302	375	29.9	4.7	6.1	36.5	14.6	35.6	0.47	69	493	2326
WQS C3 X HC33	710	409	290	306	371	29.0	5.1	7.3	38.9	17.6	35.1	0.51	73	492	2489
WQS C3 X LH198	691	391	309	301	357	26.2	6.3	7.7	39.4	17.5	38.6	0.49	72	479	3022
WQS C3 X LH332	711	409	289	306	370	27.8	4.9	7.1	33.0	16.4	33.9	0.53	72	492	2413
WQS C3 X TR7245	685	389	315	298	357	25.6	6.1	8.3	33.1	18.1	37.2	0.51	71	477	2866
TR7245/W601S	701	410	299	297	375	27.9	5.9	8.3	33.9	14.0	36.1	0.51	71	489	2869
SGI912/W601S	683	396	317	296	362	26.3	5.3	8.4	41.1	14.8	33.2	0.53	71	479	2528
B126/W601S	669	386	331	290	357	23.3	5.2	6.2	33.4	15.4	36.4	0.50	71	471	2418
B129/W601S	658	378	342	287	349	25.3	5.0	6.7	41.8	18.4	35.8	0.50	73	463	2260
TR7245/W602S	673	382	327	298	349	24.3	5.0	8.2	34.3	11.4	33.0	0.55	73	471	2382
SGI912/W602S	685	391	315	301	358	24.8	5.5	7.1	39.2	15.9	33.6	0.50	71	479	2637
B126/W602S	669	382	331	292	349	23.8	4.9	6.7	36.7	13.9	36.6	0.52	70	467	2242
B129/W602S	696	391	304	306	359	25.0	5.6	6.9	44.1	18.8	38.4	0.49	72	484	2724
TR7245/W603S	710	422	290	298	383	29.7	7.1	9.2	29.4	17.7	38.1	0.49	73	496	3516
SGI912/W603S	696	411	304	296	374	28.4	6.6	7.4	33.9	19.3	38.5	0.46	71	488	3172
B126/W603S	670	389	330	290	354	26.3	4.8	6.0	32.8	19.9	36.2	0.51	72	469	2183
B129/W603S	702	407	298	299	372	28.3	5.5	6.7	36.9	21.7	38.0	0.48	74	488	2640
TR7245/W604S	690	387	311	302	356	25.7	6.6	8.3	29.7	16.1	38.2	0.49	71	479	3169
SGI912/W604S	689	393	311	296	358	26.5	6.2	7.3	36.7	19.0	40.3	0.47	71	476	2920
B129/W604S	685	387	315	299	353	25.7	4.2	6.1	41.9	21.5	34.1	0.48	73	475	1987
B129/TR7322	701	402	299	300	368	27.8	6.5	8.5	47.4	23.1	37.2	0.49	73	486	3151
B73/Mo17	710	415	290	300	376	31.1	6.8	8.3	29.2	21.3	39.7	0.48	73	492	3463
TR7245/BS32(R)C0-249-1-02-01-01-01-B-B	721	422	279	299	389	29.6	7.1	8.9	33.8	24.3	38.1	0.49	72	500	3591
BS31(R)C0-246-1-01-01-01-01-B-B/B116	702	412	298	298	372	28.4	6.8	8.8	27.7	17.5	37.4	0.50	74	487	3346
BS31(R)C0-246-1-01-01-01-01-B-B/TR7322	708	405	292	302	368	28.6	5.7	9.2	39.0	17.1	32.1	0.52	73	487	2773
Populations and population crosses															
WQS C3 Syn2	684	387	316	299	354	25.2	4.1	4.4	35.1	14.2	42.9	0.45	72	476	1897
BS28(R)C4	711	404	289	302	374	27.2	6.1	5.4	24.0	16.6	46.0	0.42	72	496	3195
BS29(R)C4	664	380	336	293	346	25.2	6.0	5.4	39.2	24.1	45.8	0.42	75	465	2842
BSSS(R)C15/BS13(S)C10	706	404	294	304	369	26.8	6.6	7.7	37.9	18.1	39.6	0.47	71	490	3181
BSSS(R)C15/BSCB1(R)C15	717	414	283	304	378	28.2	5.7	6.8	33.2	21.6	41.0	0.46	72	496	2818

Table 1. (continued)

	Stover composition†						Yield							Stover ethanol	
Hybrid	NDF	ADF	SOL	HEM	CEL	LIG	Stover yield	Grain yield‡	Stover moist	Ear moist	Stover: TDM§	HI¶	SILK#	TEP††	TEY‡‡
	g kg^{-1}						t ha^{-1}		Percent				DOP	l t^{-1}	l ha^{-1}
Populations and population crosses															
Nokomis Gold/BS21(R)C7	720	421	280	300	382	29.5	5.0	6.2	28.8	17.9	37.3	0.50	68	497	2474
TEPR-EC6/BS33(S)C5	703	412	297	300	370	28.6	5.0	6.4	28.0	16.2	37.5	0.50	68	488	2395
BS33(S)C5/BS22(R)C7	714	418	286	303	374	29.0	4.4	6.3	28.0	18.0	35.8	0.52	68	493	2046
Inbred x population															
BS13(S)C10/B116	712	411	288	307	374	28.1	7.0	7.8	37.9	20.2	40.3	0.46	73	496	3465
BSSS(R)C16/B116	686	395	314	296	357	26.6	6.2	8.5	45.4	20.9	36.4	0.51	72	476	2952
BS32(R)C2/B129	704	409	296	300	375	28.2	6.8	8.2	40.4	26.2	39.1	0.48	75	492	3168
BS32(R)C2/B126	688	400	312	297	365	27.2	6.8	7.5	38.3	23.5	41.3	0.46	74	482	3303
BS31(R)C2/B114	702	403	298	302	368	26.6	6.7	7.8	34.7	18.3	39.9	0.45	71	487	3262
BS31(R)C2/B116	710	413	290	306	374	28.0	8.4	8.9	38.3	20.8	41.1	0.47	73	495	4138
Commercial hybrids															
LH244/LH295	696	405	304	297	371	27.5	6.2	8.9	36.3	14.6	36.8	0.52	70	487	3027
Renk 232	711	416	289	301	379	29.6	4.4	6.7	22.7	11.2	32.1	0.54	67	495	2209
N48V8 (leafy)	678	385	322	302	346	24.9	6.9	7.5	34.9	19.3	42.2	0.45	73	472	3226
Pioneer 34M93	712	408	288	308	376	27.2	5.8	8.7	31.1	17.6	34.8	0.52	71	498	2870
Mycogen F697	696	391	289	299	378	20.4	5.8	7.1	33.0	19.6	38.6	0.49	73	496	2470
DKC51-43	708	413	292	299	379	28.5	6.2	8.8	30.9	15.1	36.1	0.53	68	493	3061
Experiment mean	697	401	303	300	367	27.1	5.9	7.4	35.2	18.2	37.7	0.49	72	485	2824
Minimum mean	658	378	279	287	346	20.4	4.1	4.4	22.7	11.2	32.1	0.42	67	463	1897
Maximum mean	721	422	342	308	389	31.1	8.4	9.2	47.4	26.2	46.0	0.55	75	500	4138
LSD(0.05)	42	28	42	14	25	2.6	1.8	1.3	7.1	3.1	8.4	0.08	2	27	868
Effective error MS	667	287	658	73	234	2.5	1.3	0.6	19.0	3.7	26.4	0.00	1	267	282453

† NDF, neutral detergent fiber; ADF, acid detergent fiber; Sol, cell solubles; Hemi, hemicellulose; Cell, cellulose; Lig, lignin

‡Grain yield adjusted to a dry matter basis

§Percent of stover accounted for of total dry matter

¶ HI, harvest index

Silk, days after planting when 50% of plants showed visible silks

†† TEP, theoretical ethanol potential

‡‡ TEY, theoretical ethanol yield

Table 2. Table of means for corn stover agronomic and quality traits for the 2006 growing season, averaged over tow environments.

	Stover composition†						Yield									Stover ethanol	
Hybrid	NDF	ADF	SOL	HEM	CEL	LIG	Stover yield	Grain yield‡	Stover moist	Ear moist	Stover: TDM§	HI¶	SILK#	PH††	EH	TEP‡‡	TEY§§
	------------------------g kg^{-1}----------------------						-----t ha^{-1}-----		-------------Percent------------				DOP	-------cm--------		l t^{-1}	l ha^{-1}
F1 Experimental hybrids																	
W64A X A619	644	374	356	270	337	24.2	6.0	6.7	49.6	29.7	37.4	0.49	70	206	82	442	2632
WQS C3 X HC33	650	375	351	275	344	23.5	7.8	8.9	51.4	26.2	38.0	0.52	73	224	107	451	3486
WQS C3 X LH198	632	370	369	262	336	23.4	8.3	8.3	54.4	30.5	41.7	0.49	74	227	115	435	3626
WQS C3 X LH332	645	381	354	266	344	23.2	7.3	8.4	52.9	29.5	38.8	0.52	74	233	121	444	3260
WQS C3 X TR7245	664	386	337	274	354	23.8	7.8	9.1	54.7	18.2	37.7	0.52	74	206	121	457	3550
TR7245/W601S	634	371	366	258	336	21.8	7.9	8.7	52.3	25.0	40.1	0.52	72	218	119	433	3432
SGI912/W601S	631	373	368	248	338	20.9	7.4	8.6	52.8	24.4	37.9	0.52	71	222	122	417	3044
B129/W601S	571	329	433	238	304	21.0	7.1	9.1	58.0	25.3	33.8	0.54	73	220	121	394	2728
TR7245/W602S	623	362	377	257	328	20.9	6.7	7.7	47.0	20.0	38.3	0.53	71	216	112	426	2891
SGI912/W602S	636	374	364	256	338	21.7	7.6	8.9	50.3	24.5	37.7	0.53	72	224	117	425	3177
B126/W602S	632	364	368	263	332	21.0	5.7	7.0	45.9	22.0	36.6	0.54	71	187	86	433	2441
B129/W602S	626	373	375	255	333	23.7	6.1	8.9	52.4	24.2	31.9	0.56	73	214	113	428	2577
TR7245/W603S	689	409	312	279	370	26.3	7.9	8.6	48.9	25.6	38.3	0.52	74	223	115	472	3726
SGI912/W603S	679	405	320	275	367	25.6	7.4	8.2	51.1	29.4	38.4	0.52	73	225	106	468	3452
B126/W603S	661	394	338	264	353	21.8	5.9	7.5	48.2	28.4	36.0	0.53	71	188	83	449	2664
B129/W603S	680	408	320	270	366	26.7	5.7	8.9	48.1	29.2	30.7	0.58	73	215	107	463	2630
TR7245/W604S	658	383	344	263	350	22.2	7.4	9.6	48.7	23.8	35.1	0.55	74	236	121	437	3201
B129/TR7322	640	374	357	267	339	23.2	8.2	8.9	60.5	36.0	39.0	0.5	74	236	118	441	3624
B73/Mo17	680	400	317	274	368	26.5	8.6	9.5	48.6	27.8	38.7	0.5	75	218	123	460	3966
TR7245/BS32(R)C0-249-1-02-01-01-01-B-B	656	380	343	271	345	23.9	7.6	8.9	57.1	26.9	36.8	0.52	73	227	117	449	3384
BS31(R)C0-246-1-01-01-01-01-B-B/B116	655	389	346	256	351	25.7	9.0	11.1	53.0	25.3	36.2	0.53	74	256	132	433	3856
BS31(R)C0-246-1-01-01-01-01-B-B/TR7322	620	365	379	255	328	24.5	8.0	9.4	54.8	25.7	36.5	0.52	74	239	117	425	3438
Population and population crosses																	
WQS C3 Syn2	606	345	377	258	317	20.1	5.1	6.2	48.1	26.0	35.3	0.53	73	194	93	419	2116
BS28(R)C4	635	364	365	272	328	23.2	6.1	6.2	47.3	25.2	37.8	0.47	73	209	99	437	2648
BS29(R)C4	609	347	391	261	317	23.2	7.9	6.5	56.0	33.3	44.2	0.44	78	221	114	421	3350
BSSS(R)C15/BS13(S)C10	647	368	336	276	345	22.0	7.5	9.2	48.2	27.8	35.2	0.52	73	226	107	452	3366
BSSS(R)C15/BSCB1(R)C15	638	374	362	263	341	25.0	8.0	7.9	55.2	28.1	40.9	0.48	73	241	115	440	3510

Table 2. (continued)

	Stover composition†						Yield										Stover ethanol	
Hybrid	NDF	ADF	SOL	HEM	CEL	LIG	Stover yield	Grain yield‡	Stover moist	Ear moist	Stover: TDM§	HI¶	SILK#	PH††	EH	TEP‡‡	TEY§§
	------------------------g kg^{-1}----------------------						-----t ha^{-1}-----		-------------Percent-----------				DOP	-------cm--------		l t^{-1}	l ha^{-1}
Population and population crosses																	
Nokomis Gold/BS21(R)C7	644	377	356	262	338	23.1	5.1	6.9	41.0	23.1	33.5	0.54	70	214	90	437	2240
TEPR-EC6/BS33(S)C5	678	398	322	278	361	25.8	5.4	7.9	38.7	25.8	31.8	0.57	70	217	97	465	2493
BS33(S)C5/BS22(R)C7	673	393	327	281	358	24.4	5.8	6.6	39.9	27.2	37.7	0.51	70	199	93	465	2688
Inbred x population																	
BS13(S)C10/B116	627	367	375	259	335	24.7	9.1	9.8	54.6	25.2	38.9	0.48	75	244	116	432	3952
BSSS(R)C16/B116	638	375	361	260	342	23.9	8.4	8.3	53.3	28.7	41.0	0.48	73	248	117	438	3685
BS32(R)C2/B129	629	368	371	262	332	24.1	9.8	7.9	54.8	30.9	43.5	0.42	77	230	127	433	4272
BS32(R)C2/B126	634	372	366	259	333	23.7	9.1	7.0	54.0	28.2	45.6	0.42	77	216	112	435	3921
BS31(R)C2/B114	622	364	378	260	328	23.0	7.9	7.7	56.1	30.3	41.7	0.48	74	230	115	428	3425
BS31(R)C2/B116	605	353	376	253	319	23.7	10.6	8.6	54.8	31.4	46.1	0.45	75	249	125	416	4422
Commercial hybrids																	
LH244/LH295	662	387	338	269	356	25.2	7.0	8.1	49.5	28.9	38.5	0.53	72	218	90	455	3160
Renk 232	701	405	301	292	369	25.8	4.1	7.2	34.3	14.1	28.3	0.6	70	194	82	481	1966
N48V8 (leafy)	655	373	348	282	345	24.2	9.2	10.2	53.5	31.1	37.6	0.5	74	258	98	457	4215
Pioneer 34M93	658	385	342	275	353	23.7	7.5	10.0	51.9	29.1	34.6	0.55	72	231	106	457	3446
Mycogen F697	653	375	343	263	356	13.7	7.1	8.7	51.8	27.7	36.0	0.52	74	214	104	440	3248
DKC51-43	652	371	378	277	364	23.1	5.5	8.8	36.1	20.3	31.7	0.6	70	208	88	454	2580
Experiment Mean	645	376	355	266	343	23.4	7.3	8.4	50.5	26.7	37.5	0.51	73	222	109	441	3226
Minimum Mean	571	329	301	238	304	13.7	4.1	6.2	34.3	14.1	28.3	0.42	70	187	82	394	1966
Maximum Mean	701	409	433	292	370	26.7	10.6	11.1	60.5	36.0	46.1	0.6	78	258	132	481	4422
LSD(0.05)	37	26	41	18	22	2.1	1.2	1.4	7.2	7.1	5.0	0.05	1	21	8	28	551
Effective Error MS	521.4	244.1	639.8	115.4	176.2	1.6	0.5	0.7	19.3	18.7	9.4	0.0	0.7	161.4	24.3	287.3	113845

† NDF, neutral detergent fiber; ADF, acid detergent fiber; Sol, cell solubles; Hemi, hemicellulose; Cell, cellulose; Lig, lignin

‡Grain yield adjusted to a dry matter basis

§Percent of stover accounted for of total dry matter

¶ HI, harvest index

Silk, days after planting when 50% of plants showed visible silks

†† PH, plant height; EH, ear height

‡‡ TEP, theoretical ethanol potential

§§ TEY, theoretical ethanol yield

Table 3. NIR calibration statistics for 2005.

Trait†	Mean	SD‡	N§	R2	SEC¶	SECV#
NDF	66.4	6.1	28	0.97	0.98	1.76
ADF	37.0	4.8	30	0.98	0.69	1.00
Cellulose	34.0	4.3	30	0.99	0.51	0.89
Hemicellulose	28.4	2.7	30	0.90	0.83	1.23
Lignin	2.4	0.6	30	0.95	0.13	0.18
Solubles	33.6	6.1	28	0.97	0.98	1.76

† NDF, neutral detergent fiber; ADF, acid detergent fiber
‡SD = standard deviation.
§N = final number of data points used in NIRS calibration equation.
¶SEC = standard error of calibration.
#SECV = standard error of cross validation.

Table 4. NIR calibration statistics for 2006.

Trait†	Mean	SD‡	N§	R2	SEC¶	SECV#
NDF	62.6	5.3	29	1.00	0.33	0.52
ADF	36.3	3.8	29	0.99	0.32	0.59
Cellulose	32.4	3.2	28	1.00	0.23	0.56
Hemicellulose	26.0	2.2	30	0.99	0.18	0.49
Lignin	2.1	0.5	28	0.98	0.08	0.14
Solubles	37.6	5.3	28	0.99	0.41	0.65

† NDF, neutral detergent fiber; ADF, acid detergent fiber
‡SD = standard deviation.
§N = final number of data points used in NIRS calibration equation.
¶SEC = standard error of calibration.
#SECV = standard error of cross validation.

Table 5. Anova for stover quality and agronomic traits for 2005.

		Stover Yield		Grain Yield		NDF†		ADF		Lig		TEP‡		TEY§	
SV	DF	F	P>F	F	P>F	F	P>F	F	P>F	F	P>F	F	P>F	F	P>F
Env	1	76.02	<.0001	6.72	0.0129	27.38	<.0001	19.63	<.0001	6.45	0.0147	52.53	<.0001	58.86	<.0001
Rep	4	2.56	0.0408	9.9	<.0001	5.62	0.0003	2.2	0.0715	2.9	0.0234	5.97	0.0002	1.86	0.1208
Trt	44	2.08	0.0085	6.29	<.0001	1.18	0.2950	1.7	0.0413	5.13	<.0001	1.15	0.3210	2.49	0.0015
Trt x Env	44	1.07	0.3707	1.71	0.0089	0.76	0.8556	1.03	0.4284	0.95	0.5605	0.83	0.7629	0.91	0.6363

† NDF, neutral detergent fiber; ADF, acid detergent fiber; LIG, lignin

‡ TEP, theoretical ethanol potential

§ TEY, theoretical ethanol yield

Table 6. Anova for stover quality and agronomic traits for 2006.

		Stover Yield		Grain Yield		NDF†		ADF		Lig		TEP‡		TEY§	
SV	DF	F	P>F	F	P>F	F	P>F	F	P>F	F	P>F	F	P>F	F	P>F
Env	1	364.33	<.0001	93.65	<.0001	0.85	0.3626	11.45	0.0016	2.06	0.1583	0.31	0.5781	313.26	<.0001
Rep	4	0.82	0.5169	2.37	0.0539	8.82	<.0001	8.16	<.0001	5.12	0.0006	13.12	<.0001	1.32	0.2649
Trt	41	11.92	<.0001	5.46	<.0001	3.81	<.0001	3.61	<.0001	9.6	<.0001	3.5	<.0001	9.72	<.0001
Trt x Env	41	1.21	0.1970	1.16	0.2543	1.78	0.0054	1.79	0.0053	1.07	0.3721	1.36	0.0864	1.55	0.0276

† NDF, neutral detergent fiber; ADF, acid detergent fiber; LIG, lignin

‡ TEP, theoretical ethanol potential

§ TEY, theoretical ethanol yield

Table 7. Pearson correlation coefficients for agronomic and quality stover traits in 2005.

	NDF†	ADF	LIG	TEP‡	STOVER§	GRAIN¶	STVMST#	SILK††	TDM‡‡	TEY§§
NDF	1.00									
ADF	0.91***	1.00								
LIG	0.73***	0.86***	1.00							
TEP	0.96***	0.89***	0.62***	1.00						
STOVER	0.23	0.25	0.18	0.28	1.00					
GRAIN	0.27	0.31*	0.26	0.29	0.66***	1.00				
STVMST	-0.36*	-0.42**	-0.28	-0.39**	0.09	0.12	1.00			
SILK	-0.25	-0.30*	-0.21	-0.20	0.45**	0.15	0.46**	1.00		
TDM	0.27	0.30*	0.25	0.31*	0.88***	0.92***	0.15	0.32*	1.00	
TEY	0.34*	0.36*	0.31*	0.37*	0.98***	0.65***	0.02	0.39**	0.87***	1.00

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† NDF, neutral detergent fiber; ADF, acid detergent fiber; LIG, lignin

‡ TEP, theoretical ethanol potential

§ Stover yield

¶ Grain yield at zero percent moisture

#STVMST, stover moisture

†† SILK, silking date

‡‡ TDM, total dry matter

§§ TEY, theoretical ethanol yield

Table 8. Pearson correlation coefficients for agronomic and quality stover traits in 2006.

	NDF†	ADF	LIG	TEP‡	STOVER§	GRAIN¶	STVMST#	SILK††	TDM‡‡	TEY§§	PH¶¶
NDF	1.00										
ADF	0.95***	1.00									
LIG	0.44**	0.51***	1.00								
TEP	0.95***	0.86***	0.48**	1.00							
STOVER	-0.28	-0.21	0.12	-0.27	1.00						
GRAIN	0.12	0.17	0.14	0.06	0.50***	1.00					
STVMST	-0.52***	-0.41**	-0.11	-0.51***	0.74***	0.34*	1.00				
SILK	-0.35*	-0.31*	0.05	-0.31*	0.74***	0.21	0.70***	1.00			
TDM	-0.12	-0.05	0.20	-0.14	0.88***	0.83***	0.64***	0.60***	1.00		
TEY	-0.09	-0.04	0.19	-0.07	0.98***	0.53***	0.66***	0.71***	0.88***	1.00	
PH	-0.18	-0.11	0.24	-0.18	0.78***	0.65***	0.57***	0.50***	0.83***	0.76***	1.000

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† NDF, neutral detergent fiber; ADF, acid detergent fiber; LIG, lignin

‡ TEP, theoretical ethanol potential

§ Stover yield

¶ Grain yield at zero percent moisture

#STVMST, stover moisture

†† SILK, silking date

‡‡ TDM, total dry matter

§§ TEY, theoretical ethanol yield

¶¶ PH, plant height

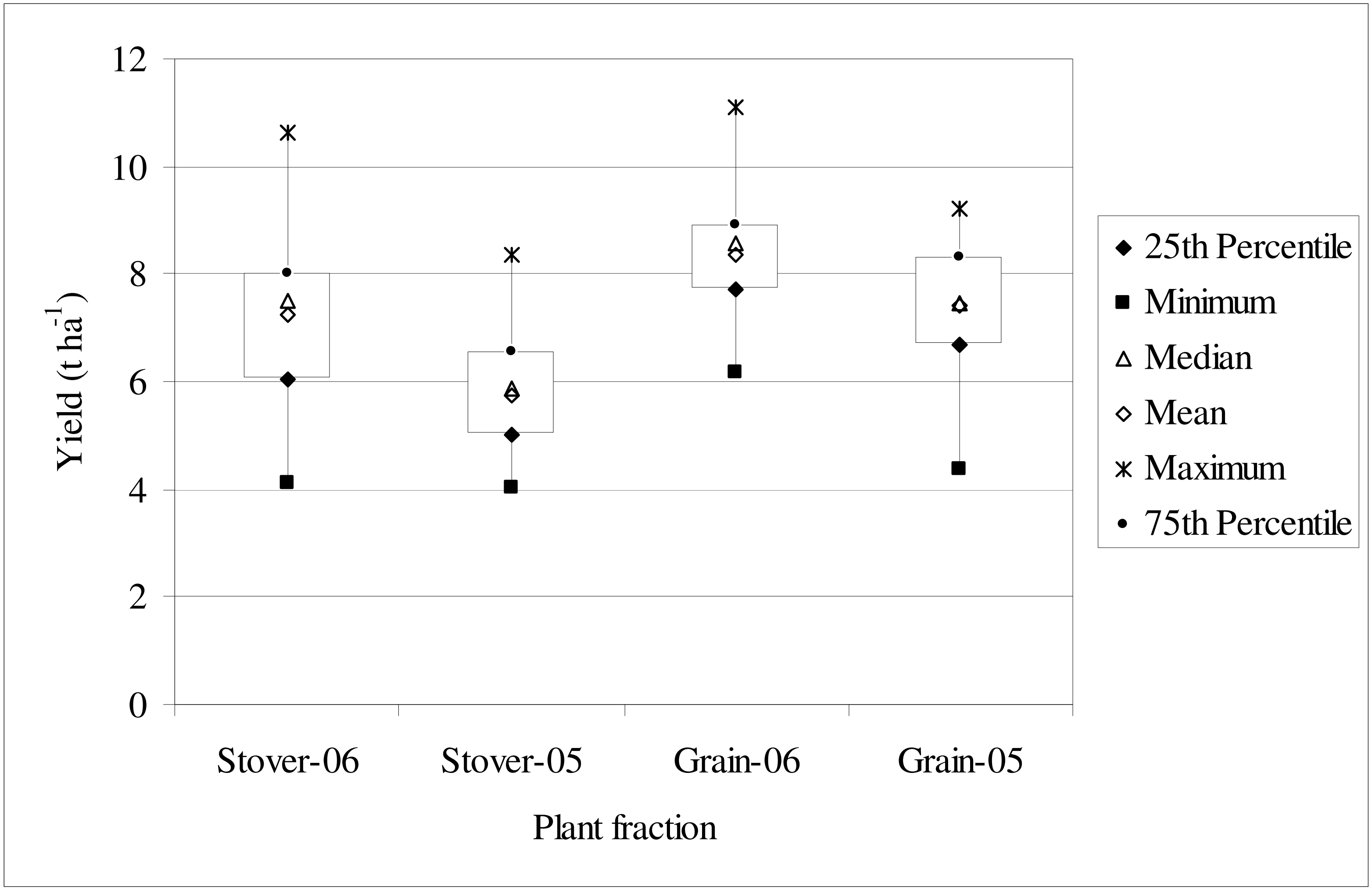

Fig. 1. Distribution of grain and corn stover dry matter yields for 2006 and 2005 growing seasons. Grain yields are adjusted to zero percent moisture.

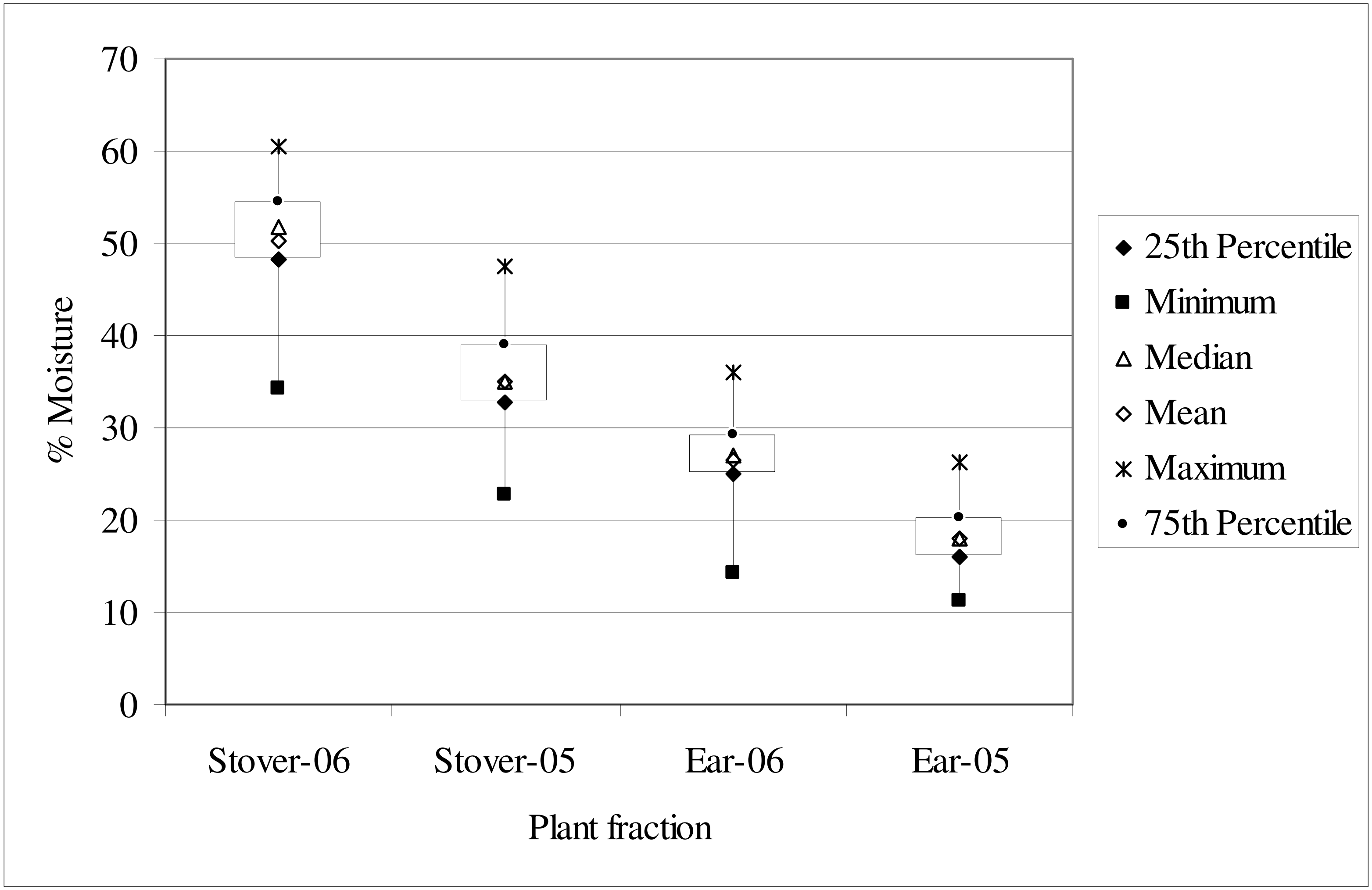

Fig. 2. Distribution of corn stover and ear moisture for the 2006 and 2005 growing seasons.

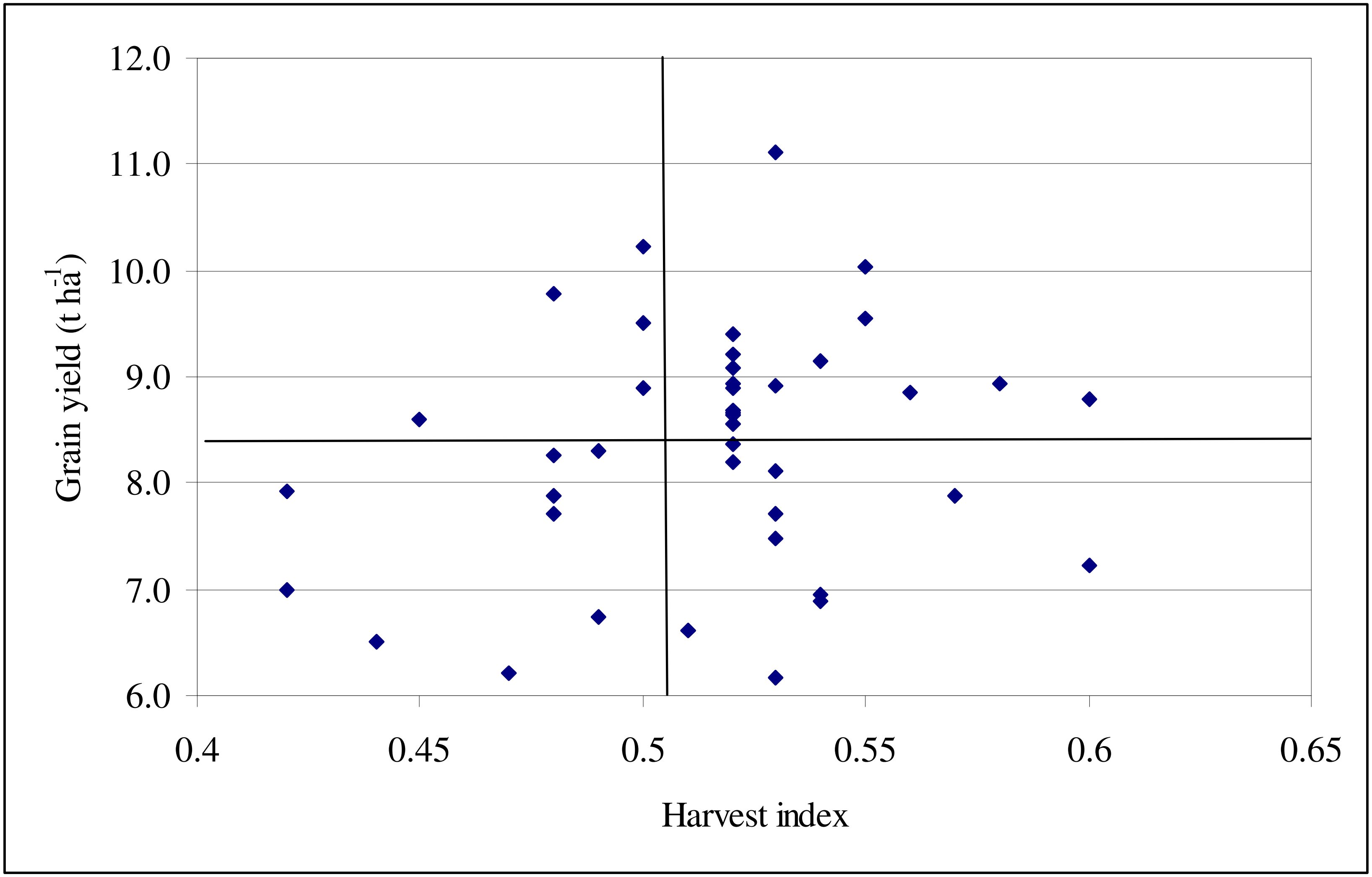

Fig. 3. Scatter plot of grain yield versus harvest index for the 2006 growing season. Grain yields are adjusted to zero percent moisture. Horizontal and vertical reference lines indicate the experiment for grain yield and harvest index, respectively.

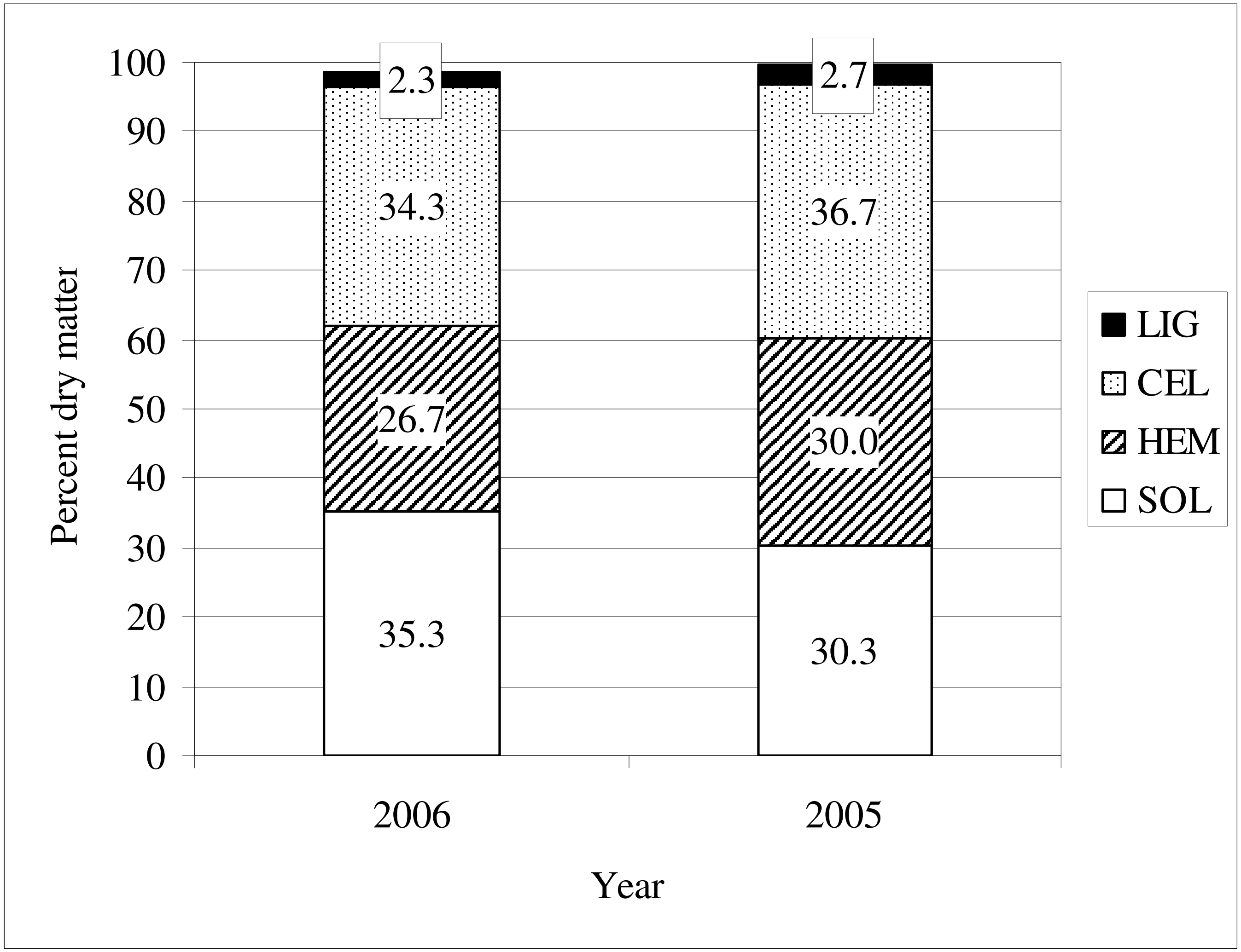

Fig. 4. Year by year comparison of corn stover chemical composition. LIG, lignin; CEL, cellulose; HEM, hemicellulose; SOL, cell solubles.

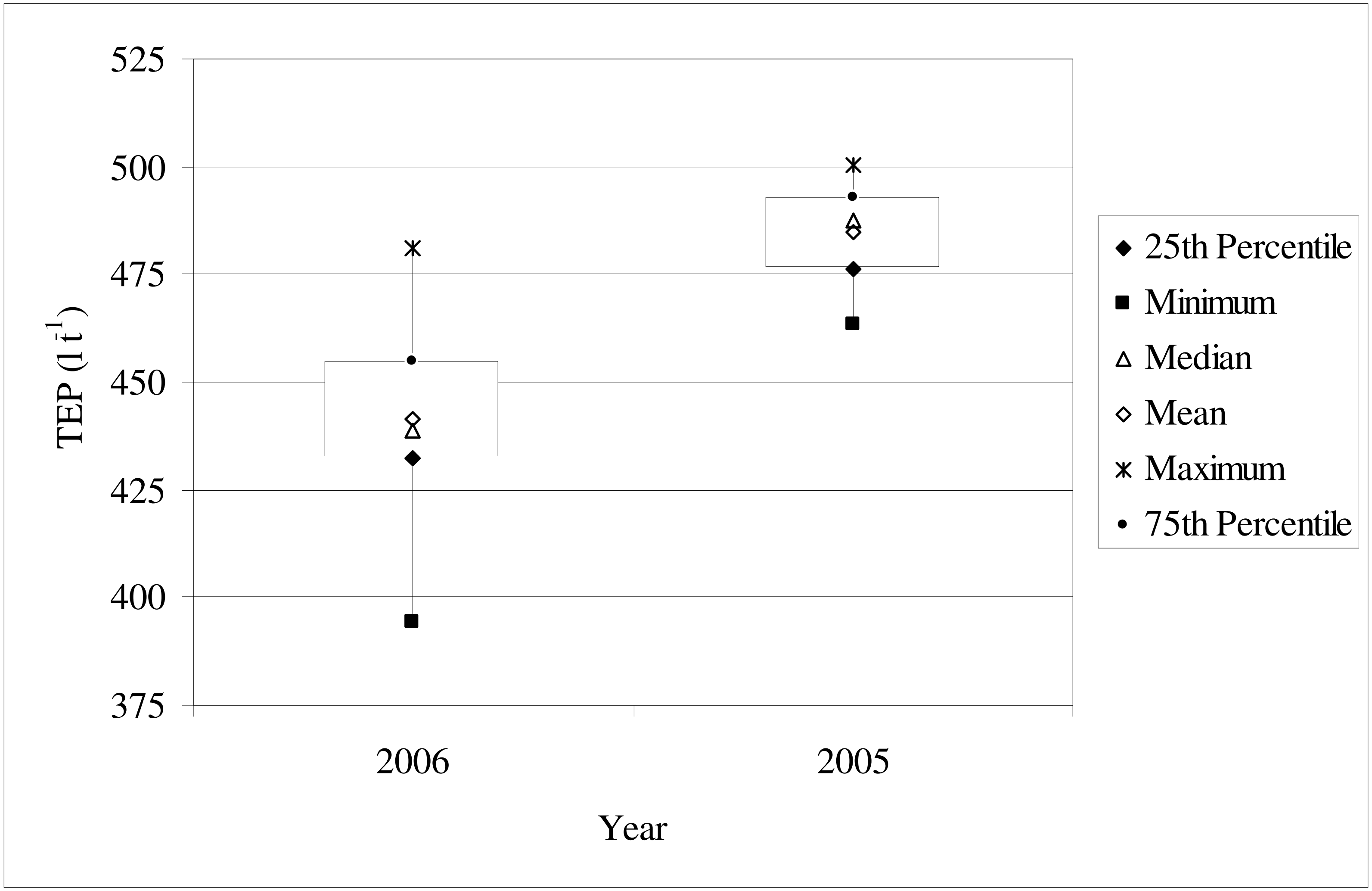

Fig. 5. Distribution of theoretical ethanol potential (TEP) of corn stover for 2006 and 2005 growing seasons.

CHAPTER 5. EVALUATION OF CORN EAR HUSK CHARACTERISTICS BENEFICIAL TO THE PRODUCTION OF CELLULOSIC ETHANOL

A paper to be submitted for publication in *Agronomy Journal*

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Abstract

Corn ear husks are a potential feedstock for cellulosic ethanol production, however little is known about husk dry matter and husk ethanol yields of corn genotypes. This study was conducted to determine agronomic and quality characteristics of corn ear husks and make predictions of potential ethanol yields. Fifty maize genotypes were evaluated for husk yield and analyzed for husk quality traits in three replications of a randomized complete block experiment, over two years and two central Iowa locations. Quality traits included cellulose (ADF-ADL), hemicellulose (NDF-ADF) and lignin (ADL). Quality traits were then used to predict theoretical ethanol potential (l t^{-1}) (TEP). On average, the genotypes yielded 0.71 t ha^{-1} of husk dry matter, ranging from 0.53 to 1.25 t ha^{-1}. Husks accounted for 6.9% of the total ear and 3.7% of the total dry matter portions of the plant. Significant variation was found among hybrids for agronomic and compositional traits. Average husk TEP was 616 l t^{-1}, ranging from 594 to 631 l t^{-1}. Theoretical ethanol yields (TEY) were calculated by multiplying husk yields (t ha^{-1}) and husk TEP (l t^{-1}). Average TEY from cobs was calculated to be 438 l ha^{-1}, ranging from 321 to 768 l ha^{-1} over all hybrids. Theoretical ethanol yield was highly correlated with husk yield ($r = 0.99$), but there was no significant correlation with TEP. Based on this data the best approach to improving ethanol yield from husks may be by selecting for higher husk dry matter yields.

Introduction

As concerns with current energy security and growth, as well as environmental and climate protection continue to escalate in the United States, alternative energy sources such as biofuels are becoming increasingly attractive (Dhugga, 2007). In the transportation sector, biofuels, such as ethanol have offered a promising solution to petroleum based fuels. However, the current starch based ethanol industry, which is reliant on corn grain alone, is not projected to meet the U.S. demand for transportation fuels (Houghton et al., 2005). Therefore, attention has recently been turned to the more abundant lignocellulosic biomass sources, such as corn stover to use as a feedstock for ethanol production.

Based on a study conducted by the USDA and DOE, if the United States were to meet the goal of displacing 30% of its petroleum usage by 2030 using sustainably available biomass, corn stover would account for 20% of it (Perlack et al., 2005). In this paper we will define corn stover as all above ground stalk and leaf dry matter. However, the idea of collecting corn stover residue has raised concern with soil quality and erosion, and ease of harvest. Collection the husk and cob fractions may alleviate some of this concern. As husks and cobs represent the highest glucose yielding residues, they may have a higher ethanol potential (Crofcheck and Montross, 2004). The remaining stalk and leaf material could be left in the field after harvest for soil erosion and quality control. Husks may also have a higher potential for ease of harvest than the remaining stover, as they represent a plant fraction already passing through the combine with regular grain harvest (Hoskinson et al., 2007). In order to maximize the potential of husks as a feedstock, agronomic and compositional characteristics should first be evaluated.

Several platforms have been proposed for the conversion of lignocellulosic material into energy, but the sugar and thermochemical methods have received the most attention (Houghton et al., 2005). The thermochemical platform involves the use of heat to break biomass into gases or liquids that can be converted into other products. The sugar platform, which has already been implemented in the starch based ethanol industry, involves the use of enzymes and acids to hydrolyze biomass into component sugars which can then be fermented. Our research focuses on the sugar platform as a means for converting biomass into energy. We hypothesize that higher component sugar yields and more accessible polysaccharides will result in higher ethanol production.

In order to evaluate ethanol potential, via the sugar platform, two methods have been evaluated. One process, developed by the National Renewable Energy Lab (NREL), Golden, CO, uses near infrared reflectance spectroscopy (NIRS) calibration equations to predict component pentose sugars: xylose, mannose, and arabinose, and component hexose sugars: glucose and galactose. Component sugar concentrations are then used to predict theoretical ethanol potential. Another possible method is based on ruminant digestion. The digestibility of forages by ruminant animals is traditionally evaluated by detergent fiber methods (Goering and Van Soest, 1970). Using these methods, measures of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) in a forage sample can be predicted. Neutral detergent fiber measures the cellulose, hemicellulose, and lignin content in the plant material, ADF measures the cellulose and lignin content in plant material, and ADL measures the lignin content of plant material (Van Soest, 1994). These three measures can then be used to estimate the structural carbohydrate content of plant material. For example, subtracting ADF from NDF gives an estimate of hemicelluose, and subtracting ADL from

ADF gives an estimate of cellulose. Cellulose and hemicellulose content can then be utilized to estimate TEP (Kirkpatrick et al., 2008a).

The objective of our study was to evaluate the suitability of husks as a lignocellulosic feedstock for the sugar platform and to assess the potential for modifying husk characteristics via plant breeding. We evaluated a group of 50 hybrids, populations, and other germplasm types to answer the following questions: What is the proportion of above ground corn biomass that husks account for and how much variation exists for husk yields? How much variation is there for husk quality traits among different hybrids and germplam sources? What is the theoretical ethanol yield potential of husks? What conclusions can we draw from this data regarding the development of selection criteria for breeders to develop maize genotypes with husks best suited for ethanol production? Material presented in this paper is a follow up to a previous study involving the evaluation of corn cobs and stover for cellulosic ethanol potential (Kirkpatrick et al., 2008b,c).

Materials and Methods

Germplasm

Fifty maize genotypes were selected to represent a range of germplasm in order to evaluate the amount of variation that may exist for agronomic and quality traits. The genotypes selected included commercial hybrids, open-pedigreed F1 hybrids, populations, population x population hybrids, and inbred x population hybrids (Table 1). Some of the genotypes were developed specifically for forage quality, while most of the genotypes were developed for grain production. Six commercial hybrids were also evaluated, including DeKalb DKC51-43, Pioneer34M93, Renk RK232, Mycogen F697 (*bm3*), Novartis N48V8

(*Lfy1*), and Holden's LH244/LH295, an open pedigreed F1 hybrid. A complete description of germplasm evaluated in this study can be found in Kirkpatrick et al. (2008b).

Field evaluation

Genotypes were evaluated in 3 replications of a randomized complete block design at 2 locations in the 2006 growing season. Hybrids were evaluated at Ames and Belmond, IA, and experimental plots consisted of two rows, 5.49 m long with 0.76 m between rows, including alley ways. Data collected on plots included silking date, ear height, plant height, root lodging, stalk lodging, grain yield, cob yield, stover yield, husk yield, total above ground dry matter yield, ear moisture at harvest, and stover moisture at harvest. See Kirkpatrick et al. (2008b) for a detailed description of the traits collected.

Plots were planted on May 3 and May 9 and harvested at physiological maturity on September 15 and September 20 for Ames and Belmond locations, respectively. Grain and stover fractions were harvested separately by hand harvesting ears from each plot. Ears were harvested from 20 plants per plot and the ear husk was harvested with the ear. A pruning shear was used to harvest ears in the husk by clipping the shank of the ear where it meets the plant. To ensure a random sample of ears, 10 ears were randomly harvested from each row of the two-row plot. Remaining ears were gleaned in the husk and discarded before stover harvest. Ears from each plot were weighed at harvest (wet weight), dried at 37.8˚C for three days, and weighed again to determine a dry weight. After drying, ears were shelled to determine total plot grain weight and cob weight. Husks were separated at the shelling stage and weighed to determine a total plot husk weight. Subsamples of grain, cobs, and husks were kept from each plot for compositional analysis.

Stover was harvested immediately after ear harvest with a commercial silage chopper modified for agronomic research, courtesy of Mycogen Seeds, Belmond, IA. Stover was chopped at a height of approximately 6 cm. Harvested stover consisted of above ground stalks and leaves. Total plot stover weight was obtained from the silage chopper. Subsamples of stover were collected from each plot, weighed, dried at 37.8˚C for four days, weighed again, and then kept for compositional analysis.

Lab evaluation

Husk samples were ground in a Hammer Mill to pass through a 1mm mesh screen. Ground samples were scanned with a NIRSystems 6500 near infrared reflectance spectrophotometer (NIRS) (FOSS NIRSystems Inc., Silverspring, MD). Standard NIRS procedures were used (Marten et al., 1989). The CENTER program was used to compute standardized *H* statistics for each sample's spectra, and the SELECT program was used to select calibration samples for wet-lab analysis using a standardized *H* of 1.5 for all husk samples.

Calibration samples were then analyzed to determine detergent fiber composition. A modified procedure of Goering and Van Soest (1970) was used for sequential analysis of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL). Modifications included the use of ANKOM^{200} fiber analyzer (Ankom Technologies Corp., Fairport, NY). Estimations of NDF, ADF, and ADL (g kg^{-1}) were obtained for the calibration samples from wet chemistry methods. Values for cell solubles, hemicellulose, cellulose, and lignin were calculated from the detergent fiber data. The results of the calibration set were used to develop prediction equations, relating the NIRS spectral data to chemical composition values. Calibration equations were developed for NDF, ADF, ADL, cell solubles, hemicellulose, cellulose, and lignin. Selection of prediction equations for each constituent was based on high R^2 values and low standard errors of calibration (SEC) and cross validation (SECV) (Marten et al., 1989). The final equations were then used to make predictions for all husk samples for all chemical constituents. Calibration statistics for compositional husk traits are shown in Table 2**.** Hemicellulose and cellulose values were used to calculate theoretical ethanol potential (TEP) for husks, (Kirkpatrick et al., 2008a):

H (hexose sugars) = (Cellulose + Hemicellulose *0.07) *172.82

P (pentose sugars) = (Hemicellulose *0.93) *176.87

TEP (l t^{-1}) = (H + P)*4.173.

Theoretical ethanol yields (TEY) (l ha^{-1}) were then calculated by multiplying TEP (l t^{-1}) by husk dry matter yield (t ha^{-1}).

Data analysis

The data for the individual environments were analyzed as a randomized complete block design and then combined over environments using the MIXED procedure (SAS Institute, 2003). A residual analysis was performed on the individual environment analyses to detect outliers, (Anscombe and Tukey, 1963). Entries were considered fixed effects in the analysis and all other effects were considered random. Entry means were used to calculate Pearson correlation coefficients between traits. An LSD (0.05) was calculated using the genotype by environment error mean square to compare hybrid means. All tests of significance were made at the $\alpha=0.05$ p level unless otherwise noted.

Results and Discussion

Husk yield

Average husk yield over all genotypes and environments was 0.71 t ha^{-1}, ranging from 0.53 to 1.25 t ha^{-1} (Table 1). The highest husk yielding genotype was BS28(R)C4, while the lowest husk yielding hybrid was Renk RK232. Husk yield showed significant differences among genotypes as well as among environments. Average husk yield for the Ames location was 0.59 t ha^{-1}, while average husk yield for the Belmond location was 0.82 t ha^{-1} (Fig. 1). This suggests that significant variation exists for husk yields within the germplasm evaluated and that husk yields are influenced by environmental growing conditions. The genotype by environment interaction effect was not significant for husk yield in the two environments evaluated. The lowest husk yielding genotype was also the lowest stover yielding genotype. Significant and positive, but relatively small correlations were observed between husk yield and stover yield, cob yield, and total dry matter yield (Table 3). No correlation was observed between husk yield and grain yield. Kirkpatrick et al. (2008b) reported average cob dry matter yield to be 1.3 t ha^{-1}. Therefore, husk dry matter yield is approximately half of what can be expected of cob dry matter yield.

Husks on average accounted for 7% of the total ear and 4% of total dry matter (Fig. 2). However, a significant amount of variation exists for these proportions among hybrids. Husk percentage of the total ear ranged from 5.4 to 14.2%, and husk percentage of TDM ranged from 2.9 to 8.0%. Shinners and Binversie (2007) reported husks to account for 8% of the nongrain above ground biomass, which is consistent with our experiment average. Pordesimo et al. (2005) reported husks to account for 7% of the total above ground dry matter. This is nearly double the average value we have reported, but still falls within our range. Type of germplasm evaluated could be a result of this discrepancy, as large differences are seen among the genotypes in our study. Differences among hybrids could be due to differences in ear length and kernel row number, resulting in more or less husk dry matter.

Since breeders have been selecting for higher grain yield in corn for decades, a positive correlation between grain yield and husk yield would be desirable. The lack of a correlation indicates that increases in grain biomass do not result in increases in husk biomass. The small correlation between husk yield and cob and stover yields indicate that husk yields are more related to plant characteristics than to grain characteristics. Ear dimensions were not measured but may be related to husk yield.

Husk quality

The theoretical ethanol potential (TEP) of husks is determined by the chemical composition of husks. Genetic variation for husk composition will be necessary for designing breeding programs to improve the TEP of husks. Genotype effects were significant for all chemical constituents of husks. Neutral detergent fiber and ADF averages were 856 and 439 g kg^{-1}, respectively (Table 1). Kuehn et al. (1999) reported husk, shank, and silk together to have NDF and ADF values of 754 and 366 g kg^{-1}, respectively. One possible explanation for the lower values in the Kuehn study is that the plants were harvested at a higher moisture for silage purposes. Therefore, lower values for lignin and structural carbohydrates may be observed when compared with our study, which was harvested at a lower moisture content and later harvest date (Russell, 1986). Environmental effects were significant for ADF, lignin, and hemicellulose values, suggesting that the growing environment has an effect on the chemical composition of husks. Genotype by environment effects were also significant for NDF, lignin, and cellulose.

Theoretical ethanol potential of husks was reported at 616 l t^{-1}, ranging from 594 to 631 l t^{-1} over both environments. The genotype with the highest TEP was DKC51-43, which also had the highest NDF value. The genotype with the lowest TEP was W64A X A619 which had below average values for NDF, ADF, cellulose, and hemicellulose. Theoretical ethanol potential was positively correlated with NDF, cellulose, and hemicellulose as expected (Table 3). Significant variation was present among genotypes for TEP, but no difference was seen between the two environments (Fig. 3). Average TEP for Ames and Belmond environments were 615 and 616 l t^{-1}, respectively. Genotype by environment effects were significant for TEP. Hames et al. (2003) reported stover samples to have TEP ranging from 438 to 496 l t^{-1}. Kirkpatrick et al. (2008b,c) reported cob TEP values to range from 588 to 627 l t^{-1} and stover TEP values to range from 394 to 500 l t^{-1}. Therefore, husks have a higher TEP than stover and are comparable to cobs in terms of their ethanol potential. Husks and cobs have higher concentration of structural carbohydrates than stalks and leaves resulting in a greater TEP (Kirkpatrick et al., 2008b,c).

Theoretical ethanol yield

Theoretical ethanol potential is a measure of the ethanol yield potential of one ton of dry matter assuming that all of the sugars can be converted to ethanol. Theoretical ethanol yield is the product of TEP and husk yield and gives ethanol yield on a per unit of land area basis. Theoretical ethanol yields for husks averaged 438 l ha^{-1}, ranging from 321 to 768 l ha^{-1}. Significant differences were observed among genotypes and among environments for TEY (Fig. 4). Average TEY for the Ames location was 363 l ha^{-1}, while average TEY for the

Belmond location was 506 l ha^{-1}. There was also a significant genotype by environment effect for TEY. Theoretical ethanol yield was significantly and positively correlated with husk yield (r = 0.99) (Fig. 5a). Theoretical ethanol yield was not correlated with TEP (Fig 5b). Therefore, husk dry matter yields, rather than husk composition, have the largest influence on ethanol yield of husks. Like husk dry matter yields, TEY was also positively correlated with stover yield, cob yield, and TDM yield (Table 3).

Conclusions

Husks, as well as cobs may offer an attractive alternative to harvesting stalks and leaves for lignocellulosic feedstocks. Husk and cob harvest may provide relief to the concerns of soil integrity, ease of harvest, and economical transportation associated with corn stover harvest (Hoskinson et al., 2007). Collection of these specific fractions may also be beneficial as they have higher ethanol potential than stalks and leaves. However, TEP was not correlated with TEY, and TEP must be attainable in order to reach the ethanol potential values reported in this study. Based on this data, the most beneficial way to increase ethanol yield from husks is to maximize husk dry matter yields, as yield was shown to be the greatest factor affecting TEY. Breeding for higher husk yields may be a possibility, as variation for dry matter yield was present among the genotypes included in this study, however it is not feasible given the current plot combine design. The correlation reported in this study suggests that there is little potential to indirectly select for husk yield as well. However, previous work has shown correlations among grain yield with stover, cobs, and TDM (Kirkpatrick et al., 2008b, c). Genotypes with higher kernel row numbers and longer ear lengths could possibly reflect greater husk yields and be interesting traits for future evaluation if husks and cobs continue to be utilized in the emerging cellulosic ethanol industry.

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Table 1. Table of means for husk quality and agronomic traits.

	Husk composition†						Yield		Husk ratios§		Husk ethanol¶	
Hybrid	NDF	ADF	SOL	HEM	CELL	LIG	Husk	Grain‡	Husk: TDM	Husk: Ear	TEP	TEY
	-----------------------g kg^{-1}------------------------						---------t ha^{-1}---------		------percent------		l t^{-1}	l ha^{-1}
F1 Experimental hybrids												
W64A X A619	854	439	146	403	412	12.9	0.54	6.74	3.3	6.4	594	321
WQS C3 X HC33	849	433	151	419	422	13.3	0.66	8.90	3.2	6.0	613	403
WQS C3 X LH198	839	419	161	431	415	10.6	0.82	8.30	4.1	7.9	617	509
WQS C3 X LH332	843	423	157	431	415	12.1	0.73	8.37	3.8	6.9	617	447
WQS C3 X TR7245	853	446	147	413	433	11.4	0.76	9.08	3.7	6.9	616	471
TR7245/W601S	859	457	141	410	442	9.2	0.58	8.67	2.9	5.4	621	362
SGI912/W601S	857	448	143	421	440	8.7	0.68	8.55	3.4	6.3	627	427
B129/W601S	854	437	146	431	427	8.6	0.75	9.14	3.7	6.5	625	472
TR7245/W602S	857	449	143	414	443	11.3	0.58	7.72	3.3	6.1	625	360
SGI912/W602S	862	444	138	420	440	10.4	0.65	8.92	3.2	5.9	627	406
B126/W602S	864	450	136	423	436	9.0	0.53	6.96	3.4	6.1	626	333
B129/W602S	856	442	144	417	431	11.4	0.65	8.85	3.4	6.0	618	402
TR7245/W603S	863	442	137	418	435	10.7	0.64	8.64	3.2	6.0	621	398
SGI912/W603S	858	429	142	425	426	10.9	0.58	8.20	3.1	5.8	619	360
B126/W603S	855	441	145	418	423	11.2	0.60	7.47	3.6	6.3	613	367
B129/W603S	858	431	142	430	420	11.2	0.71	8.93	3.9	6.4	620	441
TR7245/W604S	846	424	154	427	424	11.6	0.64	9.55	3.0	5.4	620	396
B129/TR7322	857	437	143	408	422	9.8	0.75	8.90	3.6	6.6	605	455
B73/Mo17	856	437	144	414	426	12.0	0.75	9.51	3.3	6.3	611	457
TR7245/BS32(R)C0-249-1-02-01-01-01-B-B	856	448	144	402	432	12.7	0.69	8.93	3.4	6.4	608	421
BS31(R)C0-246-1-01-01-01-01-B-B/B116	861	452	139	405	444	11.6	0.83	11.12	3.4	6.0	618	515
BS31(R)C0-246-1-01-01-01-01-B-B/TR7322	860	458	140	386	439	11.4	0.74	9.40	3.4	6.1	601	433
Populations and population crosses												
WQS C3 Syn2	839	432	161	411	417	11.2	0.75	6.17	5.4	9.5	603	455
BS28(R)C4	852	416	148	449	397	13.9	1.25	6.21	8.0	14.2	617	768
BS29(R)C4	856	421	144	437	413	12.1	0.83	6.50	4.7	9.7	620	515
BSSS(R)C15/BS13(S)C10	863	446	137	426	429	12.2	0.72	9.21	3.5	6.2	623	450
BSSS(R)C15/BSCB1(R)C15	851	428	149	428	418	13.4	0.79	7.88	4.0	7.9	612	486

Table 1. (continued)

	Husk composition†						Yield		Husk ratios§		Husk ethanol¶	
Hybrid	NDF	ADF	SOL	HEM	CELL	LIG	Husk	Grain‡	Husk: TDM	Husk: Ear	TEP	TEY
	g kg^{-1}						t ha^{-1}		percent		l t^{-1}	l ha^{-1}
Populations and population crosses												
Nokomis Gold/BS21(R)C7	863	459	137	401	442	13.6	0.66	6.88	4.3	7.5	614	407
TEPR-EC6/BS33(S)C5	862	448	138	408	424	13.1	0.69	7.88	4.2	7.1	606	415
BS33(S)C5/BS22(R)C7	846	430	154	417	406	14.2	0.63	6.62	4.1	7.5	597	373
Inbred x population crosses												
BS13(S)C10/B116	859	435	141	429	426	12.4	0.73	9.78	3.0	5.9	623	453
BSSS(R)C16/B116	860	443	140	414	430	14.2	0.69	8.26	3.4	6.7	615	425
BS32(R)C2/B129	856	411	144	441	396	11.8	0.94	7.93	4.2	9.1	610	574
BS32(R)C2/B126	862	434	138	427	415	13.8	0.78	7.00	3.9	8.6	614	479
BS31(R)C2/B114	855	433	145	422	422	10.4	0.60	7.71	3.1	6.2	615	370
BS31(R)C2/B116	860	434	140	435	418	13.2	0.84	8.59	3.6	7.6	622	519
Commercial hybrids												
LH244/LH295	864	464	136	406	437	15.0	0.63	8.12	3.4	6.4	614	386
Renk 232	866	454	134	413	434	16.0	0.53	7.22	3.7	5.8	617	326
N48V8 (leafy)	848	417	152	442	410	11.4	0.97	10.22	4.0	7.4	620	603
Pioneer 34M93	862	439	138	432	431	9.8	0.77	10.03	3.7	6.3	629	486
Mycogen F697	839	458	161	383	446	7.2	0.62	8.68	2.9	5.9	604	371
DKC51-43	870	461	130	414	452	12.9	0.61	8.78	3.5	5.9	631	386
Experiment Mean	856	439	144	419	426	11.8	0.71	8.35	3.7	6.9	616	438
Minimum Mean	839	411	130	383	396	7.2	0.53	6.17	2.9	5.4	594	321
Maximum Mean	870	464	161	449	452	16.0	1.25	11.12	8.0	14.2	631	768
LSD(0.05)	9	12	9	10	14	2.4	0.13	1.39	0.5	0.9	12	82
Effective Error MS	27.54	53.92	27.54	39.74	72.33	2.2	0.01	0.73	0.1	0.27	55.85	2498.1

† NDF, neutral detergent fiber; ADF, acid detergent fiber; Sol, cell solubles; Hemi, hemicellulose; Cell, cellulose; Lig, lignin

‡Grain yield adjusted to a dry matter basis

§Percent of husk material accounted for in the total dry matter (TDM) and ear fractions of the plant

¶ TEP, theoretical ethanol potential; TEY theoretical ethanol yield

Table 2. NIRS calibration statistics

Trait†	Mean	SD‡	N§	R2	SEC¶	SECV#
NDF	85.2	1.24	27	0.94	0.30	0.54
ADF	42.8	1.54	25	0.98	0.22	0.45
Cellulose	41.6	1.61	28	0.99	0.18	0.51
Hemicellulose	41.9	1.96	29	0.95	0.46	0.93
Lignin	1.2	0.35	30	0.95	0.08	0.19
Solubles	14.8	1.24	27	0.94	0.30	0.54

† NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.
‡SD = standard deviation.
§N = final number of data points used in NIRS calibration equation.
¶SEC = standard error of calibration.
#SECV = standard error of cross validation.

Table 3. Table of Pearson correlation coefficients fro quality and agronomic husk traits.

	NDF†	ADF	SOL	CELL	LIG	HEM	TEP‡	TEY	HUSK§	GRAIN	STOVER	COB	TDM
NDF	1.00												
ADF	0.54***	1.00											
SOL	-1.00***	-0.54***	1.00										
CELL	0.44**	0.89***	-0.44**	1.00									
LIG	0.24	-0.04	-0.24	-0.27	1.00								
HEM	-0.09	-0.78***	0.09	-0.64***	0.07	1.00							
TEP	0.40**	0.06	-0.40**	0.35*	-0.24	0.49***	1.00						
TEY	-0.20	-0.59***	0.20	-0.55***	0.10	0.59***	0.10	1.00					
HUSK	-0.22	-0.58***	0.22	-0.58***	0.11	0.55***	0.03	0.99***	1.00				
GRAIN	0.11	0.15	-0.11	0.37*	-0.32*	-0.07	0.34*	0.08	0.06	1.00			
STOVER	-0.06	-0.33*	0.06	-0.20	0.14	0.29	0.13	0.41**	0.41**	0.50***	1.00		
COB	0.10	-0.22	-0.10	-0.13	-0.07	0.18	0.08	0.31*	0.31*	0.67***	0.56***	1.00	
TDM	0.03	-0.17	-0.03	0.01	-0.21	0.17	0.22	0.36*	0.35*	0.83***	0.88***	0.76***	1.00

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

† NDF, neutral detergent fiber; ADF, acid detergent fiber; LIG, lignin

‡ TEP, theoretical ethanol potential

§ Husk yield

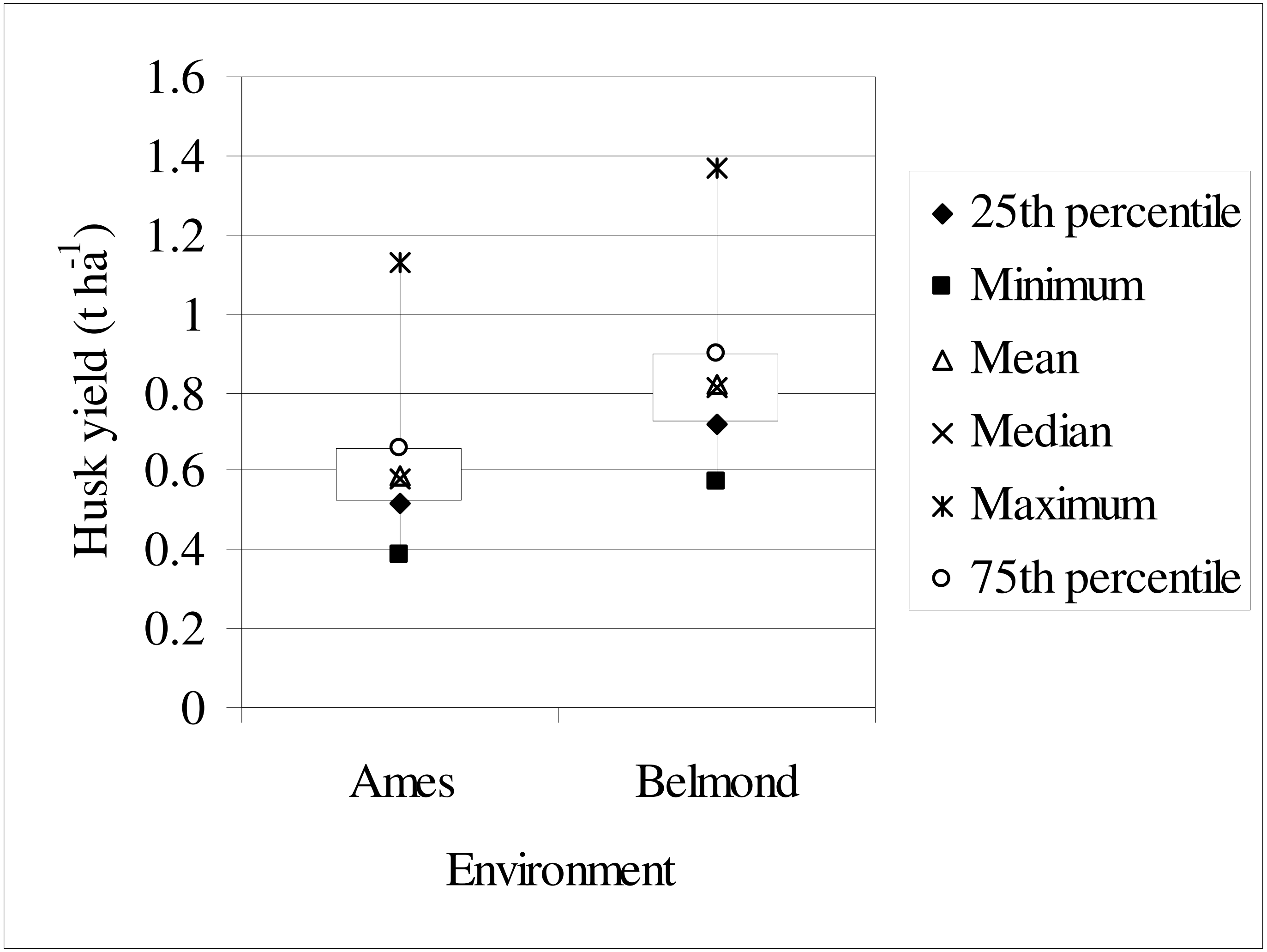

Fig. 1. Distribution of husk dry matter yield data between the two environments evaluated. Distribution of data is based on entry means of each environment.

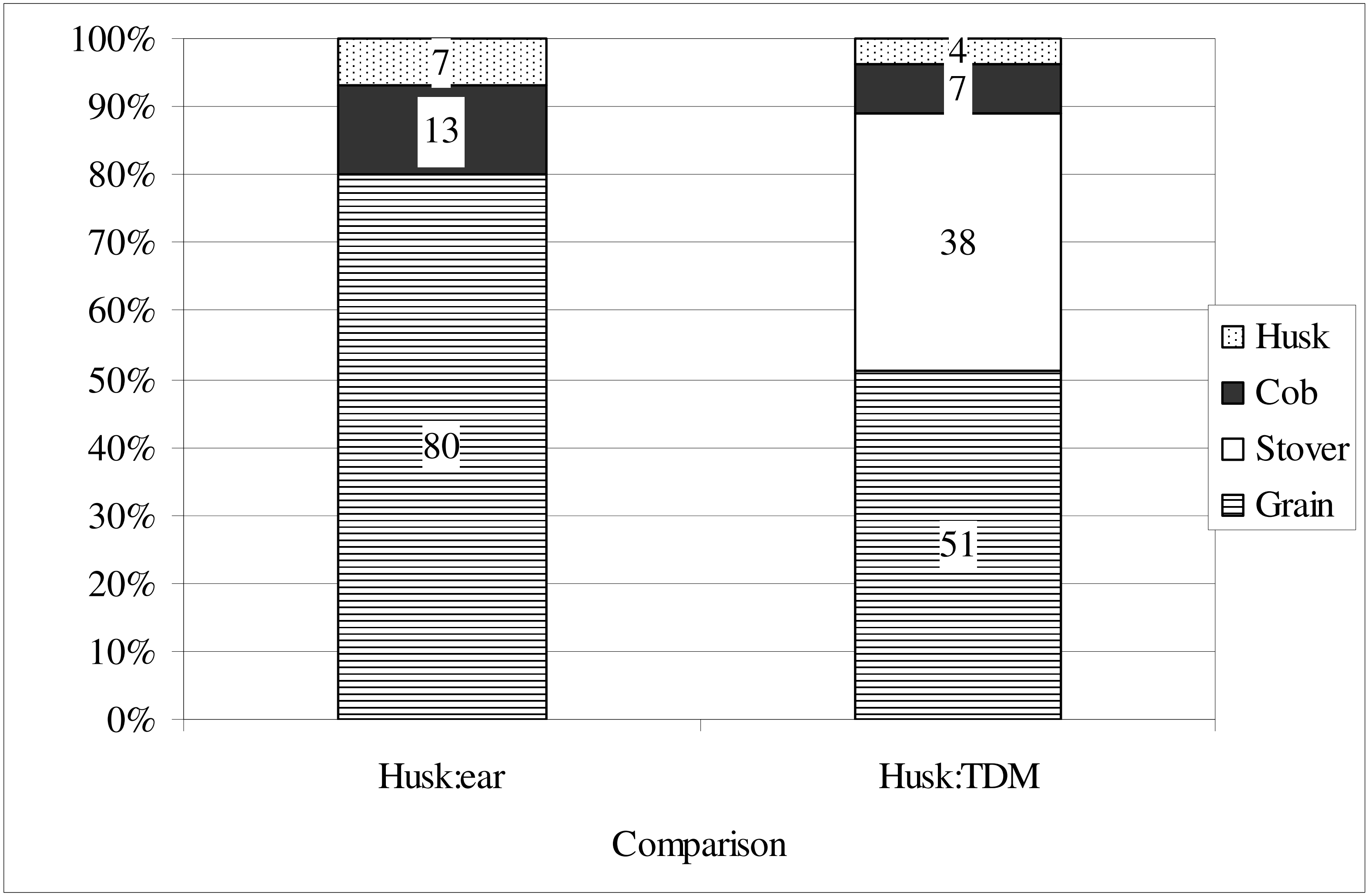

Fig. 2. Proportion of husk dry matter accounted for in the ear and total dry matter ((TDM) plant fractions, respectively.

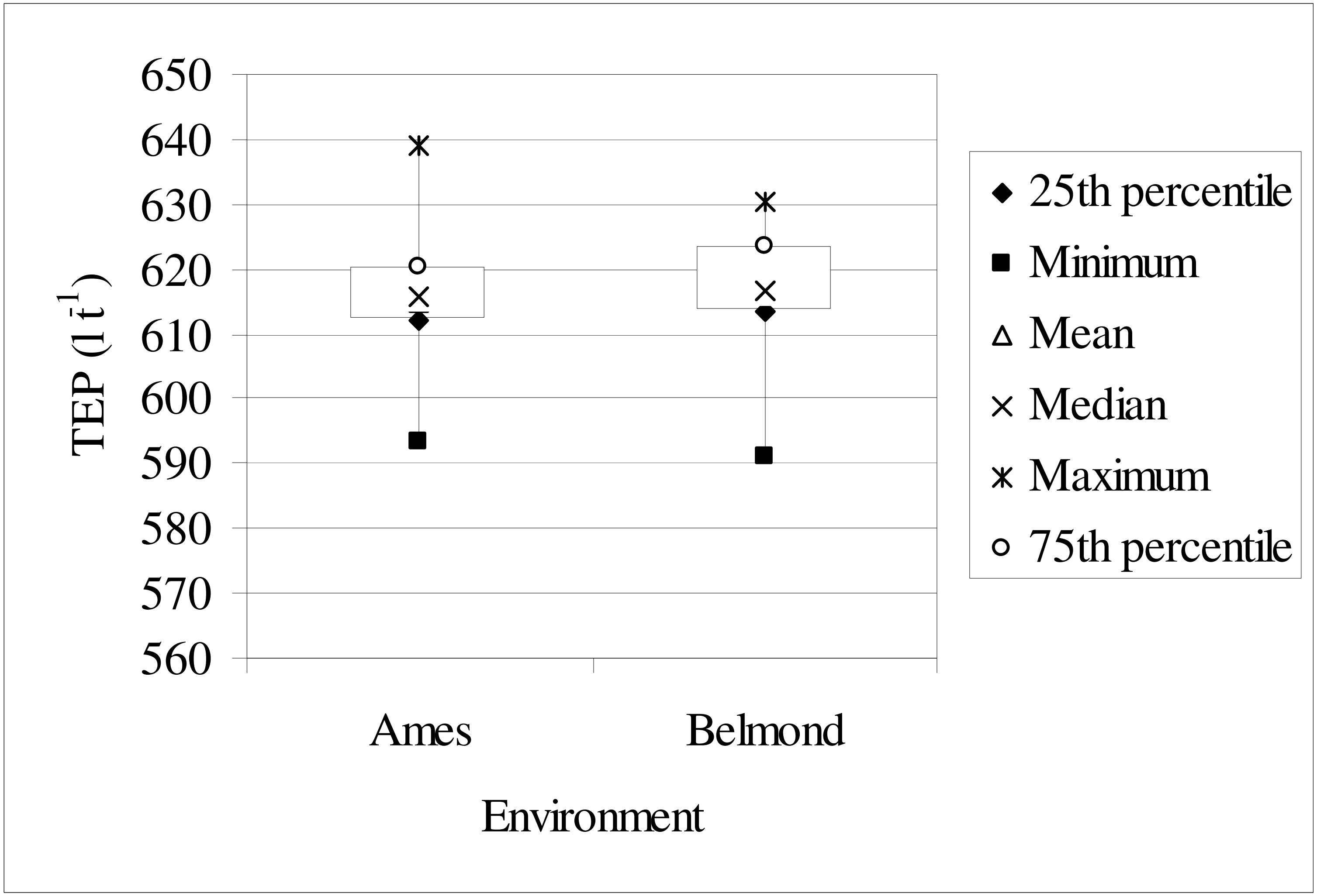

Fig. 3. Distribution of theoretical ethanol potential (TEP) data between the two environments evaluated. Distribution of data is based on entry means of each environment.

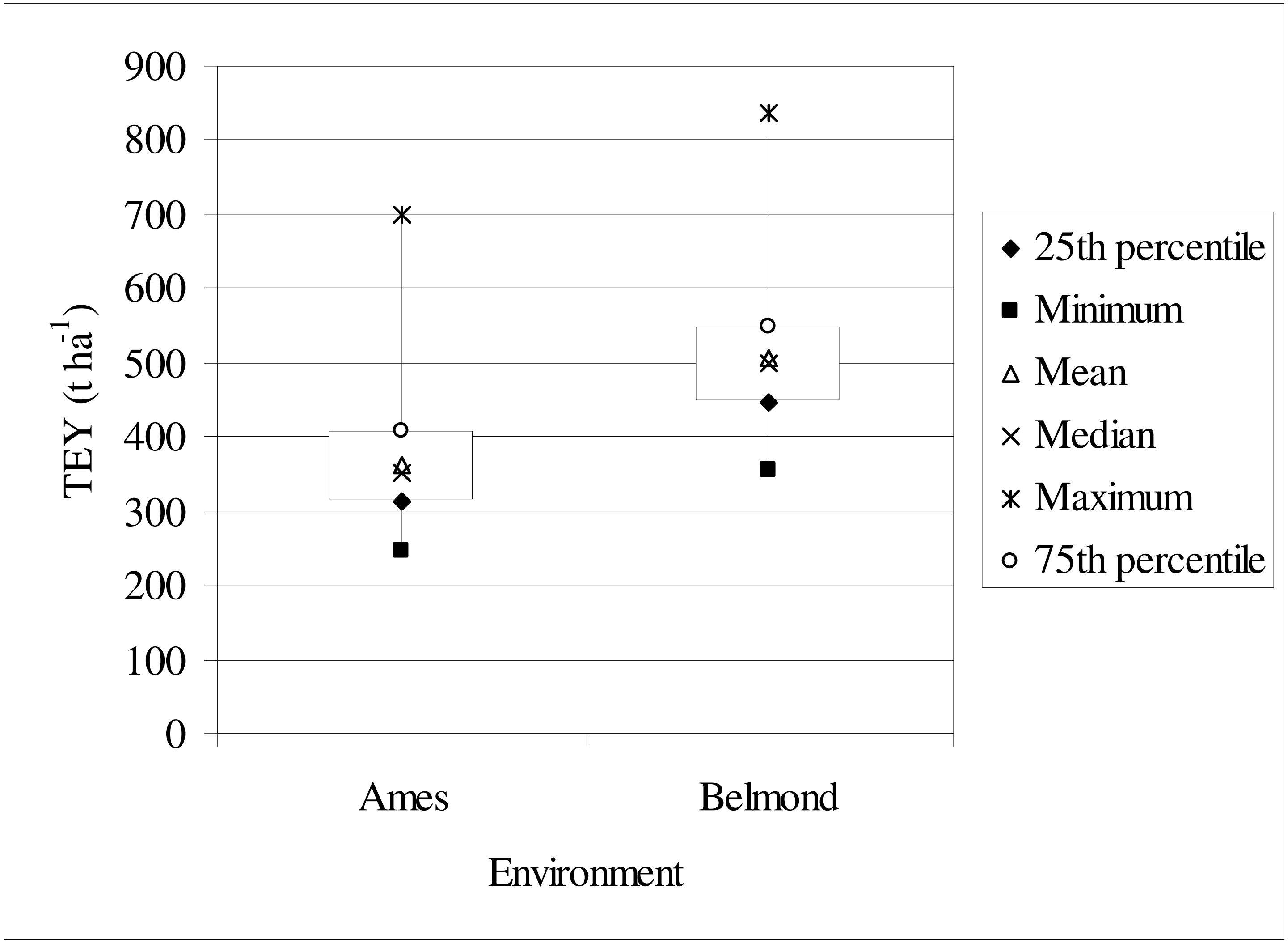

Fig. 4. Distribution of theoretical ethanol yield (TEY) data between the two environments evaluated. Distribution of data is based on entry means of each environment

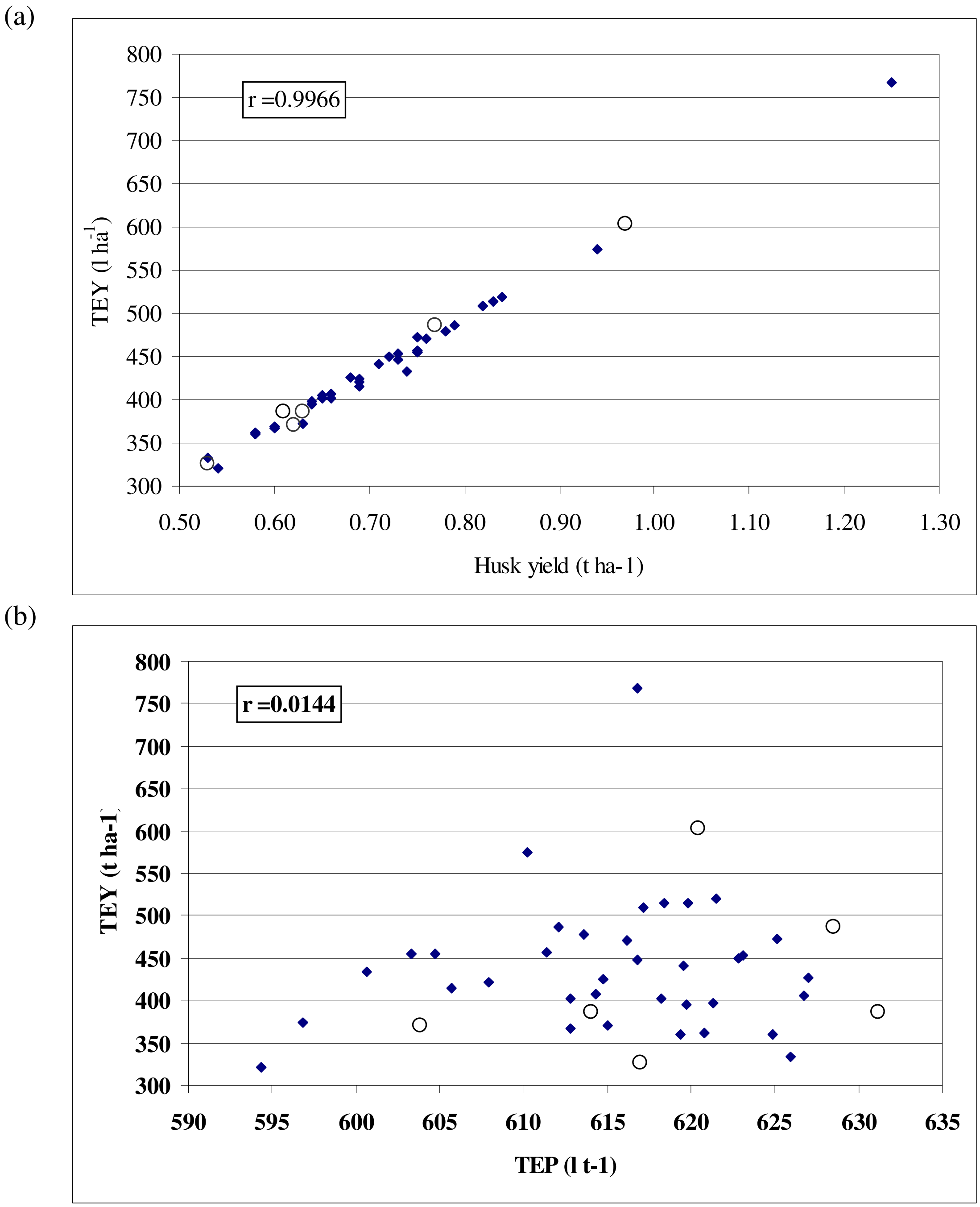

Fig. 5. Relationship between husk dry matter yield and theoretical ethanol yield (TEY) (a) and between theoretical ethanol potential (TEP) and TEY (b). Highlighted points indicate commercial hybrids.

CHAPTER 6. ESTIMATING THEORETICAL ETHANOL POTENTIAL (TEP) OF CORN STOVER BASED ON DETERGENT FIBER PREDICTIONS

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Abstract

The ability of plant breeders to rapidly analyze ethanol potential from cellulosic feedstocks, such as corn stover is becoming increasingly important in the emerging biobased industry. The National Renewable Energy Lab (NREL) has developed a theoretical yield calculator to predict ethanol potential of corn stover based on values cell wall monosaccharides. Traditionally corn stover and other forage samples have been analyzed using a combination of detergent fiber and near infrared reflectance spectroscopy (NIRS) methods. This study was conducted to determine if neutral detergent fiber (NDF) and acid detergent fiber (ADF) values could be used to predict theoretical ethanol potential (TEP), of corn stover samples. Stover samples of fifty maize genotypes over two years and husk samples of fifty maize genotypes over one year were evaluated for chemical composition characteristics using detergent fiber methods. Hemicellulose and cellulose values were predicted from NDF, ADF, and acid detergent lignin (ADL) lab measurements. The proportions of pentose and hexose sugars in the hemicellulose fraction were validated by high pressure liquid chromatography (HPLC) methods. Total pentose sugar and total hexose sugar values for each stover sample were then used to predict TEP ($l\ t^{-1}$). Average TEP for 2005 stover samples as predicted by NREL was 419 $l\ t^{-1}$, ranging from 403 to 436 $l\ t^{-1}$, TEP values predicted from detergent fiber methods resulted in an average of 485 $l\ t^{-1}$, ranging from 463 to 500 $l\ t^{-1}$. A significant correlation between the two methods for predicting TEP

was found (r = 0.75). These results suggest while the two methods gave differing results, they ranked hybrids in the same relative order. This indicates that it may be reasonable to use detergent fiber methods to predict TEP.

Introduction

The production of ethanol from lignocellulosic feedstocks, such as corn stover, is becoming an attractive option to current petroleum and starch based ethanol production for transportation fuels. Environmental concerns, as well as issues with economic and energy security have heightened in the United States, and it is becoming increasingly important to investigate alternative energy sources for transportation fuels (Dhugga, 2007). Currently, starch based ethanol, derived from corn grain, has been the main source for renewable transportation fuel. However, as the United States consumption of gasoline averages 388.6 million gallons per day (DOE, 2007), the currently implemented starch based ethanol industry is not projected to meet the fuel demand (Houghton et al., 2005). Lignocellulosic biomass feedstocks, however, are low cost, abundant, and renewable; and sources include agricultural residues, forestry residues, industrial waste, and dedicated energy crops.

One agricultural residue source, corn stover, has been shown to be a widely available feedstock. The billion ton annual supply study determined that the land resources of the United States were capable supplying enough biomass to displace 30% of the country's petroleum usage by the year 2030, and that corn stover could account for 20% of this total (Perlack et al., 2005). However, there are many concerns involving the utilization of corn stover, including soil erosion, ease of harvest, economics of transportation, and storage (Shinners and Binversie 2007, Hoskinson et al., 2007). Therefore, it is important to develop methods to evaluate corn stover traits in order to maximize ethanol potentials and create a sustainable and efficient production system.

The sugar platform is one method being investigated for the breakdown of lignocellulosic biomass, as the concepts have already been implemented in starch based ethanol production (Houghton et al., 2005). The sugar platform uses enzymes or acids to hydrolyze biomass into component monosaccharides, which can then be fermented and used in transportation fuels. It is predicted that higher component sugar yields and more accessible monosaccharides will result in higher ethanol production. Currently the only method developed to measure the ethanol potential of corn stover is by using the stover calibration developed by NREL. This method uses NIRS calibration equations to predict component monosaccharides in corn stover residue. Estimations of pentose and hexose sugars are then used to predict theoretical ethanol potential (TEP) by means of the NREL theoretical yield calculator (DOE, 2006). Using this method, Hames et al. (2003) estimated the TEP of 47 corn stover samples to range from 105 to 119 gal t^{-1} (438-496 l t^{-1}). Although this method offers one promising way to estimate TEP of corn stover, it has some drawbacks. Cost and time committed to developing as well as maintaining these calibrations are one major factor. Laboratory methods are also timely and expensive. This method is relatively new, and therefore not well researched and developed.

Forage crops are traditionally measured for cell wall characteristics that impact feed value by the Van Soest detergent fiber method (Goering and Van Soest, 1970). In this method of analysis, measures of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) are determined, from which values of cellulose, hemicellulose, and lignin can be calculated. Cellulose, hemicelluloses, and lignin are the three main components of the cell wall (Van Soest, 1994). Cellulose and hemicellulose contain pentose and hexose sugars. Cellulose is the most abundant naturally occurring biological compound on earth and is composed of six carbon glucose units. Hemicellulose surrounds the cellulose fibrils and is composed of the pentose sugars xylan and arabinan, and the hexose sugars glucan, galactan, and mannan.

Neutral detergent fiber measures the cellulose, hemicellulose, and lignin content in the plant material, ADF measures the cellulose and lignin content in plant material, and ADL measures the lignin content of plant material (Van Soest, 1994). Subtracting ADF from NDF gives an estimate of hemicelluose, and subtracting ADL from ADF gives an estimate of cellulose. Since the detergent fiber methods can be used to estimate cellulose and hemicellulose they may offer another approach to predicting TEP. The detergent fiber methods are well established and more cost effective than the NREL methods, so the ability to use them in predicting TEP would be very beneficial.

Based on these methods we hypothesized that it may be possible to modify the TEP equation developed by NREL in order to estimate ethanol potential of corn stover fractions using the cellulose and hemicellulose values obtained from detergent fiber analyses. The objective of this study was to answer the question: Can hexose and pentose sugar values obtained by means of detergent fiber analysis methods be used to calculate theoretical ethanol potential of corn stover biomass? The results of this paper have been utilized in follow-up papers involving the evaluation of cobs, stover, and husks for their potential use in cellulosic ethanol production (Kirkpatrick et al., 2008a, b, c).

Materials and Methods

Experimental field methods

Fifty maize genotypes were selected to represent a range of germplasm in order to evaluate the amount of variation that may exist for agronomic and quality traits. The genotypes selected included commercial hybrids, open-pedigreed F1 hybrids, populations, population x population hybrids, and inbred x population hybrids. Some of the genotypes were developed specifically for forage quality, while most of the genotypes were developed for grain production. Genotypes were evaluated in 3 replications of a randomized complete block design at 2 locations in each of 2 years. In 2005, genotypes were evaluated at Ames and Ankeny, IA, and in 2006, genotypes were evaluated at Ames and Belmond, IA. Agronomic data collected on plots included silking date, ear height, plant height, root lodging, stalk lodging, grain yield, cob yield, stover yield, total above ground dry matter yield, ear moisture at harvest, and stover moisture at harvest. See Kirkpatrick et al. (2008a) for a complete description of germplasm and data collection.

In 2005, the Ames and Ankeny locations were planted on May 5 and May 6, respectively and harvested on October 9. In 2006, plots were planted on May 3 and May 9 and harvested at physiological maturity on September 15 and September 20 for Ames and Belmond locations, respectively. Grain and stover fractions were harvested separately by hand harvesting ears from each plot. In 2005, ears (grain and cob) were harvested from all plants in each plot. In 2006, ears were harvested from 20 plants per plot and the ear husk was harvested with the ear. A pruning shear was used to harvest ears in the husk by clipping the shank of the ear where it meets the plant. To ensure a random sample of ears, 10 ears were randomly harvested from each row of the two-row plot. Remaining ears were gleaned in the husk and discarded before stover harvest. In both years, ears from each plot were weighed at harvest (wet weight), dried at 37.8˚C for three days, and weighed again to obtain a dry weight. After drying, ears were shelled to determine total plot grain weight and cob weight. In 2006, husks were separated at the shelling stage and weighed for a total plot husk weight. Subsamples of grain, cobs, and husks (2006 only) were kept from each plot for compositional analysis.

Stover was harvested immediately after ear harvest with a commercial silage chopper modified for agronomic research, courtesy of Mycogen Seeds, Belmond, IA. Stover was chopped at a height of approximately 6 cm. In 2005, harvested stover consisted of stalks, leaves, and husks. In 2006, harvested stover consisted of stalks and leaves only, because the husk was harvested with the ears. Total plot stover weight was obtained from the silage chopper. Subsamples of stover were collected from each plot, weighed, dried at 37.8˚C for four days, weighed again, and then kept for compositional analysis.

Experimental laboratory methods

Stover and husk samples were ground to a 1-mm particle size in a hammer mill at the University of Wisconsin, Madison WI. Cobs were first passed through a wood chipper to reduce the particle size to approximately 3 cm, and then ground to 2 mm in a Wiley mill. Ground stover and husk samples were scanned by near infrared reflectance spectroscopy (NIRS), and the scans were sent to the National Renewable Energy Lab (NREL) in Golden, CO. The stover9 equation (Hames et al., 2003) was used to predict proportions of glucose, xylose, arabinose, galactose and mannose in each stover and husk sample from the sample NIR scans. The percentage of each monosaccharide was used to calculate theoretical ethanol potential (TEP) for each sample as follows:

H (hexose sugars) = (%Glucan + %Galactan + %Mannan) *172.82

P (pentose sugars) = (%Xylan + %Arabinan) *176.87

TEP (gallons/dry ton) = H + P.

The equation successfully predicted monosaccharide values for samples collected in the 2005 growing season. However, over 40% of the 2006 stover and 40% of the husk samples were determined unpredictable by the stover equation. In addition, 8% of the stover samples and 9% of the husk samples had higher than expected prediction errors. Therefore, in order to obtain more complete and accurate data set, detergent fiber methods (Goering and Van Soest, 1970) were utilized in order to repredict all stover and husk samples for their chemical composition, as well as cob samples. All samples were scanned with a NIRSystems 6500 near infrared reflectance spectrophotometer (NIRS) (FOSS NIRSystems Inc., Silverspring, MD). Standard NIRS procedures were used (Martens et al., 1989). The CENTER program was used to compute standardized *H* statistics for each sample, and the SELECT program was used to select calibration samples for wet-lab analysis using a standardized *H* of 1.5 for all samples. A calibration set was selected for 2005 stover, 2006 stover, 2006 husk, and all cobs. Separate calibrations were performed for 2005 and 2006 stover due to the separation of the husk fraction in 2006. Calibration statistics for stover and husks can be found in Table 1.

Calibration samples were then analyzed for fiber composition. A modified procedure of Goering and Van Soest (1970) was used for sequential analysis of NDF (neutral detergent fiber), ADF (acid detergent fiber), and ADL (acid detergent lignin). Modifications included the use of ANKOM^{200} fiber analyzer (Ankom Technologies Corp., Fairport, NY). Data from the wet lab analysis was used to develop prediction equations, relating the NIRS spectral data to NDF, ADF, and ADL values. Selection of prediction equations were based on high R^2 values and low standard errors of calibration (SEC) and cross validation (SECV) (Martens et al., 1989). Neutral detergent fiber, ADF, and ADL predictions were then made for all stover, husk and cob samples. Estimations of hemicellulose and cellulose for each sample were calculated by subtracting ADF from NDF and by subtracting ADL from ADF, respectively. Since the most abundant monosaccharides that make up the cell walls of grasses are glucose, xylose, and arabinose, (Vermerris et al., 2007) it was determined that it may be logical to substitute hemicellulose and cellulose values into the TEP equation in place of each component sugar.

Validating proportions of pentose and hexose sugars in hemicellulose

In order to most accurately predict TEP based on detergent fiber predictions, proportions of pentose and hexose sugars in the hemicelluose fraction were adjusted. Lee et al. (2007) reported that for corn stover, 93% of the sugars in hemicellulose were pentoses, with the remainder hexoses. For the purposes of this paper we found it necessary to validate this proportion for stover, husk, and cob fractions. Ten cob, stover, and husk samples from the same genotype entries were randomly selected and NDF detergent analysis was performed by a modified version of Goering and Van Soest (1970). Concentrations of neutral sugars hydrolyzed from the NDF residue of each sample were determined (Gerhardt et al., 1994). Hydrolysis was performed in glass tubes fitted with Teflon-lined screw caps. Into each tube 0.1g of NDF residue was weighed, 1.25 ml of 12 M H_2SO_4 were added, and the tubes were vortexed for 45 min. Next, 13.5 ml H_20 was added and the tubes were placed in a 100˚C water bath for 3 hours. When cool, 3.2 ml 15 M ammonia was added to neutralize the solution and 10 mg myo-inositol was added to the hydrolysate. A final volume of 20 ml was reached by adding 1 M ammonia. High pressure liquid chromatography (HPLC) was used to determine component sugars.

Once proportions of each monosaccharide in the NDF residue were determined, the percentage of pentose and hexose sugars in hemicellulose could be calculated:

Xylose + Arabinose + Galactose + Mannose = Hemicellulose

(Xylose + Arabinose)/Hemicellulose = Pentose Proportion

(Galactose + Mannose)/Hemicellulose = Hexose Proportion

From these proportions, a modification of the NREL TEP equation was created to predict TEP based on detergent fiber analysis:

H (hexose sugars) = (Cellulose + Hemicellulose * Hexose Proportion) *172.82

P (pentose sugars) = (Hemicellulose) * Pentose Proportion) *176.87

TEP ($l\ t^{-1}$) = (H + P)*4.173.

Estimations of TEP were then used to calculate ethanol yield on a per land unit basis. Theoretical ethanol potential ($l\ t^{-1}$) was multiplied by the dry matter yield ($t\ ha^{-1}$) of stover, husks and cobs to obtain theoretical ethanol yield (TEY) ($l\ ha^{-1}$) of stover, husks, and cobs, respectively.

Data analysis

The data for the individual environments were analyzed as a randomized complete block design and then combined over environments using the MIXED procedure (SAS Institute, 2003). A residual analysis was performed on the individual environment analyses to detect outliers, (Anscombe and Tukey, 1963). Entries were considered fixed effects in the analysis and all other effects were considered random. Entry means were used to calculate Pearson correlation coefficients between traits. An LSD (0.05) was calculated using the genotype x environment error mean square to compare genotype means. All tests of significance were made at the $\alpha=0.05$ p level unless otherwise noted.

Results and Discussion

The average ratio of pentose and hexose sugars in the hemicellulose for all plant fractions was estimated at 93:7, ranging from 89:11 to96:4, where pentose sugars accounted for 93% of the hemicellulose and hexose sugars accounted for 7%. This was expected, as Lee et al. (2007) estimated xylose and arabinose to account for 93% of the hemicellulose fraction. This is further validated as Vermerris et al. (2007) reported that the primary monosaccharides in grasses, other than cellulose are the pentose sugars, xylose and arabinose.

Average TEP predictions from NREL were 419, 371, and 495 l t^{-1}, for 2005 stover, 2006 stover, and 2006 husk samples, respectively (Table 2). Average TEP predictions from detergent fiber calculations were 485, 445, and 615 l t^{-1}, respectively. Average TEP values for the detergent fiber predictions were higher than the NREL predictions for all plant fractions. Therefore, it can be assumed that the detergent fiber method for calculating TEP overestimates the values when compared to the NREL method. This could be largely due to laboratory error associated with the NREL predictions as well as with the detergent fiber predictions. Hames et al. (2003), reported corn stover TEP to range from 438 to 496 l t^{-1}, which is more comparable to the detergent fiber predictions than the NREL predictions of our stover samples. Significant variation was observed among hybrids and among environments evaluated for TEP values predicted by both NREL and detergent fiber methods.

Correlations were significant among the two methods for calculating TEP for all plant fractions evaluated (Table 3). Stover fractions from 2005 had the highest correlation between methods (r = 0.75), while husks had the lowest correlation (r = 0.49). The two methods for calculating TEP had a correlation of r = 0.68 when stover samples from the 2006 growing season were evaluated. In a study done by Vogel et al. (1999), the filter bag system of forage analysis was evaluated and compared to traditional in vitro dry matter digestibility (IVDMD). They found that the two methods produced similar results and ranked samples in relatively the same order. Significant Pearson correlation coefficients between the two methods in the study ranged from r = 0.73 to r = 0.98. The correlations found in our data among TEP calculations from NREL and TEP calculations based on detergent fibers suggest that there is a relationship between the two methods, and that genotype ranks for TEP predictions between the two methods are comparable. However, the correlations for the 2006 stover and husks are significant, but lower. The rejection rate of the stover and husk samples from 2006 was 40% using the NREL calibration, suggesting that the NREL calibration was unable to account for the amount of variability in the 2006 samples. This may account for the lower correlations between prediction methods observed in 2006.

The TEP predictions for both methods of calculation were significantly correlated with NDF, ADF, and lignin constituents for both years of stover harvest, except for lignin in 2006. This is important, as neutral detergent fiber estimations have a significant relationship with both TEP methods. Therefore, it may be possible to predict TEP using detergent fiber methods. Since the relationships are positive, it may also be possible to increase TEP by targeting higher NDF and ADF values. Theoretical ethanol predictions for both methods were not significantly correlated to any chemical constituents for husk samples. This may be a result of decreased number of entries included in the analysis. In this analysis only 67% of the entries were included due to the lack of data reported from NREL. In the original husk analysis, when all entries were included, significant correlations were found among TEP calculated with detergent fiber methods with NDF, cellulose, and hemicellulose (Kirkpatrick et al., 2008c).

Conclusions

From these data it can be concluded that detergent fiber methods may be a convenient way to estimate component sugars needed to calculate TEP. The predictions from detergent fiber methods tended to over estimate TEP compared to the NREL method. This should not be of concern to the breeder as we are more interested in genotype rank than in precise values. The TEP estimates are in fact “theoretical” and depending on the infrastructure, ethanol potential may never be reached. Although, the predictions from the two methods differ, they bear a significant and positive correlation. Therefore, genotype rank is relatively the same when calculating TEP using either method. The stover samples analyzed from 2005 resulted in the highest correlation between the two methods. Samples from 2006 showed lower correlations between methods, possibly due to the high number of samples that were determined to be unpredictable by the NREL methods. The two methods for predicting TEP are related, but it is not possible to assume that one method more accurately predicts TEP than the other. The best way to determine the accuracy of the TEP methods may be to compare TEP calculations to an actual bench-top fermentation process.

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Table 1. NIR calibration statistics for 2005 stover samples (a), 2006 stover samples (b), and husk samples (c).

(a)

Trait†	Mean	SD‡	N§	R2	SEC¶	SECV#
NDF	66.4	6.13	28	0.97	0.98	1.76
ADF	37.0	4.84	30	0.98	0.69	1.00
Cellulose	34.0	4.33	30	0.99	0.51	0.89
Hemicellulose	28.4	2.66	30	0.90	0.83	1.23
Lignin	2.4	0.57	30	0.95	0.13	0.18
Solubles	33.6	6.13	28	0.97	0.98	1.76

(b)

Trait†	Mean	SD‡	N§	R2	SEC¶	SECV#
NDF	62.6	5.30	29	1.00	0.33	0.52
ADF	36.3	3.80	29	0.99	0.32	0.59
Cellulose	32.4	3.21	28	1.00	0.23	0.56
Hemicellulose	26.0	2.15	30	0.99	0.18	0.49
Lignin	2.1	0.48	28	0.98	0.08	0.14
Solubles	37.6	5.29	28	0.99	0.41	0.65

(c)

Trait†	Mean	SD‡	N§	R2	SEC¶	SECV#
NDF	85.2	1.24	27	0.94	0.30	0.54
ADF	42.8	1.54	25	0.98	0.22	0.45
Cellulose	41.6	1.61	28	0.99	0.18	0.51
Hemicellulose	41.9	1.96	29	0.95	0.46	0.93
Lignin	1.2	0.35	30	0.95	0.08	0.19
Solubles	14.8	1.24	27	0.94	0.30	0.54

† NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.
‡SD = standard deviation.
§N = final number of data points used in NIRS calibration equation.
¶SEC = standard error of calibration.
#SECV = standard error of cross validation.

Table 2. Mean theoretical ethanol potential (TEP) (l t^{-1}) values for NREL and detergent fiber methods.

	Detergent fiber 2005 stover	NREL 2005 stover	Detergent fiber 2006 stover	NREL 2006 stover	Detergent fiber husk	NREL husk
N†	45	45	22	22	29	29
Mean	485	419	445	370	615	495
Minimum Mean	463	403	417	349	594	477
Maximum Mean	500	436	481	412	631	507
σ^2	100	70	302	284	60	41

† Number of entries included in the analysis

Table 3. Table of Pearson correlation coefficients for quality traits in 2005 stover samples (a), 2006 stover samples (b), and husk samples (c).

(a)

	NDF†	ADF	LIG	TEPDF	TEPNREL
NDF	1.0				
ADF	0.91***	1.0			
LIG	0.72***	0.85***	1.0		
TEPDF	0.96***	0.89***	0.61***	1.0	
TEPNREL	0.68***	0.77***	0.59***	0.75***	1.0

(b)

	NDF	ADF	LIG	TEPDF	TEPNREL
NDF	1.0				
ADF	0.94***	1.0			
LIG	0.59**	0.63**	1.0		
TEPDF	0.93***	0.79***	0.61**	1.0	
TEPNREL	0.64**	0.51*	0.38	0.68***	1.0

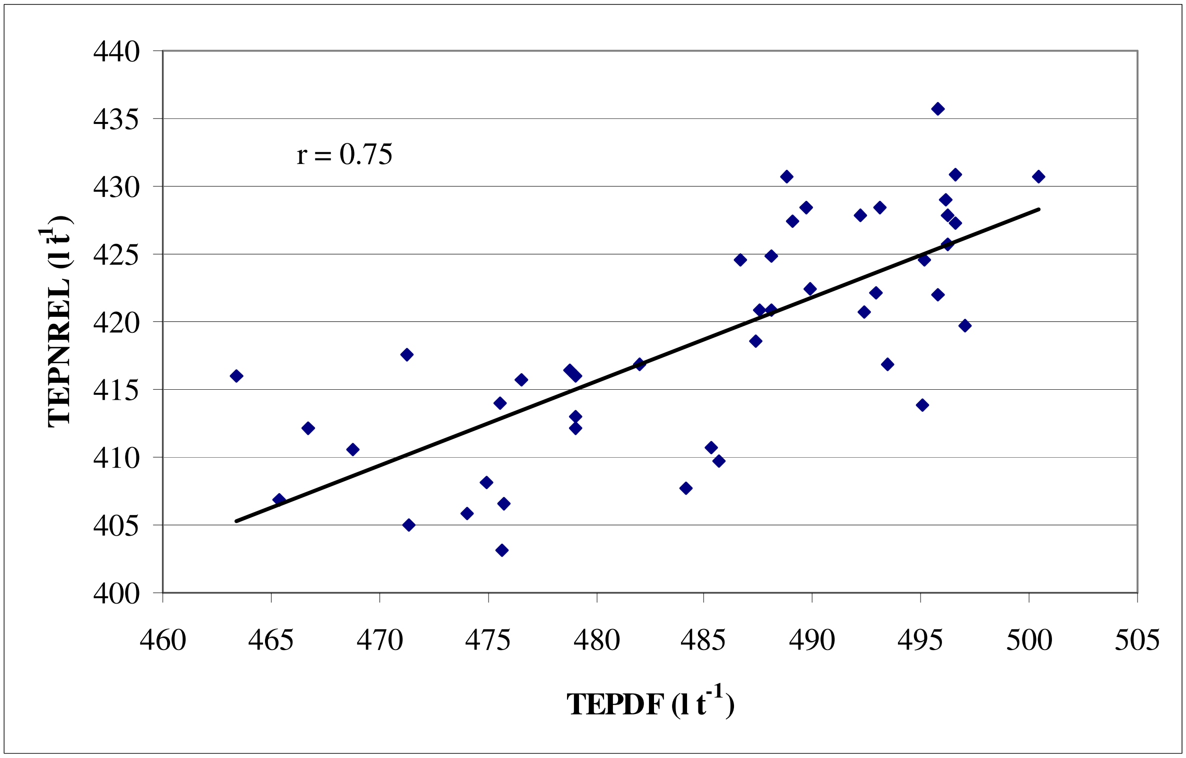
(c)

	NDF	ADF	LIG	TEPDF	TEPNREL
NDF	1.0				
ADF	0.74***	1.0			
LIG	0.37	0.29	1.0		
TEPDF	0.33	-0.03	-0.13	1.0	
TEPNREL	0.30	0.12	-0.04	0.49**	1.0

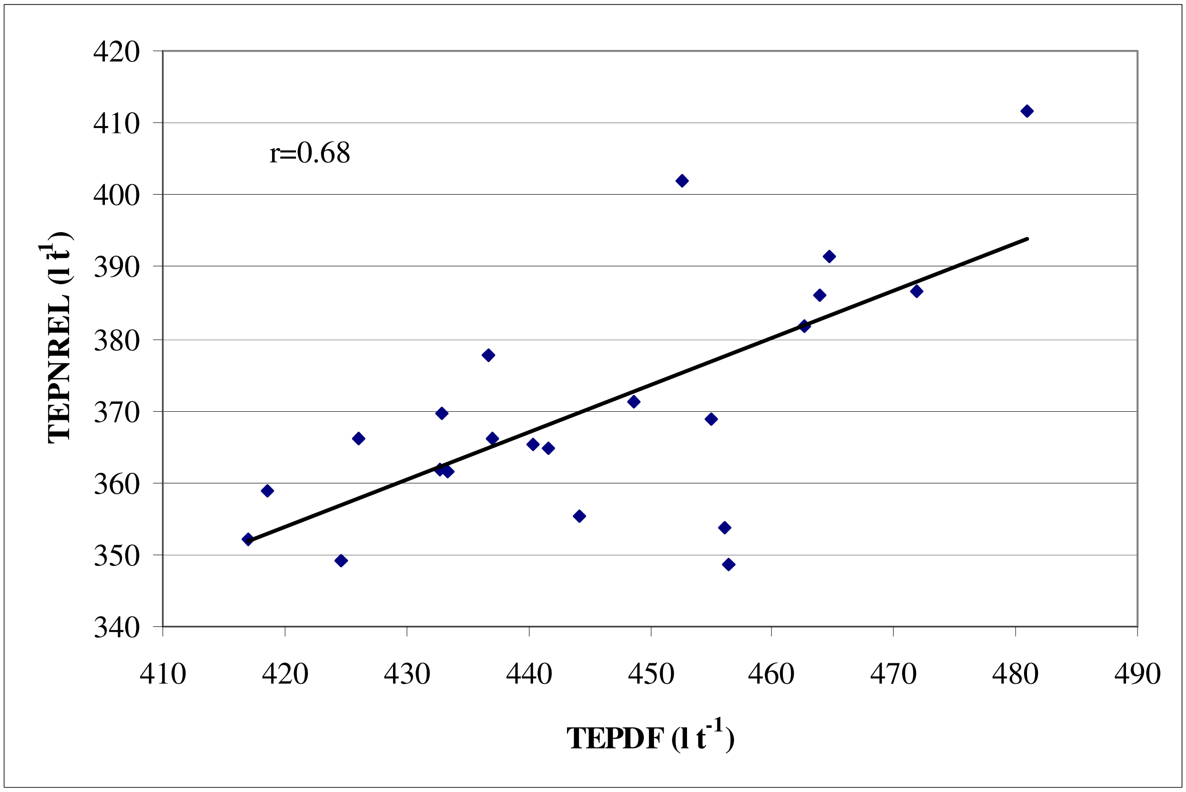
*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively

† NDF, neutral detergent fiber; ADF, acid detergent fiber; LIG, lignin; TEPDF, theoretical ethanol potential calculated by detergent fiber method; TEPNREL, TEP calculated by National Renewable Energy Lab methods.

(a)


r = 0.75
TEPNREL (l t-1)
TEPDF (l t-1)
440
435
430
425
420
415
410
405
400
460
465
470
475
480
485
490
495
500
505

(b)


r=0.68
TEPNREL (l t-1)
TEPDF (l t-1)
420
410
400
390
380
370
360
350
340
410
420
430
440
450
460
470
480
490

(c)

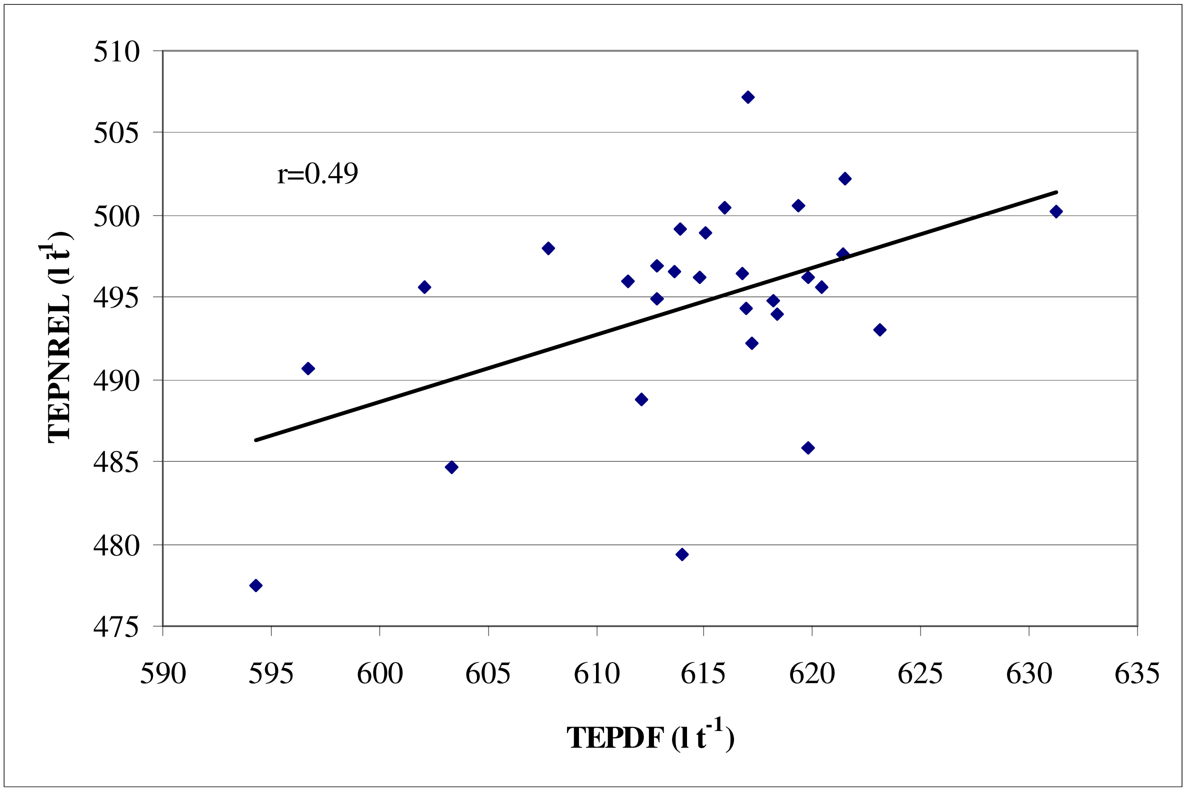


Fig. 1. Scatter plots of the National Renewable Energy Lab (NREL) method for calculating theoretical ethanol potential (TEPNREL) versus the detergent fiber method for calculating TEP (TEPDF) for 2005 stover samples (a), 2006 stover samples (b), and husk samples (c).

CHAPTER 7. GENERAL CONCLUSIONS

In order to evaluate corn stover fractions for their potential use in the cellulosic ethanol industry, a relatively quick and inexpensive method is needed to measure chemical composition. The results from our study indicate that detergent fiber methods may be just as efficient if not better than the stover equation developed by the National Renewable Energy Lab (NREL). Since detergent fiber methods (Goering and Van Soest, 1970) have been widely used to evaluate forage digestibility, they are already well established. Coupling the wet chemistry with Near Infrared Reflectance Spectroscopy (NIRS) calibrations, creates a relatively quick and easy way to predict the chemical composition of corn stover, husks, and cobs, which can then be used to estimate their ethanol potential.

Theoretical ethanol potential values for the plant fractions that are presented in this research may not be attainable without 100% conversion efficiency of an ethanol plant. The theoretical ethanol potential values presented assume that all available sugars can be converted into ethanol. However, in reality, this may not be the case. Since the TEP data has never been compared to pretreatment and fermentation methods implemented on a large scale, projected numbers may not be attainable. They do have some value, however, as variation among genotypes can be detected and genotypes can be ranked.

Corn cob and husk fractions may be the best fractions of corn residue to utilize as a feedstock for large scale cellulosic ethanol production. As indicated by Hoskinson et al. (2007), these fractions may be easier to harvest, and require fewer modifications to current harvest equipment, as they already pass through combines that harvest corn grain. Cobs and husks have lower dry matter yields than stover and therefore, less dry matter removal is associated with their harvest. Transportation of cob material may also be more efficient than other corn residue, as cobs have a higher bulk density than stalks, leaves, and husks. Finally, as indicated by our results, cobs and husks have higher theoretical ethanol potential than stover (stalks and leaves). However, based on our results, dry matter yields have been shown to have much more influence on potential ethanol yields over a per land unit area, than chemical composition. From this perspective, the stover fraction seems to be the most beneficial, yielding about four times as much potential ethanol per hectare. However, the economics and sustainability of implementing corn stover harvest may not be possible. All plant fractions were influenced by environment and growing conditions. Stover and cob fractions had higher dry matter yields when harvested at an earlier harvest date and higher moisture content. However, TEP increased when plants were harvested at a later harvest date and lower moisture content. These observations reflect regular senescence and field dry down.

The best option for the utilization of corn stover feedstocks for cellulosic ethanol production, based on our data and the literature, is from corn cobs and husks. Cob dry matter yields showed variation among genotypes and were correlated to grain yields. Since high grain yield is already a main trait of interest for corn breeders, it is likely that genotypes with high cob yields have been selected in conjunction. In addition, average shelling percentages are fairly constant, indicating that cob yield can be estimated from grain yield. There are some exceptions to this correlation, however. These exceptions may be further examined by evaluating kernel row numbers and ear diameters and comparing them to cob dry matter yields. Ear husks yield about half the amount of dry matter that can be expected from cobs. However, they are similar to cobs in ethanol potential and harvest advantages.

References

Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). Agric. Handb. 379. U.S. Gov. Print. Office, Washington, DC.

Hoskinson, R.L., D.L. Karlen, S.J. Birrell, C.W. Radtke, and W.W. Wilhelm. 2007. Engineering, nutrient removal, and feedstock conversion evaluations of four corn stover harvest scenarios. Biomass and Bioenergy 31:126-136.

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