MANAGEMENT PRACTICE EFFECTS ON CORN GRAIN ETHANOL YIELD AND ETHANOL BYPRODUCT QUALITY

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

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And hereby certify that in their opinion it is worthy of acceptance.

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LITERATURE REVIEW

Introduction

Corn (*Zea mays* L.) is one of the most important food and feed commodities in the world. The United States accounted for approximately 41% of world corn production during fiscal year 2009 (ERS/USDA, 2010). In 2009, the United States produced 333.3 million megagrams of corn on over 32.2 million hectares, making corn the number one commodity in both area planted and quantity produced nationally (USDA, 2010). Missouri farmers produced 11.4 million megagrams of corn in 2009, ranking the state tenth in total production nationally (USDA, 2010).

Due to the Renewable Fuel Standard (RFS1) that was established under the Energy Policy Act of 2005 (EPAct), corn used for ethanol has significantly increased. Nearly 35% of the total domestic crop was used to produce ethanol for fuel in 2009 (ERS/USDA, 2010). The revision of the Renewable Fuel Standard (RFS2) under the Energy Independence and Security Act of 2007 (EISA) requires the production and use of 136.08 billion liters of renewable fuels by the year 2022, quadrupling the requirement of 34.02 billion liters for 2008 (EPA, 2010). Although portions of the increase will come from other sources of biofuel, a significant portion will still be produced using corn.

History of Corn

Corn is a warm-season, monoecious grass plant of the family Poaceae and is thought to have originated in the Tehuacán Valley in Mexico. The use of the word “corn” is a loose term used in the United States for the plant maize. The word corn was originally used to mean grain and was given to the most common grain of a given area

(Walden, 1966). In England and Germany, wheat (*Triticum spp* L.) is given the name corn because it is the most common grain in both countries (Blake and Cutler, 2001). Corn has also been used as a word for oats (*Avena sativa* L.), rye (*Secale cereale* L.) and barley (*Hordeum vulgare* L.) to name a few. For the purpose of this thesis, corn will be used as a reference to maize.

It is believed that corn originated in west central Mexico because of the large populations of teosinte (*Z. mexicana*) and *Tripsacum* that were abundant in that area (Blake and Cutler, 2001). The oldest dated archaeological finding of corn remnants was found in the Tehuacán Valley in Mexico. Prior to 1984, these remnants were believed to date back to about 5000 BC (Mangelsdorf, 1974) using date by association because of a small sample size. However, this date has been determined to be inaccurate through the use of the more recent accelerator (AMS) dating technique. Using this technique, the sample from the Tehuacán Valley was dated at no older than 3600 BC (Long et al., 1989). By this date, corn had already been specialized to the extent that it was dependent upon humans for seed dissemination (Benson and Pearce, 1987)

After domestication in Mexico, corn spread north to what is now Canada, and south to what is now Argentina (Benson and Pearce, 1987). Native American populations served as a tool for the migration of corn into North America. Carl Sauer's map from 1941 shows the movement of the Native Americans northward through Mexico and into the future USA states of Arizona and New Mexico before heading east (Blake and Cutler, 2001). In 1492, two members of Christopher Columbus's crew returned with corn after exploring the interior of Cuba (Walden, 1966). Following the discovery of the Americas, corn moved quickly to Europe, Africa, and Asia (Benson and Pearce, 1987).

Most of the modern corn races can be traced back to Mexico, Central America, and South America. However, one race can be traced solely to postcolonial North America: Yellow Dent Corn (Benson and Pearce, 1987). Yellow Dent Corn is the primary race planted in the US Corn Belt, Canada, and much of Europe.

Uses of Corn

For many years, corn has been the most important crop in the USA (Wallace and Brown, 1988). Its volume of production and value leads all other crops. Corn is also one of the more versatile crops. When corn was first domesticated, its primary use was for food. Today corn is used in a wide variety of ways. It has industrial uses, such as plastics, paints and adhesives. It also has food, drug and cosmetic uses, including sweeteners along with the manufacture of antibiotics and aspirin. But, the primary use of corn in the USA is animal feed. In 2009, feed and residual use consumed approximately 41% of the total supply of corn in the United States (ERS/USDA, 2010).

One corn product that, in recent years, has increased its share of total use is ethanol. In the ten years from 1997 to 2007 corn use for ethanol increased from approximately 14.3 million megagrams to 115.7 million megagrams (ERS/USDA, 2010), an increase of approximately 800%. Nearly 35% of the total domestic crop was used to produce ethanol for fuel in 2009 (ERS/USDA, 2010).

Currently, there are two primary methods of producing ethanol from corn, wet milling and dry grind. In the wet milling process, ethanol is not the only high-value product produced. Corn is first separated into several component parts, including starch, fiber, gluten, and germ (RFA, 2008). The separated parts can then be further processed into products including corn oil from the germ, a fiber feed product, gluten meal and one of several starch based products, including ethanol (RFA, 2008).

Most recently built ethanol plants are the dry grind type. This is due to the reduced initial cost of the processing facility and the increased ethanol produced per bushel of corn (Bothast and Schlicher, 2005). Unlike wet milling, in dry grind the entire kernel is ground into meal (RFA, 2008). Enzymes, such as alpha-amylase, produce dextrose from the starch in the meal. Yeast converts the sugars to ethanol that is then distilled and dehydrated, creating fuel grade ethanol. The remaining solids are dried producing distillers' dried grains with solubles (DDGS). Dry grind ethanol production yields 30% of the initial corn weight in DDGS (Bothast and Schlicher, 2005). DDGS are marketed primarily for use in animal feed rations as a source of crude protein, crude fat and crude fiber. Ruminant animals, such as cattle, are the primary market for DDGS because cellulose present in the fiber component is not digestible by monogastric livestock such as poultry and swine. Swine fed DDGS in their diets may require additional supplements of lysine, typically the first limiting amino acid in swine diets, due to the relatively low lysine content in the crude protein component of DDGS.

There has been controversy on whether or not ethanol from corn produces a net gain in energy. To determine the energy balance of ethanol from corn, the United States Department of Agriculture (USDA) calculates the amount of energy in ethanol, subtracts energy used to produce and apply all inputs used for corn production on the farm, subtracts energy used for the transportation of corn to the ethanol facility, subtracts energy for ethanol production, subtracts energy used for ethanol distribution, and adds a credit for estimated energy values of the coproducts. In a report from the USDA, Shapouri, et al. (2002) estimated that production of ethanol from corn yields 34% more energy than it takes to produce it. This is similar to the results of Hill, et al. (2006) who reported a net positive energy balance of 25%. However, Pimentel (2003) reported a net negative energy balance of about 29%, meaning that it took more energy to produce ethanol than the amount of energy gained. Pimentel's study also included energy required in the production of steel, cement and other materials used to construct the ethanol plant, which are not components included in most studies. He also used lower corn yields and higher nitrogen rates than most other investigators. One of the most significant factors in determining the net energy value of corn-derived ethanol is the amount of ethanol produced from each kilogram of corn. As the amount of ethanol produced from a kilogram of corn increases, the net energy value of the production of ethanol from corn also increases.

Kernel Physical Characteristics

A kernel of corn is comprised of four main parts, embryo, endosperm, pericarp and tip cap. Endosperm makes up the majority of the kernel, at approximately 82% of kernel dry weight. The embryo, pericarp and tip cap account for approximately 10, 5 and 1% of kernel dry weight, respectively (Earle et al., 1946).

The endosperm is composed of cells containing starch granules surrounded by a protein matrix that contains protein bodies. Cells are the largest in the center of the kernel and get progressively smaller toward the outer part of the kernel. There are two types of endosperm, vitreous (hard) endosperm and floury (soft) endosperm (Watson, 1987).

Soft endosperm is located in the central core of the kernel and is foggy to white in appearance. Duvick (1961) suggested this coloration is the result of the shrinking and tearing of the protein matrix around starch granules as the kernel dries leaving air pockets in which light is refracted. The starch granules in the soft endosperm are round in shape and are surrounded by a thin and non-continuous protein matrix (Watson, 1987).

The hard endosperm is located on the outer part of the kernel and surrounds the central core of soft endosperm. It is more translucent to light than soft endosperm. Because of a thick and fully intact protein matrix after drying, the starch granules take on a polyhedral shape as they compress during drying (Watson, 1987). According to Hamilton, et al (1951), genetic and environmental influences can have significant effects on the ratio of hard to soft endosperm. An example of an environmental effect is soil nitrogen. Corn produced on soils high in nitrogen produce kernels with a higher than normal hard to soft endosperm ratio (Hamilton et al., 1951). The ratio of hard to soft endosperm can also vary depending on the position of the kernels on the ear (Watson, 1987).

During the dry grind process, the two endosperm types react differently. The soft endosperm is more likely to break at the protein matrix releasing starch granules free of protein; whereas, the hard endosperm tends to break across the starch granules resulting in damaged granules that are highly associated with protein from the protein matrix (Watson, 1987). This may explain why hybrids with high ethanol yield contain starch granules that are loosely packed with a protein matrix that is smooth, continuous and fragile. Low ethanol yielding hybrids have starch granules that are tightly packed with a protein matrix that is irregular, thicker and has a high density of globular structures (Ubach et al., 2007). Singh and Graeber (2005) observed a weak negative correlation between ethanol concentration and corn kernel density. Kernel density has been shown to be positively correlated with the ratio of hard to soft endosperm (Li et al., 1996).

The embryo, pericarp and tip cap make up a relatively small portions of the kernel dry weight. The embryo stores nutrients and hormones and consists of a single cotyledon (scutellum) and the embryo axis. The pericarp is the thin outermost kernel membrane that originated from the ovary wall. In grass plants such as corn, the pericarp is fused tightly to the seed coat and cannot be separated. The pericarp extends to the end of the kernel were it is connected to the tip cap. The tip cap connects the kernel to the cob through vascular bundles. Although the embryo, pericarp and tip cap contain a small percentage of a kernel's total starch content, they may have a significant effect on ethanol efficiency and yield. Murthy, et al. (2006) found that the removal of the embryo and the pericarp from the kernel before fermentation, using a dry degerm, defiber (3D) process, resulted in reduced ethanol efficiency and yields. They suggested that lipid content, which is abundant in the embryo, is important to the health of the yeast responsible for fermenting corn into ethanol and an abundant supply of lipids can improve fermentation rates and final ethanol concentrations.

Kernel Chemistry

The corn kernel, botanically known as a caryopsis, is a dry, one-seeded fruit in which the fruit wall and the seed are fused to form a single grain. The composition of a normal dent corn kernel consists of four primary substances: starch, protein, lipid and fiber.

In the United States, corn is the most important and economical source of starch. Starch can be easily converted to glucose and fermented into ethanol, making corn a good source for ethanol production. Starch is the major carbohydrate storage product in the corn kernel, comprising approximately 71% of kernel dry weight (Inglett, 1970). Although this percentage can vary, starch content may not be the primary factor that determines ethanol yield or ethanol production efficiency from corn. Ethanol yield varied as much as 7.4% in sorghums (*Sorghum bicolor* L.) with similar starch contents, and Wu, et al. (2007) concluded that factors other than starch content affected the conversion process to ethanol. Dien, et al. (2002) also concluded that ethanol yield is not exclusively dependent on starch content and that the chemical structure of starch and the starch-protein matrix may affect starch availability to enzymes responsible for conversion to dextrose. Singh, et al. (2005) found a positive correlation between starch percentage and percent extractable starch. However, no correlation was observed between starch extractability and final ethanol concentration by Singh and Graeber (2005).

Approximately 98% of the kernel's total starch content is located in the endosperm (Earle et al., 1946). A starch granule is made up of two glucan polymers, amylose and amylopectin. Amylopectin is a branched molecule that may contain 40,000 or more glucose units. Amylose is a straight chain of about 1000 glucose units (Inglett, 1970).

In typical dent corn, amylose comprises approximately 27% of the starch granule while amylopectin constitutes the remaining 73% (Senti and Dimler, 1959). Hybrids differ for amylose to amylopectin ratios. Starch granules with greater than 35% amylose are called high-amylose starch, whereas; granules with less than 5% amylose are referred to as waxy (Tester et al., 2004). The amylose content of starch has significant effects on ethanol conversion efficiency and ethanol yield. As amylose content increased from 18 to

52%, fermentation efficiencies decreased (Wu et al., 2006). There was a correlation between increased amylose content and increased insoluble particles from corn starch (Wu et al., 2006). With low amylose content, the use of waxy corn for ethanol production may decrease processing time and increase ethanol yields (Sharma et al., 2007). However, Wu et al. (2006) concluded that there was no significant effect on fermentation efficiency when amylose content was less than 30% in starch.

Corn provides a significant amount of protein in many livestock and poultry diets, but it is low in feed value due to inadequate quantities of two essential amino acids, lysine and tryptophan. For this reason, corn is typically considered an energy type feed and is supplemented with protein from other sources that contain lysine and tryptophan (Perry, 1988).

Protein constitutes approximately 10% of total kernel dry weight (Perry, 1988). Seventy-four percent of the protein in a kernel of corn is located in the endosperm (Earle et al., 1946). In the endosperm, protein is located in both an amorphous protein material, called the protein matrix, and granular protein bodies embedded in the protein matrix (Duvick, 1961). Protein bodies are largest in the subaleurone cells and get progressively smaller from the outer portion to the inner part of the endosperm.

Protein bodies are a major site of zein according to early studies on zein development completed by Duvick (1961). Nearly 47% of the total protein in the kernel is comprised of zein (Perry, 1988). Zein is a protein fraction that is extremely low in lysine and tryptophan, but forms a moisture-resistant barrier making it useful in coatings for various products, such as pharmaceuticals. Zein may prevent starch from dissociating itself from protein through non-covalent bonding and hydrophobic interactions resulting in less starch exposed for enzymatic conversion to dextrose (Jurado and Das, 2008).

In studies using corn or small grains, several investigators have found that as kernel protein content increases, kernel starch content decreases (Fox et al., 1992; Lacerenza et al., 2008; Singh et al., 2005; Wu et al., 2007). Although a change in protein content may not have an effect on fermentation efficiency (Wu et al., 2007), it may reduce the final ethanol yield (Lacerenza et al., 2008; Wu et al., 2007). However, a study completed by Singh and Graeber (2005) on various corn hybrids at multiple locations showed no significant correlation between ethanol yield and protein content.

Lipids, or corn oil, comprise approximately 4.3% of a kernel's dry weight, although this percentage can vary as much as 0.6% depending on the kernel position on the ear (Lambert et al., 1967; Weber, 1978). Approximately 80% of lipids in a corn kernel are found in the embryo, constituting around 32.5% of the embryo's dry weight (Weber, 1978). A much smaller percentage of a kernel's lipids are found in the endosperm (Weber, 1978). Lipid content showed no significant correlation ($r = 0.129$) with ethanol concentration (Singh and Graeber, 2005). However, as stated earlier, removal of the embryo resulted in reduced ethanol efficiency and yield (Murthy et al., 2006). Murthy, et al. (2006) suggested lipids in the embryo maintained yeast cell membrane integrity under anaerobic conditions.

Fiber makes up a small percentage of total kernel composition. Fiber along with protein and oil are considered non-fermentables and are recovered at the end of the dry grind process as a mixture called DDGS. Corn fiber content does not have an effect on ethanol conversion efficiency (Wu et al., 2006), but may affect ethanol yields (Wu et al., 2007). This reduction in ethanol yield may be due to a reduction in the percentage of total fermentables per kernel. Fiber yield was positively correlated with grain protein content ($r = 0.57$), which may be due to poor separation of the endosperm from the bran (Fox et al., 1992).

Corn Management

The primary motivation for changing management practices, for most producers, is to maximize yield and profits. This may include adjusting planting population, planting date, nitrogen rate, and hybrid selection, among others. Little consideration has been given by producers on how their management practice decisions affect physical and chemical components of a corn kernel or ethanol production efficiency.

Corn plant densities have more than doubled from approximately three plants per square meter in the 1930's to approximately seven plants per square meter in the 1990's (Duvick et al., 2004). There are no set recommendations on plant population applicable to the entire corn growing region of the USA because optimum densities vary depending on the soil type, water and nutrient availability, hybrid selection and planting date, among others (Sangoi, 2001). In studies completed in various locations throughout the corn belt, grain yields increase as plant population increase when favorable growing conditions are present (Ahmadi et al., 1993; Porter et al., 1997; Staggenborg et al., 1999). However, yields were reduced by increased population when adverse growing conditions were present (Porter et al., 1997).

Increasing planting density reduces the amount of area a plant has in which to develop. This may reduce the amount of water available per plant increasing the chance of a water deficit during the growing season. Water deficits may decrease yield and affect kernel characteristics related to ethanol yield. When a deficit occurs during flowering and the kernel development stage, a significant reduction in yield, kernel number per ear and kernel size can be expected (Claassen and Shaw, 1970). A reduction in kernel growth affects the apical portion of the ear more significantly than it affects the middle and basal portions of the ear (Claassen and Shaw, 1970). Starch accumulation in the endosperm of the apical kernels was significantly reduced when the plant was exposed to a water deficit during the first 15 days after pollination. Kernels on other positions of the ear were changed to a lesser extent (Ober et al., 1991).

Several studies have investigated the effects of planting population on kernel physical and chemical properties, but results have been contradictory. Ruffo et al. (2007) found that an increased plant population can have a negative effect on protein concentration while increasing grain extractable starch. However, Singh et al. (2005) reported no significant effects of planting population on protein content, extractable starch, oil content, or starch content. An increase in population can have a negative effect on final kernel weight (Baenziger and Glover, 1980; Borrás and Otegui, 2001). Kernel weight has been shown to be correlated with a decrease in starch concentration (Borrás et al., 2002).

For the entire corn belt, the initiation of corn planting is currently two weeks earlier than it was in the 1970's (Kucharik, 2006). In Missouri, during the years spanning 2001 to 2005, the average for 10% of the corn crop being planted was April 4 (Kucharik, 2008). Planting date can have a significant impact on grain yield. Ahmadi, et al. (1993) reported a decrease in grain yield as planting date was delayed. Similar results were observed by Staggenborg, et al. (1999), however, a significant increase in yield was present between the first and second planting date as a result of drought and heat stress that occurred in late June and early July for one location-year. Few data exists which document an effect on kernel composition. Ahmadi, et al (1993) reported an increase in protein content as yield was decreased by delayed planting.

Corn that is planted early in the season may experience delayed emergence and slow growth due to less than ideal temperatures available early in the year. A longer growing season allows for planting of full-season hybrids that tend to produce higher yields than shorter-season hybrids (Lauer et al., 1999). Higher yields may increase the amount of ethanol produced for a given area and unit of corn.

Delayed planting may necessitate the harvesting of grain at a higher moisture level, requiring high temperature drying before storing. High drying temperatures may impact ethanol yield through a reduction in extractable starch available from the kernel (Paulsen et al., 2003). However, some hybrids were found to be more sensitive than others regarding harvest conditions and post harvest processes (Murthy and Singh, 2005).

Earlier planting reduces the risk of corn hybrids flowering when midsummer heat stress is likely to occur and allows plants to reach full maturity before a killing frost (Duvick, 1989). Temperature after pollination and during the kernel development stage can have a significant impact on kernel physical and chemical properties along with grain yield. High temperatures during the kernel-fill stage decreased the number and size of starch granules in the corn endosperm (Jones et al., 1985) and reduced kernel weight and density (Lu et al., 1996). In a similar study, a reduction in kernel weight was associated with a decrease in protein and zein content (Monjardino et al., 2005). However, an earlier study by Lu et al. (1996) found no significant changes in protein and starch in the kernel from high temperatures.

The amount of nitrogen fertilizer applied to a field can significantly impact corn kernel chemical properties and yield. An increase in nitrogen fertilizer rates is correlated with an increase in protein content and a decrease in extractable starch (Ruffo et al., 2007). Although kernel protein content may not have shown an effect on ethanol yield in a dry grind facility (Singh and Graeber, 2005), extractable starch is the method used to estimate ethanol production for a wet milling facility. Haefele, et al. (2004) reported a relatively small decrease in total fermentables for every increase in applied nitrogen compared to grain yield response. This suggests it is more appropriate to select a nitrogen rate based on maximizing grain yield than kernel properties when trying to maximize ethanol yield per acre.

Hybrid selection and planting location can impact corn kernel characteristics. In a study on grain sorghum, Wu, et al. (2008) observed a significant effect of location on physical properties, chemical composition and ethanol yield, but no effects on fermentation efficiency was present. Wu, et al. (2008) attributed higher starch content and lower protein content in sorghum to cooler climate extending the grain-filling period in Kansas versus Texas locations. Hybrid selection is probably the most significant and easiest to adjust factors involved when trying to grow corn for ethanol production. Singh and Graeber (2005) reported that corn hybrid had more of an impact on ethanol production than planting location. This suggests that selecting a corn hybrid that is marketed for high ethanol yield is more important than the planting location.

Summary

Most studies, until recent years, have focused on the nutritional aspect of corn production, in relation to food and feed instead of ethanol production. With a high percentage of corn being processed in ethanol plants, factors that affect corn ethanol efficiency and yield have become important topics. The objective of this study is to determine whether management practice methods of planting date, planting density or location affects corn grain yield, ethanol yield and kernel characteristics important in ethanol byproduct quality.

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CHAPTER II

THE EFFECT OF PLANTING DATE ON CORN GRAIN ETHANOL YIELD AND ETHANOL BYPRODUCT QUALITY

ABSTRACT

Management practice strategies for corn [*Zea mays L.*] have traditionally been focused on maximizing grain yield. Due to the dramatic increase in corn grain usage for ethanol production it is important to understand how corn management strategies affect ethanol yield and byproduct quality. Four hybrids in 2007 and six hybrids in 2008 were grown at Bradford Research and Extension Center near Columbia, MO using five planting dates to determine whether planting date has an impact on grain yield, ethanol yield and corn kernel characteristics. Whole kernel samples were measured for seed composition using a FOSS Infratec 1241 Grain Analyzer. Averaged across all hybrids, the variation between the highest and lowest yielding planting dates were 89% in 2007 and 50% in 2008. The June planting date recorded the lowest yields for both years. Planting date affected dry grind and wet mill ethanol yield along with starch, protein, and lipid concentrations; however the effect of delaying planting was inconsistent between years. Ethanol yield varied by 5.9% and 3.5% in 2007 and 2008 between all combinations of hybrids and planting dates using the dry grind method while wet mill ethanol yield varied less than 3% for both years. Kernel starch concentration was not a good predictor of ethanol yield while kernel protein concentration was positively correlated with the dry grind and negatively correlated with the wet mill ethanol yield for both years.

INTRODUCTION

Corn [*Zea mays* L.] is one of the top food and feed commodities in the world. Production of ethanol from corn has significantly increased partly because of the Renewable Fuel Standard (RFS1) that was established under the Energy Policy Act of 2005 (EPAct). The revision of the Renewable Fuel Standard requires the production and use of 136.08 billion liters of renewable fuels by the year 2022. A significant portion of the requirement will be accomplished by the production of ethanol from corn.

Two processes are primarily used to produce ethanol from corn, the dry grind process and the wet mill process. The most important distinction between the two processes is that the entire kernel is ground and processed during dry grind, whereas, the embryo is removed before wet mill processing. Because only starch is used to produce ethanol, protein and lipid constituents of the corn kernel remain after processing and are constituents of valuable byproducts. Distillers dried grains with solubles (DDGS) is the primary byproduct of dry grind. Gluten meal and gluten feed are byproducts of wet milling. All three products are marketed to the livestock industry as a source of protein and energy.

There is some evidence that kernel physical and chemical properties can affect ethanol yield and production efficiency. Starch is easily converted to glucose and fermented into ethanol. However, ethanol yield is often more dependent on characteristics of the starch-protein matrix and chemical structure of starch than on total amount of starch in the kernel (Dien, et al., 2002). Low ethanol yielding hybrids have starch granules tightly packed with a protein matrix that is irregular, relatively thick and possessing a high density of globular structures (Ubach, et al., 2007). In the dry grind process, tightly packed starch granules are often damaged and remain highly associated with protein from the protein matrix, which limits access of enzymes to starch (Watson, 1987). Nearly 47% of the total protein in the kernel is comprised of zein (Perry, 1988). Zein may prevent starch from dissociating from protein through non-covalent bonding and hydrophobic interactions resulting in less starch exposed for fermentation (Jurado and Das, 2008). Murthy, et al. (2006) suggested lipids maintain yeast cell membrane integrity under anaerobic conditions after observing a decline in ethanol efficiency and yield when the kernel embryo was removed before fermentation.

Corn management practices have primarily been focused on maximizing yield of grain per hectare with little attention given to corn kernel characteristics. Planting date is an important corn management strategy. Grain yield can be significantly reduced when planting date is delayed (Ahmadi, et al., 1993; Staggenborg, et al., 1999). High yields resulting from early planting have been associated with decreased protein content (Ahmadi et al., 1993). Earlier planting may also reduce the risk of corn hybrids flowering when midsummer heat stress is likely to occur (Duvick, 1989). High temperatures during the kernel-fill stage of ear development, decreases the number and size of starch granules in the corn endosperm (Jones, et al., 1985) while reducing kernel weight and density (Lu, et al., 1996).

Increasing ethanol yield from corn is essential to the ability of farmers to fulfill ethanol mandates while also meeting other important demands for corn. In order to develop appropriate corn management strategies we need to understand how those decisions affect ethanol yield from corn. The objective of this study was to determine the effects of an important corn management practice, planting date, on ethanol production and by product quality.

MATERIALS AND METHODS

This experiment was conducted in 2007 and 2008 at the University of Missouri Bradford Research and Extension Center near Columbia, Missouri (N38º53’36.80”, W92º12’53.21”). Mexico silt loam (fine, smectitic, mesic, Aeric Vertic Epiaqualfs) is the predominant soil type in the plot area at this location.

Four yellow dent corn hybrids were selected for this study in 2007, and six hybrids were used in 2008. The 2007 hybrids were Mycogen brand 2T787, Pioneer brand 34A16, Garst brand 8353, and NK brand N70-T9. The 2008 study included these hybrids and two additional hybrids; Garst brand 83C55-3000GT and NK brand N70C-3000GT. The Garst and NK hybrids added in 2008 came from the same lineage as the Garst and NK hybrids used in 2007 with the addition of a corn rootworm (*Diabrotica spp.*) resistance event, MIR604. All hybrids were resistant to glufosinate (Liberty Link®) and possessed one of several genes for resistance to European corn borer (*Ostrinia nubilalis*, Hübner).

Planting dates in 2007 were 2 April, 20 April, 13 May, 29 May, and 13 June. Planting dates in 2008 were 7 April, 17 April, 6 May, 20 May and 12 June. The experimental design was a split plot with whole plots arranged in randomized complete block. Whole plots were planting dates and split plots were hybrids. Blocks were replicated four times.

Plots were planted without tillage using a four-row planter modified for planting plots. For both years, the previous crop was soybean [*Glycine max (L.) Merr.*]. Plots were four rows wide and 7.6 m long. Inter-row spacing was 0.76 m. Plots were overplanted and thinned to 59,305 plants/ha at the V3 stage of growth. Nitrogen fertilizer was surface broadcasted as ammonium nitrate at a rate of 185 kg N/ha. Application was made just prior to the third planting date in both years.

Before the first planting date in 2007, a tank mix of glyphosate [N-(phosphonomethyl) glycine], S-metolachlor [acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-,(S)], atrazine [2-chloro-4-ethylamino-6-isopropylamino-s-triazine], and AMS [sprayable ammonium sulfate/polymer combination] was applied for burndown and preemergence weed control. In 2008, the same tank mix was used with the addition of mesotrione [2-[4-(methylsulfonyl0-2-nitrobenzoy]-1, 3-cyclohexanedione]. In both years, glufosinate-ammonium (1-methoxy-2-propanol) was applied, as needed, for post emergence weed control.

No irrigation was necessary in either year. An automated weather station, located within one kilometer of the test site recorded daily high air temperature, low air temperature and precipitation. Before harvest, the center two rows of each plot were trimmed to 6.1 m in length. Plots were harvested using a plot combine and yield was adjusted to 15% moisture. During harvest, 0.5 kg samples were collected from each plot.

Laboratory Analysis and Ethanol Estimation

Grain samples were sent to Monsanto Company for analysis. Whole kernels were analyzed using a FOSS Infratec 1241 spectrometer that used near-infrared transmission to determine kernel composition. Each sample was tested three times and averaged for composition. This instrument was calibrated to Monsanto Company’s proprietary specifications to determine protein, starch, extractable starch and lipid concentrations. An additional variable was used by Monsanto Company personnel to provide an estimate for ethanol yield from the dry grind process. Ethanol yield for the wet milling process was estimated using Patzek's (2006) formula, which uses extractable starch to determine the theoretical ethanol yield. Ethanol yield from both processes are presented on a grain weight basis (L kg^{-1}).

Data Analysis

Analyses of variance were conducted using PROC GLM in SAS. Means were compared using an LSD ($P = 0.05$) if a significant F-test was found. Pearson correlation coefficients, using PROC CORR in SAS, were used to determine associations between two variables. Because the two years differed for hybrids and planting dates, data were not combined over the two years.

RESULTS AND DISCUSSION

Grain Yield

There were no hybrid by planting date interactions for grain yield in either 2007 (Table 2.1) or 2008 (Table 2.2), meaning hybrids reacted similarly to planting dates. In 2007, the greatest yield was obtained from the first two planting dates (2 April and 20 April), and yield declined with each later planting date. Ahmadi, et al. (1993) reported similar observations of reduced yields as planting date was delayed. Garst brand 8353 yielded more than the other hybrids, averaging 11086 kg ha^{-1} for the five planting dates, whereas, Pioneer brand 34A16 yielded the lowest average yield of 10083 kg ha^{-1}, a difference of about 10%.

In 2008, the 5 May planting date produced a greater yield than the 6 April planting date. The lower yields of the 6 April planting date may be attributed to cool and wet weather encountered after planting. Cool, wet soil may inhibit seedling emergence resulting in a reduced plant population. However, this was avoided in our study by overplanting plots and thinning to a consistent population across all planting dates. Swanson and Wilhelm (1996) attributed reduced yield in early planting dates to slowed emergence, plant growth and development due to cooler soil temperatures. As in 2007, the June planting date produced the least yield. Averaged across all planting dates, Garst brand 8353 produced the greatest yield in 2008, but its yield was not significantly different from Mycogen brand 2T787 or Garst brand 83C55-3000GT. As in 2007, Pioneer brand 34A16 produced the lowest average yield. No significant differences were observed between hybrids of the same genetic background with and without the corn rootworm resistance trait.

Changing planting date had a large affect on yield. Averaged across hybrids in 2007 and 2008, yield increased 89 and 50% from the planting date with the least yield to the planting date with the greatest yield. These large changes in yield indicate that the environment and the corn plants' reactions to that environment were affected by planting dates. This confirmed that planting date was an appropriate selection for determining if cultural practices affect ethanol production from corn grain.

Ethanol Yield

Dry Grind

The amount of ethanol produced per unit of grain weight is an important component for calculating energy balance, and it greatly influences efficiency and profitability of ethanol production facilities. The USDA uses a corn grain to ethanol conversion rate of 0.395 L kg^{-1} (Shapouri, et al., 2002). Our estimated ethanol yield from dry grind, averaged across all planting dates and hybrids, was 0.418 and 0.408 L kg^{-1} in 2007 and 2008, respectively. These yields are similar to (6 and 3% higher) the USDA figures.

A significant planting date by hybrid interaction for ethanol yield from the dry grind process was observed in 2007 (Table 2.3). Both planting date and hybrid affected dry grind ethanol yield. The difference for ethanol yield between the highest and lowest combinations of hybrids and planting dates was 5.9%. So, effects from planting date and hybrid were relatively small and much less than effects on grain yield. Ethanol yields for the two earliest planting dates were significantly less than ethanol yields from any of the later planting dates for all hybrids. The two planting dates, 2 April and 20 April, produced the least ethanol yields while producing the greatest grain yields. Differences among the four hybrids for ethanol yield were small, 2% or less, for each planting date. For one planting date, 14 May, hybrids did not differ. Pioneer brand 34A16, the lowest grain yielding hybrid, was among the hybrids with the greatest ethanol yield for each of the other planting dates.

In 2008, planting dates and hybrids did not interact for dry grind ethanol yield (Table 2.4). As in 2007, both planting date and hybrid affected ethanol yield, but the effects were small. The difference for ethanol yield between the highest and lowest combinations of planting dates and hybrids was only 3.5%. The planting date with the greatest ethanol yield (16 April) was only 0.006 L kg^{-1} higher than the planting date with the least ethanol yield (10 June). Pioneer 34A16, the hybrid with the least yield, produced the greatest amount of ethanol per kilogram.

Dry grind ethanol yield was negatively correlated with precipitation during the grain filling period in 2007, but not in 2008 (Table 2.5). The negative correlation in 2007 was unexpected. As planting date was delayed, precipitation accumulated during grain fill decreased to less than 50 mm for the last two planting dates (29 May and 13 June), potentially creating a water deficiency. Ober, et al (1991), reported a substantial decrease in starch accumulation when plants were subjected to water deficiency during the grain filling period. Less starch might reduce ethanol yield. In our study, larger ethanol yield was associated with less precipitation. Apparently, other factors that differed among planting dates were influencing ethanol yield in 2007.

Wet mill

The expected ethanol yield from corn grain using the wet mill process is approximately 0.372 L kg^{-1} (Bothast and Schlicher, 2005). Our estimated ethanol yields from the wet mill process, averaged over all hybrids and planting dates, were 0.371 and 0.381 L kg^{-1} in 2007 and 2008. Because our results were similar to numbers reported in the literature, we believe that use of the Patzek (2006) formula accurately estimates ethanol yield from the wet mill process.

Both planting date and hybrid affected estimated ethanol yield from the wet mill process, however, interactions were found between planting date and hybrid in both 2007 (Table 2.6) and 2008 (Table 2.7). The effects from both planting date and hybrid were small. Differences in ethanol yields between the highest and lowest planting date and hybrid combinations were only 2.5% and 2.9% in 2007 and 2008.

In 2007, all hybrids produced the lowest ethanol yield when planted on 13 June, the latest planting date in 2007. Ethanol yields for Mycogen brand 2T787, Garst brand

8353, and NK brand N70-T9 tended to decrease with delayed planting, although not every planting date was different from its preceding planting date. This trend differed from what we found for dry grind ethanol yield. In 2007, delayed planting tended to increase dry grind ethanol yield. Wet mill ethanol yields for Pioneer 34A16 increased through the first three planting dates then declined for the last two planting dates. In 2008, no hybrid exhibited a consistent effect of planting date on ethanol yield. In both 2007 and 2008, hybrids differed for ethanol yield for all planting dates, but no consistently low ethanol producing hybrid was evident.

Kernel Composition

Starch and Extractable Starch

In 2007, starch concentration increased with delayed planting (Table 2.8). Grain yield decreased with delayed planting, so factors associated with delayed planting that decreased grain yield resulted in small, but significant, increases in starch concentrations in the kernel. In 2008, the planting date with the greatest starch concentration was 5 May, the date that exhibited the largest yield (Table 2.9). The relationship between kernel starch concentration and grain yield in 2008 was much different than the relationship in 2007. Correlations between dry grind ethanol yield and starch concentration differed between years with a positive correlation in 2007 and a negative correlation in 2008 (Table 2.5). The negative correlation in 2008 was unexpected because starch molecules are long chains of sugars that are fermented into ethanol. Dien, et al. (2002) concluded that ethanol yield is not exclusively dependent on starch content. Wu, et al. (2007) reported grain sorghum hybrids produced different ethanol amounts even though they did not differ for kernel starch content. It is clear from our results and those from other researchers that starch concentration alone cannot predict the ethanol output of a grain sample.

Extractable starch is an estimate of the amount of starch available for ethanol production in the wet milling process. Because extractable starch is the only variable used in the Patzek (2006) equation, we chose not to present extractable starch data, because planting date and hybrid effects would be identical to those presented for ethanol yield from the wet mill process.

Negative correlations were found between extractable starch and ethanol produced in the dry grind process in 2007 and 2008 (Table 2.5). Singh and Graeber (2005) found no correlation between extractable starch and ethanol yield from the dry grind process ($r = 0.06$). Significant negative correlations have not been reported in the literature.

<u>Lipids</u>

Most of the kernel's lipids are found in the embryo and are removed with the embryo during the wet mill process, but pass through to the DDGS in the dry grind process. Lipid in DDGS contributes substantially to its energy content so changes in kernel lipid composition may affect value of DDGS. In 2007, the earliest two planting dates (2 April and 20 April) produced higher amounts of lipids than any of the other planting dates (Table 2.10). Mycogen brand 2T787 and NK brand N70-T9 hybrids produced the highest amounts of lipids, and Pioneer 34A16 produced the least. The effects of planting date on lipid concentration in 2008 differ from the effects in 2007. Lipid concentrations were highest for the two latest planting dates (19 May and 10 June) (Table 2.11). NK brand N70-T9, NK brand N70C-3000GT, and Mycogen brand 2T787 were the highest lipid producing hybrids. So, although the two years differed for planting date effects on lipid concentration hybrid rankings were consistent between the two years.

Murthy, et al (2006) suggested lipid content may improve final ethanol concentrations and fermentation rates due to the increased health of yeast responsible for fermenting corn grain into ethanol. In 2007, we found a negative correlation between dry grind ethanol yield and lipid content (Table 2.5). In 2008, the correlation was not significant. Our data do not support the conclusion of Murphy, et al. (2006).

Protein

Protein is a non-fermentable that passes through to important byproducts used to feed livestock. Protein composition of these byproducts may impact their values. A significant date by hybrid interaction for kernel protein concentration was found in 2007 (Table 2.12). All hybrids produced the highest concentration of protein when planted on the latest planting date (13 June). This was the planting date that exhibited the least grain yield. Pioneer brand 34A16, the lowest yielding hybrid, produced kernels with the greatest protein concentration for planting dates 2 April and 20 April. The difference between the planting date/hybrid combination with the greatest protein concentration and the combination with the least protein was 19.0 g kg^{-1} or 23.7%. This large variation could significantly affect protein content of byproducts.

In 2008, the earliest two planting dates (6 April and 16 April) produced higher concentrations of protein in the kernel compared to the latest planting date (10 June) (Table 2.13). Pioneer brand 34A16 exhibited a greater average protein concentration than all other hybrids. Pioneer brand 34A16 was also the lowest yielding hybrid. The average kernel protein concentration for all hybrids and planting dates in 2007 and 2008 were 88.6 g kg^{-1} and 66.5 g kg^{-1}, respectively. This sizable difference in protein concentration between years may have been due to the above average precipitation during the growing season in 2008. In 2008, 407 mm of rainfall occurred between nitrogen application and September 1, compared to the 196 mm in 2007. Excessive rainfall can lead to the leaching and denitrification of nitrogen resulting in less nitrogen available for plant uptake. Nitrogen available in the soil is positively correlated with kernel protein concentration (Ruffo, et al., 2007). Most production practices that increase yield tend to decrease the protein concentration of grain (Sander, et al., 1987).

An unexpected positive correlation was found between dry grind ethanol yield and protein concentration in both years (Table 2.5). Singh and Graeber (2005) found no correlation between the two (r = -0.072). In studies on corn and small grains, as protein content increases, starch content decreases (Fox, et al., 1992; Lacerenza, et al., 2008; Singh, et al., 2005; Wu et al., 2007). A similar relationship was found in this study in 2008 (r = -0.75) but not in 2007 (r = -0.11).

A significantly negative correlation was present for both years between protein concentration and wet mill ethanol yield (Table 2.5). A similar relationship was observed by Singh, et al. (2005) with a negative correlation (r = -0.87) between kernel protein content and extractable starch. The negative correlation may be due to the association of starch and protein in the protein matrix of the endosperm. In the wet mill process, a portion of the total starch may be removed with protein before fermentation, resulting in less starch available for the production of ethanol. Fox, et al. (1992) reported a negative correlation (r = -0.63) between protein concentration and starch yield after wet milling. In this experiment, correlations with wet mill ethanol yield and starch concentration varied between years with no correlation in 2007 and a positive correlation in 2008 (Table 2.5). A positive correlation between extractable starch and starch was observed by Singh, et al. (2005).

CONCLUSION

Planting date can have a significant impact on grain yield, ethanol yield and kernel composition. Grain yield was highly affected by a change in planting date while ethanol yield and kernel composition were affected to a lesser extent. When favorable conditions follow planting, early planting dates are preferred as grain yield can be significantly reduced when planting is delayed into June. The effect of planting date was inconsistent between years for dry grind and wet mill ethanol yield along with starch, protein and lipid concentrations.

In this experiment, starch was not a good predictor of ethanol production. Protein, however, showed more consistent relationships with ethanol yield with positive correlations with dry grind ethanol yield and negative correlations with wet mill ethanol yield for both years. This suggests the association of starch with protein in the starch-protein matrix may be more important than kernel starch concentration alone. An inconsistent relationship was present for dry grind ethanol yield and kernel lipid concentration between years.

Hybrid selection is an important management practice decision when selecting for grain yield, ethanol yield and kernel composition. Differences between hybrids of the same genetic background with and without the corn rootworm resistance trait were inconsistent for ethanol yield and kernel composition while no significant effect was present for grain yield. However, corn rootworm pressure may be minimal at the experiment location.

In order to more effectively meet the requirements of ethanol production under RFS2, choosing a planting date and hybrid based on maximizing grain yield, would be most effective. Selection of hybrids with high ethanol yield potential should also be considered.

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Table 2.1. Grain yields of four hybrids planted on five planting dates in 2007.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	NK N70-T9	Mean
	------------------------------ kg ha^{-1} ------------------------------				
2 April	14060	12674	13856	13672	13565a†
20 April	13075	12835	14105	13988	13501a
14 May	11832	11394	12818	12738	12196b
29 May	9201	9267	9433	8254	9039c
13 June	7052	6837	7988	6765	7160d
Mean	10290bc‡	10083c	11086a	10436b	

† Means within this column followed by the same letter are not significantly different (LSD 0.05).

‡ Means within this row followed by the same letter are not significantly different (LSD 0.05).

Table 2.2. Grain yields of six hybrids planted on five planting dates in 2008.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	Garst 83C55-3000GT	NK N70C-3000GT	NK N70-T9	Mean
	----------	----------	----------	kg ha^{-1}	----------	----------	----------
6 April	10905	9507	11181	11758	10670	9601	10604b†
16 April	11600	10095	11956	11753	10999	11561	11327ab
5 May	11162	11910	12280	11648	11611	11840	11742a
19 May	11727	10319	11794	11228	11716	12040	11471ab
10 June	7803	7368	8278	8383	7991	7492	7886c
Mean	10639ab‡	9840c	11098a	10954ab	10597b	10507b	

† Means within this column followed by the same letter are not significantly different (LSD 0.05).

‡ Means within this row followed by the same letter are not significantly different (LSD 0.05).

Table 2.3. Estimated ethanol yield using the dry grind process on grain from four hybrids planted on five planting dates in 2007.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	NK N70-T9	Mean
	------------------------- L kg^{-1} -------------------------				
2 April	0.411†	0.414	0.409	0.407	0.410
20 April	0.410	0.414	0.407	0.407	0.410
14 May	0.420	0.421	0.420	0.419	0.420
29 May	0.424	0.430	0.424	0.431	0.427
13 June	0.423	0.431	0.422	0.422	0.425
Mean	0.418	0.422	0.416	0.417	

† LSD (0.05) to compare any two planting date and hybrid combinations is 0.004 L kg^{-1}.

Table 2.4. Estimated ethanol yield using the dry grind process on grain from six hybrids planted on five planting dates in 2008.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	Garst 83C55-3000GT	NK N70C-3000GT	NK N70-T9	Mean
	----------	----------	----------	L kg^{-1}	----------	----------	----------
6 April	0.411	0.412	0.406	0.406	0.406	0.408	0.408ab†
16 April	0.410	0.415	0.411	0.408	0.408	0.408	0.410a
5 May	0.407	0.414	0.408	0.405	0.407	0.410	0.409ab
19 May	0.410	0.412	0.406	0.406	0.405	0.407	0.408b
10 June	0.408	0.407	0.401	0.401	0.402	0.404	0.404c
Mean	0.409b‡	0.412a	0.407cd	0.405d	0.406d	0.407c	

† Means within this column followed by the same letter are not significantly different (LSD 0.05).

‡ Means within this row followed by the same letter are not significantly different (LSD 0.05).

Table 2.5. Pearson correlation coefficients between estimated ethanol yield from dry mill and wet mill processes and kernel composition and weather during grain fill in 2007 and 2008.

	2007		2008	
	Dry mill	Wet mill	Dry mill	Wet mill
Kernel composition				
Extractable starch	-0.55***	1.00***	-0.50***	1.00***
Starch	0.56***	-0.06	-0.45***	0.61***
Lipid	-0.69***	0.18	-0.09	-0.15
Protein	0.70***	-0.81***	0.86***	-0.71***
Weather during grain fill†				
Average temperature	0.00	0.23*	0.45***	-0.07
Total precipitation	-0.61***	0.34**	-0.04	0.06

* Significance of P= 0.05.

** Significance of P= 0.01.

*** Significance of P= 0.001.

† Dates used to calculate environmental conditions during grain fill period were 50% silk date and expected physiological maturity according to company literature and accumulated Growing Degree Days (GDD).

Table 2.6. Estimated ethanol yield using the wet mill process on grain from four hybrids planted on five planting dates in 2007.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	NK N70-T9	Mean
	----------	----------	$L\ kg^{-1}$	----------	----------
2 April	0.375†	0.370	0.375	0.374	0.374
20 April	0.374	0.371	0.375	0.371	0.373
14 May	0.374	0.373	0.373	0.370	0.372
29 May	0.371	0.372	0.373	0.366	0.371
13 June	0.367	0.366	0.373	0.366	0.368
Mean	0.372	0.370	0.374	0.369	

† LSD (0.05) to compare any two planting date and hybrid combinations is 0.001 $L\ kg^{-1}$.

Table 2.7. Estimated ethanol yield using the wet mill process grain from six hybrids planted on five planting dates in 2008

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	Garst 83C55-3000GT	NK N70C-3000GT	NK N70-T9	Mean
	$L\ kg^{-1}$						
6 April	0.382†	0.379	0.381	0.381	0.381	0.379	0.380
16 April	0.384	0.375	0.379	0.380	0.381	0.379	0.379
5 May	0.382	0.379	0.386	0.385	0.383	0.379	0.382
19 May	0.379	0.376	0.384	0.382	0.379	0.376	0.379
10 June	0.386	0.381	0.382	0.377	0.380	0.382	0.381
Mean	0.382	0.378	0.382	0.381	0.381	0.379	

† LSD (0.05) to compare any two planting date and hybrid combinations is 0.001 $L\ kg^{-1}$.

Table 2.8. Kernel starch concentrations of four hybrids planted on five planting dates in 2007.

	Hybrid				
Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	NK N70-T9	Mean
	---------- $g\ kg^{-1}$ ----------				
2 April	714	713	718	716	716c
20 April	715	714	718	715	716c
14 May	720	721	724	720	722b
29 May	724	728	722	719	724ab
13 June	723	726	729	723	726a
Mean	719b	721ab	723a	719b	

† Means within this column followed by the same letter are not significantly different (LSD 0.05).

‡ Means within this row followed by the same letter are not significantly different (LSD 0.05).

Table 2.9. Kernel starch concentrations of six hybrids planted on five planting dates in 2008.

Planting date	Hybrid						Mean
	Mycogen 2T787	Pioneer 34A16	Garst 8353	Garst 83C55-3000GT	NK N70C-3000GT	NK N70-T9	
	---------- g kg^{-1} ----------						
6 April	736	730	738	739	743	740	738b
16 April	740	736	741	740	747	740	741ab
5 May	742	738	751	743	748	740	744a
19 May	739	740	742	747	744	737	742ab
10 June	740	736	744	739	742	738	740ab
Mean	740bc	736a	744a	742ab	745a	739bc	

† Means within this column followed by the same letter are not significantly different (LSD 0.05).

‡ Means within this row followed by the same letter are not significantly different (LSD 0.05).

Table 2.10. Kernel lipid concentrations of four hybrids planted on five planting dates in 2007.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	NK N70-T9	Mean
	------------	------------	g kg^{-1}	------------	------------
2 April	35.0	32.0	32.7	34.8	33.6a†
20 April	34.0	32.0	32.8	35.3	33.5a
14 May	32.3	28.8	28.0	32.3	30.3b
29 May	31.5	26.5	29.0	29.8	29.2c
13 June	33.0	28.0	30.0	33.5	31.1b
Mean	33.2a‡	29.5c	30.5b	33.1a	

† Means within this column followed by the same letter are not significantly different (LSD 0.05).

‡ Means within this row followed by the same letter are not significantly different (LSD 0.05).

Table 2.11. Kernel lipid concentrations of six hybrids planted on five planting dates in 2008.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	Garst 83C55-3000GT	NK N70C-3000GT	NK N70-T9	Mean
	$g\ kg^{-1}$						
6 April	42.0	39.3	37.5	38.0	39.8	40.5	39.5b†
16 April	40.0	37.8	38.3	37.8	39.8	41.5	39.2b
5 May	41.0	38.0	37.3	39.3	40.3	41.8	39.6b
19 May	41.8	38.8	39.5	38.5	42.5	42.8	40.6a
10 June	42.5	40.5	39.0	40.5	42.5	42.8	41.3a
Mean	41.5ab‡	38.9c	38.3c	38.8c	41.0b	41.9a	

† Means within this column followed by the same letter are not significantly different (LSD 0.05).

‡ Means within this row followed by the same letter are not significantly different (LSD 0.05).

Table 2.12. Kernel protein concentrations of four hybrids planted on five planting dates in 2007.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	NK N70-T9	Mean
	--	---------- g kg^{-1} ----------		--	
2 April	85.0	91.5	83.3	80.3	85.0
20 April	86.5	92.0	81.8	81.5	85.4
14 May	84.8	88.5	85.5	85.8	86.1
29 May	88.3	89.5	88.8	95.5	90.5
13 June	97.3	99.3	91.5	96.0	96.0
Mean	88.4	92.2	86.2	87.8	

† LSD (0.05) to compare any two planting date and hybrid combinations is 5.3 g kg^{-1}.

Table 2.13. Kernel protein concentrations of six hybrids planted on five planting dates in 2008.

Planting date	Mycogen 2T787	Pioneer 34A16	Garst 8353	Garst 83C55-3000GT	NK N70C-3000GT	NK N70-T9	Mean
	g kg^{-1}						
6 April	70.5†	74.5	69.8	67.3	64.5	66.8	68.9a†
16 April	69.3	78.0	70.8	68.8	64.3	67.3	69.7a
5 May	65.3	75.0	61.3	62.8	60.5	68.3	65.5ab
19 May	68.5	70.5	65.0	62.8	61.3	67.0	65.8ab
10 June	64.8	65.5	61.0	62.0	59.0	61.8	62.3b
Mean	67.7b‡	72.7a	65.6bc	64.7cd	61.9d	66.2bc	

† Means within this column followed by the same letter are not significantly different (LSD 0.05).

‡ Means within this row followed by the same letter are not significantly different (LSD 0.05).

CHAPTER III

PLANTING DENSITY AND ENVIRONMENTAL EFFECTS ON CORN GRAIN ETHANOL YIELD AND ETHANOL BYPRODUCT QUALITY

ABSTRACT

Four locations in 2007 and six locations in 2008 were selected from important corn [*Zea mays L.*] growing regions in Missouri to determine whether planting density and environment (location and year) significantly affected corn grain yield, ethanol yield, and kernel characteristics. Corn planting densitiess ranged from 44460 seeds ha^{-1} to 98800 seed ha^{-1}. Whole kernel samples were measured for seed composition using a FOSS Infratec 1241 Grain Analyzer. Best linear unbiased predictions (BLUP) were used to estimate random effects of environment and replications. Increasing planting density had a positive effect on grain yield, wet mill ethanol yield, and starch concentrations for most environments. Negative effects were observed when planting density increased for dry grind ethanol yield and kernel protein concentration. Lipid concentration was not affected by a change in planting density. Averaged across all environments, grain yield increased 42% from the planting density that produced the least yield to the planting density that produced the greatest yield. Less variation was observed for dry grind ethanol yield (1%), wet mill ethanol yield (2.2%), starch (1.2%), and protein (13.4%). Environmental differences were also observed. Variation between the highest yielding environments and the lowest yielding environments averaged across all planting densitys were 130%, 3.9%, 2.5%, 4.5%, 46%, and 14% for grain yield, dry grind ethanol yield, wet mill ethanol yield, starch, lipid, and protein, respectively.

INTRODUCTION

The establishment of the Renewable Fuel Standard (RFS1) under the Energy Policy Act of 2005 (EPAct) has contributed to a significant increase in corn [*Zea mays* L.] production in the United States. RFS2, the revision of RFS1 under the Energy Independence and Security Act of 2007 (EISA), requires the use of 136.08 billion liters of renewable fuels by the year 2022. Ethanol from corn is expected to provide a significant portion of the requirement.

Two primary methods are used to produce ethanol from corn grain, dry grind and wet mill. In recent years, byproducts from ethanol production have become important commodities. Distillers' dried grains with solubles (DDGS) is a byproduct of dry grind ethanol production; whereas, gluten meal, gluten feed, and corn oil are the byproducts of wet mill production of ethanol. DDGS, gluten meal and gluten feed are marketed primarily to the cattle industry as a source of protein and energy. Corn oil is used primarily in the food industry.

Ethanol is the result of the fermentation of starch; however kernel starch concentration is not a good indicator of the amount of ethanol that can be produced from corn grain. The physical properties of the starch-protein matrix and the chemical structure of starch may be better indicators of potential ethanol yield from corn (Dien, et al., 2002).

Crop management practices can have a significant impact on kernel chemical and physical properties. Corn plant populations have more than doubled between the 1930's to the 1990's (Duvick, et al., 2004) with the primary goal to increase corn yield. A recommendation for optimum plant population varies depending on the soil type, water and nutrient availability, hybrid selection and planting date, among others (Sangoi, 2001). Changes in planting population effects kernel protein and extractable starch concentration (Ruffo, et al., 2007) along with final kernel weight (Baenziger and Glover, 1980; Borrás and Otegui, 2001).

With the increased demand for ethanol from corn, it is important to determine how management practice decisions effect corn kernel characteristics, ethanol yield and byproduct quality. The objective of this study was determine if planting density and location affect kernel chemistry and ethanol production from corn grain.

MATERIALS AND METHODS

This experiment was conducted at four locations (Charleston, LaGrange, Lamar, Oran) in 2007 and at six locations (Albany, Charleston, Lamar, Novelty, Oran, Portageville) in 2008. Albany, Novelty, and Portageville locations are University of Missouri research centers. All other locations are operated by farmer cooperators. Soil types, latitude, and longitude for each location are listed in Table 3.1. Weed control and fertilizer applications varied somewhat among the locations, but in each instance followed University of Missouri recommendations. Plot areas at Charleston, Oran and Portageville were irrigated.

The experimental design was a randomized complete block with four replications. DeKalb brand DKC60-19 was planted at all locations in both years. Treatments were six seeding densities: 44460, 54340, 64220, 79040, 88920 and 98800 seeds per hectare. Plots were four rows wide and 8.3 m long. Row spacing was 0.76 m.

Before harvest, the center two rows of each plot were trimmed to 7.6 m once ears reached dent stage or later and final plant density was recorded. Plots were harvested using a plot combine and yields were adjusted to 15% moisture. During harvest, 0.5 kg samples were collected from each plot sent to Monsanto Company for analysis. NIR analyses were as described in Chapter 2.

Data Analysis

The experimental design at each location was a randomized complete block with four replications and the six planting densities as treatments. In the model used for data analyses, replications and environments (locations and years) were random and seeding densities were fixed. The SAS procedure PROC MIXED was used to produce best linear unbiased predictions (BLUP), a method used for estimating random effects of a mixed model. Final plant stand was used as a covariate.

RESULTS AND DISCUSSION

Corn Yield

Significant and positive linear effects of planting densities on grain yield were found for all environments (Table 3.2). As reported by other researchers (Ahmadi, et al., 1993; Porter, et al., 1997; Staggenborg, et al., 1999), the effects of planting densities were quite large. Averaged across all environments, yield increased 42% from the planting rate that produced the least yield to the planting rate that produced the greatest yield. This large effect confirmed that planting rate was an appropriate selection for determining if cultural practices affect ethanol production from corn grain. Quadratic effects were significant at all environments except 2007 LaGrange. This indicates that the increase in grain yield was greatest at lower planting densities and the rate of increase in yield diminished at higher planting densities. Calculating an optimum planting rate was beyond the scope of this study.

Environment also strongly affected grain yield (Table 3.3). Average grain yield ranged from a low of 6545 kg ha^{-1} at 2008 Lamar to a high of 15069 kg ha^{-1} at 2007 Oran, a difference of 130%. Lamar produced the lowest grain yield in both years. Three locations in southeast Missouri (Charleston, Oran, Portageville) were among the highest yielding. These three locations were provided supplemental irrigation during the growing season, whereas all other locations were not irrigated.

Ethanol Yield (L/Kg)

Dry grind

Ethanol yield from the dry grind process was estimated to be 0.418 L kg^{-1} averaged across all planting densities and environments, similar to figures used by the USDA (Shapouri, et al., 2002). Significant linear effects from planting densities on ethanol yield from dry grind were found for all environments (Table 3.4). In general, ethanol yield decreased with increasing planting density, but the effects were very small. Changes in ethanol yield were 1% or less within each environment. Quadratic effects were significant for six of the 10 environments. Haefele, et al. (2004) observed an increase in total fermentables, a measurement of dry grind yield, as plant density increased to an optimum level followed by a decrease as plant density increased over the optimum level.

Significant differences for ethanol yield were observed among planting environment (Table 3.3). Dry grind ethanol yield ranged from a low average yield of 0.409 L kg^{-1} for 2007 Oran to a high average yield of 0.425 L kg^{-1} 2008 Albany and 2008 Lamar, a difference of approximately 4%. In an experiment on grain sorghum, ethanol yield differed by 2.1% on a comparison of two growing locations (Zhan, et al., 2003). Singh and Graeber (2005) observed varying effects of location on ethanol concentration with a significant effect in one experiment and no significant effect on another.

Wet milling

The average estimated ethanol yield from wet milling in this study was 0.397 L kg^{-1}, approximately 6% higher than the expected average of 0.372 L kg^{-1} (Bothast and Schlicher, 2005). Significant linear effects from planting densities on ethanol yield from wet milling occurred at all environments (Table 3.5). Unlike the relationship found for dry mill, the relationship was positive. Increased seeding density increased ethanol yield from wet milling. But, changes in ethanol yield were less than 3.6% within all environments. Ruffo, et al. (2007) observed a similar relationship with an increase in extractable starch concentrations as plant density increased. Quadratic effects from planting density were found for all environments except two, 2007 Charleston and 2007 LaGrange.

Within each year, small, but significant differences for wet mill ethanol were found among locations (Table 3.3). Across all environments average wet mill ethanol production ranged from 0.394 L kg^{-1} at 2008 Lamar to 0.404 L kg^{-1} at 2008 Novelty, a difference of only 2.5%.

Kernel Composition

Starch

Linear effects of planting density on starch composition were found for all environments (Table 3.6). Quadratic effects were found at six of 10 environments. An increase in starch concentration was observed for all environments as planting density increased; however differences within an environment were small, less than 1.5%. A similar response to planting density was observed in grain yield and wet mill ethanol yield. In the production of ethanol, starch is converted to sugars, which are then fermented, distilled and dehydrated, creating fuel grade ethanol. Dien, et al (2002), Haefele, et al (2004), and Singh and Graeber (2005), suggest starch concentration alone cannot predict the ethanol output of a sample.

Environments differed for kernel starch composition (Table 3.3). Starch concentrations varied from 713 g kg^{-1} for 2007 Charleston to 745 g kg^{-1} for 2008 Novelty, a difference of 4.5%.

Less variation was observed by Reicks, et al. (2009) with a difference of less than 1% across environments.

Lipids

Lipids, considered a non-fermentable portion of the kernel, are primarily found in the embryo. In the dry grind process, the embryo is not removed and lipids pass through to the DDGS. Lipids contribute a substantial amount of energy to DDGS from the dry grind process. Changes in kernel lipid content would change energy content of DDGS.

Planting density had almost no effect on kernel lipid concentrations (Table 3.7). Linear effects of planting density on lipid concentration were found at only two environments, 2008 Albany and 2008 Novelty. A significant quadratic effect was found only at 2008 Novelty. Relatively large differences among environments were found for kernel lipid concentration. Lipid concentration varied from 29.1 g kg^{-1} at 2007 Lamar to 42.5 g kg^{-1} at 2008 Novelty, a difference of 46%. Jellum and Marion (1966) observed significant differences in lipid content between years and locations within a year.

Protein

Like lipids, protein is considered a non-fermentable in the production of ethanol. Linear effects of planting density on kernel protein concentration were found for all environments, while quadratic effects were significant at six of the 10 environments (Table 3.8). Ahmadi, et al. (1993) also observed linear effects between planting density and kernel nitrogen concentration, an estimate of protein concentration. The protein concentration in this experiment ranged from a high of 96.8 g kg^{-1} in 2008 Charleston at the lowest planting density to a low of 73.5 g kg^{-1} in 2008 Novelty at the highest planting density.

With the exception of the lowest planting density in Oran in 2008, kernel protein concentration decreased as planting density increased in all environments. Planting densities within an environment varied for kernel protein concentration as much as 17%. This observation may be the result of less nitrogen available per plant as planting density is increased. An increase in nitrogen fertilizer rate has been shown to increase kernel protein content (Ruffo, et al., 2007).

Average protein concentration was significantly different in three environments while comparisons between all other environments were not significant (Table 3.3). Lamar in 2007 and Novelty and Portageville in 2008 produced lower average concentration of protein (82.5 g kg^{-1}) compared to the other seven environments (88.4 g kg^{-1}), a difference of 7.2%. Novelty in 2008 was significantly lower than all other environments.

CONCLUSION

Planting density and growing environment can have a significant impact on corn grain yield, ethanol yield and kernel characteristics. In most environments, increasing planting density had a positive effect on grain yield, wet mill ethanol yield, and starch concentrations while it negatively affected kernel protein concentration and dry grind ethanol yield. Planting density had little effect on the variations in lipid concentration. Significant differences between environments were common for grain yield, dry grind ethanol yield, starch concentration, and lipid concentration. Environments had less of an effect on wet mill ethanol yield and protein concentration.

The relatively small variation of ethanol yields across all planting densities and environments is good for ethanol production facilities. Selecting a planting density most suitable to the growing environment for economically maximizing grain yield is desired to more effectively meet the ethanol production and use requirements under the RFS2.

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Table 3.1. Soil type, latitude and longitude for locations tested in 2007 and 2008.

Location	County	Latitude	Longitude	Soil
Albany	Gentry	40º 14’ 37”	94º 21’ 18”	Grundy Silt Loam (Fine, smectitic, mesic Aquertic Argiudolls)
Charleston	Mississippi	36º 53’ 4”	89º 19’ 59”	Dundee Silt Loam (Fine-silty, mixed, active, thermic Typic Endoaqualfs)
LaGrange	Lewis	40º 1’ 25”	91º 29’ 33”	Westerville Silt Loam (Fine-silty, mixed, superactive, acid, mesic Aeric Fluvaquents)
Lamar	Barton	37º 30’ 34”	94º 22’ 10”	Parsons Silt Loam (Fine, mixed, active, thermic Mollic Albaqualfs)
Novelty	Knox	40º 0’ 52”	92º 10’ 40”	Putnum Silt Loam (Fine, smectitic, mesic Vertic Albaqualfs)
Oran	Scott	37º 6’ 8”	89º 39” 14”	Commerce Silt Loam (Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts)
Portageville	New Madrid	36º 24’ 34”	89º 38’ 45”	Portageville Clay (Fine, smectitic, calcareous, thermic Vertic Endoaquolls)

Table 3.2. BLUP of grain yields for six planting densities at multiple locations in 2007 and 2008.

	2007				2008					
Planting Density	Charleston	LaGrange	Lamar	Oran	Albany	Charleston	Lamar	Novelty	Oran	Portageville
Seeds/ha	---------- kg ha^{-1} ----------									
44460	10742	10664	6642	11641	7976	11164	5151	6795	9211	8310
54340	12523	12298	7386	12997	8808	12391	6448	7731	10405	9829
64220	13265	13040	8128	13739	9904	13134	7190	8473	11147	10571
79040	14522	13660	8507	15669	10416	15343	6933	8926	12915	11776
88920	15735	14756	8548	16321	10634	15065	7021	9009	13004	11245
98800	16568	15322	8194	17901	10717	14944	7535	8692	13209	11856
Mean	14035	13237	7822	15069	9756	13643	6545	8154	11692	10527
Linear	**	**	**	**	**	**	**	**	**	**
Quadratic	*	n.s.	**	**	*	**	**	**	**	**

* = single-degree-of-freedom comparison significant (P=0.05, F-test)

** = single-degree-of-freedom comparison significant (P=0.01, F-test)

n.s. = not significant

Table 3.3. BLUP comparisons of means for various yield components at multiple locations in 2007 and 2008.

	Yield Components					
Comparisons	Yield	Dry grind	Wet mill	Starch	Lipid	Protein
2007 Charleston vs 2007 LaGrange	ns	**	**	**	ns	ns
2007 Charleston vs 2007 Lamar	**	**	ns	**	**	*
2007 Charleston vs 2007 Oran	ns	ns	*	**	*	ns
2007 Charleston vs 2008 Albany	**	**	ns	**	**	ns
2007 Charleston vs 2008 Charleston	ns	**	ns	**	**	ns
2007 Charleston vs 2008 Lamar	**	**	ns	**	**	ns
2007 Charleston vs 2008 Novelty	**	**	**	**	**	**
2007 Charleston vs 2008 Oran	**	**	ns	**	**	ns
2007 Charleston vs 2008 Portageville	**	**	ns	**	**	ns
2007 LaGrange vs 2007 Lamar	**	ns	**	**	**	ns
2007 LaGrange vs 2007 Oran	**	**	ns	ns	**	ns
2007 LaGrange vs 2008 Albany	**	**	**	**	**	ns
2007 LaGrange vs 2008 Charleston	ns	**	**	ns	**	ns
2007 LaGrange vs 2008 Lamar	**	**	**	**	**	ns
2007 LaGrange vs 2008 Novelty	**	**	ns	**	**	**
2007 LaGrange vs 2008 Oran	**	ns	**	ns	**	ns
2007 LaGrange vs 2008 Portageville	**	ns	*	**	**	ns
2007 Lamar vs 2007 Oran	**	**	*	**	ns	*
2007 Lamar vs 2008 Albany	**	**	ns	**	**	**
2007 Lamar vs 2008 Charleston	**	**	ns	**	**	**
2007 Lamar vs 2008 Lamar	*	**	ns	**	**	*
2007 Lamar vs 2008 Novelty	ns	**	**	**	**	*
2007 Lamar vs 2008 Oran	**	ns	ns	ns	**	**
2007 Lamar vs 2008 Portageville	**	*	ns	ns	**	ns
2007 Oran vs 2008 Albany	**	**	*	**	**	ns
2007 Oran vs 2008 Charleston	*	**	*	ns	**	ns
2007 Oran vs 2008 Lamar	**	**	**	**	**	ns
2007 Oran vs 2008 Novelty	**	**	*	**	**	**
2007 Oran vs 2008 Oran	**	**	**	**	**	ns
2007 Oran vs 2008 Portageville	**	**	ns	**	**	ns
2008 Albany vs 2008 Charleston	**	**	ns	**	*	ns
2008 Albany vs 2008 Lamar	**	ns	ns	ns	ns	ns
2008 Albany vs 2008 Novelty	**	**	**	**	ns	**
2008 Albany vs 2008 Oran	**	**	ns	**	**	ns
2008 Albany vs 2008 Portageville	ns	**	ns	**	**	*
2008 Charleston vs 2008 Lamar	**	**	ns	**	**	ns
2008 Charleston vs 2008 Novelty	**	ns	**	**	ns	**
2008 Charleston vs 2008 Oran	**	**	ns	**	**	ns
2008 Charleston vs 2008 Portageville	**	**	ns	**	**	*
2008 Lamar vs 2008 Novelty	**	**	**	**	**	**
2008 Lamar vs 2008 Oran	**	**	ns	**	**	ns
2008 Lamar vs 2008 Portageville	**	**	ns	**	**	ns
2008 Novelty vs 2008 Oran	**	*	**	**	**	**
2008 Novelty vs 2008 Portageville	**	ns	**	**	**	**
2008 Oran vs 2008 Portageville	*	ns	ns	**	ns	**

* = single-degree-of-freedom comparison significant (P=0.05, F-test)

** = single-degree-of-freedom comparison significant (P=0.01, F-test)

n.s. = not significant

Table 3.4. BLUP of estimated ethanol yields using the dry grind process on grain from six planting densities at multiple locations in 2007 and 2008.

Planting Density	2007				2008					
	Charleston	LaGrange	Lamar	Oran	Albany	Charleston	Lamar	Novelty	Oran	Portageville
Seeds/ha	L kg^{-1}									
44460	0.412	0.418	0.417	0.411	0.427	0.423	0.427	0.422	0.419	0.420
54340	0.412	0.417	0.416	0.410	0.426	0.423	0.426	0.421	0.418	0.419
64220	0.412	0.417	0.416	0.410	0.426	0.423	0.426	0.421	0.418	0.419
79040	0.411	0.416	0.415	0.409	0.425	0.421	0.425	0.419	0.417	0.418
88920	0.410	0.415	0.414	0.408	0.424	0.420	0.424	0.418	0.415	0.417
98800	0.409	0.414	0.413	0.407	0.423	0.419	0.423	0.418	0.415	0.416
Mean	0.411	0.416	0.415	0.409	0.425	0.422	0.425	0.420	0.417	0.418
Linear	**	**	**	**	**	**	**	**	**	**
Quadratic	n.s.	n.s.	**	**	ns	ns	**	**	**	**

* = single-degree-of-freedom comparison significant (P=0.05, F-test)

** = single-degree-of-freedom comparison significant (P=0.01, F-test)

n.s. = not significant

Table 3.5. BLUP of estimated ethanol yields using the wet mill process on grain from six planting densities at multiple locations in 2007 and 2008.

Planting Density	2007				2008					
	Charleston	LaGrange	Lamar	Oran	Albany	Charleston	Lamar	Novelty	Oran	Portageville
Seeds/ha	$L\ kg^{-1}$									
44460	0.392	0.394	0.390	0.393	0.389	0.390	0.388	0.397	0.390	0.391
54340	0.392	0.397	0.393	0.395	0.395	0.394	0.391	0.399	0.392	0.393
64220	0.394	0.400	0.396	0.398	0.396	0.396	0.393	0.402	0.395	0.395
79040	0.398	0.402	0.398	0.402	0.399	0.399	0.396	0.406	0.397	0.400
88920	0.400	0.405	0.400	0.404	0.401	0.400	0.397	0.408	0.399	0.401
98800	0.402	0.407	0.401	0.407	0.401	0.401	0.400	0.409	0.398	0.402
Mean	0.397	0.401	0.396	0.400	0.397	0.396	0.394	0.404	0.395	0.397
Linear	**	**	**	**	**	**	**	**	**	**
Quadratic	n.s.	n.s.	**	**	**	*	**	**	**	**

* = single-degree-of-freedom comparison significant (P=0.05, F-test)

** = single-degree-of-freedom comparison significant (P=0.01, F-test)

n.s. = not significant

Table 3.6. BLUP of kernel starch concentrations of six planting densities at multiple locations in 2007 and 2008.

Planting Density	2007				2008					
	Charleston	LaGrange	Lamar	Oran	Albany	Charleston	Lamar	Novelty	Oran	Portageville
Seeds/ha	---------- g kg^{-1} ----------									
44460	709	716	722	713	731	713	731	739	720	724
54340	711	719	724	715	733	717	733	742	722	727
64220	713	721	726	717	736	720	736	745	724	729
79040	714	723	727	719	737	720	737	746	725	730
88920	715	722	727	720	737	720	738	748	725	732
98800	716	725	730	722	740	722	740	751	726	733
Mean	713	721	726	718	736	718	736	745	724	729
Linear	**	**	**	**	**	**	**	**	**	**
Quadratic	n.s.	n.s.	**	**	n.s.	n.s.	**	**	**	**

* = single-degree-of-freedom comparison significant (P=0.05, F-test)

** = single-degree-of-freedom comparison significant (P=0.01, F-test)

n.s. = not significant

Table 3.7. BLUP of kernel lipid concentrations of six planting densities at multiple locations in 2007 and 2008.

Planting Density	2007				2008					
	Charleston	LaGrange	Lamar	Oran	Albany	Charleston	Lamar	Novelty	Oran	Portageville
Seeds/ha	g kg^{-1}									
44460	33.9	34.7	29.1	32.2	41.2	43.0	40.5	43.6	38.5	37.8
54340	34.4	34.0	28.9	33.9	42.4	42.6	40.7	43.6	38.7	38.0
64220	34.6	34.2	29.1	34.1	41.9	42.8	40.9	43.7	38.9	38.2
79040	34.2	34.4	29.1	32.9	41.2	42.8	41.2	42.9	38.9	38.4
88920	34.4	34.3	29.1	33.1	41.3	42.3	40.2	41.8	39.7	37.9
98800	34.2	33.9	28.6	33.1	40.4	42.5	40.1	41.3	39.8	38.2
Mean	34.4	34.2	29.1	33.2	41.5	42.7	40.6	42.5	39.2	38.2
Linear	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	**	n.s.	n.s.
Quadratic	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.

* = single-degree-of-freedom comparison significant (P=0.05, F-test)

** = single-degree-of-freedom comparison significant (P=0.01, F-test)

n.s. = not significant

Table 3.8. BLUP of kernel protein concentrations of six planting densities at multiple locations in 2007 and 2008.

Planting Density	2007				2008					
	Charleston	LaGrange	Lamar	Oran	Albany	Charleston	Lamar	Novelty	Oran	Portageville
Seeds/ha	g kg^{-1}									
44460	92.2	93.3	89.1	95.0	96.3	96.8	94.6	86.0	86.4	91.7
54340	91.4	90.0	86.2	93.1	93.3	92.3	92.0	83.7	93.5	89.0
64220	88.5	87.1	83.3	90.1	89.9	89.4	89.0	80.7	90.5	86.0
79040	85.0	84.5	81.1	85.9	87.5	87.5	85.6	76.4	87.9	83.1
88920	83.8	83.0	80.0	83.7	86.4	86.6	84.9	75.2	87.2	81.1
98800	82.4	80.6	77.8	81.1	85.1	84.5	81.8	73.5	86.2	80.2
Mean	87.1	86.5	83.3	87.8	89.7	89.8	88.0	79.2	90.2	85.1
Linear	**	**	**	**	**	**	**	**	**	**
Quadratic	n.s.	n.s.	**	**	n.s.	n.s.	**	**	**	**

* = single-degree-of-freedom comparison significant (P=0.05, F-test)

** = single-degree-of-freedom comparison significant (P=0.01, F-test)

n.s. = not significant