

**THE EFFECT OF ENVIRONMENT ON SEED COMPOSITION OF  
TOFU AND NATTO SOYBEAN CULTIVARS**

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Master of Science

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by  
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**THE EFFECT OF ENVIRONMENT ON SEED COMPOSITION OF TOFU AND  
NATTO SOYBEAN CULTIVARS**

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# **CHAPTER I**

## **LITERATURE REVIEW**

### **Introduction**

Soybean [*Glycine max* (L.) Merr.] is the leading oilseed crop produced and consumed in the world today (Wilcox, 2004). In 2005, according to the Food and Agricultural Organization of the United Nations (2005), 91.4 million hectares of soybean were harvested in the world, and 28.9 million hectares were harvested in the United States. The USA is the leading country for soybean production with 40% of the world total (USDA, 2005). In 2004, 85.5 million metric tons of soybean were produced in the USA and 29.9 million metric tons were exported (USDA, 2005). The USA is the largest exporter of soybean in the world, with China and Japan as the two major buyers (ASA, 2005). In Missouri, soybean is the number one crop in hectares planted. In 2005, 2.02 million hectares of soybean were planted, and valued at 1 billion dollars (USDA, 2005).

### **Soybean History**

Soybean was first domesticated in the eastern half of north China in the 11<sup>th</sup> century B.C. It was grown mainly for the seeds that were used for fresh, fermented, and dried food products (Whigham, 2004). The first introduction of soybean into the USA occurred when Henry Yonge, the Surveyor General of the Colony of Georgia, planted soybean on his farm at the request of Samuel Bowen in 1765 (Hymowitz, 2004). Bowen, a former seaman employed by the East India Company, brought soybean to Savannah from China via London. In 1766, Bowen planted soybean on his plantation “Greenwich” located at Thunderbolt, a few miles east of Savannah, Georgia (Hymowitz, 2004). Another early introduction of soybean into the United States was by Benjamin Franklin.



In 1770, Franklin sent seeds from London to the botanist John Bartram who planted them near Philadelphia, Pennsylvania (Hymowitz, 2004).

In 1851, soybean was introduced to Illinois and subsequently throughout the Corn Belt (Hymowitz, 2004). Throughout the 1800's additional soybean cultivars were introduced to the USA and soybean was grown primarily as a forage crop. Soybean was harvested for a single crop of hay or plowed under as a green manure crop to improve soil structure and fertility (Whigham, 2004).

In 1904, George Washington Carver began studying soybean at the Tuskegee Institute in Alabama. His discoveries helped to change soybean plants from a forage crop to a grain crop with seeds that were a valuable oil and protein source (AgEBB, 1996). By the late 1920's, thousands of new cultivars had been brought to the United States, mostly from China by William Morse, who later helped form the American Soybean Association and became its first president (Liu, 1997).

Prior to World War II, the USA imported 40% of its edible fats and oils (AgEBB, 1996). By 1940, the U.S. soybean crop had grown to 2.12 million metric tons of grain harvested on 2 million hectares, and the USA became a net exporter of soybean and soybean products. Today, farmers in 31 U.S. states grow soybean, making soybean the second largest crop in cash sales (USDA, 2005).

### **Traditional Uses of Soybean**

Soybean is produced today mainly for its protein and oil components. Commodity soybean seeds consist of approximately 40% protein and 20% oil on a dry weight basis (Liu, 1997). One metric ton of soybean yields an average of 183 kg of oil and 800 kg of protein meal (Snyder, 1987).

Soybean meal contains 44% or 48% protein depending on the type of processing used to produce the soybean meal (Maier et al., 1998). Most soybean protein, in the form of defatted meal, is used as animal feed for poultry and swine. In 2004, the USDA (2005) reported that 67% of protein meal consumed in the world was produced from soybean. Food products enhanced by soybean protein for human consumption are also widely used and accepted. Textured soy protein, soy flour, soy concentrates, and soy isolates can be found in many baked goods, meat products, and dairy products.

Soybean oil is the predominant vegetable oil in the world, making up approximately 30% of the total vegetable oil consumed worldwide (USDA, 2005). In 2004, soybean oil accounted for 80% of the total edible fats and oils consumed in the United States (ASA, 2005). Soybean oil is used to manufacture salad and cooking oils, baking and frying fats, and margarine (Huth, 1995).

Soybean cultivars grown in the USA are traditionally used for protein and oil products. Farmers grow these commodity soybean cultivars and are mainly concerned about maximizing yield. Buyers of commodity soybean grain desire uniform seeds with little variation in protein and oil contents. In the past, protein and oil have been the only components of soybean seeds that have been of major importance in the USA. Now, as soybean is becoming a major component of foods used for human consumption, the focus is shifting from commodity soybean cultivars and maximizing yield to specialty soybean cultivars and maximizing profits. In specialty soybean cultivars used for human consumption, soybean seed composition is important. Protein and oil are still important, but other components, such as carbohydrates, become important in food grade soybean cultivars.

## **Soy Foods**

Soybean has been a major food source in Asian countries for thousands of years. According to the 1998 Japanese National Nutrition Survey, approximately 70 grams of soybean and soybean products are consumed daily per person in Japan (Taira, 2001).

While only 10% of the world's soybean is used for food, with 90% of this being consumed in Asian countries, consumption of soy foods in North America has grown by about twenty percent annually since 1998 (Poysa et al., 2002). A survey conducted by the Soyfoods Association of America (1995) revealed that 26 million Americans consume foods made from soybean. Soy foods, in general, have been proven to benefit human health by helping to prevent diseases such as cancer, heart disease, and osteoporosis. In 1999, the United States Food and Drug Administration (FDA) approved the health claim that consumption of 25 grams of soybean protein per day can help lower cholesterol and prevent heart disease (Bachmann, 2001). In 2003, one in six Americans consumed soy foods or soy beverages once a week or more (Rao et al., 2002). Because of the proven health benefits, soy foods have become readily available in large supermarket stores in the USA.

There are many different types of soy foods available to consumers. Fermented whole soybean products include, miso (soup base), natto (topping), tempeh (meat substitute), and soy sauce (flavoring). Roasted soybean products are soy nuts and soy flour. Products made from soymilk include soymilk itself, tofu (meat and dairy substitute), soy cheese, soy yogurt, nondairy frozen desserts, and okara (fiber additive) (Kroll, 2000). Fresh soybeans called edamame and soybean sprouts are also common soy foods.

## **Tofu and Natto**

One of the most well known and accepted soy foods is tofu. Tofu is one of the major soybean products in Japan; 47 grams of tofu and processed tofu products are consumed daily per person (Taira, 2001). According to the Soyfoods Center in California, an organization that follows consumption trends of soybean products, USA shoppers spend an average of \$130 to \$150 million on tofu each year and they are doubling their consumption of tofu every three to four years (Pollock and St. Martin, 2002).

There are two main types of tofu available. Hard or firm tofu is dense and solid and is higher in protein, fat and calcium than other types of tofu (Indiana Soyfood Board, 1998). Soft or silken tofu has a creamy custard-like texture. In tofu, the amount and type of protein largely determines the yield and texture of the finished product (Poysa et al., 2002). The protein content in soybean seeds used for tofu is usually greater than 45%. Large amounts of available protein and oil in the seed will result in high tofu yields. A high protein-to-oil ratio produces hard or firm tofu and a high oil-to-protein ratio makes soft or silky tofu (Bachmann, 2001).

The carbohydrate content contributes to the flavor of tofu and soymilk. Soybean seeds contain approximately 35% carbohydrates. Half of the carbohydrates are insoluble polysaccharides, which include pectin, cellulose, hemicellulose, and trace amounts of starch (Wilson, 1999; Liu, 1997). The other half is soluble carbohydrates, of which the major components are monosaccharides, disaccharides, and oligosaccharides (Liu, 1997). The average soybean seed contains 9 to 12% total soluble carbohydrates, of which 4 to 5% is sucrose, 1 to 2 % is raffinose, and 3.5 to 4.5% is stachyose (Wilson, 1995).

Sucrose, a disaccharide, is formed from glucose and fructose. It is easily digested and is the main source for the sweet flavor of soybean products. Raffinose and stachyose are oligosaccharides. Raffinose, a trisaccharide, is formed from galactose, fructose, and glucose. Stachyose, a tetrasaccharide, is formed from two galactose units, fructose, and glucose. In raffinose and stachyose, galactose must be removed from fructose and glucose by the enzyme alpha-galactosidase in order to be digested (Messina, 1999). Human intestines do not contain alpha-galactosidase. Raffinose and stachyose are undesirable in soy food products because they are not digestible by humans. These oligosaccharides pass into the large intestine where bacteria metabolize them and form large amounts of carbon dioxide, hydrogen, and sometimes methane (Messina, 1999). Although oligosaccharides cause flatulence and are undesirable in large quantities in soy food products, in small amounts they are considered beneficial prebiotics and can improve colon health (Gibson, 1995).

The process of making tofu is similar to making cheese. Tofu is made from soymilk curd that is separated from the whey by a calcium or magnesium salt. The curd used to make tofu is pressed to remove excess whey. The length of pressing time determines the type of tofu produced (Golbitz, 1994). In the process of making tofu, most of the soluble carbohydrates are retained in the whey (Liu, 1997). Oligosaccharides are found only in small amounts in tofu, but they are still capable of causing intestinal distress.

Tofu is perishable and should be kept cold. The type of packaging used determines the shelf life of the product. By using aseptic packages, tofu can have a shelf

life of up to six months. Tofu acts like a sponge and will soak up any flavor added to it. Tofu can be used in most recipes as a replacement for eggs and dairy products.

Most USA tofu production is aimed at international markets, such as Japan. The USDA (2005) estimates that the Japanese consume 540,000 metric tons of tofu soybean per year. Tofu production represents only about 1% of total U.S. soybean production, yet demand for tofu products is increasing in the USA as well as internationally (Pollock and St. Martin, 2002).

Another important soy food that is gaining popularity is natto. Natto is made by fermenting whole soybean seeds with bacteria called *Bacillus natto*. Natto is commonly used in Japan as a flavoring agent with rice or a sauce used at breakfast. Natto is not as popular as tofu in the USA because of its sticky, slimy appearance and strong ammoniated odor. The finished fermented beans are grayish and covered with a sticky polymer produced by the bacterium (Hesseltine, 1989). Because natto is made from whole soybean seeds, it is one of the most nutritious soy foods available.

When natto is fermented, the soluble carbohydrates serve as an initial carbon and energy source for the natto bacteria, *Bacillus natto* (Liu, 1997). High levels of soluble oligosaccharides are preferred in natto soybean seeds (Cober et al., 1997). Higher soluble carbohydrate content can also make the finished product taste sweeter. Since *Bacillus natto* feeds on soluble carbohydrates during the fermentation process, the problem of flatulence is greatly reduced.

Hard seed is a problem in natto soybean seeds grown in the USA. Hard natto seeds will not absorb water during the fermentation process. Geater et al. (2000) reported that the hardness of natto was negatively correlated with the total carbohydrate content of

small-seeded genotypes developed for the natto industry. Increasing the amount of total carbohydrate in natto soybean seeds grown in the USA will reduce the number of hard seeds and make soybean grain grown in the USA more desirable in the Japanese natto industry.

Soybean seeds used for human consumption are chosen based on the physical and chemical composition of the seed. Visual appearance of the seed is important to buyers of food grade soybean (Bachmann, 2001). The seeds should be uniform in size and color. Shrunken, discolored and split seeds can lead to price reductions or rejection of the grain by food grade soybean buyers. The seed coats should be colorless and the hilum should be clear or very light in color. Seed size is also important for certain types of soy foods. Seeds of soybean cultivars used for tofu are generally 25% to 50% larger than those of commodity soybean cultivars. The small surface-to-volume ratio reduces the amount of material lost as okara during tofu processing. Seeds of natto soybean seeds are less than one-half the size of seeds of commodity soybean cultivars, which creates the large surface-to-volume ratio needed during the fermentation process (Poysa et al., 2002).

Specific soybean seed composition is desired for both tofu and natto production, and determining what factors affect composition of soybean seed while the plants are growing in the field is important to growers and buyers of tofu and natto soybean cultivars.

### **Soybean Seed Composition**

There have been numerous studies conducted on the composition of soybean seeds. The major components that have been studied extensively are protein and oil. As USA farmers begin to switch from commodity soybean cultivars to specialty soybean

cultivars, more information will be needed on the carbohydrate content of specialty soybean seeds used for human consumption.

The relationship of oil, protein, and carbohydrate (sugar) of 60 soybean lines was studied by Hymowitz et al. (1972). They found that the total sugar content of a soybean seed is positively associated with the total oil content and both are negatively associated with the total protein content. Hymowitz et al. (1972) also found that total sugar was positively correlated with sucrose and raffinose, but it is not significantly correlated with stachyose content. A positive relationship between stachyose and protein was found and this could cause difficulty for plant breeders who wish to reduce the stachyose content while maintaining a high protein content.

Hymowitz and Collins (1974) evaluated 194 Maturity Groups 00 through IV soybean cultivars for total sugars, sucrose, raffinose, and stachyose. Range in grams of carbohydrate per 100g dry seed weight for sucrose, raffinose, and stachyose were 3.0 to 10.2, 0.4 to 1.8, and 1.2 to 3.8, respectively. They concluded that sufficient variability existed to permit selection of lines with varying concentrations of total sugars or soluble sugar concentration.

Hartwig, et al. (1997) looked at Maturity Group V, VI, and VII high protein and high oil soybean lines to determine the variation in soluble sugar content of the seed. The range in values in grams of carbohydrate per 100 g of dry seed weight was sucrose 2.9 to 7.0; raffinose 0.7 to 1.3; and stachyose 3.3 to 4.8. Hymowitz et al. (1972) reported a positive and significant correlation ( $r = 0.41$ ) between protein and stachyose in Maturity Group 00 through IV soybean seeds. Hartwig et al. (1997) found a nonsignificant correlation of 0.11, which would suggest development of high protein lines with lower



levels of stachyose is possible within Maturity Groups V through VII. They also found a low nonsignificant correlation between protein and stachyose + raffinose. Hymowitz et al. (1972) and Hartwig et al. (1997) both found a significant negative correlation between protein and sucrose.

In these studies, carbohydrate content has a wide range of variability. There may be other reasons for variation in composition of soybean seeds, such as environmental factors.

### **Environmental Effects on Soybean Seed Composition**

Soybean seed composition is affected by both genetic and environmental variables, as well as the interaction of the two (Honeycutt et al., 1989; Piper and Boote, 1999). For most commodity soybean growers, the crop is managed to produce the highest possible yield. When growing tofu and natto soybean seeds, high yield is sometimes not the end goal. If a grower has a contract with a buyer of tofu or natto soybean grain, it may state that the soybean seeds must have a certain chemical composition or uniform size and color to qualify for any premium above commodity prices. Although yield is still an important factor, having the right seed composition may result in the greatest profit. Many factors, such as yield, temperature, and drought stress can affect the final composition of soybean seeds.

Changes in soybean yield can affect the composition of the seed. Filho et al. (2004) conducted a soybean breeding study in Brazil to determine if cultivars can be selected for high protein and still maintain high yield. They found that as yield increases, the protein content of the seed decreases in most instances. In a similar study by Escalante and Wilcox (1993), it was also concluded that protein content decreases as

yield increases. Filho et al. (2004) crossed high protein and high yield lines to produce seed, and six out of the eight lines studied maintained high protein and high yield. In a soybean breeding study by Wilcox and Shibbles (2001), no relationships were found between concentrations of carbohydrates and seed yield for 43 Maturity Group III lines planted in Iowa and Indiana. This would indicate that concentrations of either total or component carbohydrates could be altered without affecting seed yield. Wilcox and Shibbles (2001) also found that increases in protein caused decreases in oil and carbohydrates. Of the carbohydrates, sucrose decreased the most with an increase in protein. They concluded that protein could be increased and carbohydrate decreased to increase the nutritional value of soybean meal. The results in the yield studies are inconsistent, and more research is needed to determine if carbohydrate content is affected by yield.

Temperature during the growing season has an effect on the composition of soybean seeds. In controlled growth chamber studies, Piper and Boote (1999) and Thomas et al. (2003) found that changes in temperature caused changes in soybean oil and carbohydrate contents, but protein was not affected by temperature changes. Starch and soluble sugar contents decreased with increased temperature, and dramatic decreases occur when the mean air temperature is greater than 20° C (Piper and Boote, 1999; Thomas et al., 2003). In a growth chamber study conducted by Wolf et al. (1982), sucrose concentration decreased by 56% with a 15° C temperature increase and stachyose showed a slight reduction while other sugars remained unchanged for the Maturity Group V cultivar studied. In the same study, oil content was found to be positively correlated

with temperature resulting in a negative correlation between sugar and oil content, which is opposite of the conclusions made by Hymowitz et al. (1972).

In a growth chamber study using three cultivars, air temperature did not affect seed yield or protein content (Howell and Cartter, 1958). Although air temperature alone did not affect seed yield, environmental stress caused by high air temperature and drought stress combined can dramatically reduce seed yield, seed size and seed number (Dornbos and Mullen, 1992). In a greenhouse study of two soybean cultivars, Dornbos and Mullen (1992) found that drought conditions affected soybean seed composition by increasing the amount of protein and decreasing the amount of oil in the seed of the severely drought stressed plants. In a study conducted by Poysa and Woodrow (2002), five food grade soybean cultivars and five breeding lines were grown at three locations over two years in Ontario, Canada. In a hot dry year, all cultivars had smaller seed size, lower protein content, and higher seed oil, free sugar, and sucrose content compared with the same soybean grown in an average year. In a field and greenhouse study of two Maturity Group II cultivars by Egli and Bruening (2004), soybean seed sucrose levels were not affected by water stress in field or greenhouse trials. The primary effect of water stress during seed-filling was to accelerate leaf senescence, which shortened the seed-filling period, reducing seed size and yield. The effect of drought stress on soybean composition has been researched, but the conclusions are different for each study. The composition of soybean seeds can be affected by environment and more specific studies are needed to determine which environmental factors are causing the variation.

## **Planting Date Effects on Seed Composition**

Although farmers cannot control many environmental factors, there are some factors that can be controlled that may affect soybean seed composition. One way to change the environment that a soybean plant grows in is to change the planting date. In the Midwestern United States, soybean is typically planted from mid-April to mid-July. Early plantings are subject to late spring frosts and low soil temperatures, while late plantings may be subject to early autumn frosts and late summer droughts (Keim et al., 1999). Soybean cultivars respond much differently to planting date than other crops because flowering is closely related to photoperiod. The shift from vegetative to reproductive stages in soybean is caused by changes in length of darkness. Most soybean cultivars will flower soon after the dark period begins to lengthen in late June. Soybean flowering is also influenced by temperature. High temperatures during the vegetative period can cause earlier flowering (Elmore and Flowerday, 1984). Many studies have examined the effects of planting date on soybean yield. Later plantings in most instances caused reduced grain yields (Anderson and Vasilas, 1985; Trostle and Bean, 2001). Planting date can also influence the composition of soybean seeds.

In a four year study in Wisconsin conducted by Pedersen and Lauer (2003), planting date was found to influence oil content in three Maturity Group II soybean cultivars. Oil content was higher in the early planting date at one location and was not affected by planting date at the other location studied. They also found that protein content was not influenced by planting date at either location. However, planting date studies by Kane et al. (1997) and Helms et al. (1990) found that planting date did influence soybean seed protein content. Kane et al. (1997) studied six cultivars in the

Maturity Group 00 through IV range in Kentucky and Helms et al. (1990) studied three cultivars in North Dakota. Both studies found delayed planting increased protein content and reduced oil content. Results of studies on the influence of planting date on protein and oil composition are inconsistent. Soybean seed carbohydrate content was not measured in any of these planting date studies.

Planting date has been studied to determine the best time to plant for optimum yields. When planting specialty soybean such as tofu or natto, the composition of the soybean seed may be more important than maximizing yield, so studying the effects of planting date on the composition of food grade soybean cultivars is necessary for producing soybean seed with a specific composition.

### **Location Effects on Seed Composition**

The location in which soybean is grown can affect the composition of the soybean seed. Soil types, average temperatures and rainfall can vary among locations within a state. Helms et al. (1998) studied two Maturity Group 0 tofu cultivars at four locations in North Dakota and Minnesota over a two year period. They found that grain yield and protein and oil content varied among locations in the same year. These results show that the effect of location within the same year can have an influence on chemical composition of soybean seeds. The reason for this effect could be variation in soil type and soil properties among locations; however the exact cause is unknown.

In a study conducted at eight locations in Iowa by Geater and Fehr (2000), there were significant differences among cultivars for total sugar, but the cultivar by location interaction was not significant for total sugar content, which indicated that the differences among the cultivars were consistent across the locations. This study included three small

seeded cultivars in the total of 23 cultivars, and found that all cultivars in the study had a significant negative correlation between total sugar and oil, but when the three small seeded cultivars were excluded, a positive correlation between sugar and oil was found for the remaining cultivars. When looking at the correlation of protein to total sugars, including the three small seeded cultivars showed no significant relationship, while excluding these three cultivars caused the correlation to become significantly negative. Protein + oil had the greatest association with total sugar and was not influenced by the seed size of the cultivars. The results of this study conclude that using protein or oil analysis of soybean to estimate total sugar content may not be reliable and analysis for sugar content alone is necessary when trying to determine accurate amounts of sugars in diverse soybean cultivars.

At three locations in Iowa, 16 small seeded cultivars and lines used for natto were studied for two years (Geater, et al. 2000). Significant differences among locations were observed for all composition measurements, except stachyose. They determined the sugar content of natto soybean cultivars by using HPLC analysis. The range among environments in grams of carbohydrate per 100 g of dry seed weight was 6.2 to 7.1 for sucrose, 0.49 to 0.58 for raffinose, and 4.7 to 4.9 for stachyose. Stachyose was not significantly correlated with any natto quality traits. In this study, a major percentage of the variation in total sugar, free sugar, and sucrose was accounted for by the variation in protein, protein + oil, and protein + oil + fiber. Analysis of protein, oil, and fiber can be done using near infrared (NIR), which is much faster than sugar analysis methods. Geater et al. (2000) concluded that using these three traits for indirect selection in specific soybean cultivars, such as natto, is a possibility.

In a study conducted at five locations in Canada by Cober et al. (1997), two data sets of 7 and 15 natto lines were planted over two years. In both data sets, there were no location effects, and year effects were significant for sugar content. These natto soybean lines contained lower sugar levels than what is desired by processors, which has been a problem for natto soybean growers in North America (Wilson, 1995). Cober et al. (1997) found a significant positive correlation between sugar and oil content in natto soybean seeds. Since it is desirable to have high sugar content but low oil content for the production of natto, this may prove to be problematic.

In research done on soybean composition and location effects, it has been determined that location can affect the composition of soybean seeds. Location has been found to affect protein and oil content by Helms et al. (1998). The location effect on sugar content of soybean seeds was not significant in the studies by Geater and Fehr (2000) and Cober et al. (1997), however in the study by Geater et al. (2000) location significantly affected sugar content. More research is needed to determine how location affects soybean composition, specifically the carbohydrate content of soybean seeds.

### **Summary**

Soybean seed composition has been studied to gain knowledge about the protein and oil content of the seed. There have not been many studies focusing on the carbohydrate content of soybean seeds related to planting date or growing location. It is important to producers of soy foods to have proper carbohydrate composition in soybean cultivars used to make tofu and natto products for human consumption.

The objective of this study is to determine if environment, altered by planting date and location, affects seed composition of tofu and natto soybean cultivars.

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## **CHAPTER II**

### **PLANTING DATE EFFECTS ON SOYBEAN SEED COMPOSITION**

#### **ABSTRACT**

Farmers traditionally manage soybean [*Glycine max* (L.) Merr.] to maximize yield. To produce specialty soybean seed for use in manufacturing tofu and natto products, a specific seed composition is desired. The objective of this study was to determine if planting date affects sucrose, raffinose, and stachyose composition in seeds of tofu and natto soybean cultivars. Planting date effects on protein and oil content and yield were also studied to determine if desirable combinations of all important traits are possible. Four tofu, two natto, and four commodity soybean cultivars were planted on three dates near Columbia, MO in 2004 and 2005. Seed composition was measured by near infrared (NIR) and high performance liquid chromatography (HPLC). In 2004 and 2005 the planting date by cultivar interaction was significant for protein, sucrose, and raffinose. For two tofu cultivars, 5346BP22 and 53462163, yields were not significantly different from the commodity cultivars. Natto cultivars yielded less than tofu and commodity cultivars for both years. Two tofu cultivars, 5346BP22 and 534545, had high percentages of sucrose. For natto cultivars, although the soluble carbohydrates were lower than desired, protein was high and oil was lower than average.

## INTRODUCTION

Current soybean management strategies emphasize the importance of yield as the predominant factor affecting profitability. However, growing interest in specialty crop management has focused attention on soybean seed composition (Poysa et al., 2002). Producing soybean grain with the desired chemical composition is essential to meeting end-user needs and may be as critical to profitability of specialty soybeans as yield.

The US Food and Drug Administration approved a health claim in 1999 that stated 25 g of soybean protein per day can lower cholesterol and reduce incidence of heart disease (Bachmann, 2001). So, food grade soybean cultivars may be an important niche for USA farmers. Tofu is a product made from large-seeded soybean cultivars. The consumption of tofu in the USA and internationally has been steadily increasing (Pollock and St. Martin, 2002). Natto is made from small-seeded soybean cultivars. Although the demand for natto in the USA is not as great as the demand for tofu, Asian countries are not able to produce enough natto soybean to satisfy their own demands (Taira, 2001).

The two soybean seed components that have garnered the most interest are protein and oil. However, carbohydrate content is also important in soybean seeds used for tofu and natto production. High amounts of the soluble carbohydrate sucrose are desired in tofu for flavor and in natto for fermentation. The oligosaccharides raffinose and stachyose are not desirable in tofu because they are not digestible by humans and can cause intestinal distress. Developing soybean management strategies that produce the desired seed composition for tofu and natto is important for maximizing profitability and attracting growers.

Planting date is an important component of any soybean management strategy. It has been well documented that soybean yield is affected by planting date (Keim et al., 1999; Anderson and Vasilas, 1985). Several studies have determined the effects of planting date on soybean seed composition (Pedersen and Lauer, 2003; Kane et al., 1997; Helms et al., 1990). These studies focused on protein and oil and did not measure carbohydrate content. The influence of planting date on important soybean seed carbohydrates is not well understood. The objective of this study was to determine if planting date affects sucrose, raffinose, and stachyose composition in seeds of tofu and natto soybean cultivars. Planting date effects on protein and oil content and yield were also studied to determine if desirable combinations of all important traits are possible.

## **MATERIALS AND METHODS**

This experiment was conducted in 2004 and 2005 at the University of Missouri Bradford Research and Extension Center near Columbia, Missouri (N38°53'36.80", W92°12'53.21"). The predominant soil type at this location is Mexico silt loam (fine, smectitic, mesic, Aeric Vertic Epiaqualfs).

Ten soybean cultivars were selected for this study: two small-seeded natto (IA 4002 and 534022), four large-seeded tofu (533559A, 53462163, 534545, and 5346BP22), and four commodity soybean cultivars. Tofu and natto seeds were obtained from the Missouri Soybean Programs office, except for IA 4002 which was provided by Dr. Walt Fehr, Iowa State University. The commodity cultivars were Dekalb brand DKB 38-52, Asgrow brand AG4403, Pioneer brand 93M50, and Pioneer brand 94M70. Planting dates in 2004 were 29 April, 2 June, and 22 June and in 2005 were 18 April, 10 May, and 17 June. The experimental design was a split plot with whole plots arranged in randomized

complete block with four replications. Whole plots were the three planting dates and split plots were the 10 cultivars.

Plots were planted without tillage using a four-row Kinze planter with a planting depth of 3.8 cm. The previous crop each year was corn (*Zea mays*, L.). Seeding rate was 430,000 seeds/ha, and it was not adjusted for planting date. Plot size was four 0.76 m-rows wide and 7.6 m long. Before planting a broadcast application of 23 kg P/ha and 57 kg K/ha was made.

In both years, a tank mix of S-metolachlor/benoxacor [Acetamide, 2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)-(S)], chloransulam-methyl [N-{2-carboxymethyl-6-chlorophenyl}-5-ethoxy-7-fluoro(1,2,4)triazolo-{1,5-C}pyrimidine-2-sulfonamide], and sulfentrazone [N-[2,4-dichloro-5-[4-difluoromethyl]-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl] methanesulfonamide] was sprayed for pre-emergence weed control. Tofu and natto cultivars were not glyphosate resistant. Fomesafen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-(methylsulfonyl)-2-nitrobenzamide, sodium salt], quizalofop P-ethyl [ethyl(r)-2-[4-(6-chloroquinoxalin-2-yloxy)phenoxy]propionate], and aciflourfen [Sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate] were used for post emergence weed control. In 2005, chloransulam-methyl was added to the tank mix.

Daily high and low air temperatures and precipitation measurements were collected by an automated weather station located at the Bradford Research and Extension Center. Plots were irrigated on 1 July and 21 July with 2.54 cm of water and with 3.18 cm on 4 August in 2005. No irrigation was necessary in 2004.

Emerging plants in 6.1 m of each of the center two rows of each plot were counted. Plants were counted at the V1 growth stage and stand densities were calculated. After plants matured, the center two rows of each plot were trimmed to 6.1 m. Plants were harvested with a small plot combine. Yield was corrected to 13% moisture.

### **Laboratory Analysis**

Protein and oil percentages were determined using near infrared (NIR) on whole soybean seeds. Soybean seed samples were tested using a Pacific Scientific scanning monochromator, model 5000 (NIRSystems, Silver Springs, MD) and software developed by Infracore International (Port Matilda, PA). Soybean samples were scanned with near infrared radiation from 1110 to 2490 nm, and log 1/reflectance was recorded at 2-nm intervals.

Sucrose, raffinose, and stachyose were determined by high performance liquid chromatography (HPLC). From each plot, 100 g of cleaned, whole soybean seeds were randomly sampled and ground with a Retsch ZM-100 grinder employing a 0.5 mm sieve. The ground soybean samples were stored at 4.4° C, 35% RH.

Duplicate 12.5 mg samples were weighed from each ground sample and placed in 96 well plates. Each sample was digested in 1 mL of 50% ETOH. The plates were then placed on a shaker for 10 minutes before incubating at 70° C for 30 minutes. The samples were allowed to settle for 1 hour. After settling, 200 µl were removed by pipette from each sample and placed in a 96 well filter plate. The filter plates were centrifuged for 5 minutes at 3000 rpm. Sample plates were covered with rubber caps and stored at -20° C prior to HPLC analysis.



An Agilent 1100 series HPLC (Wilmington, DE) and an ESA Coulochem III detector (Chelmsford, MA) were used to measure carbohydrates. Sucrose, raffinose, and stachyose were separated using a Dionex (Sunnyvale, CA) Carbo Pac PA 10 analytical column (250 mm x 4 mm, 10  $\mu$ m) connected to a Carbo Pac PA 10 guard column (50 mm x 4 mm). The mobile phase was 90 mM NaOH with a flow rate of 1.5 ml/min, maintained at 30° C and sparged with helium. A standard curve was used to convert the HPLC area measurements to grams of carbohydrate per 100 g seed weight.

### **Data Analysis**

Analyses of variance were calculated using PROC GLM in SAS. Means were compared using an LSD of 0.05 if a significant F-test was found. Single-degree-of-freedom comparisons were calculated to compare 1) the mean of two natto cultivars to the mean of four commodity cultivars, 2) the mean of four tofu cultivars to the mean of four commodity cultivars, and 3) the mean of two natto cultivars to the mean of four tofu cultivars. Data were not combined over the two years.

## **RESULTS AND DISCUSSION**

### **Yield**

In 2004 and 2005 there were no planting date by cultivar interactions for yield. Planting date affected yield in 2004, but not 2005 (Table 2.1). In 2004, yield was highest for the earliest (29 April) planting date and decreased with each later planting date. The results in 2004 are in agreement with planting date studies conducted by Anderson and Vasilas (1985) and Trostle and Bean (2001). In 2005, yield did not differ across planting dates. In 2005, two tofu cultivars, 534545 and 533559A, produced yields for the first two planting dates that were much less than expected. These low yields may be attributed to

poor stands (Table 2.2). Weather conditions in 2005 following the first planting date were cold and wet. The average high temperature for the week after planting was 12.9°C and the low was 3.9°C. Also, the plots received 4.27 cm of precipitation during the week of planting and an additional 1.32 cm in the first week after planting. Temperatures did not return to normal until approximately three weeks after the first planting date. Normally, yield is not affected by decreased stands. However, soybean stands that are under 125,000 plants/ha can have significant decreases in yield (Conley, 2006).

In both years, the average yield of the two natto cultivars was less than average yield of the commodity cultivars on all three planting dates (Table 2.1). Soybean yield components are seed size and number of seeds. To produce yields equivalent to commercial cultivars, small-seeded cultivars must compensate by increasing seed number. Apparently, the two natto cultivars used in this study did not increase seed number enough to produce yields equal to the commodity cultivars.

The average yield of the four tofu cultivars was less than the average yield of the commodity cultivars for five of the six planting date and year combinations. However, the means of two tofu cultivars, 5346BP22 and 53462163, were not significantly different than any of the commodity cultivars in 2004 and 2005. These results indicate that it is possible to select tofu cultivars with high yield potential.

### **Protein**

A significant planting date by cultivar interaction for seed protein was found in each year. So, planting date means across all cultivars were not compared (Table 2.3). In 2004, 29 April and 2 June planting dates were significantly different for three of the 10 cultivars. The two natto cultivars, IA 4002 and 534022, and one tofu cultivar, 5346BP22,

increased in protein content. Studies conducted by Helms et al. (1990) and Kane et al. (1997) also found that later planting increased protein content of soybean seeds. However, four cultivars, 5346BP22, 533559A, and two commodity cultivars exhibited a decrease in seed protein content when planting was delayed from 2 June to 22 June. When comparing 29 April planting date with the 22 June planting date, there were no significant differences for any of the tofu and commodity cultivars, but the two natto cultivars increased in protein content.

In 2005, there was less variation among planting dates for seed protein content. Protein content of natto cultivars decreased when planting was delayed from 18 April to 17 June, which is opposite of results in 2004. Two tofu cultivars, 53462163 and 533559A, exhibited greater protein when planted on 17 June than when planted on 18 April.

High concentrations of protein in seeds of tofu and natto soybean cultivars are desired. An average soybean seed contains about 40% protein on a dry weight basis (Liu, 1997). Most tofu cultivars contain at least 45% protein (Bachmann, 2001). For all three planting dates in 2004 and 2005, natto cultivars had higher seed protein concentrations than all of the commodity cultivars (Table 2.3). Both natto cultivars, IA 4002 and 534022, produced seeds with protein contents over 45% in 2004 for the 2 June and 22 June planting dates and in 2005 for the 18 April planting date. The four tofu cultivars differed for seed protein content for each planting date in both years. One cultivar, 53462163, was the lowest of all cultivars, including the commodity cultivars, for seed protein. Another tofu cultivar, 533559A, had a greater seed protein content than all of the commodity cultivars in 2004. This cultivar had a protein content above 45% only on the

2 June planting date in 2004. While tofu cultivars, on average, were higher in protein than commodity cultivars in 2004 and on 18 April 2005, they were low when compared to the standard protein content of 45% for tofu.

## **Oil**

Soybean seeds contain 20% oil, on average (Liu, 1997). For tofu and natto soybean cultivars, low oil content is desired to reduce the saturated fat content of the finished product (Messina, 1999). In 2004, there was not a planting date by cultivar interaction for seed oil concentrations and planting dates did not differ (Table 2.4). The two natto cultivars had average oil contents of 17.4 and 16.2%, respectively. All tofu cultivars had lower oil content than the commodity cultivars with the exception of one cultivar, 53462163, which was the highest among all cultivars for oil content with an average of 22.2%.

For 2005, there was a planting date by cultivar interaction for oil content. The two natto cultivars, but none of the other cultivars, increased in oil when planting was delayed from 18 April to 10 May. All tofu and commodity cultivars exhibited a decrease in oil content, when planting was delayed until 17 June. These tofu and commodity cultivar results are consistent with previous planting date studies that found delayed planting reduced oil content (Pedersen and Lauer, 2003; Kane et al., 1997; Helms et al. 1990).

## **Soluble Carbohydrates**

### **Sucrose**

Sucrose is desired in high amounts in tofu and natto soybean cultivars because it is easily digested. Sucrose influences the flavor of tofu and is used during the

fermentation process when making natto. On average, soybean seeds of commercial cultivars contain 4 to 5% sucrose (Wilson, 1995). The planting date by cultivar interaction was significant in both 2004 and 2005 (Table 2.5). In 2004, planting date affected seed sucrose content of only one of the 10 cultivars, the commodity cultivar 94M70. The average sucrose contents of the two natto cultivars were lower than the average of the four commodity cultivars for all three planting dates in 2004. Tofu cultivars differed among themselves for sucrose. Two tofu cultivars, 5346BP22 and 534545, had much higher amounts of sucrose than other cultivars measured. These cultivars had 6 to 7% sucrose and would be more desirable for producing tofu because of the high sucrose content.

In 2005, planting date affected sucrose content for six of the 10 cultivars (Table 2.5). For each of these 10 cultivars, the latest planting date produced the largest sucrose content. Unlike 2004, sucrose content for natto cultivars was not consistently lower than sucrose content of commodity cultivars. The two tofu cultivars identified as high sucrose in 2004 also had high amounts of sucrose in 2005 except for 534545 planted on 18 April.

### Raffinose

Raffinose content of soybean seeds averages 1 to 2% (Wilson, 1995). Raffinose is not desired in tofu cultivars because it is not digestible by humans and can cause intestinal distress. Natto soybean cultivars can have higher amounts of raffinose because it is metabolized during the fermentation process (Cober et al., 1997). Because of the small amount of raffinose in soybean seeds, it is difficult to measure. In 2004, all cultivars contained less than 1% raffinose (Table 2.6). In 2005, raffinose was lower than 1% for one natto, one tofu, and one commodity cultivar on all planting dates.

In 2004 and 2005, there was an interaction between planting date and cultivar for raffinose. For natto cultivars there were no effects from planting date in both years. For tofu cultivars in 2004 the 29 April and 2 June planting dates did not differ for raffinose content, but raffinose content decreased with a delay in planting from 2 June to 22 June. The only difference among planting dates in 2005 for raffinose were the 18 April to 10 May planting dates of tofu cultivars 53462163 and 533559A.

### Stachyose

Stachyose is an oligosaccharide that is found in soybean seeds in the range of 3.5 to 4.5%, on average (Wilson, 1995). High amounts of stachyose are desirable in natto cultivars to feed the bacteria during the fermentation process, and low amounts of stachyose are desired in tofu cultivars because it is not digestible by humans. In 2004, there was an interaction between planting date and cultivar (Table 2.7). There was no planting date by cultivar interaction in 2005. However, planting date affected stachyose content of only one cultivar, the commodity cultivar 93M70.

For all planting dates in 2004 and for the 18 April and 10 May planting dates in 2005, the average stachyose content of the two natto cultivars was not significantly different than the average of the four commodity cultivars. Tofu cultivars, on average, were lower for stachyose than the average of the commodity cultivars for 29 April and 22 June planting dates in 2004 and all planting dates in 2005. In 2005, planting date averages across all cultivars were not significantly different. Although tofu cultivars were lower in stachyose when compared to the commodity cultivars, the percentage of stachyose for most cultivars in 2005 was above the average range of 3.5 to 4.5%.

## **CONCLUSIONS**

In 2004 and 2005 the planting date by cultivar interaction was significant for protein, sucrose, and raffinose. The planting date by cultivar interaction was also significant for stachyose in 2004 and oil in 2005.

Planting date had an effect on tofu soybean composition. Oil decreased for later plantings of tofu cultivars in 2005. Sucrose increased for later plantings of tofu cultivars in 2005. These results may indicate that planting tofu cultivars later may improve the composition of the seed by increasing sucrose and decreasing oil content. Two high yielding tofu cultivars, 5346BP22 and 53462163, were greater than or not significantly different than the commodity cultivars for yield in 2004 and 2005.

Natto cultivars had higher protein and lower oil than commodity cultivars which is desired by manufacturers of natto. In 2004, yield and raffinose decreased with later planting dates, and protein increased with later planting dates for natto cultivars. Sucrose in natto cultivars was lower than commodity cultivars, but was in the average range of 4 to 5%. To produce quality seed desired for natto production, sucrose content needs to be increased. Increasing the yield of natto cultivars would also be beneficial to farmers growing natto soybean cultivars.

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Table 2.1. Yields of 10 soybean cultivars planted on three dates in 2004 and 2005.

		2004				2005			
Cultivar	Type	29 April	2 June	22 June	Mean	18 April	10 May	17 June	Mean
-----kg/ha-----									
IA4002	natto	3852†	3215	2541	3203d‡	3560†	3514	3050	3388b‡
534022	natto	3375	3279	2588	3081d	3443	3583	3260	3429b
5346BP22	tofu	4645	3773	3195	3871abc	4658	4559	3801	4339a
534545	tofu	4357	3652	2748	3586c	2368	2887	3438	2898c
53462163	tofu	4871	4092	3282	4082a	4623	4332	3847	4267a
533559A	tofu	3334	3578	2793	3235d	1572	2840	3123	2512d
DKB38-52	commodity	4441	4238	3349	4010a	4183	4530	3642	4118a
93M50	commodity	4579	3904	3264	3916ab	3482	3664	3821	3656b
AG4403	commodity	4285	4057	3301	3881abc	4285	4298	3984	4189a
94M70	commodity	4421	3608	2869	3633bc	4424	4146	3843	4138a
Mean		4216a§	3740b	2993c		3364a§	3835a	3581a	
natto vs. commodity		**	**	**		**	**	**	
tofu vs. commodity		n.s.	*	*		**	**	**	
natto vs tofu		**	**	**		n.s.	n.s.	**	

†FLSD(0.05) to compare any two numbers within a year are 593 and 575 kg/ha in 2004 and 2005, respectively.

‡Cultivar means within a column and within a year followed by the same letter are not significantly different (FLSD 0.05).

§Planting date means within a row and within a year followed by the same letter are not significantly different (FLSD 0.05).

\* = single-degree-of-freedom comparison significant at P=0.05 (F-test).

\*\* = single-degree-of-freedom comparison significant at P=0.01 (F-test).

n.s. = not significant.

Table 2.2. Stand densities of 10 soybean cultivars planted on three dates in 2004 and 2005.

		2004				2005			
Cultivar	Type	29 April	2 June	22 June	Mean	18 April	10 May	17 June	Mean
-----plants/ha -----									
IA4002	natto	320090†	426338	375769	374067a‡	200123†	263603	419613	294440¶
534022	natto	331118	415848	361244	369428ab	196896	228098	270059	231691
5346BP22	tofu	331925	395943	344298	357389bc	153320	225946	366355	248540
534545	tofu	310945	362589	317669	330402e	105979	159238	362589	209269
53462163	tofu	303414	381148	319283	334616de	164080	162466	334077	220200
533559A	tofu	253113	317400	248540	273019f	61320	132878	323856	172687
DKB38-52	commodity	324932	404819	353444	361065abc	217876	282970	416924	305931
93M50	commodity	323048	407778	369582	336802ab	318476	293191	409930	340532
AG4403	commodity	331925	398094	364472	364831ab	397019	331925	422303	383742
94M70	commodity	314710	388412	344298	349139cd	195282	284584	409392	296419
Mean		314522§c	389838a	339860b		201038¶	236490	373510	
natto vs. commodity		n.s.	**	n.s.		**	*	*	
tofu vs. commodity		**	**	**		**	**	**	
natto vs tofu		*	**	**		**	**	n.s.	

†FLSD(0.05) to compare any two numbers within a year are 30,198 and 75,746 plant/ha in 2004 and 2005, respectively.

‡Cultivar means within a column in 2004 followed by the same letter are not significantly different (FLSD 0.05).

§Planting date means within a row in 2004 followed by the same letter are not significantly different (FLSD 0.05).

¶Planting date x cultivar interaction was significant ( P=0.05, F-test).

\* = single-degree-of-freedom comparison significant at P=0.05 (F-test).

\*\* = single-degree-of-freedom comparison significant at P=0.01 (F-test).

n.s. = not significant.

Table 2.3. Protein concentrations in seeds of 10 soybean cultivars planted on three dates in 2004 and 2005.

Table 2.5: Protein concentrations in seeds of 16 soybean cultivars planted on three dates in 2004 and 2005.									
Cultivar	Type	2004				2005			
		29 April	2 June	22 June	Mean	18 April	10 May	17 June	Mean
-----%									
IA4002	natto	43.8†	45.3	46.0	45.0‡	45.2†	44.1	43.9	44.4‡
534022	natto	43.4	46.6	46.0	45.3	45.3	44.4	43.9	44.5
5346BP22	tofu	41.2	43.1	41.3	41.9	42.1	41.4	42.7	42.1
534545	tofu	41.5	42.7	41.9	42.0	40.9	40.7	41.3	41.0
53462163	tofu	39.6	39.8	39.6	39.7	39.2	39.8	40.9	40.0
533559A	tofu	44.4	45.6	43.3	44.4	42.4	42.9	44.4	43.2
DKB38-52	commodity	40.1	39.8	39.8	39.9	42.5	42.3	41.1	42.0
93M50	commodity	41.0	40.3	40.8	40.7	41.4	40.4	42.2	41.4
AG4403	commodity	40.7	42.1	40.2	41.0	41.4	39.6	41.1	40.7
94M70	commodity	41.7	42.9	41.3	42.0	41.4	41.7	43.0	42.0
Mean		41.7‡	42.8	42.1		42.2‡	41.7	42.4	
natto vs. commodity		**	**	**		**	**	**	
tofu vs. commodity		**	**	**		*	n.s.	n.s.	
natto vs tofu		**	**	**		**	**	**	

<sup>†</sup>FLSD(0.05) to compare any two numbers within a year are 1.5 and 1.2% in 2004 and 2005, respectively.

<sup>‡</sup> Planting date x cultivar interaction was significant (P=0.05, F-test).

\* = single-degree-of-freedom comparison significant at P=0.05 (F-test).

\*\* = single-degree-of-freedom comparison significant at P=0.01 (F-test).

n.s. = not significant.

Table 2.4. Oil concentrations in seeds of 10 soybean cultivars planted on three dates in 2004 and 2005.

		2004				2005			
Cultivar	Type	29 April	2 June	22 June	Mean	18 April	10 May	17 June	Mean
-----%									
IA4002	natto	18.1†	17.3	16.8	17.4ef‡	18.8†	19.9	19.2	19.3¶
534022	natto	17.1	15.7	15.8	16.2f	18.9	19.9	19.0	19.3
5346BP22	tofu	19.5	20.3	18.5	19.4cd	20.6	21.3	19.0	20.3
534545	tofu	18.4	20.8	19.3	19.5cd	21.8	21.6	20.6	21.4
53462163	tofu	21.9	22.8	21.8	22.2a	23.1	22.7	21.1	22.3
533559A	tofu	18.6	19.1	19.5	19.1cd	21.4	21.1	18.9	20.4
DKB38-52	commodity	20.3	21.9	21.2	21.2ab	21.4	21.4	20.9	21.2
93M50	commodity	21.0	21.5	21.5	21.3ab	22.8	22.9	20.6	22.1
AG4403	commodity	20.6	20.5	20.8	20.6abc	21.0	21.9	20.3	21.0
94M70	commodity	20.1	20.0	20.1	20.0bcd	21.2	21.5	20.2	21.0
Mean		19.7a§	20.0a	19.1a		21.1¶	21.4	20.0	
natto vs. commodity		**	**	**		**	**	**	
tofu vs. commodity		n.s.	n.s.	n.s.		n.s.	n.s.	*	
natto vs tofu		**	**	**		**	**	**	

†FLSD(0.05) to compare any two numbers within a year are 2.8 and 0.9% in 2004 and 2005, respectively.

‡Cultivar means within a column in 2004 followed by the same letter are not significantly different (FLSD 0.05).

§Planting date means within a row in 2004 followed by the same letter are not significantly different (FLSD 0.05).

¶Planting date x cultivar interaction was significant (P=0.05, F-test).

\* = single-degree-of-freedom comparison significant at P=0.05 (F-test).

\*\* = single-degree-of-freedom comparison significant at P=0.01 (F-test).

n.s. = not significant.

Table 2.5. Sucrose concentrations in seeds of 10 soybean cultivars planted on three dates in 2004 and 2005.

Table 2.5: Sucrose concentrations in seeds of 10 soybean cultivars planted on three dates in 2004 and 2005.									
Cultivar	Type	2004				2005			
		29 April	2 June	22 June	Mean	18 April	10 May	17 June	Mean
-----%									
IA4002	natto	4.30†	4.14	4.73	4.39‡	4.50†	4.71	4.86	4.69‡
534022	natto	4.69	3.90	4.73	4.44	4.34	5.00	5.30	4.88
5346BP22	tofu	6.98	6.44	6.06	6.49	6.09	6.13	7.05	6.42
534545	tofu	6.05	6.81	7.03	6.62	4.73	5.98	7.35	6.02
53462163	tofu	3.83	4.45	4.14	4.14	4.70	4.40	4.99	4.70
533559A	tofu	4.31	4.36	4.06	4.25	4.09	4.65	4.59	4.44
DKB38-52	commodity	5.08	5.69	4.96	5.24	4.28	5.13	6.24	5.21
93M50	commodity	5.70	4.58	5.08	5.12	4.91	4.94	5.91	5.25
AG4403	commodity	5.75	4.95	5.93	5.54	5.08	5.54	6.15	5.59
94M70	commodity	5.40	4.30	6.23	5.31	5.43	5.11	5.38	5.30
Mean		5.21‡	4.96	5.29		4.81‡	5.16	5.78	
natto vs. commodity		**	**	**		**	n.s.	**	
tofu vs. commodity		n.s.	**	n.s.		n.s.	n.s.	n.s.	
natto vs. tofu		**	**	**		*	*	**	

†FLSD(0.05) to compare any two numbers within a year are 1.3 and 0.8% in 2004 and 2005, respectively.

‡ Planting date x cultivar interaction was significant (P=0.05, F-test).

\* = single-degree-of-freedom comparison significant at P=0.05 (F-test).

\*\* = single-degree-of-freedom comparison significant at P=0.01 (F-test).

n.s. = not significant.

Table 2.6. Raffinose concentrations in seeds of 10 soybean cultivars planted on three dates in 2004 and 2005.

Table 2.6: Raffinose concentrations in seeds of 16 soybean cultivars planted on three dates in 2004 and 2005.									
Cultivar	Type	2004				2005			
		29 April	2 June	22 June	Mean	18 April	10 May	17 June	Mean
-----%									
IA4002	natto	0.54†	0.54	0.63	0.57‡	0.95†	1.05	0.76	0.92‡
534022	natto	0.50	0.55	0.53	0.53	0.86	0.80	0.76	0.81
5346BP22	tofu	0.81	0.71	0.46	0.66	1.13	1.05	1.26	1.15
534545	tofu	0.44	0.59	0.35	0.46	0.74	0.84	0.68	0.75
53462163	tofu	0.80	0.93	0.49	0.74	1.48	1.10	1.21	1.26
533559A	tofu	0.70	0.66	0.48	0.61	1.20	0.96	0.83	1.00
DKB38-52	commodity	0.88	0.88	0.68	0.81	1.29	1.29	1.15	1.24
93M50	commodity	0.78	0.48	0.61	0.62	1.50	1.36	0.91	1.26
AG4403	commodity	0.65	0.61	0.41	0.56	0.96	0.90	0.83	0.90
94M70	commodity	0.69	0.56	0.45	0.57	1.15	0.95	0.86	0.99
Mean		0.68‡	0.65	0.51		1.13‡	1.03	0.93	
natto vs. commodity		**	**	n.s.		**	**	**	
tofu vs. commodity		n.s.	**	**		n.s.	**	n.s.	
natto vs. tofu		**	**	**		**	n.s.	**	

†FLSD(0.05) to compare any two numbers within a year are 0.18 and 0.22% in 2004 and 2005, respectively.

‡ Planting date x cultivar interaction was significant (P=0.05, F-test).

\* = single-degree-of-freedom comparison significant at P=0.05 (F-test).

\*\* = single-degree-of-freedom comparison significant at P=0.01 (F-test).

n.s. = not significant.

Table 2.7. Stachyose concentrations in seeds of 10 soybean cultivars planted on three dates in 2004 and 2005.

Table 2.7: Stachyose concentrations in seeds of 16 soybean cultivars planted on three dates in 2004 and 2005.									
Cultivar	Type	2004				2005			
		29 April	2 June	22 June	Mean	18 April	10 May	17 June	Mean
-----%									
IA4002	natto	4.29†	4.05	4.50	4.28¶	5.01†	4.98	4.59	4.86cd‡
534022	natto	4.40	3.78	4.29	4.15	4.64	4.60	4.84	4.69de
5346BP22	tofu	4.20	3.61	3.19	3.67	4.66	4.39	4.70	4.58de
534545	tofu	3.54	3.93	3.58	3.68	3.94	4.39	4.65	4.33e
53462163	tofu	3.54	3.98	3.88	3.80	4.78	4.18	5.10	4.68de
533559A	tofu	4.39	3.91	3.68	3.99	4.65	4.84	4.70	4.73de
DKB38-52	commodity	4.20	4.20	3.79	4.06	4.48	4.78	5.59	4.95bcd
93M50	commodity	4.70	3.56	4.08	4.11	5.16	5.26	5.40	5.28abc
AG4403	commodity	5.00	4.40	4.16	4.52	5.24	5.21	5.80	5.42a
94M70	commodity	4.58	3.68	4.54	4.26	5.36	5.31	5.19	5.29ab
Mean		4.28¶	3.91	3.97		4.79a§	4.79a	5.06a	
natto vs. commodity		n.s.	n.s.	n.s.		n.s.	n.s.	**	
tofu vs. commodity		**	n.s.	**		**	**	**	
natto vs. tofu		*	n.s.	**		n.s.	n.s.	n.s.	

†FLSD(0.05) to compare any two numbers within a year are 1.03 and 0.87% in 2004 and 2005, respectively.

‡Cultivar means within a column in 2005 followed by the same letter are not significantly different (FLSD 0.05).

§Planting date means within a row in 2005 followed by the same letter are not significantly different (FLSD 0.05).

¶Planting date x cultivar interaction was significant (P=0.05, F-test).

\* = single-degree-of-freedom comparison significant at P=0.05 (F-test).

\*\* = single-degree-of-freedom comparison significant at P=0.01 (F-test).

n.s. = not significant.



# **CHAPTER III**

## **LOCATION EFFECTS ON SOYBEAN SEED COMPOSITION**

### **ABSTRACT**

Environmental factors such as precipitation and temperature can affect the composition of soybean seeds. It is important to plant soybean cultivars in the appropriate region in Missouri to produce seed composition desired by manufacturers of tofu and natto food products. The objective of this study was to determine if planting tofu and natto soybean cultivars in different soybean-producing regions throughout Missouri affects the composition of the soybean seeds. Four tofu and one natto cultivars were planted at seven locations in 2004 and five tofu and one natto cultivars were planted at ten locations in 2005 in north, central, southwest, and southeast Missouri. Sucrose, raffinose, and stachyose were measured using high performance liquid chromatography (HPLC) and protein and oil were measured using near infrared (NIR). Significant location differences in sucrose, raffinose, and stachyose contents were found. The central Missouri location, Truxton, had the highest sucrose concentrations for all cultivars in 2004. Two central Missouri locations, Columbia and Grand Pass, had high sucrose contents for all six cultivars in 2005. For 2004 and 2005, sucrose was negatively correlated with August average high temperatures. Raffinose was low at Grand Pass, and stachyose was low at Oran for both years.

## **INTRODUCTION**

Planting the same soybean cultivars in different geographic locations has been found to vary yields (Helms et al., 1998). The cause for this variation is usually unknown. Temperature, precipitation, and soil type are variables that can affect the yield of soybean cultivars. Farmers may not be able to change location, but they can choose cultivars that are known to perform well at a specific geographic location.

When growing specialty soybean seeds for specific uses, composition is important. If the location can affect the composition of the seed, then planting the right cultivar in the best location is important to maintain the required seed composition. Two studies were conducted in 2000 to determine the effects of location on composition of soybean seeds (Geater and Fehr, 2000; Geater, et al., 2000). Natto soybean cultivars were included in these studies. One study found that location did not affect sugar content, and the other study found that protein, oil, fiber, sucrose, raffinose, free sugar, and total sugar were significantly different among locations. These results indicate that more research is needed to determine how location can affect the composition of soybean seeds.

Managing tofu and natto soybean for composition may require specific cultivars to be planted in locations known to produce the desired composition. The objective of this study is to determine if planting tofu and natto soybean cultivars in different soybean-producing regions throughout Missouri affects the composition of the soybean seeds.

## **MATERIALS AND METHODS**

### **Study Area**

This experiment was conducted at seven locations in 2004 and at 10 locations in 2005. Locations were distributed throughout soybean-producing regions of Missouri.

Soil types and geographic coordinates for each location are listed in Table A.1.

Locations in North Missouri were the Hundley-Whaley Research Center, Albany (Gentry Co.) and the Bill Cason farm, Macon (Macon Co.). These locations will be referred to as Albany and Macon.

Central Missouri locations were the Ryland Utlaut farm, Grand Pass (Saline Co.), the Bradford Research and Extension Center, Columbia (Boone Co.), the Roy Cope farm, Truxton (Montgomery Co.) and the Bob Burkemper farm, Annada (Pike Co.). These locations will be referred to as Grand Pass, Columbia, Truxton, and Annada.

Southwest Missouri locations were the Kurt Gretzinger farm, Urich (Henry Co.), and the Wally Norton farm, Lamar (Barton Co.), and Southeast Missouri locations were the Glen Nothdruff farm, Oran (Scott Co.), Delta Research Center-Loam and Delta Research Center-Clay, Portageville (Pemiscot Co.). These locations will be referred to as Urich, Lamar, Oran, Portageville loam, and Portageville clay.

### **Planting Procedures**

In 2004, four tofu and one natto cultivars were planted at each location. The tofu cultivars were 533559A, 534545, 5346BP22, and 53462163 and the natto cultivar was 534022. These were the same cultivars used in Chapter II. In 2005, five tofu cultivars and one natto cultivar were planted. The tofu cultivars were 5346, 5347, 5349, 53465445, and 533559A. The natto cultivar was 534022. Seed was provided by the

Missouri Soybean Programs office. Maturities for cultivars planted in 2004 ranged from 3.5 to 4.8. In 2005, the range was 3.9 to 4.9.

All tests were planted using commercial equipment modified for small plot work. Row spacing for locations in the North, Central, and Southwest Regions was 0.38 m. Row spacing for the locations in the Southeast Region was 0.76 m. Seeding rates for the 0.38 and 0.76 m row spacing were 374,000 and 430,000 seeds/ha, respectively. Planting dates are listed in Table A.2. Plot size at all locations was four rows wide and 7.62 m long.

### **Plot Treatments**

Pre-emergence and post emergence herbicide treatments for weed control are in Table A.3. In Columbia, fertilizer applications of 23 kg P/ha and 57 kg K/ha were made both years. In 2004, 28 kg K/ha was applied at Truxton. In 2005, 29 kg P/ha and 74 kg K/ha was applied at Albany. No fertilizer applications were made at the other locations.

Daily high and low air temperature and precipitation measurements were collected by automated University of Missouri Extension weather stations located near each location. Irrigation of 20.3 cm in 2004 and 30.5 cm in 2005 was applied at Portageville loam and Portageville clay locations. In 2005, Columbia was irrigated with 7.4 cm of water. All other locations did not have irrigation.

### **Harvest Procedures**

Harvest dates are listed in Table A.2. All four rows were harvested for plots with a 38 cm row spacing. The center two rows were harvested for plots with a 76 cm row spacing. Yield was corrected to 13% moisture.

Seed concentrations of protein and oil were determined using NIR analyses as described in Chapter II. Sucrose, raffinose, and stachyose were determined using HPLC procedures described in Chapter II.

Data were analyzed for each cultivar separately using location as the treatment. Location means were compared using an F-test protected LSD. Pearson correlation coefficients were calculated using location means averaged across all cultivars.

## **RESULTS AND DISCUSSION**

### **Sucrose**

Sucrose is desired in high amounts in tofu and natto soybean seeds. Sucrose influences the flavor of tofu and is used in the fermentation of natto. An average soybean seed contains 4 to 5% sucrose (Wilson, 1995). In 2004, seed sucrose concentration differed among locations for all five cultivars (Table 3.1). Truxton was the highest or not different than the highest location for sucrose for all cultivars except 533559A for which no grain was available for testing. Columbia was another high-sucrose location. It was highest among all locations for three cultivars, equal to the highest location for one cultivar, and ranked second for the natto cultivar, 534022. Oran had the lowest amount of sucrose for all cultivars. Lamar was also a low-sucrose location for two cultivars, 533559A and 53462163. The two locations with low sucrose also had low yields in 2004 (Table 3.2). However, average sucrose was not significantly correlated with average yield in 2004 ( $r = 0.103$ ; Table 3.3).

In 2005, the locations Lamar, Oran, Portageville loam, and Portageville clay had low sucrose contents for all six cultivars (Table 3.4). Columbia and Grand Pass had high sucrose content for all six cultivars. Average sucrose was positively correlated to average

yield in 2005 ( $r = 0.724$ ; Table 3.3). Apparently, environmental characteristics conducive to high yield were also conducive to high seed sucrose concentration in 2005 (Table 3.5).

Sucrose concentrations were negatively correlated to August average high temperatures in both 2004 and 2005 (Table 3.3). These results are in agreement with studies conducted by Thomas et al. (2003), Piper and Boote (1999), and Wolf et al. (1982). Sucrose was positively correlated to precipitation in 2004, but not in 2005. This may be due to the differences between years for total precipitation. Precipitation amounts ranged among location from 18 to 51 cm in 2004 and only 30 to 41 cm in 2005.

### **Raffinose and Stachyose**

Raffinose and stachyose are oligosaccharides that are not digestible by humans. Oligosaccharides are desired in low quantities in soybean seeds used to make tofu, but are desired in high amounts in natto for fermentation. Average raffinose and stachyose contents for soybean seeds are 1 to 2% and 3.5 to 4.5%, respectively (Wilson, 1995). All cultivars studied, except 53462163 and 5346BP22, were below the average range for raffinose for all locations in 2004.

In 2004, Lamar was the highest location for raffinose for the four tofu cultivars (Table 3.6). Truxton was the highest location for raffinose for the natto cultivar. Grand Pass had the lowest raffinose for all five cultivars. Location ranking for stachyose differed somewhat from location ranking for raffinose. Lamar was the lowest location or not different from the lowest location for all cultivars for stachyose, but not raffinose (Table 3.7). Columbia, a central Missouri location, and Portageville clay, a southeast Missouri location, were among the highest locations for stachyose.

In 2005, Lamar was again a high-raffinose location for all six cultivars (Table 3.8). Similar to 2004, Grand Pass was the lowest or equal to the lowest location for raffinose. Oran had low raffinose for five of the six cultivars. As in 2004, the location ranking for stachyose was different than the location ranking for raffinose. Lamar was among the lowest locations for stachyose for five of the six cultivars (Table 3.9). Grand Pass was among the highest locations for four of the six cultivars. Other high locations for stachyose were Columbia and Annada.

Apparently, environmental parameters conducive to high seed raffinose are different from parameters conducive to high seed stachyose. In 2004, raffinose was negatively correlated to August precipitation but stachyose was positively correlated to season-long precipitation. In 2005, stachyose was positively correlated to August precipitation and raffinose was not correlated to any of the weather parameters.

### **Protein and Oil**

Protein and oil are also important in tofu and natto soybean production. High amounts of protein and low amounts of oil are desired. Average soybean seeds contain 40% protein and 20% oil (Liu, 1997). For tofu and natto it is desirable to have protein contents greater than 45% (Bachmann, 2001). Helms et al. (1998) found that protein and oil contents were affected by location. In 2004, Grand Pass had the highest amount of protein for all cultivars, but none of the cultivars were above 45% (Table 3.10). Oil content in 2004 was low at Columbia and Grand Pass, with below average oil content for the natto cultivar 534022, and the tofu cultivar 533559A (Table 3.11). Oil content was highest at Oran and Lamar.

In 2005, protein content was above 45% for 5347 grown at all locations except Columbia, Albany, and Grand Pass (Table 3.12). The natto cultivar 534022 had protein content above 45% at Macon, Columbia, Urich, and Lamar. Oil content in 2005 was low at Macon and Urich (Table 3.13). The natto cultivar 534022 had oil content below 20% at Macon, Columbia, Annada, and Urich.

## **CONCLUSIONS**

Location did affect the composition of the soybean cultivars planted in 2004 and 2005. Significant differences among locations for composition were observed, which is in agreement with a study conducted by Geater et al. (2000). Sucrose was consistently high at Columbia for both years. Stachyose was low for both years at Oran. Knowing that sucrose is negatively correlated to August temperatures may help to determine where cultivars grown for tofu and natto production will contain a desired composition.

While there is not a definite conclusion on which location has the best overall composition for the cultivars studied, the data collected in this study may be helpful to determine growing locations when a specific component is desired in a soybean seed.



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Table 3.1. Sucrose concentrations in seeds of five cultivars planted at seven locations in Missouri in 2004.

Location	533559A	534545	53462163	5346BP22	534022
	----- % -----				
Columbia	4.50a†	6.33a	4.65a	5.45ab	4.75b
Grand Pass	3.39b	5.01bc	3.26bc	4.58bc	3.76c
Truxton	na	5.81ab	4.01ab	5.31ab	5.83a
Oran	2.40c	3.46d	2.84c	3.78c	2.78d
Lamar	3.96ab	4.54c	4.48a	4.28c	3.22cd
Portageville clay	3.69b	5.41bc	4.48a	5.74a	3.75c
Portageville loam	2.56c	5.58ab	3.83ab	4.69bc	3.54cd

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.2. Yields of five soybean cultivars planted at seven locations in Missouri in 2004.

Location	533559A	534545	53462163	5346BP22	534022
	-----kg/ha-----				
Columbia	3131b†	3726bc	3563ab	4302b	2958abc
Grand Pass	3196b	3913ab	3650ab	3860c	3204a
Truxton	220d	3721bc	3826a	3663c	2369d
Oran	2673c	3041d	2898bc	3223d	2352d
Lamar	2451c	3148cd	2513c	3014d	2616bcd
Portageville clay	2609c	3496bcd	3875a	4565ab	3071ab
Portageville loam	3678a	4362a	4058a	4753a	2563cd

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.3. Correlation between various weather parameters and sucrose, raffinose, and stachyose in 2004 and 2005.

	Season‡			August			Yield
	Precipitation	High	Low	Precipitation	High	Low	
2004							
Sucrose	0.793*	-0.690†	-0.460	0.337	-0.683†	-0.415	0.103
Raffinose	0.133	0.104	0.293	-0.708†	0.306	0.540	-0.339
Stachyose	0.757*	-0.351	-0.077	0.210	-0.357	0.009	0.511
2005							
Sucrose	0.319	-0.080	-0.388	0.596†	-0.608†	-0.545†	0.724*
Raffinose	-0.374	0.333	0.348	-0.263	0.543†	0.519	-0.711*
Stachyose	-0.022	0.024	-0.195	0.618†	-0.415	-0.239	0.480

†, \*, \*\* significance of P= 0.10, 0.05, 0.01, respectively.

‡ Dates used to calculate season parameters were planting date through 15 September.

Table 3.4. Sucrose concentrations in seeds of six soybean cultivars planted at 10 locations in Missouri in 2005.

Location	533559A	5346	5347	53465445	534022	5349
	----- % -----					
Macon	3.14de†	4.13d	3.98cd	4.21d	3.10d	4.40cde
Albany	4.40ab	5.94bc	5.73ab	6.61ab	4.89abc	5.43bcd
Columbia	4.25abc	7.10a	5.94a	6.96ab	5.15ab	6.60ab
Grand Pass	4.83a	6.63ab	5.76ab	7.13a	5.01abc	6.59ab
Annada	3.90bc	6.25ab	5.31ab	5.89bc	5.90a	7.65a
Urich	3.64cd	6.08abc	4.99b	4.34d	4.00bcd	4.93cde
Lamar	2.70ef	4.26d	4.13c	5.14cd	3.06d	4.19de
Oran	2.58ef	4.73d	3.26d	4.05d	3.15d	3.71e
Portageville clay	2.50f	4.18d	3.45cd	4.59d	3.83cd	4.40cde
Portageville loam	2.63ef	5.03cd	3.84cd	4.58d	3.95bcd	5.81bc

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.5. Yields of six soybean cultivars planted at 10 locations in Missouri in 2005.

Location	533559A	5346	5347	53465445	534022	5349
	-----kg/ha -----					
Macon	2132c†	2349b	2356abc	1979d	2256de	2578cde
Albany	3069a	2556ab	2888a	3011b	2604bcd	2441de
Columbia	2276bc	3108a	2576a	2893b	2788ab	3810a
Grand Pass	3034a	2618ab	2856a	3965a	3074a	3171b
Annada	2731ab	1337cd	1932bcd	2876b	2321cde	2264de
Urich	2893a	2214b	2438ab	3228b	2297cde	2132e
Lamar	1209d	732e	1084e	1382e	1045f	1642f
Oran	2036c	812de	1814cd	2398cd	2086e	2377de
Portageville clay	1782c	1606c	1657d	2783bc	2884ab	2983bc
Portageville loam	1839c	1192cde	1394de	3061b	2693abc	2616cd

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.6. Raffinose concentrations in seeds of five soybean cultivars planted at seven locations in Missouri in 2004.

Location	533559A	534545	53462163	5346BP22	534022
	----- % -----				
Columbia	0.76b†	0.46d	1.09a	0.68cd	0.63b
Grand Pass	0.51c	0.36d	0.61c	0.54d	0.48cd
Truxton	na	0.66bc	0.84bc	0.74bc	0.79a
Oran	0.73b	0.60c	0.80bc	0.80bc	0.39d
Lamar	0.95a	0.93a	1.19a	0.99a	0.61b
Portageville clay	0.73b	0.71bc	1.10a	1.04a	0.59bc
Portageville loam	0.55c	0.74b	0.98ab	0.88ab	0.53bc

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.7. Stachyose concentrations in seeds of five soybean cultivars planted at seven locations in Missouri in 2004.

Location	533559A	534545	53462163	5346BP22	534022
	----- % -----				
Columbia	4.75a†	3.81b	4.74a	3.76b	4.89a
Grand Pass	3.14c	2.75c	3.10cd	2.90cd	3.60bc
Truxton	na	2.94c	3.14cd	2.89cd	4.89a
Oran	2.05d	1.85d	2.48d	2.21d	2.46d
Lamar	3.64b	3.15c	3.26cd	3.33bc	3.28c
Portageville clay	4.58a	4.26a	4.53ab	4.49a	4.24ab
Portageville loam	3.44bc	4.09ab	3.86bc	3.74b	3.29c

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).



Table 3.8. Raffinose concentrations of six soybean cultivars planted at 10 locations in Missouri in 2005.

Location	533559A	5346	5347	53465445	534022	5349
	----- % -----					
Macon	1.03b†	1.01d	1.24bc	0.88bc	1.00cd	0.96d
Albany	0.98b	1.44abc	1.49ab	1.04bc	0.98cd	0.93d
Columbia	1.10ab	1.45abc	1.23bc	1.09bc	1.00cd	1.00cd
Grand Pass	0.90b	1.20cd	1.19bc	0.90bc	0.90d	1.08cd
Annada	0.94b	1.25cd	1.36ab	0.81c	1.30abc	1.53ab
Urich	1.05b	1.55ab	1.26ab	0.89bc	1.10bcd	1.18bcd
Lamar	1.34a	1.65a	1.55a	2.29a	1.44ab	1.36abc
Oran	0.98b	1.73a	0.94c	1.01bc	0.86d	0.88d
Portageville clay	0.94b	1.65a	1.35ab	1.18bc	1.51a	1.05cd
Portageville loam	1.18ab	1.68a	1.34ab	1.29b	1.48a	1.55a

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.9. Stachyose concentrations in seeds of six soybean cultivars planted at 10 locations in Missouri in 2005.

Location	533559A	5346	5347	53465445	534022	5349
	----- % -----					
Macon	3.99abc†	2.90d	3.30bc	3.66bc	3.78cd	3.55cd
Albany	4.64ab	3.71bcd	3.96abc	4.48ab	4.54bcd	3.36cd
Columbia	4.86a	4.84a	4.30a	5.00a	5.79ab	4.68bc
Grand Pass	4.43ab	4.14ab	4.06ab	4.30abc	4.64bcd	4.18bcd
Annada	4.48ab	4.34ab	4.31a	4.48ab	6.61a	6.78a
Urich	4.60ab	4.71a	4.33a	3.59bc	4.91bc	4.20bcd
Lamar	3.28cd	3.60bcd	3.18cd	4.36ab	4.15cd	3.31cd
Oran	3.04d	3.09cd	2.29d	3.13c	3.24d	3.16d
Portageville clay	3.28cd	2.84d	3.16cd	4.20abc	4.70bcd	3.56cd
Portageville loam	3.86bcd	3.94abc	3.63abc	4.19abc	5.20abc	5.11b

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.10. Protein concentrations in seeds of five soybean cultivars planted at seven locations in Missouri in 2004.

Location	533559A	534545	53462163	5346BP22	534022
	----- % -----				
Columbia	43.5b†	40.8bc	38.6c	40.3a	42.3b
Grand Pass	44.6a	42.1a	41.0a	40.3a	44.0a
Truxton	na	41.2abc	39.8b	41.2a	43.3ab
Oran	43.2b	41.7ab	40.1ab	41.4a	40.1c
Lamar	43.3b	40.7bc	39.9b	41.0a	43.9a
Portageville clay	42.0c	40.4c	39.8b	41.1a	42.4b
Portageville loam	44.7a	40.6c	39.6b	40.9a	43.0ab

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.11. Oil concentrations in seeds of five soybean cultivars planted at seven locations in Missouri in 2004.

Location	533559A	534545	53462163	5346BP22	534022
	----- % -----				
Columbia	19.2d†	20.7d	21.9ef	20.8d	19.5de
Grand Pass	19.1d	20.7d	21.6f	21.0d	19.0e
Truxton	na	21.9c	23.2c	21.7bc	20.1cd
Oran	22.7a	23.0b	23.9b	22.1b	22.8a
Lamar	23.2a	24.2a	25.2a	23.2a	21.0b
Portageville clay	21.3b	21.6c	22.3de	20.9d	20.3bc
Portageville loam	20.0c	21.3cd	22.5d	21.3cd	20.8bc

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.12. Protein concentrations in seeds of six soybean cultivars planted at 10 locations in Missouri in 2005.

Location	533559A	5346	5347	53465445	534022	5349
	----- % -----					
Macon	45.5a†	45.3ab	46.8ab	42.3a	45.8a	43.5a
Albany	42.5e	44.0cd	44.5c	41.2bcde	43.9cd	43.3ab
Columbia	43.4de	44.0cd	44.1c	40.9de	45.1ab	42.3abc
Grand Pass	44.3bcd	44.0cd	44.6c	40.3e	43.9cd	40.4d
Annada	43.9bcd	44.5abcd	45.9b	41.0cde	44.4bc	43.6a
Urich	45.0ab	45.0abc	46.7ab	42.3ab	45.5a	43.2abc
Lamar	44.5abcd	45.7a	47.1a	41.8abcd	45.5ab	43.3ab
Oran	43.6cde	43.4d	46.5ab	42.2abc	43.8cd	42.2bc
Portageville clay	44.7abc	44.4bcd	46.2ab	42.5a	43.6cd	41.9c
Portageville loam	45.0ab	43.9cd	45.8b	41.5abcd	43.0d	42.1bc

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

Table 3.13. Oil concentrations in seeds of six soybean cultivars planted at 10 locations in Missouri in 2005.

Location	533559A	5346	5347	53465445	534022	5349
	----- % -----					
Macon	20.1e†	19.1f	18.5fg	22.1bc	19.8de	20.8de
Albany	21.8bc	20.9bc	20.3abc	22.2bc	20.1bcd	20.8de
Columbia	21.2cd	19.7def	19.7cde	21.5c	19.3ef	20.2e
Grand Pass	20.3e	20.0de	19.1efg	21.6c	20.2bcd	22.3ab
Annada	20.5de	19.4ef	19.4def	21.7c	19.8cde	21.2cd
Urich	19.8e	19.5ef	18.4g	21.7c	18.6f	20.3e
Lamar	21.9bc	20.5cd	19.9bcde	22.3bc	20.3bcd	22.0bc
Oran	22.6ab	22.0a	20.0bcd	22.9ab	20.5bc	22.7ab
Portageville clay	22.0abc	21.5ab	20.7ab	21.9c	20.8b	22.6ab
Portageville loam	22.8a	22.1a	21.1a	23.4a	21.7a	23.1a

† Numbers within a column followed by the same letter are not significantly different (FLSD 0.05).

## **APPENDIX**

Table A.1. Latitude, longitude, and soil type for each location.

Location	Region	Latitude	Longitude	Soil type
Macon	North	39°44'32.23"	92°28'21.21"	Mexico silt loam (Vertic Epiaqualfs)
Albany	North	40°15'04.61"	94°19'37.10"	Grundy silt loam (Aquertic Argiudolls)
Columbia	Central	38°53'36.80"	92°12'53.21"	Mexico silt loam (Vertic Epiaqualfs)
Grand Pass	Central	39°12'23.13"	93°26'33.15"	Haynie silt loam (Mollic Udifluvents)
Truxton	Central	39°00'07.09"	91°14'23.12"	Mexico silt loam (Vertic Epiaqualfs)
Annada	Central	39°15'39.99"	90°49'39.05"	Tice silt loam (Fluvaquentic Hapludolls)
Urich	Southwest	38°27'34.83"	94°00'01.89"	Hartwell silt loam (Typic Argialbolls)
Lamar	Southwest	37°29'41.94"	94°16'35.03"	Parsons silt loam (Mollic Albaqualfs)
Oran	Southeast	37°05'05.83"	89°39'19.27"	Commerce silt loam (Fluvaquentic Endoaquepts)
Portageville clay	Southeast	36°25'41.24"	89°41'57.06"	Portageville clay (Vertic Endoaquolls)
Portageville loam	Southeast	36°25'41.24"	89°41'57.06"	Tiptonville silt loam (Oxyaquic Argiudolls)



Table A.2. Planting and harvest dates for locations in 2004 and 2005.

Location	2004		2005	
	Planting date	Harvest date	Planting Date	Harvest Date
Albany	-	-	9 May	8 Oct
Macon	-	-	10 May	10 Oct
Columbia	28 Apr	6 Oct	18 May	14 Oct
Grand Pass	3 June	8 Nov	19 May	11 Oct
Truxton	13 May	7 Nov	-	-
Annada	-	-	9 May	3 Oct
Urich	-	-	4 May	17 Oct
Oran	28 May	8 Nov	13 May	19 Oct
Lamar	10 May	25 Oct	3 May	7 Oct
Portageville clay	7 May	7 Oct	9 May	6 Oct
Portageville loam	6 May	4 Nov	3 May	7 Oct

Table A.3. Pre-emergence and post-emergence herbicide applications for locations in 2004 and 2005.

Location	2004		2005	
	Pre-emergence	Post emergence	Pre-emergence	Post emergence
Albany	-	-	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	-
Macon	-	-	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	quizalofop P-ethyl, benzothiadiazole, aciflourfen
Columbia	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	aciflourfen, sethoxydim	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	benzothiadiazole, clethodim
Grand Pass	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	clethodim	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	quizalofop P-ethyl, benzothiadiazole, aciflourfen
Truxton	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	-	-	-
Annada	-	-	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	fomesafen, clethodim
Urich	-	-	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	quizalofop P-ethyl, benzothiadiazole, aciflourfen
Lamar	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	aciflourfen, clethodim	glyphosate, sulfentrazone, chloransulam-methyl, S-metolachlor	quizalofop P-ethyl, benzothiadiazole, aciflourfen
Oran	S-metolachlor, imazaquin	fomesafen, sethoxydim	S-metolachlor, imazaquin, glyphosate	fomesafen, clethodim
Portageville clay	S-metolachlor, imazaquin	fomesafen, clethodim	S-metolachlor, imazaquin, glyphosate	fomesafen, clethodim
Portageville loam	S-metolachlor, imazaquin	fomesafen, clethodim	S-metolachlor, imazaquin, glyphosate	fomesafen, clethodim

Table A.4. Sources of variation for planting date study in 2004.

Source of Variation	Stand	Yield	Ratio S:RS	Sucrose	Raffinose	Stachyose	Protein	Oil
Date	**	**	**	0.763	*	0.583	0.067	0.186
Cultivar	**	**	**	**	**	**	**	**
Date*Cultivar	0.272	0.237	**	**	**	**	**	0.119

\* Significant interaction occurs at alpha = 0.05

\*\* Significant interaction occurs at alpha = 0.01

Table A.5. Sources of variation for planting date study in 2005.

Source of Variation	Stand	Yield	Ratio S:RS	Sucrose	Raffinose	Stachyose	Protein	Oil
Date	**	**	**	**	*	0.335	0.085	**
Cultivar	**	**	**	**	**	**	**	**
Date*Cultivar	**	0.181	**	**	**	0.322	**	**

\* Significant interaction occurs at alpha = 0.05

\*\* Significant interaction occurs at alpha = 0.01