8-2009

Soybean Enhancement for Improved Biodiesel Production

Benjamin David Fallen
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Benjamin David Fallen entitled "Soybean Enhancement for Improved Biodiesel Production." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Vincent R. Pantalone, Major Professor

We have read this thesis and recommend its acceptance:

Dean A. Kopsell, Carl E. Sams

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Soybean Enhancement for Improved Biodiesel Production

A Thesis

Presented For The

Master of Science

Degree

The University of Tennessee, Knoxville

Benjamin David Fallen

August 2009

Acknowledgments

I would like to extend all my appreciation and thanks to all those that have made this thesis possible, for without their kind support, hard work and dedication none of this would have been possible.

I would like to start off by thanking my family and friends, for without them I would not be who or where I am today. To my mom Cindy Fallen and my dad David Fallen, I am thankful everyday to have such kind and caring parents. I don't know how they were able to raise a child who is as stubborn and determined as I am, but some how they did. To my "little bro" Jonathan Fallen and "big sis" Bridgett Fallen, they were right there with me growing up in the tobacco field (well maybe not everyday), it means the world to me to have such great siblings.

I am also thankful for the trenchant critiques, the probing questions and the remarkable patience of my advisor- Dr. Vincent Pantalone. I cannot think him enough. I am also grateful for the continued guidance of my committee members, Dr. Carl Sams and Dr. Dean Kopsell.

I would also like to express a big thanks to the Beam Team: Deborah Ellis, Beth Meyers, Amanda Pierce, Catherine Nyinyi, Jennifer Lane and Sam Plank for accepting me with open arms and their continued support, even though at times I'm sure it was not easy.

Support provided by the Tennessee Agricultural Experiment Station of the University of Tennessee, the Soybean Tennessee Promotion Board and support by the Better Bean Initiative of the United Soybean Board, which led to the development of the mid-oleic soybean line that made this research possible is greatly appreciated.

"For me life is continuously being hungry. The meaning of life is not simply to exist, to survive, but to move ahead, to go up, to achieve, to conquer. Failure is not an option."

- Arnold Schwarzenegger

“Trust in the Lord with all your heart and lean not on your own understanding; in all your ways acknowledge him, and he will make your paths straight.” – Proverbs 3:5-6

Abstract

As energy prices continue to rise, concern grows about the economy and about petroleum supplies. On January 1, 2009 The Energy Independence and Security Act of 2009 was enacted. It states that 500 million gallons of biomass-based biodiesel must be produced in 2009 and 1 billion gallons by 2012. In the United States 90 % of the biodiesel is produced from soybean oil, despite its shortcomings. The biggest problem facing the soy diesel industry is the American Society of Testing and Materials (ASTM) specifications for Biodiesel and Biodiesel Blends. The two categories that are in need of immediate improvement to enhance test results and produce a better burning fuel are cloud point and oxidation stability.

Monounsaturated fatty acid methyl ester (FAME) are reported to strike the best balance between cold flow properties and oxidative stability to enhance biodiesel test results and produce a better burning fuel. In addition, treating fuels derived from fatty acid alkyl esters with oxidation inhibitors (antioxidants) has been reported to increase resistance to oxidation. Fuel properties: acid value, cloud point, iodine value, pour point, peroxide value, induction period, onset temperature, and kinematic viscosity were used to evaluate a newly developed Roundup Ready® soybean recombinant inbred line with a novel oil profile, exhibiting an elevated level of monounsaturated FAME and the possibility of using selenium as a natural antioxidant for use in the biodiesel industry. We were able to demonstrate higher polyunsaturated content lead to lower IP values, lower PV values were indicative of increased monounsaturated FAME content and elevated levels of saturated FAME content resulted in higher CP and PP values.

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Introduction and Literature Review

Introduction

The genus *Glycine* Wild is divided into two subgenera, *Glycine* and *Soja*. The subgenus *Soja* (Moench) includes the cultivated soybean, *Glycine max* (L.) Merrill and the wild soybean, *Glycine soja* Sieb. & Zucc. *Glycine soja* is the wild ancestor of *Glycine max* and grows in China, Japan, Korea, Taiwan and Russia. *Glycine Soja* is an annual, weed-like, climbing pioneer of secondary seccessions whose pods contain black seeds that shatter a maturity (Chung, et. Al, 2008).

The taxonomic classification of the soybean is as follows (USDA Plants Database):

Kingdom Plantae
Division Magnoliophyta
Class Magnoliopsida
Order Fabales
Family Fabaceae
Genus and species *Glycine max* (L.) Merrill

The soybean [*Glycine max* (L.) Merrill] was first cultivated over 3,000 years ago in China. However, soybeans didn't come to America until almost 2,800 years later. Today more soybeans are grown in the United States than anywhere else in the world. In 2007, 25.7 million hectares of soybeans were planted in the United States, producing 70.4 million metric tons of soybeans. The total value of the crop exceeded US$ 26.8 billion. In 2007, worldwide soybean production reached an astonishing 219.8 million metric tons (Soy Stats, 2008).

Part of the tremendous success of the soybean comes from the unusually large nitrogen (N) producing nodules found on the roots of soybean plants. Once it was discovered that soybeans were N-fixing legumes and could replenish soil N, research began to find new uses for the crop.

Today soy can be found in nearly every industry. Food uses for soybean products include: tofu, soymilk, tempeh, miso, vegetable oil, margarine, bread, candy, doughnut mix, frozen desserts, milk, pancake flour, pan grease, sweet goods, etc. Health and beauty products include: shampoo, conditioner, hand soap, lotion, etc. Industrial products include: biodiesel caulking compounds, electrical insulation, fungicides, herbicides, ink, paints, adhesives, carpet, etc. As interest continues to grow in soy's health, economical benefits, and industrial applications, the soybean market will continue to grow (Soy Products Guide, USB).

For most soy products, excluding some edible uses, soybeans have to be processed before they can be used. During processing, the oil and meal components of the soybean are separated. Soybean meal is often referred to as the "gold standard" in that all other protein sources are generally compared to it. On a global basis, soybean meal accounts for approximately 69 % of protein meal consumption, followed by rapeseed (canola) meal (12 %), cottonseed meal (7 %), sunflower seed meal (5 %), fish meal (2 %), peanut meal (2 %), palm kernel (2 %) and copra (1 %). In 2007, the United States produced 43.8 million metric tons of soybean meal, at a value of $369 per metric ton. In the United States, the soybean meal produced is utilized approximately 50 % for poultry, 27 % for swine, 11 % for beef, 6 % for dairy, 3 % for pet food and 3 % for other sources (Soy Stats, 2008). The popularity of soybean meal in swine and poultry feeds is largely due to its high concentration of protein (44 % to 48 %) and its excellent profile of highly digestible amino acids and isoflavones.

Soybeans represented 56 % of 2007 world oilseed production and 32 % of those soybeans were produced in the United States. An estimated 9.6 million metric tons of soybean oil was produced in the United States in 2007 at an average price of $1,213 per ton. United States soybean oil consumption accounts for 49 % of salad and cooking oil, 27 % of baking and frying fats, 18 % of industrial products, 5 % of margarine, and 1 % of other edible products (Soy Stats,

2008). The popularity of soybean oil is due in part to the health benefits offered by using soybean oil. New soybean cultivars contain low linoleic and high oleic fatty acid levels. Lower linoleic acid produces oil that requires very little hydrogenation, and higher oleic acid increases stability and shelf life.

Literature Review

Soy Biodiesel

In the mid 1930's J. Walton suggested "to get the utmost value from vegetable oils as fuel it is academically necessary to split off the triglycerides and to run on the residual fatty acid." While Walton may have had the right idea, it was G. Chavanne who would take that concept and turn it into a developmental approach to producing biodiesel. On August 31, 1937 G. Chavanne of the University of Brussels in Belgium was granted a patent for the 'Procedure for the transformation of vegetable oils for their uses as fuels', which constitutes the first report on what is today known as biodiesel. The report described the use of ethyl esters of palm oil as diesel fuel. However, the real interest in vegetable oil fuels did not begin until the 1970s, during the OPEC oil embargo, and it wasn't until the late 1970s that extensive research took place on using soybeans as diesel fuel (Knothe, 2001a).

Biodiesel Chemistry

Vegetable oils are triglycerides, meaning it is made of the combination of three fatty acids (which can be of different types) and a glycerin backbone. So, while some esters consist of just one acid, vegetable oil molecules have three acids combined with an alcohol. When fatty acids and glycerin are added to make triglycerides, the glycerin loses the three hydrogens on the

end, and the acids lose the hydroxyl group on the end, making water. The extra oxygens on the triglyceride are attached to the carbon on the end of the fatty acid's alkyl group with a double bond, one oxygen atom has a double bond to the carbon atom at the end of the fatty acid alkyl group, the other oxygen has one bond to that carbon atom, and its second bond is to one of the carbons in the glycerol itself. The fatty acids involved are R_1OOH, R_2OOH, and R_3OOH, where "R" represents a chain of carbon atoms with hydrogen atoms attached, but with the carbon on one end being a carboxyl group (carbon with OOH attached).

In the case of biodiesel, during transesterification a vegetable oil ester is combined with a simple alcohol and a catalyst, resulting in the breakup of the triglyceride ester and the joining of the fatty acids with the added simple alcohols. The reaction can proceed both ways, so it is necessary to add an excess of methanol to shift the equilibrium to the product side. Since it is not desirable to have free methanol in the biodiesel fuel, it is then necessary to recover the methanol either by water washing, or a pressure-condensing method, but the glycerin must be removed first. If you remove the surplus methanol while the glycerin is still present with the biodiesel, the process will start gradually reversing – biodiesel and glycerin combining to re-make vegetable oil and methanol. The glycerin is more dense than the biodiesel, so it will gradually settle to the bottom in the reactor, simplifying separation (Knothe, 2001b).

Soy Biodiesel Production

To begin the production process, soybeans must first be cleaned and dried to a low moisture level. Next, the soybeans are put inside an extruder, where the soybeans are compressed into a paste. After the extruder, either a mechanical or solvent extraction process is used to separate the oil from the rest of the soybean. Then, the soybean oil is pumped into a screening tank where any solid particles are removed. Finally, the oil is passed through another

filter and the oil is ready to be stored, or used directly to produce biodiesel (Figure 1.1). The remaining soybean powder is referred to as meal and is the most commonly used protein supplement in North America because of its high energy and protein content (NBB, 2005).

Once the oil is ready to be converted into biodiesel, alcohol and catalyst are added to the oil and agitated briefly. The agitation disperses the oil into small droplets, allowing the catalyst to concentrate on individual droplets. As the reaction proceeds, glycerol collects in small droplets with the catalyst, and the surrounding substance is biodiesel. After the glycerol is removed, the biodiesel is washed, purified, and flash vaporized, leaving only pure biodiesel (NBB, 2005).

While production can play a major role on any material, the nature of the fuel components ultimately determines the fuel properties. Some of the properties that are included as specifications in American Society for Testing and Materials (ASTM) for biodiesel are based on the structure of the fatty esters comprising the soybean used to produce the biodiesel. Two properties controlled by the structure of fatty esters that are in need of improvement are cold flow and oxidative stability (Knothe, 2005).

Cold Flow Properties

In North America, one of the biggest problems facing the biodiesel industry is poor flow properties at low temperatures. Most poor temperature flow properties are caused by crystallization. Crystallization can completely shut down an engine because it can lead to clogged fuel lines and filters. Soy diesel has a crystallization onset temperature of 3.7 °C and develops operability problems when ambient temperatures drop below 0 °C, a significantly higher temperature than diesel fuel (Hammond et al., 1996). Diesel fuel develops similar

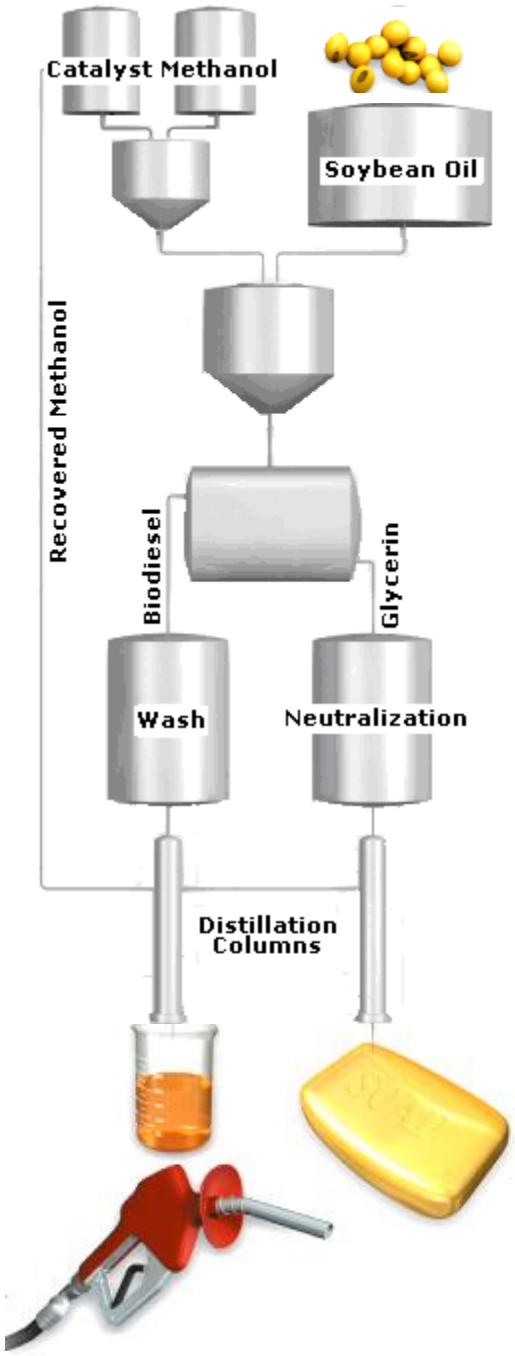
Mixing of Methanol and Catalyst
A catalyst, typically sodium hydroxide is dissolved in methanol.

Reaction
The methanol/catalyst mix and soybean oil are added together and heated, producing a reaction called transeterification, which results in two major products: glycerin and biodiesel.

Settling
Glycerin is much denser than biodiesel and the two can be gravity separated, with glycerin simply drawn off the bottom of the settling vessel.

Wash
Biodiesel must be washed with water to remove contaminants. Water is heavier than biodiesel and absorbs the excess methanol, sodium hydroxide and soap suspended in it.

Methanol Recovery
Excess methanol remaining in the biodiesel and glycerin are removed through distillation and recycled for reuse.



Glycerin Neutralization
The glycerol byproduct contains unused catalyst and soaps that are neutralized with an acid. Water and methanol are removed to produce 80% to 88% pure glycerin, which is ready to be sold as crude glycerin or further refined pharmaceutical grade.

Glycerin Uses
Glycerin has numerous uses: preserving food, as an emulsifier in butter, margarine and mayonnaise, as a base for lotions, in some printing inks, in cake and candy making, as antifreeze, and making clear soaps.

Figure 1.1 Transesterification of Vegetable Oil to Biodiesel. Photo by Craig Johnson, Copyright 2007, The Des Moines Register and Tribune Company. Reprinted with permission.

problems when temperatures drop into the range of -10 to -15 °C (Bagby et al, 1996). In order for soy diesel to be attractive as an alternative fuel, the low-temperature filterability properties have to be dealt with.

Low temperature flow properties can be measured by cloud point (CP), pour point (PP), low-temperature flow test (LTFT) and cold filter plugging point (CFPP). CP is the temperature at which a fuel sample first becomes cloudy due to the formation of crystals and solidification of saturates. The PP is the lowest temperature at which the fuel sample continues to flow (Knothe, 2005). The LTFT determines the lowest temperature at which a fuel can be expected to pass through an engine fuel filtration system. CFPP is used mainly outside of North America as a European standard (EMA, 2006).

Low-temperature flow tests have been used as the primary test in the past because they were believed to demonstrate a better correlation with operability tests than CP or PP. However, research by Bagby et al. (1996) showed cold temperature operability limits predicted by LTFT were a linear function of CP. Since then other studies have reported similar findings (Knothe, 2005; Hammond et al, 1996). So emphasis on reducing CP is now considered to be the key to developing soy biodiesel with improved low-temperature flow properties.

Many different approaches have been made to fix the low temperature problems of soy diesel. One method is adding diesel fuel cold flow additives to biodiesel, but only slight improvements in filterability with respect to CP were observed. Additives to typical soy diesel have been tested to reduce CP by a maximum of 2-5 °C and to reduce LTFT by 5 °C-6 °C (Bagby et al., 1996).

Another solution to correct this problem is winterization. Winterization is a process in which saturated methyl esters are removed by inducing crystallization. Today, food processing companies are already using winterization to produce refrigerator-stable salad dressing. To

winterize soy diesel, frequent filtrations are required to prevent the methyl esters from congealing and it is difficult to separate the liquid esters from the crystals; resulting in poor yield fractions of the remaining soy diesel. The liquid yield was only about 26 % after winterization had taken place, with about a 9 % decrease in saturated esters. However, thermograms of the winterized liquid fraction showed a lower crystallization onset temperature than the unwinterized soy oil. Winterization can reduce CP to -16 °C (Hammond et al., 1996).

The extent of crystallization, and the temperature at which crystallization occurs, depends largely on the percent of saturated fatty acids the soybean oil contains. A follow up study by Bagby et al. (1996) showed a nearly linear relationship between CP and low-temperature filterability of distillate/methyl ester blends; proving that low-temperature flow properties of blends may be dominated by the presence of methyl esters. For soy biodiesel, CP may be directly correlated with crystallization onset temperature (Bagby et al., 1996). So perhaps the best solution to crystallization is producing varieties that are low in saturated fatty acids, palmitic and stearic acid. Since typical soybeans already contain a lower percentage of stearic acid than palmitic acid, selecting lines with lower palmitic acid will have a greater effect on reducing crystallization. Winterization of low-palmitic soybean oil yields better than typical soybean oil because less saturated esters have to be removed to achieve lower crystallization onset temperatures. However, even with higher yields, winterizing low-palmitic acid soybean oil will increase the price of liquid methyl soyate by about 16 % over the price of conventional soybean oil, not including the cost of the winterization process itself (Hammond et al., 1996).

When the cost of producing soy diesel is already considerably more expensive than the cost of producing diesel fuel, any after production solutions do not seem very cost effective.

Therefore one solution to fixing the low temperature flow properties of soy diesel while still making it cost competitive with diesel fuel seems to be using a soybean with lower levels of

saturated fatty acids to produce soy diesel.

Oxidation Stability

Another major concern is that soybean oil, like all natural oils, is slowly oxidized. When oxidation occurs, fuel will gum up and form sediments. Oxidation is primarily a concern during extended storage, but if oxidation occurs in an engine, the fuel filter can clog or if allowed to continue could cause permanent engine damage. Due to the high rate of oxidation, soy diesel has a shorter shelf life compared to petroleum diesel (Dunn, 2005).

Oxidation stability is used to predict the amount of time that fuel can be stored before the production of acids indicates that the fuel is becoming unstable. Fuel that meets the specified oxidative stability set forth by the ASTM is expected to provide six months of storage capability before degradation occurs (EMA, 2006). Air, heat, light, traces of metal, peroxides and other factors all can facilitate oxidation. However, the compound structure of fatty esters has been shown to have the greatest catalyzing effect.

Oxidative instability comes from the fact that soybean oil contains high amounts of polyunsaturated fatty acids. Unsaturated fatty acids are more susceptible to oxidation than saturated fatty acids. The rate at which unsaturated fatty compounds oxidize depends on the number and position of double bonds. Linolenic acid and linoleic acid are polyunsaturated and have three and two double bonds, respectively, making them highly susceptible to oxidation. Monounsaturated fatty acids on the other hand, are two or more times less reactive as polyunsaturated fatty acids because they have only one double bond. This is important to note because soybeans produced to promote better cold flow properties have a high concentration, around 85 %, of polyunsaturated and monounsaturated fatty acids. So to improve cold flow properties and increase oxidative stability a soybean should have high oleic acid content. Oleic

acid, a monounsaturated fatty acid, has the lower crystallization temperatures seen in polyunsaturated fatty acids, but still has a relatively low oxidation rate (Knothe, 2005; Dunn, 2005).

The Ideal Chemical Structure of Biodiesel

In search of the ideal biodiesel composition, high presence of monounsaturated fatty acids, reduced presence of polyunsaturated acids, and controlled saturated acids content are recommended. In this sense, oleic and palmitoleic are the best-fitting acids in terms of oxidative stability and cold weather behavior, among many other properties. However, if the composition of unsaturated fatty acids gets to high, however, biodiesel tends to be vulnerable to oxidation.

In a study conducted by Hiroaki et. al (2005) the correlations of fuel properties of biodiesel with its fatty acid composition were investigated. Methyl esters of palmitic, stearic, oleic, and linoleic acids were mixed at various ratios to make binary or multi-component mixture as a model of actual biodiesel fuel. CP was found to be -13 °C for pure methyl oleate and increased largely with methyl palmitate added even in its low mole fraction (0.11). Similar behaviors were also observed for binary mixtures of other saturated and unsaturated esters. However, CPs were almost the same, when the mole fractions of methyl oleate and methyl linoleate changed in their compostion. These data suggest that CP can be mainly determined by the amount of saturated esters, reguardless of the compositon of unsaturated ones.

Knothe (2008) investigated various fatty esters for fuel properties contained in the biodiesel standards ASTM D6751 and EN 14214. Knothe reported saturated fatty esters are very oxidatively stable and increase cetane number (CN). The data on oxidative stability showed that any biodiesel fuel based on unsaturated esters requires an antioxidant to meet the requirements of the ASTM D6751 and EN 14214 standards, because even the most oxidatively stable

monounsatered fatty ester, methyl oleate, did not meet the ASTM or EN oxidative stability specifications in the neat form. So, while enriching the oil as much as possible in one of the desirable fatty acids, small amounts of the other fatty acids complete the fatty acid profile.

Agronomic Traits

Not only is it important for soybean breeders to develop cultivars with desired characteristics for the biodiesel industry, but it is also important that these cultivars maintain agronomic traits needed for successful crop production. In addition, the fatty acid profile must be proven to be stable over the entire region where the soybean is expected to grow.

Multiple studies have shown that alleles that determine fatty acid content can have a negative influence on agronomic traits that are vital to successful crop production. Studies conducted by Ablett et al. (2002) showed different factors affect different fatty acid profiles. Palmtic acid, a saturated fatty acid, was shown to be the most stable fatty acid in soybeans. On the other hand stearic acid, another saturated fatty acid, was shown to be the most subjective to environmental changes. Growing environment was shown to have the most effect on cultivars with altered unsaturated fatty acid content, and climate was shown to have the most impact on oleic acid content, a monounsaturated fatty acid. In some trials, all mutant soybean genotypes were lower yielding in comparison to their parents (Albett et al., 2002; Wilcox et al. 1993; McClure 1999).

In a study by Dornbos and Mullen (1992), soybeans exposed to a high daily temperature showed an increase in oleic content, but yields were slightly lower. Whereas, other studies have found that soybeans containing higher oleic acid content did not affect yield (Carver et al., 1986; Clemente and Kinney, 2005). In fact, Carver et al. (1986) also found selection for increased oleic acid content may lead to an increase in seed size and an earlier maturity date. Changing

weather patterns are believed to cause the differences between different locations and years (Albett, 2002). These observations suggest that researchers need to not only develop a soybean for enhanced biodiesel production, but prove those soybeans can compete with the agronomic traits of local cultivars in order to be acceptable to farmers.

Selenium

Selenium (Se) is a trace mineral present in the soil in varying amounts around the world. The amount in soils depends mainly upon the parent material, climate, topography and soil weathering. Selenium can also be derived from other sources which include: percolating ground or surface waters, plant and animal residues, mining and other industrial activities, volcanic exhalations, industrial smoke and fumes, fertilizers, wastes such as sewage sludges, and fly ash applied to soils and marine aerosol (Ihnat, 1989).

However, many soils are incapable of providing to the plants growing on them the adequate amount needed by animals. Soils are considered Se deficient when there is less than 0.5 mg of Se per kg of soil, and seleniferous when there is greater than 2.0 mg of Se per kg of soil. Selenium content of most soils varies between 0.5 and 2.0 mg kg^{-1} depending on geographical area (Mayland 1994, Dhillon and Dhillon, 2003).

The concentration, chemical form, the soil redox potential, pH, sulfate, and phosphate content determine the bioavailability of Se in the soil. In acid soils (pH 4.5 to 6.5), Se is usually bound as a basic ferric selenite of extremely low solubility and is unavailable to plants. In alkaline soils (pH 7.5 to 8.5), Se may be oxidized to more soluble selenate ions which are readily available to plants. In the soil, Se may be present in four different oxidation states: selenate, selenite, elemental Se and as inorganic selenide (McNeal and Balistreri, 1989).

Selenate vs Selenite: Uptake, Assimilation and Volatilization

Selenate

Selenate (SeO_4^{-2}) is the predominant form of Se taken up into plant roots from the soil. The uptake of selenate into roots and its distribution in plants is much faster than that of selenite (White et al., 2004; Cartes et al., 2005). De Souza et al. (1998) reported that total Se accumulation in a plant was about 10-fold higher from selenate than from selenite. It was proposed that selenate, chemically analogous to the sulphate ion, is actively transported into roots via sulphate transporters and subsequently quickly transported into shoots.

Selenite

Selenite (SeO_3^{-2}) is not normally accumulated in the root. Selenite is less available to plants than selenate because it is absorbed more strongly by iron oxide surfaces and soil clays. However, liming will decrease the sorption of selenite by oxide surfaces and thus, increase the solubility in the soil and the uptake of Se (Mikkelsen et al., 1989; Garlston et al., 1991). In addition, selenite seems to be accumulated through passive diffusion and can be inhibited by phosphate (Yläranta, 1985)

In a field experiment, Archer (1983) sprayed pasture crops with 70 and 140 g Se ha^{-1} as selenate and as sodium selenite. Selenate had a more pronounced, but shorter lasting effect on the Se concentration on the plants than did selenite. Yiaranta (1995) concluded from foliar applications of selenite and selenate that selenate is somewhat more effective than selenite and that the effect depends on the stage of development of the crop at the time of spraying.

Selenium in plants

Selenium concentration in plants depends on the chemical form of Se, its concentration and bioavailability in soils and the accumulation capacity of the plant. Higher plants can be

divided into different categories depending on their capacity to accumulate and tolerate selenium. They are classified into non-accumulators, indicators, accumulators and hyperaccumlators. The ability to accumulate and tolerate high Se levels is related to differences in Se metabolism, rooting depth and genetic traits. In non-accumlator and indicator plants the non-specific integration of the selenoamino acids into proteins is believed to be the major contributor of Se toxicity. The ability of Se hyperaccumlator plants to accumulate and tolerate high concentrations of Se is thought to be associated with a distinct metabolic capacity that enables them to divert Se away from being incorporated into proteins (Brown and Shrift, 1981).

Soybean crops have a much higher Se concentration than cereal or forage crops (Gupta and Macloed, 1994). Selenium concentrations in soybean seeds vary significantly with their different genotypes (Li et al., 2000). To address this issue Zhang et al. (2003) conducted an experiment to study the accumulation and transport of two Se species, selenite and selenate, by two soybean cultivars, TA and QG. Tong-ai 405 (TA) and Qidong Green-skin (QG) are two widely grown soybeans in low-Se soils in Jiangsu Province, China. In the time sequential experiment, uptake of selenate by both roots and shoots had a tendency to reach a plateau while the linear uptake of selenite by shoots and roots exist within the time used. In the concentration sequential experiments, a significantly linear tendency was observed in Se uptake by both roots and shoots. A majority of the selenite taken up by the soybean seedling was retained in roots, while much of the selenate was transported to the shoots. This led them to conclude that selenite had a much faster passive uptake and selenate had an active uptake. Therefore, not only forms of Se supply, but also the genotype differences affect the Se bioavailability by different cultivars (Zhang et al., 2003).

Selenium in Vegetable Oils

Antioxidants are added to fats and oil to retard oxidation of unsaturated fatty acids and to decrease the development of rancidity (Sherwin, 1976). Many naturally occurring flavonoids in plants are recognized as important compounds in conferring stability against autooxidation to vegetable oils. The effectiveness of flavonids in retarding lipid oxidation in fat-containing foods is related to their ability to act as free-radical acceptors (Das & Pereira, 1990). Today, natural antioxidant flavonids are being looked at to reduce synthetic food additives.

Wanasundara and Shahidi (1993) tested the antioxidant activity of flavonoids and synthetic antioxidants in refined-bleached canola oil at a 200- mg L^{-1} addition level. They concluded through chemical analysis of the oil, subjected to accelerated oxidation, that all antioxidants treated oil samples showed a delayed induction period as compared with that of the control. However, five of the eleven flavonoids were more effective than the two synthetic antioxidants in retarding the formation of primary and secondary oxidation products of canola oil. Futhermore, it was evident that the antioxidant activity of flavonoids was generally governed by their chemical structures.

Selenium in Biodiesel

As previously mentioned, methyl soyate (biodiesel) when exposed to air during storage can undergo oxidation, which can cause fuel quality degradation by adversely affecting properties such as viscosity, acid value and peroxide value. Today, biodiesel is stored under inert nitrogen atmosphere to retard oxidation. However, this type of treatment is expensive and can be dangerous.

One solution to increase the resistance of fuels derived from fatty acid alkyl esters against oxidation is to treat them with oxidation inhibitors (antioxidants). In a study by Dunn (2005) he

examined the effectiveness of synthetic antioxidants tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PrG) and a natural antioxidant, α – tocopherol in mixtures with soybean oil fatty acid methyl esters (SME). Dunn's results showed all antioxidants improved oxidative stability. However, PrG, BHA and BHT were most effective and α – Tocopherol was least effective at improving oxidative stability.

In previous studies, TBHQ and BHT have been shown to retard effects of oxidation on viscosity, acid value and peroxide value of biodiesel (Canakci et al., 1999; Dunn, 2000, 2002). Tocopherols, BHA and PrG have also been shown to improve resistance to oxidation of vegetable oils (Chu et al., 1999; Tan et al., 2002).

Experimental Objectives

The purpose of this study is to evaluate newly developed soybean lines with increased oleic acid concentration for enhanced biodiesel oxidative stability and cold flow performance. In addition, we will be evaluating the effects of different Se applications on soybean biodiesel properties. In the current study, we will: 1) test the hypothesis that increased oleic soybean lines produce oil with significantly improved oxidative stability for biodiesel; 2) test the hypothesis that there is no difference in the environmental stability of oleic acid concentration for normal soybeans compared with that for increased oleic soybeans when grown at multiple environments in the southeast and mid-South regions of USA; 3) evaluate the agronomic and seed quality attributes of increased oleic acid soybeans; and 4) evaluate the possibility of using Se as a natural antioxidant for use in the biodiesel industry.

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Chapter 2

Increased Oleic Acid Concentration of Soybean for Enhanced Biodiesel Production

Abstract

As energy prices continue to rise, concern grows about the economy and about petroleum supplies. On January 1, 2009 The Energy Independence and Security Act of 2009 was enacted. It states that 500 million gallons of biomass-based biodiesel must be produced in 2009 and 1 billion gallons by 2012. In the United States 90 % of the biodiesel is produced from soybean oil, despite its shortcomings. The biggest problem facing the soy diesel industry is the American Society of Testing and Materials (ASTM) specifications for Biodiesel and Biodiesel Blends. The two categories that are in need of immediate improvement to enhance test results and produce a better burning fuel are cloud point and oxidation stability.

Research was conducted in 2007-2008 to evaluate six newly developed Roundup Ready® soybean recombinant inbred lines with novel oil profiles, exhibiting elevated levels of monounsaturated fatty for enhanced biodiesel oxidative stability and cold flow performance. Analysis of variance was preformed to test the effects of location, replication and their interactions on the levels of five fatty acids in the seed oil of the six new inbred lines and two commercial checks. The six inbred lines averaged 38.1 % oleic acid, 4.7 % linolenic acid and yielded 3485 kg ha^{-1}, while the two commercial checks (AG3906 and AG4103) averaged 23.8 % oleic acid, 8.3 % linolenic acid and yielded 3614 kg ha^{-1}.

Introduction

For most soy products, excluding some edible uses, soybeans have to be processed before they can be used. During processing, the oil and meal components of the soybean are separated. In 2007, soybeans represented 56 % of world oilseed production, and 32 % of those soybeans

were produced in the United States. In 2007, 9.6 million metric tons of soybean oil was produced in the United States at an average price of $1,213 per ton. Of the oil produced, only 18 % went into industrial products and less than 5 % went into biodiesel production. However, in 2007 the United States produced an estimated 1.7 billion L of biodiesel (Soy Stats, 2008). While 1.7 billion L is small, it represents significant growth over the 950 million L produced in 2006 and the 284 million L produced in 2005; a trend that has been on the rise since 1999 when only 1.9 million L of biodiesel were produced. According to the National Biodiesel Board, an additional 6.4 billion L of capacity may come online if current plants in construction are completed (National Biodiesel Board, 2007). Moreover, on January 1, 2009 The Energy Independence and Security Act of 2009 was enacted. It states that 500 million gallons of biomass-based biodiesel must be produced in 2009 and 1 billion gallons by 2012.

Biodiesel is a very attractive alternative fuel. Biodiesel is made from a renewable feedstock, it is environmentally friendly, relatively safe to handle, and has reduced exhaust emissions. In addition, soy diesel has enhanced biodegradation, increased flash point, increased lubricity, reduced toxicity, and reduced emissions. It also has an energy content, specific gravity, kinematic viscosity, and cetane number comparable to petroleum diesel (Clemente and Kinney, 2005). In the United States approximately 90 % of the biodiesel being production comes from soybeans (Dunn, 2005). Biodiesel can be produced from a number of other feedstocks, but using soybeans to produce biodiesel in the United States does have advantages over other sources for producing biodiesel. Soybeans are Roundup Ready®, N-fixing legumes, that are well established in the United States.

Problems Facing the Soy Biodiesel Industry

Today, one of the biggest problems facing soy biodiesel in the United States is meeting American Society of Testing and Materials (ASTM) specifications for oxidative stability and cold flow performance. Cloud Point (CP) is designed to evaluate the lowest temperature a fuel can tolerate without causing problems. The lowest temperature at which the fluid will pour is defined as the pour point (PP). At low temperatures, diesel fuel can gel or crystallize and cause engine damage. According to ASTM specifications for Tennessee, the maximum CP temperature shall be equal to or lower than -10 °C. Current soy diesel has a cloud point of 0 °C. Oxidation stability is a prediction of the amount of time a fuel can be stored before becoming unstable. Today, the soybean oil being produced has a relatively high oxidative reactivity that compromises the storage life of soybean oil biodiesel (EMA, 2006).

Improvements to the Fatty Acid Profile of Soybean to Improve Biodiesel Production

Some characteristics that determine how well a soybean will perform for biodiesel applications are based on the soybean's oil composition. The fatty acid profile and the alcohol moieties of the oil determine such characteristics as cetane number, cold flow, oxidation stability, lubricity and viscosity (Knothe, 2005). Soybean oil is composed of five fatty acids: palmitic acid (~11 %), stearic acid (~4 %), oleic acid (~24 %), linoleic acid (~54 %), and linolenic acid (~7 %). Each fatty acid differs in the number of carbon and hydrogen atoms it contains, giving each acid varying characteristics. Palmitic acid and stearic acid are saturated fatty acids which have a better shelf life, but tend to crystallize more rapidly in cold weather. Linoleic acid and linolenic acid are polyunsaturated fatty acids which help increase cold flow performance, but reduce shelf life. Oleic acid is a monounsaturated fatty acid which helps balance the tradeoff between cold flow performance and shelf life stability (Clemente and

Kinney, 2005).

The fatty acid distribution in the oil can be controlled through traditional breeding or genetic modification. Soybeans with high oleic acid usually display lower levels of polyunsaturated fats because less oleic acid undergoes desaturation. The high content of oleic acid and low content of polyunsaturated fatty acids result in an oil that has improved oxidative stability. The higher the oxidative stability, the more stable the oil and the more heat and pressure it can stand without gelling up. However, as the level of polyunsaturated fatty acids decreases so does the oil's ability to perform in cold weather. This inverse relationship between cold flow performance and oxidation stability poses a major problem for producing the ideal soybean for biodiesel production in all environments (Clemente and Kinney, 2005).

Currently research is being done to develop two different feedstocks, one with increased oxidative stability due to elevated oleic acid coupled with reduced polyunsaturated fatty acid content, and the other with enhanced fuel performance, but reduced cold flow properties due to an increase in stearic acid in addition to high oleic acid content. Enhanced soybean for improved biodiesel production could have a huge impact on the economy, the environment, and could decrease the United States dependence on foreign oil. A 2 % or 5 % blend of soy diesel with petroleum diesel could also generate new job opportunities and market expansion (Clemente and Kinney, 2005).

Materials and Methods

Background of the Genetic Material

At the University of Tennessee, crosses we made between Roundup® Ready early generation lines from which 'Allen' was later derived (♀) and early generation lines from which N98-4445A was later derived (♂). For simplicity, the parents will be referred to as Allen and N98-4445A. The germplasm N98-4445A was developed by the USDA-ARS, in cooperation with the North Carolina Agriculture Research Service at NC State University (Burton et al., 2006). N98-4445A has a high concentration of oleic acid, approximately 560 g kg^{-1}. This concentration is more than 100 g kg^{-1} greater than any oleic acid concentration in the United States germplasm collection and around 340 to 380 g kg^{-1} (i.e. nearly 3x) greater than the oleic acid concentration in commercial soybean cultivars.

Allen is the Roundup® Ready version of the cultivar 5601T, developed at the University of Tennessee. Allen was among the top performing cultivars for its maturity class in the Roundup® Ready Tennessee State Variety Tests in 2008, and the top performing cultivar in 2007. In 2001, 5601T was released because of its high yield over geographically diverse regions of southern United States (Pantalone et al., 2003). Since that time it has been shown to have excellent estimated processor value (EPV) (Graef 2006; 2005; 2004). The quality of the protein meal is near ideal for the poultry feed industry (Brake et al., 2005), and it is one of the highest isoflavone containing commercial cultivars (Charron et al., 2005). The agronomic stability of 5601T has been well documented: it was among the top performing cultivars for its maturity class in Tennessee State Variety Tests in 2008, 2004, 2003, 2002, 2001 and was the top performing cultivar in 2007, 2006, 2005, and has been a top yielder in KY, NC, and AR. 5601T currently serves as a high yield check cultivar in the USDA Southern Regional Tests Program.

Population Development

In the summer of 2001, researchers at the University of Tennessee began work on developing a high yielding soybean with increased oleic acid. When Allen was crossed with the high oleic acid germplasm, N98-4445A, the pedigree-selection procedure was used to select single plants based on oleic acid content until the F_6 generation. An example, in correspondence with the pedigree-selection method used in this study is presented as Appendix Figure A.1, followed by a detailed description of an example of specific row selection from 2001-2007.

Experimental Design

2007 Preliminary Yield Trial

In 2007, 67 Roundup Ready® soybean recombinant inbred lines were entered into four Preliminary Yield Trials (PYTs) at Knoxville, TN. The lines used in the study range from maturity group III to IV. In addition, other TN lines and commercial checks were also included in the PYT tests for overall agronomic comparisons. A randomized complete block design was used for each of the PYTs. Each line was planted with three replications in 2 row plots, with 76 cm spacing between rows and 6 m in length.

2008 Multiple Environment Yield Trial

In 2008, 6 Roundup Ready® soybean recombinant inbred lines were entered into yield trials at 4 locations: East Tennessee Research and Education Center (Knoxville, TN), the Highland Rim Research and Education Center (Springfield, TN), the Research and Education Center at Milan (Milan, TN) and the Eastern Virginia Agricultural Research and Extension Center at Warsaw (Warsaw, VA) representing major physiographic regions of East Tennessee, Middle Tennessee, West Tennessee and Eastern Virginia. The experimental design was a randomized complete block design with replication. At each location, three replications of 8

soybean lines were grown as 4 row plots, 6 m in length, with 76 cm spacing between rows. The 8 lines consisted of 6 $F_{6:9}$ increased oleic acid soybeans from Allen x N98-4445A selected from 2007 and two commercial checks (AG4103, AG3906).

Experimental Field Procedures

After planting, all the plots were monitored for agronomic traits. Flowering was recorded as the number of days since planting when 95 % of the plants had bloomed. At the same time flower color was taken as well (purple, white or segregating). At maturity, plant height was taken as an estimation of the distance from the soil surface to the tip of the main stem in cm. Lodging was scored on a scale from 1-5; with 1 being all the plants in the plot were erect and 5 being all the plants in a plot were prostrate. Maturity was recorded as the date, according to the Julian calendar, when 95 % of the pods achieved their mature color. At that time pubescence was also recorded. Seed yield was estimated from the two inside rows in the four row plots and both rows in the two row plots after the plots had been end trimmed to 4.88 m in length. Yield was recorded in kg ha^{-1} at 13 % maturity basis. Seed size was taken as the weight in g from a random 100 seed sample.

Laboratory Procedures

Fatty Acid Composition Analysis by Gas Chromatography

Prior to harvest, 5 pods per line were hand collected at mid-node height. This was done to minimize error variance from sampling at different nodes. The pods were shelled by hand and the seed was collected. Five seeds from each line were randomly selected, crushed and placed in a test tube. Then 1 mL of seed extraction solvent consisting of chloroform, hexane, and methanol (8:5:2 v/v) was added. After the seeds were left to extract overnight, 100 µL of the

extracted oil supernatant was transferred to a 1.5 mL auto sampler vial to which 0.75 ml of a methylating reagent [a mixture of 50 mL sodium methoxide 20 mL petroleum ether, 10mL ethyl ether, and 1 mL Hexane] was added to the autosampler vial before capping. The resulting fatty acid methyl ester was analyzed using a Hewlett Packard HP 6890 gas chromatograph (Palo Alto, CA) equipped with model 7673 autosampler, flame ionization detector, and an immobilized 30m long x 0.53 mm inner diameter All-Tech AT-Silar capillary column with 0.5µL fused stationary phase. Operating conditions were set to: carrier He (20mL/Min), 20:1 (v/v) split injection, injection temperature 250ºC, detector temperature 275ºC and column temperature 240ºC. The RM-1 standard was used to determine relative fatty acid concentration of the selected lines.

Sample Preparation for Protein, Oil, and Amino Acid Composition via NIR Analysis

Approximately 20 g of soybean seed collected from plot seed samples were ground in a water-cooled Knifetec 1095 Sample Mill (FOSS Tecator, S-26321, Hogana, Sweden) for 20 s. This produced soybean flour that is uniform in particle size. The samples were analyzed using a FOSS 6500 near infrared spectrometer (NIR). A dehumidifier was used throughout the analysis to reduce the humidity to 40 %, and room temperature was maintained at approximately 20ºC.

First the NIR was warmed up for 2 h after turning on the lamp. Then auto diagnostics was run for instrument response, wavelength accuracy and NIR repeatability. Ground soybean samples were scanned to get the predicted concentrations of oil, protein, and all amino acids using ISIscan (System II version 2.80 software (FOSS, State College, PA). The instrument was left on for the whole period of analyses, and diagnostics was performed every day until the scanning was finished.

Extraction and Processing

The 2 $F_{6:9}$ increased oleic acid soybeans from Allen x N98-4445A with the highest oleic acid content and the two commercial checks used in the 2008 multiple environment yield trial were tested according to AOCS methods at NP Analytical Laboratories in St. Louis, MO. The samples were tested for oxidative stability and peroxide value.

Peroxide Value

Peroxide value determination was made in agreement with AOCS official Method Cd 8-53. Crude oil was extracted from the sample by dissolution in petroleum ether. The ether was volatilized, and a 5 g of oil was redissolved in 30 ml of a 3:2 acetic acid-chloroform solution. 0.5 ml of potassium iodide was added and was oxidized by any peroxide present, liberating the iodine. The liberated iodine was titrated with 0.1 N sodium thiosulfate, using a starch indicator solution. Peroxide value was calculated from the volume of titer consumed.

Oxidative Stability Index

Oxidative stability determination was made in agreement with AOCS Method Cd 12b-92. Incubator tubes were heated for 1 h at 110ºC (to drive off any volatiles that might increase conductivity of the water). A 15 g milled sample was then placed in the Oxidative Stability Index (OSI) at 110ºC. The conductivity of each probe in the instrument was monitored and a plot of water conductivity vs. time was obtained from the OSI. The OSI reflection point was determined by a micro-processor-computed slop/change algorithm.

Experimental Analysis

Proc GLM model analysis was done using SAS (ver. 9.1) on each genotype in PYT3LateRR, PYT3Late2RR, PYTOle3RR and PYT4earlyRR in 2007 to test for significant genotype effects for oleic acid, linolenic acid and seed yield.

Analysis of variance was conducted for the 2008 Multiple Environment Yield Trial using PROC MIXED in SAS (ver. 9.1.3) to 1) test the hypothesis that increased oleic soybean lines produce oil with significantly improved oxidative stability for biodiesel, 2) test the hypothesis that there is no difference in the environmental stability of oleic acid concentration for normal soybean compared with that for increased oleic soybean when grow at multiple environments in the southeast and mid-South regions of United States, 3) evaluate the agronomic and seed quality attributes of increased oleic acid soybeans compared to two commercial check cultivars. Least significant differences were used to compare fatty acid content (palmitic, stearic, oleic, linoleic, and linolenic acid), yield and biodiesel properties at the 0.05 significance level.

Results and Discussion

Selection of Increased Oleic Acid Genotypes in 2007

In 2007, 67 Roundup Ready® soybean recombinant inbred lines were entered into four Preliminary Yield Trails (PYTs) at Knoxville, TN. The yields reported are below average because 2007 was the worst drought year in east Tennessee in three decades. According to the U.S. Drought Monitor (2007) most of east Tennessee exhibited exceptional drought, the highest intensity of the drought monitor scale, by early September. Never the less selection of lines for further study was made based on seed yield and oleic acid concentration.

Four lines were selected from PYT3LateRR (Appendix Table A.1) TN07-022RR had the highest yield of the increased oleic lines (1402.65 kg ha^{-1}) with an oleic acid content of 45.8 % and a linolenic acid content of 2.5 %. TN07-021RR yielded 1350.13 kg ha^{-1} with an oleic acid content of 40.5 % and a linolenic acid content of 2.6 %. TN07-015RR yielded 1326.66 kg ha^{-1}

with an oleic acid content of 48.0 % and 3.8 % linolenic acid. TN07-024RR yielded 1219.76 kg ha^{-1} with an oleic acid content of 48.9 % and 3.5 % linolenic acid. Although, TN07-026RR had the highest oleic acid content at 55.2 %, it yielded too low to be selected.

Only one line was selected from PYT3Late2RR (Appendix Table A.2). TN07-186RR had the highest oleic acid content of the increased oleic lines (55.3 %) with a yield of 959.63 kg ha^{-1} and the lowest linolenic acid content (2.0 %). No other lines in the test were selected because of low yields.

Seven lines were selected from PYTOle3RR (Appendix Table A.3) TN07-96RR had the highest yield of the increased oleic lines (1488.84 kg ha^{-1}) with an oleic acid content of 54.3 % and a linolenic acid content of 2.2 %. TN07-93RR yielded 1284.36 kg ha^{-1} and had the highest oleic acid content of 59.1 % and a linolenic acid content of 2.0 %. TN07-102RR yielded 1160.59 kg ha^{-1} with an oleic acid content of 50.7 % and 3.3 % linolenic acid. TN07-95RR yielded 1129.45 kg ha^{-1} with an oleic acid content of 52.3 % and 2.3 % linolenic acid. TN07-130RR yielded 1124.31 kg $ha^{-1}$with an oleic acid content of 52.7 % and 2.2 % linolenic acid. TN07-98RR yielded 1090.44 kg ha^{-1} an oleic acid content of 57.1 % and 3.2 % linolenic acid. TN07-106RR yielded 1050.87 kg ha^{-1} with an oleic acid content of 56.6 % and a linolenic acid content of 1.9 %.

Three lines were selected from PYT4earlyRR (Appendix Table A.4) TN07-236RR had the highest yield of the increased oleic lines (2730.13 kg ha^{-1}) with an oleic acid content of 40.3 % and a linolenic acid content of 4.6 %. TN07-238RR yielded 2626.30 kg ha^{-1} with an oleic acid content of 42.1 % and a linolenic acid content of 5.7 %. TN07-239RR yielded 2236.03 kg ha^{-1} with an oleic acid content of 43.4 % and the lowest linolenic acid of 2.6 % linolenic acid.

In the fall of 2007, 15 increased oleic acid genotypes (Appendix Table A.5) were selected to send to Homestead, Florida to be increased for the 2008 growing season. In 2008, only the

top 6 genotypes were used to standardize the experiment and to optimize costs and resource allocation. The top 6 lines were selected on yield and oleic acid content (Appendix Table A.6).

2008 Multiple Environment Yield Trial: Fatty Acid Profile Analysis of Variance

While air, heat, light, traces of metals, antioxidants, peroxides and other factors all can facilitate oxidation, the compound structure of fatty esters have been shown to have the greatest catalyzing effect. A soybean should have high oleic acid content to improve cold flow properties and increase oxidative stability. Oleic acid, a monounsaturated fatty acid, has the lower crystallization temperatures seen in polyunsaturated fatty acids, but still has a relatively low oxidation rate (Knothe, 2005; Dunn, 2005). All 6 $F_{6:9}$ increased oleic acid lines (TN07-236RR, TN07-238RR, TN07-93RR, TN07-022RR, TN07-96RR, and TN07-015RR) had significantly higher oleic acid content then both commercial checks (Table 2.1). The 6 $F_{6:9}$ increased oleic acid lines averaged 38 % oleic acid, while AG4103 and AG3906 had oleic acid contents of 23.9 % and 23.8 %, respectively. All 6 $F_{6:9}$ increased oleic acid lines also had significantly lower linolenic acid content then both commercial checks. The 6 $F_{6:9}$ increased oleic acid lines averaged 4.7 % linolenic acid, while AG4103 and AG3906 had a linolenic acid content of 8.3 % and 8.4 %, respectively. The six experimental lines averaged a 59 % increase in oleic acid and 44 % decrease in linolenic acid over the two commercial checks.

The 58 % increase in oleic acid of the 6 $F_{6:9}$ increased oleic acid lines when compared to a traditional soybean of 24 % oleic acid, is a significant breeding accomplishment. To develop soybeans lines with 38 % oleic acid with a Roundup Ready® background with high yield could have significant impacts on the soybean market and the biodiesel industry. Genetic gains represent more than half of all improvements combined for U.S. crop productivity. In 2008, total value of soybean production in Tennessee was 434 million dollars, over 100 million dollars

Table 2.1 Descriptive statistics of mean seed yield, maturity, lodging, height, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid content of 6 $F_{6:9}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

	Agronomic Traits				**Fatty Acid Concentration**				
	Seed Yield	**Maturity**	**Lodging**	**Height**	**Palmitic**	**Stearic**	**Oleic**	**Linoleic**	**Linolenic**
Genotype	**kg ha^{-1}**	**Day of Year**	**1-5 Scale**	**cm**	**% Total Lipid**				
AG4103	3730	261	1.3	81	11.5	4.0	23.9	52.0	8.3
TN07-236RR	3680	263	1.3	85	10.7	5.0	38.2	41.5	4.6
TN07-238RR	3601	262	1.2	85	10.8	4.9	37.1	42.8	4.4
TN07-93RR	3433	261	1.7	87	10.0	4.7	39.1	40.9	5.2
AG3906	3398	261	1.1	75	10.5	4.8	23.8	52.4	8.4
TN07-022RR	3370	264	1.7	112	10.4	4.6	35.7	44.5	4.7
TN07-96RR	3365	261	1.7	88	10.0	4.8	39.2	41.2	4.8
TN07-015RR	3173	264	2.2	108	10.1	4.2	39.0	42.3	4.4
LSD (0.05)	268	1.5	0.36	6.38	0.27	0.22	2.00	1.97	0.89

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
Lodging was scored on a scale from 1-5; with 1 being all the plants in the plot were erect and 5 being all the plants in the plot were prostrate.

higher than the past ten years, due in part to Tennessee genetics (NASS, 2009).

2008 Multiple Environment Yield Trial: Agronomic Traits Analysis of Variance

Various studies have reported the effects of altered fatty acid composition on agronomic traits. In this study we found TN07-236RR (3680 kg ha^{-1}) and TN07-238RR (3601 kg ha^{-1}) both had significantly higher yields than AG3906 (3398 kg ha^{-1}), and did not have significantly different yields than AG4103 (3730 kg ha^{-1}). TN07-93RR (3433 kg ha^{-1}), TN07-96RR (3365 kg ha^{-1}), TN07-015RR (3173 kg ha^{-1}), and TN07-022RR (3370 kg ha^{-1}) had yields similar to AG3906 (3398 kg ha^{-1}). The 6 $F_{6:9}$ increased oleic acid lines averaged 3485 kg ha^{-1}, while the two commercial checks (AG4103, AG3906) averaged 3614 kg ha^{-1}. The yield of the six experimental lines averaged 96 % of the yield of the two commercial checks (Table 2.1).

This data supports results obtained by Kinney and Knowlton (1998), who found the high oleic trait did not have negative effects on yield or other agronomic traits. Carver et al. (1986) reported that increased oleic acid content in soybean did not affect yield. Therefore, it should be possible to develop soybean lines with elevated oleic acid content and high yields, among other desirable agronomic traits.

2008 Multiple Environment Yield Trial: Correlation Coefficients among Agronomic Traits and Fatty Acid Content

Relationships among yield, lodging, maturity, height and fatty acid content of soybean are important in order to produce a marketable soybean. In general, maturity, plant height, lodging, yield and fatty acid content of the increased oleic acid soybean genotypes were not strongly correlated, except for stearic acid. Simple correlation analysis (Table 2.2) showed maturity and stearic acid had a strong negative relationship ($r=-0.76$, $P<0.0001$), height

Table 2.2 Phenotypic correlations coefficients among agronomic traits of 6 $F_{6:9}$ increased oleic acid soybeans from 'Allen' x N98-4445A grown in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

Trait	Maturity	Height	Lodging	Yield	Palmitic	Stearic	Oleic	Linoleic
Height	****0.79							
Lodging	****0.53	****0.52						
Yield	****0.78	****0.47	**0.33					
Palmitic	**-0.36	***-0.38	**-0.36	ns				
Stearic	****-0.76	****-0.65	**-0.29	****-0.71	****0.49			
Oleic	****0.48	**0.32	**0.33	****0.56	****-0.60	****-0.50		
Linoleic	**-0.35	ns	ns	****-0.48	****0.56	****-0.44	****-0.88	
Linolenic	ns	ns	*-0.27	ns	*-0.27	**-0.33	ns	**-0.31

*, **, ***, **** Significant at P=0.05, P=0.01, P=0.001, P=0.0001, ns=non-significance

and yield and stearic acid had a strong negative relationship (r=-0.71, P<0.0001). This suggests that stearic acid has a strong impact on maturity and yield, as stearic acid increases maturity and yield decreases. This data supports the results obtained by Hartmann et al. (1997) and Lundeen et al. (1987) who found the soybean lines with the most stearic acid had significantly lower yields and shorter plant heights than normal stearic acid soybean lines. Hartmann et al (1997) also reported the maturity of the elevated stearic acid soybean lines were significantly earlier, and significantly later maturing than the normal stearic acid soybean lines. Although, in this study there were significant differences between stearic acid content among genotypes, and the genotype with the lowest stearic acid had the highest yield; the genotype with the second lowest stearic acid content had the lowest yield. This study was not designed to test the influence of stearic acid on yield and therefore, the results are inconclusive.

The increased oleic acid content in the 6 $F_{6:9}$ soybeans did not have a strong effect on any agronomic traits. However, oleic acid and yield did have a moderately positive relationship (r=0.56, P<0.0001). This does suggests increased oleic acid may slightly increase yield. These simple correlations also suggest oleic acid, stearic acid and palmitic acid are correlated. Oleic acid and palmitic acid had a moderate negative relationship (r=-0.60, P<0.0001). Oleic acid and stearic acid had a moderate negative relationship (r=-0.50, P<0.0001). Linoleic acid and oleic acid had a strong negative relationship (r=-0.88, P<0.0001). Similar correlated changes have been observed in other studies. Carver et al. (1986) showed a gradual decrease in palmitic acid after recurrent selection for increased oleic acid contents. Negative associations between palmitic and oleic acid content have also been reported in oats (*Avena sativa* L.; Forsberg et al., 1974) and rapeseed (*Brassica napus L.;* Auld et al., 1992). Hartmann et al. (1997) and Lundeen et al. (1987) found soybean lines with elevated stearic acid exhibited a significant reduction in oleic acid. The strong correlation between oleic acid and linoleic acid supports Bhattacharyya et

al. (2007) who reported that in the fatty acid pathway, oleic acid is converted to linoleic acid by the fatty acid desaturase, FAD2, in the endoplasmic reticulum.

2008 Multiple Environment Yield Trial: Mean Oleic acid Content across Four Locations

The usefulness of a soybean with an altered fatty acid profile in a breeding program, is very dependent upon the stability of the profile across multiple environments. In this study there were significant differences among the locations ($P<0.05$) for oleic acid content (Table 2.3 and Figure 2.1). However, two increased oleic acid soybean lines and the two commercial checks had an oleic acid content that was stable across all four locations.

The two commercial checks (AG3906, AG4103), TN07-238RR, and TN07-93RR each had an oleic acid content that was significantly similar across all four locations. TN07-015RR had higher oleic acid content in Milan, TN (41.4 %) then in Warsaw, VA (37.1 %) and Knoxville, TN (36.9 %). TN07-022RR had higher oleic acid content in Milan, TN (40.3 %) then in Warsaw, VA (34.4 %), Springfield, TN (35.5 %) and Knoxville, TN (32.8 %). TN07-0236RR (44.0 %) had higher oleic acid content in Milan, TN than in Warsaw, VA (38.7 %), Springfield, TN (36.5 %) and Knoxville, TN (33.5 %). TN07-96RR had higher oleic acid content in Milan, TN (43.3 %) than in Warsaw, VA (36.5 %) and in Knoxville, TN (36.8 %). While these differences were significant, most differences were less than 5 % across locations.

Primomo et al. (2002) studied the effect of environment on three normal soybean cultivars and 14 soybean lines with modified fatty acid composition at four locations in Ontario, Canada. They found that the soybean lines with modified fatty acid content, except for soybean lines with increased oleic and stearic acid content, had more stable fatty acid profiles over locations. Oliva et al. (2006) reported mid-oleic acid genotypes, N97-3363-4 and N98-4445A, were less stable for oleic acid content across environments than genotypes with normal oleic acid

Table 2.3 Descriptive statistics of mean oleic acid content of 6 $F_{6:9}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

	Line							
Location	AG3906	AG4103	TN07-015RR	TN07-022RR	TN07-236RR	TN07-238RR	TN07-93RR	TN07-96RR
EVAREC	22.09	21.31	37.13	34.36	38.73	37.79	38.47	36.54
HRREC	21.92	24.74	40.34	35.51	36.50	35.84	38.59	40.53
ETREC	25.87	24.93	36.94	32.81	33.53	36.55	37.91	36.79
MREC	25.43	24.69	41.42	40.32	44.03	38.30	41.53	42.95
LSD (0.05)	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;

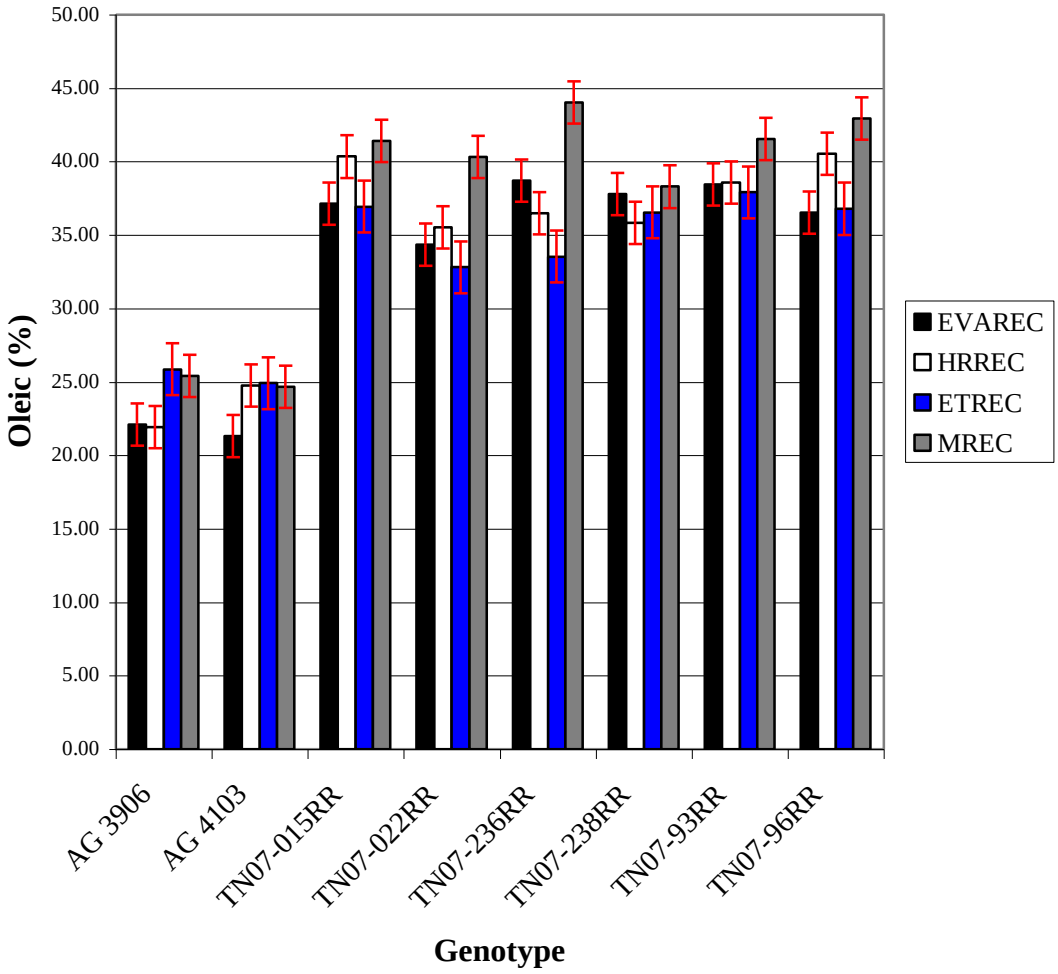

Figure 2.1 Descriptive statistics of mean oleic acid content of 6 $F_{6:9}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

content. However, similar to this study, Oliva et al. (2006) also reported two other mid-oleic genotypes were stable across environments. These results suggest the stability of oleic acid may be determined by the genotype not the level of oleic acid.

2008 Multiple Environment Yield Trial: Mean Linolenic Acid Content across Four Locations

Although there were differences among the locations ($P<0.05$) for linolenic content (Table 2.4 and Figure 2.2), we found that the lower linolenic acid levels in soybeans were more stable than the normal levels. The two commercial checks (AG3906, AG4103) had a linolenic acid content that was significantly different across locations. AG3906 had significant differences in linolenic acid content between HRREC (6.9 %), ETREC (9.9 %), and MREC (7.7 %) and between EVAREC (9.1 %) and HRREC (6.9 %). AG4103 had significant differences in linolenic acid content between EVAREC (9.6 %) and HRREC (6.9 %) and between HRREC (6.9 %) and ETREC (8.7 %). TN07-015RR, TN07-022RR, TN07-236RR, TN07-238RR, and TN07-93RR had significant differences in linolenic acid content between ETREC and the other three locations. TN07-96RR had no significant differences in mean linolenic acid content across all four locations. This data supports the results obtained by Oliva et al. (2006) and Primomo et al. (2002) who found reduced linolenic acid genotypes are more stable across environments than progressively higher linolenic acid genotypes.

To try to explain the increase in linolenic acid in ETREC and the increase in oleic acid in MREC, average temperature, average maximum temperature, average minimum temperature, and average rainfall for ETREC, MREC, HREC, and EVAREC were obtained for May, June, July, August, and September in 2008 (Table 2.5). MREC and ETREC were irrigated. Correlation analysis for average temperature, average maximum temperature, average minimum

Table 2.4 Descriptive statistics of mean linolenic acid content of 6 $F_{6:9}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

	Line							
Location	AG3906	AG4103	TN07-015RR	TN07-022RR	TN07-236RR	TN07-238RR	TN07-93RR	TN07-96RR
EVAREC	9.06	9.61	3.58	4.23	4.00	3.77	3.93	5.45
HRREC	6.86	7.08	3.50	3.42	3.88	3.34	3.82	3.97
ETREC	9.91	8.74	6.81	7.10	6.49	6.29	8.63	5.31
MREC	7.68	7.58	3.75	4.07	4.15	4.15	4.48	4.29
LSD (0.05)	2.06	2.06	2.06	2.06	2.06	2.06	2.06	2.06

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level

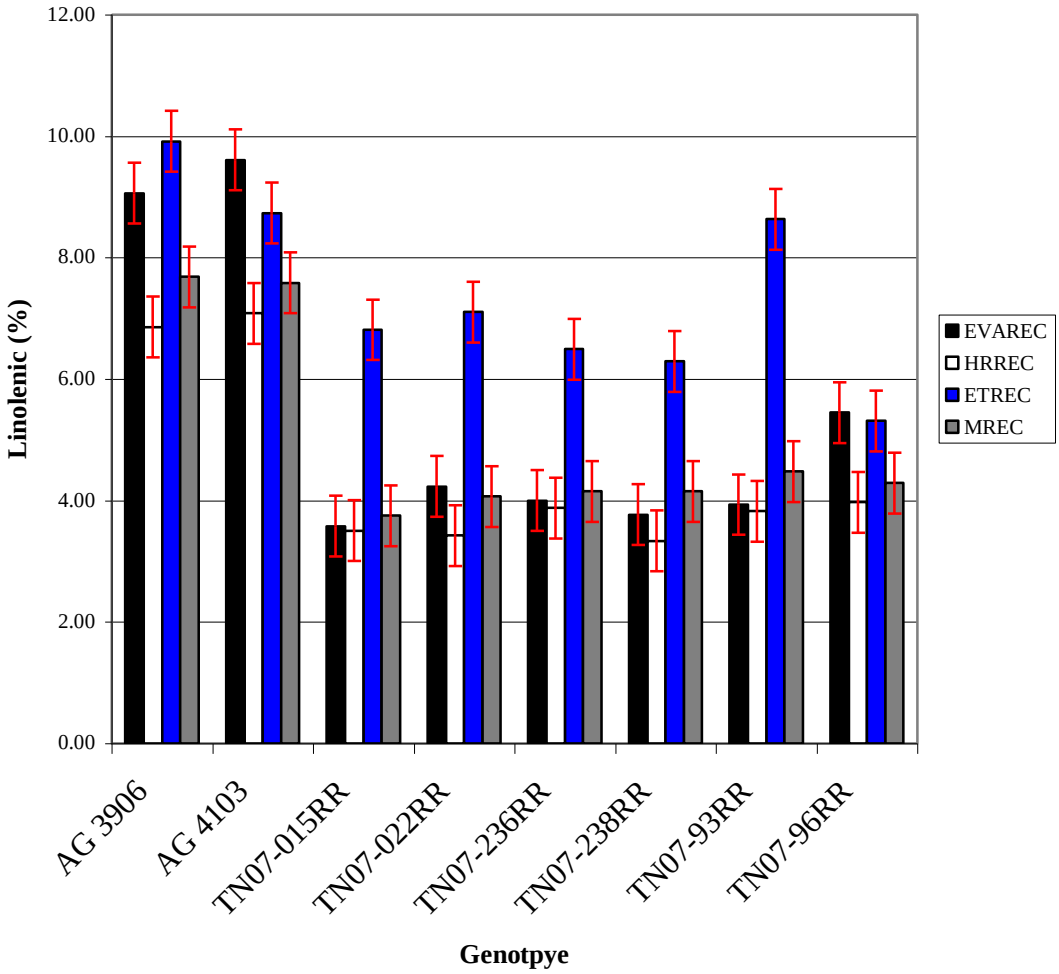

Figure 2.2 Descriptive statistics of mean linoleic acid content of 6 F_9 increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

Table 2.5 Average temperature, average maximum temperature, average minimum temperature, and average rainfall for May, June, July, August, and September for Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

Location	Month	Average Temperature (°C)	Average Maximum Temperature (°C)	Average Minimum Temperature (°C)	Average Rainfall (cm)
EVAREC	May	18.5	25.0	12.0	20.50
HREC	May	17.8	23.9	11.1	15.04
KPSF	May	18.4	24.7	12.2	Irrigated
MREC	May	19.2	25.9	12.6	Irrigated
EVAREC	June	25.2	31.6	18.9	12.52
HREC	June	24.7	30.7	18.8	4.60
KPSF	June	24.3	31.0	17.0	Irrigated
MREC	June	25.5	31.5	19.4	Irrigated
EVAREC	July	25.8	31.8	19.7	13.39
HREC	July	25.5	31.5	19.5	14.81
KPSF	July	24.7	30.8	18.5	Irrigated
MREC	July	26.5	33.2	19.8	Irrigated
EVAREC	August	24.3	30.4	18.2	6.73
HREC	August	17.8	30.2	24.0	2.79
KPSF	August	24.1	30.3	17.8	Irrigated
MREC	August	25.2	31.8	17.4	Irrigated
EVAREC	September	22.3	22.3	16.7	12.52
HREC	September	22.2	28.3	16.0	7.44
KPSF	September	22.3	28.8	15.9	Irrigated
MREC	September	22.7	29.9	15.4	Irrigated

temperature, and average rainfall for each location averaged over all five months and mean oleic and linolenic acid for each location showed no significant correlations (Table 2.6).

Other studies have shown high temperatures are associated with an increase in oleic acid content and a decrease in linolenic acid content (Howll and Collins, 1957; Cherry et al., 1985; Wilcox and cavins, 1992; Primomo et al., 2002). Oliva et. al (2006) reported three mid-oleic genotypes showed inconsistent responses to irrigation for oleic and linolenic acid content.

2008 Multiple Environment Yield Trial: Essential and Non-Essential Amino Acid and Total Protein and Oil Content Analysis of Variance

The 6 $F_{6:9}$ increased oleic acid soybeans had significantly lower oil content and significantly higher protein content than the two commercial checks (Table 2.7). TN07-015RR (42.4 %), TN022 RR (41.9 %), TN07-236RR (41.3 %), TN07-238RR (42.0 %), TN07- 93 RR (41.7 5%), and TN07-96RR (41.8 %) all had significantly higher protein content than AG3906 (40.3 %) and AG4103 (39.5 %). TN07-015RR had the highest protein content with 42.4 %. TN07-015RR (22.2 %), TN022 RR (20.9 %), TN07-236RR (21.9 %), TN07-238RR (21.9 %), TN07- 93 RR (21.8 %), and TN07-96RR (21.6 %) all had significantly lower oil content than AG3906 (24.4 %) and AG4103 (23.3 %). In addition, there were significant differences ($p < 0.05$) among all essential and non-essential amino acids in the 6 $F_{6:9}$ increased oleic acid soybeans.

Studies on other modified fatty acid soybeans have had similar results. Ndzana et al. (1994) showed reduced palmitic acid soybean populations consistently produce less oil content content than normal palmitic soybean lines. Lundeen et al. (1987) observed soybean lines with elevated stearic acid content had significantly greater protein content than those with normal stearic acid. However, it has been previously reported that soybean lines with elevated stearic

Table 2.6 Correlations coefficients among mean oleic and linolenic acid content of 6 $F_{6:9}$ increased oleic acid soybeans from 'Allen' x N98-4445A and average temperature, average maximum temperature, average minimum temperature, and average rainfall averaged over May, June, July, August, and September in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

	Average Temperature	**Average Maximum Temperature**	**Average Minimum Temperature**	**Average Rainfall**	**Linolenic Acid**
Average Maximum Temperature	ns				
Average Minimum Temperature	ns	ns			
Average Rainfall	ns	ns	ns		
Linolenic Acid	ns	ns	ns	ns	
Oleic Acid	ns	ns	ns	ns	ns

ns=non-significance

Table 2.7 Descriptive statistics of essential and non-essential amino acid and total protein and oil concentration of 6 $F_{6:9}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

LINE	PRO[1]	OIL	ALA[a]	ARG[c]	ASP[a]	CYS[c]	GLU[a]	GLY[a]	HIS[c]	ILE[b]	LEU[b]	LYS[b]	MET[b]	PHE[b]	PRO[a]	SER[a]	THR[b]	TRP[b]	TYR[c]	VAL[b]
AG3906	40.26	24.44	2.44	3.59	5.19	0.68	6.96	3.07	2.11	2.14	3.28	1.72	0.71	2.28	2.67	2.72	2.11	0.53	2.01	2.85
AG4103	39.46	23.28	2.37	3.48	5.12	0.68	7.01	2.83	1.98	2.12	3.32	1.85	0.71	2.25	2.59	2.55	2.04	0.51	1.95	2.75
TN07-015RR	42.36	22.20	2.46	3.88	5.35	0.60	7.84	2.86	1.95	2.31	3.56	2.03	0.69	2.37	2.75	2.56	2.02	0.48	1.96	2.90
TN07-022RR	41.89	20.92	2.45	3.78	5.32	0.64	7.54	2.86	1.94	2.26	3.46	2.00	0.72	2.34	2.72	2.61	2.02	0.47	1.95	2.91
TN07-236RR	41.27	21.85	2.44	3.77	5.28	0.61	7.53	2.82	1.94	2.25	3.48	1.97	0.71	2.32	2.70	2.53	2.01	0.45	1.93	2.87
TN07-238RR	42.00	21.90	2.47	3.80	5.33	0.61	7.62	2.91	1.96	2.29	3.50	1.96	0.72	2.34	2.76	2.63	2.04	0.46	1.97	2.93
TN07-93RR	41.65	21.81	2.44	3.83	5.34	0.58	7.60	2.87	2.01	2.26	3.52	1.93	0.70	2.36	2.70	2.54	2.02	0.47	1.95	2.88
TN07-96RR	41.80	21.59	2.43	3.83	5.33	0.61	7.70	2.82	1.93	2.27	3.53	1.98	0.71	2.34	2.70	2.52	1.99	0.46	1.92	2.86
LSD (0.05)	0.36	0.25	0.02	0.05	0.06	0.05	0.18	0.07	0.09	0.02	0.06	0.07	0.01	0.05	0.03	0.07	0.02	0.01	0.03	0.03

Pro[1]: protein

[a]Non-essential amino acids.

[b]Essential amino acids.

[c]Essential as well as non-essential, depending upon classification based on animal requirement.

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level.

acid had no effect on protein content, elevated stearic acid content had significantly greater oil content (Hartmann et al., 1997), and genetic correlations between palmitic acid content and oil and protein content were typically nonsignificant ($P>0.05$). Therefore, it may be hard to determine exactly what effects modified fatty acid concentrations may have on the protein and oil content of soybean; such changes may be genotype specific.

2008 Multiple Environment Yield Trial: Biodiesel Properties

The term rancidity refers to the off odors and flavor resulting from lipid oxidation. Lipid oxidation occurs via a self-sustaining free radical mechanism that produces hydroperoxides that undergo scission to form various secondary products including aldehydes, ketones, organic acids and hydrocarbons. Peroxide value is one of the most common tests for lipid oxidation. High-quality, freshly deodorized fats and oils will have a peroxide value of zero. For soybean oil, peroxide values of 1-5, 5-10 and >10 correspond to low, medium and high levels of oxidation, respectively (Nielsen, 2003). As seen in Table 2.1, AG3906 and AG4103 contained the lowest percentage of monounsaturated FAME (23.8 % and 23.9 %, respectively). This helps explain the difference between the PV of these samples (Table 2.8); higher PV values are indicative of lower levels of monounsaturated FAME and greater amounts of oxidation. However, peroxide value measures a transient product of oxidation. A low value may represent either the beginning of oxidation, or advanced oxidation. The rate at which unsaturated fatty compounds oxidize depends on the number and position of double bonds. Linolenic acid and linoleic acid are polyunsaturated and have three and two double bonds respectively, making them highly susceptible to oxidation. Monounsaturated fatty acids on the other hand, are two or more times less reactive as polyunsaturated fatty acids because they have only one double bond (Knothe, 2005; Dunn, 2005).

Table 2.8 Descriptive statistics of induction period (IP) and peroxide value (PV) of 2 $F_{6:9}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in Milan, TN, Springfield, TN, Knoxville, TN, and Warsaw, VA in 2008.

	Oxidative Stability	
Genotype	**IP (110°C, h)**	**PV (meq/kg)**
AG3906	4.5	1.2
AG4103	4.8	1.2
TN07-93RR	6.3	0.3
TN07-96RR	6.4	0.5
LSD (0.05)	0.21	0.18

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level.
IP = induction period
PV = peroxide value

Dunn (2002) reported during the initial stages in the oxidation of fatty derivatives, primary products such as hydroperoxides increase in concentration, causing PV to increase. The length of time before detectable rancidity is defined as the induction period. Following the induction period, decomposition of the hydroperoxides yields a mixture of secondary products. The Rancimat method and OSI method are two nearly identical methods for determining induction period. According to the European standard (EN 14214) the minimum limit for IP is greater than six hours. In this study, AG3906 and AG4103 did not meet the minimum limit for IP, with an IP of 4.5 h and 4.8 h, respectively. However, TN07-93RR and TN07-96RR were able to meet IP requirements, with an IP of 6.3 h and 6.4 h, respectively. According the less stringent American standard (ASTM D6751), all samples were satisfactory for IP (>3 h). Neither EN 14112 nor ASTM D6751 has limits for peroxide value.

Conclusions

In conclusion, this study has provided an evaluation of the environmental and agronomic performance, seed quality and biodiesel properties of oxidative stability and peroxide value of a set of $F_{6:9}$ derived increased oleic acid soybean lines. It was found that while the $F_{6:9}$ derived increased oleic acid soybean lines had significantly higher oleic acid and lower linolenic acid concentrations than the two commercial checks, yield was not affected. Stability analysis revealed the $F_{6:9}$ derived increased oleic acid soybean lines could be grown in different environments without severely compromising their fatty acid profile. The reduced linolenic acid concentrations were found to be more stable across environments than the higher linolenic acid concentrations seen in the two commercial checks. However, while the correlation of the increased oleic acid lines did have some variation across environments there was data that

suggested stability maybe determined by the genotype and not by the concentration of oleic acid. We were also able to show that increased oleic acid concentrations can decrease oxidation degradation and improve biodiesel performance of soybean based biofuels. Therefore, it should be possible to produce a marketable soybean that has desirable agronomic traits and biodiesel properties.

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Chapter 3

The Effects of Different Selenium Applications on Soybean Biodiesel Properties

Abstract

Monounsaturated fatty acid methyl ester (FAME) are reported to strike the best balance between cold flow properties and oxidative stability to enhance biodiesel test results and produce a better burning fuel. In addition, treating fuels derived from fatty acid alkyl esters with oxidation inhibitors (antioxidants) has been reported to increase resistance to oxidation. Fuel properties: acid value, cloud point, iodine value, pour point, peroxide value, induction period, onset temperature, and kinematic viscosity were used to evaluate a newly developed Roundup Ready® soybean recombinant inbred line with a novel oil profile, exhibiting an elevated level of monounsaturated FAME and the possibility of using selenium as a natural antioxidant for use in the biodiesel industry. TN07-93RR was determined as the most desirable for biodiesel fuel, based on its fatty acid profile and fuel properties. AG3906 was deemed too high in undesirable polyunsaturated FAME content. As a result of high polyunsaturated FAME content, AG3906 had a high iodine value (IV) and low oxidative stability and was unsatisfactory with regard to these properties according to the European biodiesel standard, EN 14214. TN07-93RR and AG3906 both were considered satisfactory with regard to the properties measured in this study according to the less stringent American biodiesel standard, ASTM D6751.

Introduction

Selenium (Se) is an essential trace element for fish, birds, mammals, microorganisms and humans (Birringer et al., 2002). Although Se has not been shown to be essential to plants, the ability of some plants to accumulate and transform Se into bioactive compounds has important implications for human nutrition, health and for the environment.

Organisms that require Se produce an array of selenoproteins. In 1973, Rotruck et al. (1973) established the biological function of Se as an integral component of glutathione peroxidase, an important antioxidative enzyme that inhibits the oxidation of lipid membranes by free radicals, thus preventing pathologies brought on by oxidative stress such as inflammation, atherosclerosis, and cardiovascular diseases. Since then, Se was identified as an essential component in cells of thioredoxin reductase (Tamura abs Stadman, 1996), type I, II, III iodothyronine deiodinases (Authur et al., 1990; Pallud et al., 1997) and for a number of other selenoproteins (Gladyshev et al., 1998; Allan et al., 1999; Kryukov et al., 2003). Thioredoxin reductase and deiodinase enzymes are essential for cell growth and survival. They are involved in the production of the active thyroid hormone, in muscle function, in the reproduction process, and in the immune response to some infections.

The Se requirement for most farm animals is between 0.1 and 0.3 mg kg^{-1} of feed (Mayland, 1994). The recommended daily intake for human adults is 50-70 µg day^{-1} and for children it is 20-30 µg day^{-1}. The Food and Nutrition Board has set the tolerance upper intake levels for Se at 400 µg day^{-1} for adults and 100 µg day^{-1} for children. However, several studies (Combs et al., 1984; Clark et al., 1996) have demonstrated Se intakes greater than those set forth by RDA may be beneficial to humans. This has sparked interest in increasing studies of Se and its effects on plants, particularly in soybeans.

When properly processed for specific applications, both soybean protein and soybean oil can be well utilized by virtually all classes of animals and by humans. World-wide in 2007, 69 % of the protein meal consumed and 30 % of vegetable oil consumed came from soybeans. Domestically, soybeans provided 71 % of the edible consumption of fats and oils in the United States (Soy Stats, 2008). In addition, in 2007, soybeans represented 56 % of world oilseed production and 32 % of those soybeans were produced in the United States. In 2007, 9.6 million

metric tons of soybean oil was produced in the United States at an average price of $1,213 per metric ton, and of the oil produced only 18 % went into industrial products and < 5 % went into biodiesel production. However, in 2007 the United States produced an estimated 1.7 billion L of biodiesel (Soy Stats, 2008). The addition of Se to the soybean market will not only offer added health benefits, but it may also allow Se to be established into an already well developed market.

Problems Facing Soybean Oil

Cloud point (CP) is designed to evaluate the lowest temperature at which a fuel can tolerate without causing problems. The lowest temperature at which the fluid will pour is defined as the pour point (PP). At low temperatures, diesel fuel can gel or crystallize and cause engine damage. According to ASTM Specifications for Tennessee, the maximum cloud point temperature shall be equal to or lower than -10 °C. Current soy diesel has a cloud point nearly 10 °C higher than Low-Temperature Flow Test (LTFT) specifications. Oxidation Stability is a prediction of the amount of time a fuel can be stored before becoming unstable. Today, the soybean oil being produced has a relatively high oxidative reactivity that compromises the storage life of soybean oil biodiesel (EMA, 2006).

Not only does the high oxidative reactivity compromise the use of soybean oil as biodiesel, but also as a vegetable oil. To retard oxidation of unsaturated fatty acids and to decrease the development of rancidity, the oil has to be hydrolyzed or synthetic antioxidants have to be added to soybean oil (Sherwin, 1976). These methods can be costly and unhealthy.

Improvements Being Made to Soybean Oil Using Additives

Many naturally occurring flavonoids in plants are recognized as important compounds in conferring stability against autooxidation to vegetable oils. The effectiveness of flavonids in

retarding lipid oxidation in fat-containing foods is related to their ability to act as free-radical acceptors (Das & Pereira, 1990). Today, natural antioxidant flavonids are being looked at to reduce synthetic food additives.

Wanasundara and Shahidi (1993) tested the antioxidant activity of flavonoids and synthetic antioxidants in refined-bleached canola oil at 200- mg L^{-1} addition level. They concluded through chemical analysis of the oil, subjected to accelerated oxidation, that all antioxidant treated oil samples showed a delayed induction period as compared with that of the control. However, five of the eleven flavonoids were more effective than the two synthetic antioxidants in retarding the formation of primary and secondary oxidation products of canola oil. Futhermore, it was evident that the antioxidant activity of flavonoids was generally governed by their chemical structures.

Antioxidants added to Biodiesel

As previously mentioned, methyl soyate (biodiesel) when exposed to air during storage can undergo oxidation, which can cause fuel quality degradation by adversely affecting properties such as viscosity, acid value and peroxide value. Today, biodiesel is stored under inert nitrogen atmosphere to retard oxidation. However, this type of treatment is expensive and can be dangerous.

One solution to increase the resistance of fuels derived from fatty acid alkyl esters against oxidation is to treat them with oxidation inhibitors (antioxidants). In a study by Dunn (2005), researchers examined the effectiveness of synthetic antioxidants tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PrG) and a natural antioxidant, α-tocopherol in mixtures with soybean oil fatty acid methyl esters (SME). Results showed all antioxidants improved oxidative stability. However, PrG,

BHA and BHT were most effective and α-Tocopherol was least effective at improving oxidative stability.

In previous studies, TBHQ and BHT have been shown to retard effects of oxidation on viscosity, acid value and peroxide value of biodiesel (Canakci et al., 1999; Dunn, 2000; Dunn 2002). Tocopherols, BHA and PrG have also been shown to improve resistance to oxidation of vegetable oils (Chu et al., 1999; Tan et al., 2002).

Materials and Methods

Experimental Objectives

At the University of Tennessee, crosses we made between Roundup® Ready early generation lines from which 'Allen' was later derived (♀) and early generation lines from which N98-4445A was later derived (♂). For simplicity the parents will be referred to as Allen and N98-4445A. The germplasm N98-4445A was developed by the USDA-ARS, in cooperation with the North Carolina Agriculture Research Service at NC State University (Burton et al., 2006). N98-4445A has a high concentration of oleic acid, approximately 560 g kg^{-1}. This concentration is more than 100 g kg^{-1} greater than any oleic acid concentration in the United States germplasm collection and is around 340 to 380 g kg^{-1} (i.e. nearly 3x) greater than the oleic acid concentration in commercial soybean cultivars. '5601T' was developed at the University of Tennessee. In 2001, it was released because of its high yield, over geographically diverse regions of southern United States (Pantalone et al., 2003). Since that time it has been shown to have excellent estimated processor value (EPV) (Graef 2006; 2005; 2004). The quality of the protein meal is near ideal for the poultry feed industry (Brake et al., 2005) and it is one of the highest

isoflavone containing commercial cultivars (Charron et al., 2005). The agronomic stability of 5601T has been well documented: it was among the in the top performing cultivars for its maturity class in Tennessee State Variety Tests in 2008, 2004, 2003, 2002, 2001 and was the top performing cultivar in 2007, 2006, 2005 as well as a top yielder in KY, NC, and AR. 5601T currently serves as a high yield check cultivar in the USDA Southern Regional Testing Program. Allen is the Roundup® Ready version of 5601T. Allen was among the top performing cultivars for its maturity class in the Roundup® Ready Tennessee State Variety Tests in 2008 and 2006 and was the top performing cultivar in 2007.

Selenium applications were made directly to the plant to evaluate the antioxidant properties of selenium on the oil produced. Antioxidants are added to fats and oil to retard oxidation of unsaturated fatty acids and to decrease the development of rancidity (Sherwin, 1976). The effectiveness of antioxidants in retarding lipid oxidation in vegetable oils is related to their ability to act as free-radical acceptors (Das & Pereira, 1990). Other studies have shown the effects of adding antioxidants to vegetable oils to improve oxidative stability (Wanasundara et. al, 1993; Canacki et al., 1999; Dunn, 2000, 2002). The purpose of this study was to investigate how much selenium is accumulated into the oil and the meal and if oxidative stability of the oil is improved.

Population Development

In the summer of 2001, researchers at the University of Tennessee began work on developing a high yielding soybean with increased oleic acid. When Allen was crossed with the increased oleic germplasm, N98-4445A the pedigree-selection procedure was used to select single plants based on oleic acid content until the F_6 generation. An example, in correspondence with the pedigree-selection method used in this study is presented as Appendix Figure A.1.

Experimental Design

In 2008, a $F_{6:9}$ increased oleic acid soybean (TN07-93RR) from Allen x N98-4445A and a commercial check (AG3906) were entered into yield trials at three locations: East Tennessee Research and Education Center (Knoxville, TN), the Highland Rim Research and Education Center (Springfield, TN), and the Research and Education Center at Milan (Milan, TN) representing major physiographic regions of East Tennessee, Middle Tennessee, and West Tennessee. The experimental design was a randomized complete block design factorial with replication. At each location three replications of TN07-93RR and AG3906 were grown under four selenium treatments as 4 row plots, 6 m in length, with 76 cm spacing between rows.

Selenium applications

Selenium foliar application concentrations were as follows: 0 mg L^{-1} (control), 3 mg L^{-1} (low), 6 mg L^{-1} (medium) and 12 mg L^{-1} (high) as sodium selenate (Table 3.1). Experimental design was a randomized complete block design with a factorial arrangement of treatments, including a nontreated control for comparison. Each treatment was replicated three times. Factors were Se concentrations at four levels. Applications were made with a compressed-air sprayer calibrated to deliver 654 L/ha at 344 kPa and a nonionic surfactant was applied at 0.25 % v/v. Applications were made twice, once at flowering (R1) and again at seed development (R5).

Experimental Field Procedures

After planting, all the plots were monitored for agronomic traits. Flowering was recorded as the number of days since planting when 95% of the plants had bloomed. At the same time flower color was taken as well (purple, white or segregating). At maturity, plant height was taken as an estimation as the distance from the soil surface to the tip of the main stem in cm.

Table 3.1 Selenium was applied as sodium selenate at four concentrations: 0 mg L^{-1} (control), 3 mg L^{-1} (low), 6 mg L^{-1} (medium) and 12 mg L^{-1} (high). Applications were made twice, once at flowering (R1) and again at seed development (R5).

Location	Line	Plot	Rep	Treatment	Selenium Conc.	Planting Date	First Application	Second Application
Knoxville	TN07-93RR	90051	1	high	12 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90052	1	med	6 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90053	1	low	3 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90054	1	high	12 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90055	1	control	0 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90056	1	control	0 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90057	1	med	6 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90058	1	low	3 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90059	2	high	12 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90060	2	control	0 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90061	2	low	3 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90062	2	med	6 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90063	2	med	6 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90064	2	high	12 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90065	2	control	0 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90066	2	low	3 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90067	3	control	0 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90068	3	high	12 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90069	3	med	6 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90070	3	high	12 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90071	3	control	0 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90072	3	med	6 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	TN07-93RR	90073	3	low	3 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Knoxville	AG3906	90074	3	low	3 mg L^{-1}	5/6/2008	6/19/2008	7/28/2008
Highland Rim	TN07-93RR	91051	1	low	3 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91052	1	low	3 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91053	1	control	0 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91054	1	high	12 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91055	1	control	0 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91056	1	med	6 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91057	1	med	6 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91058	1	high	12 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91059	2	low	3 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91060	2	control	0 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91061	2	med	6 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91062	2	med	6 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008

Table 3.1 Continued.

Location	Line	Plot	Rep	Treatment	Selenium Conc.	Planting Date	First Application	Second Application
Highland Rim	AG3906	91063	2	control	0 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91064	2	high	12 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91065	2	high	12 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91066	2	low	3 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91067	3	high	12 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91068	3	low	3 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91069	3	control	0 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91070	3	med	6 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91071	3	low	3 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91072	3	med	6 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	AG3906	91073	3	high	12 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Highland Rim	TN07-93RR	91074	3	control	0 mg L^{-1}	5/19/2008	7/2/2008	8/14/2008
Milan	AG3906	92051	1	low	3 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92052	1	med	6 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92053	1	control	0 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92054	1	low	3 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92055	1	control	0 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92056	1	high	12 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92057	1	med	6 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92058	1	high	12 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92059	2	med	6 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92060	2	high	12 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92061	2	control	0 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92062	2	med	6 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92063	2	control	0 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92064	2	high	12 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92065	2	low	3 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92066	2	low	3 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92067	3	low	3 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92068	3	high	12 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92069	3	med	6 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92070	3	low	3 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92071	3	med	6 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92072	3	control	0 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	TN07-93RR	92073	3	high	12 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008
Milan	AG3906	92074	3	control	0 mg L^{-1}	6/4/2008	7/15/2008	8/27/2008

Lodging was scored on a scale from 1-5; with 1 being all the plants in the row were erect and 5 being all the plants in a plot were prostrate. Maturity was recorded as the date, according to the Julian calendar, when 95 % of the pods achieved their mature color. At that time pubescence was also recorded. Seed yield was estimated from the two inside rows in the four row plots and both rows in the two row plots after the plots had been end trimmed to 4.88 m in length. Yield was recorded in kg ha^{-1} at 13 % maturity basis. Seed size was taken as the weight in grams from a random 100 seed sample.

Laboratory Procedures

Fatty Acid Composition Analysis by Gas Chromatography

Prior to harvest, 5 pods per line were hand collected at mid-node height this was done to minimize error variance from sampling at different nodes. The pods were shelled by hand and the seed was collected. Five seeds from each line were selected randomly, crushed and placed in a test tube. Then 1 mL of seed extraction solvent consisting of 8:5:2 (by volume) ratio of chloroform, hexane, and methanol was added. After the seeds were left to extract overnight, 100 µL of the extracted oil supernatant was transferred to a 1.5 mL auto sampler vial to which 0.75 ml of a methylating reagent [a mixture of 50 mL sodium methoxide 20 mL petroleum ether, 10mL ethyl ether, and 1 mL Hexane] was added to the autosampler vial before capping. The resulting fatty acid methyl ester was analyzed using a Hewlett Packard HP 6890 gas chromatograph (Palo Alto, CA) equipped with model 7673 autosampler, flame ionization detector, and an immobilized 30m long x 0.53 mm inner diameter All-Tech AT-Silar capillary column with 0.5µL fused stationary phase. Operating conditions were set to: carrier He (20mL/Min), 20:1 (v/v) split injection, injection temperature 250ºC, detector temperature 275ºC and column temperature 240ºC. The RM-1 standard was used to determine relative fatty acid

concentration of the selected lines.

Sample Preparation for Protein, Oil, and Amino Acid composition via NIR Analysis

Approximately 20 g of soybean seed collected from plot seed samples were ground in a water-cooled Knifetec 1095 Sample Mill (FOSS Tecator, S-26321, Hogana, Sweden) for 20 s. This produced soybean flour that is uniform in particle size. The samples were analyzed using a FOSS 6500 near infrared spectrometer (NIR). A dehumidifier was used throughout the analysis to reduce the humidity to 40 %, and room temperature was maintained at approximately 20ºC. First the NIR was warmed up for 2 h after turning on the lamp. Then auto diagnostics was run for instrument response, wavelength accuracy and NIR repeatability. After that the ground soybean samples were scanned to get the predicted concentrations of oil, protein, and all amino acids using ISIscan system II version 2.80 software. The instrument was left on for the whole period of analyses, and diagnostics were performed every day until the scanning was finished.

Elemental Analysis

To determine the Se concentration in the meal a ground 0.3 g meal sample from the above procedure was mixed with 10 ml of 70 % concentrated nitric acid (HNO_3) and digested in a microwave accelerated reaction system (MARS5, CEM Corp., Matthews, N.C.). The digested solution was cooled to room temperature and 0.1 ml of the solution was added to 9.9 mL solution of RO H_2O, 2 % nitric acid (HNO_3) and 0.5 % hydrochloric acid (HCL) for a final volume of 10 ml. Elemental analysis was determined by ICP-MS (Inductively Coupled Plasma – Mass Spectrometry; 7500, Agilent Technologies Inc., Wilmington, Del.)

To determine the Se concentration in the oil, a ground 0.5 g meal sample was mixed with 1 ml extraction solvent consisting of an 8:5:2 ratio of chloroform, hexane and methanol and allowed to set overnight. Any remaining solvent was evaporated using a Meyer NEVAP. Then

the extracted oil sample was digested using 10 ml of 70 % concentrated nitric acid (HNO_3) and allowed to set overnight. 0.1 ml of the digested solution was added to 9.9 mL solution of RO H_2O, 2 % nitric acid (HNO_3) and 0.5 % hydrochloric acid (HCL) for a final volume of 10 ml. Elemental analysis was determined by ICP-MS (Inductively Coupled Plasma – Mass Spectrometry; 7500, Agilent Technologies Inc., Wilmington, Del.)

A nitric acid reflux was used for soil analysis (Chang et al., 1984). Soil samples were ground by mortar and pestle and sieved through a 2 mm screen. A 3.5 g ground sample was placed into a 50-ml tube with 21 ml of 4M nitric acid (HNO_3) and placed on a heating block (Thermolynem, Model 2200, Dubuque, Iowa) under reflux overnight at 75ºC. Tubes were allowed to cool to room temperature and brought to a final volume of 35 ml with deionized water. The solutions were filtered through Whatman #42 filter paper (Maidstone, England) before total elemental analysis was determined by ICP-MS (Inductively Coupled Plasma –Mass Spectrometry; 7500, Agilent Technologies Inc., Wilmington, Del.)

Extraction and Processing

The untreated control and the high Se treatment for both the increased oleic acid soybean (TN07-93RR) and the commercial check (AG3906) were tested according to ASTM standards at the USDA National Center for Agricultural Utilization Research in Peoria, IL. The samples were tested for the following:

Fatty acid profile by GC

Fatty acid methyl esters (FAME) of SME were separated using a Varian 8400 GC equipped with an FID detector and a SP2380 (Supelco) column (30 m x 0.25 mm i.d., 0.20 μm film thickness). Carrier gas was He at 1 mL min^{-1}. The oven temperature was initially held at 150 °C for 15 min, increased to 210 °C at 2 °C/min, increased to 220 °C at 50 °C/min, then held

for 10 minutes. The injector and detector temperatures were 240 °C and 270 °C, respectively. FAME peaks were identified by comparison to the retention times of reference standards. Each FAME determination was run in triplicate and average values are reported.

NMR and FT-IR spectroscopy

^{1}H-NMR data were recorded using a Bruker AV-500 spectrometer (Billerica, Mass.) operating at 500 MHz using a 5-mm broadband inverse Z-gradient probe in $CDCl_3$ (Cambridge Isotope Laboratories, Andover, Mass.) as solvent. FT-IR spectra were recorded on a Thermo-Nicolet Nexus 470 FTIR spectrometer (Madison, Wis.) with a Smart ARK accessory containing a 45 ZeSe trough in a scanning range of 650-4000 cm^{-1} for 64 scans at a spectral resolution of 4 cm^{-1}.

Low temperature operability

CP and PP determinations were made in agreement with ASTM D5773 (ASTM, 2007) and ASTM D5949 (ASTM, 2001), respectively, using a Phase Technology Analyzer model PSA-70S (Richmond, B.C., Canada). Cloud and pour points were rounded to the nearest whole degree (^{o}C). For a greater degree of accuracy, PP measurements were done with a resolution of 1 ^{o}C instead of the specified 3^{o}C increment. Each sample was run in triplicate and mean values are reported.

Kinematic viscosity

Kinematic viscosity (υ, mm^2/s) was determined with Cannon-Fenske viscometers (Cannon Instrument Co., State College, Penn.) at 40^{o}C in accordance to ASTM D445 (ASTM, 2006). All experiments were run in triplicate and mean values are reported.

Oxidative stability

Induction period (IP, h) was measured in accordance to EN 14112 (European Committee

for Standardization, 2003) utilizing a Metrohm USA, Inc. (Riverview, Fla.) model 743 Rancimat instrument. The flow rate of air through 3 ± 0.01 g of methyl esters was 10 L/h with a block temperature of 110 °C and a correction factor (ΔT) of 1.5 °C. IP was mathematically determined as the inflection point of a computer-generated plot of conductivity (μS/cm) of distilled water versus time (h). The glass conductivity measuring vessel contained 50 ± 0.1 mL of deionized water. Each sample was run in triplicate and mean values are reported.

Oxidation onset temperature (OT, ^{o}C) was determined using a DSC 2910 thermal analyzer from TA Instruments (Newcastle, Del.). Typically, a 2 μL sample, resulting in a film thickness of < 1mm, was placed in an aluminum pan hermetically sealed with a pinhole lid and oxidized with pressurized (1378.95 kPa; 200 psi) dry air (Gateway Airgas, St. Louis, Mo.) in the module with a heating rate of 10 ^{o}C/min from 50 to 350 ^{o}C. A computer-generated plot of heat flow (W/ g) versus temperature (^{o}C) was used to graphically determine OT. Each sample was run in triplicate and average values rounded to the nearest tenth of a degree are reported.

Acid, iodine and peroxide values

Acid value (AV, mg KOH/g) titrations were performed as described in AOCS official method Cd 3d-63 (8) using a Metrohm 836 Titrando (Westbury, NY) autotitrator equipped with a model 801 stirrer, a Metrohm 6.0229.100 Solvotrode, and Tiamo 1.1 Light software. However, the official method was modified for scale to use 2 g of sample and 0.02 M KOH. The titration endpoint was determined by the instrument and visually verified using a phenolphthalein indicator. Each sample was run in triplicate and mean values are reported.

Iodine value (IV) was calculated from the fatty acid profile according to AOCS official method 1c-85(AOCS, 1999a).

Peroxide value (PV, meq. of peroxide/kg sample) of SME samples was determined using the modified ferric thiocyanate method of Shantha and Decker (AOCS, 1999b). Hydroperoxides were calculated using a standard curve made from solutions of ferric chloride (0-15 μg Fe^{3+}). Each sample was run in triplicate and average values are reported.

Experimental Analysis

Analysis of variance was conducted using PROC MIXED in SAS (ver. 9.1.3) to 1) test the hypothesis that increased oleic soybean lines with Se applications produce oil with significantly improved oxidative stability for biodiesel, 2) evaluate the possibility of using Se as a natural antioxidant for use in the biodiesel industry, 3) evaluate the agronomic and seed quality attributes of increased oleic acid soybeans and Se applications. Least significant differences were used to compare fatty acid content (palmitic, stearic, oleic, linoleic, and linolenic acid), yield and biodiesel properties at the 0.05 significance level. In addition, least significant differences were used to compare the differences between fatty acid content, yield, essential and non-essential amino acids, protein and oil content, Se levels in the meal compared with the oil and biodiesel properties under four Se treatments at the 0.05 significance level.

Results and Discussion

Fatty Acid Profile Analysis of Variance

While air, heat, light traces of metal, antioxidants, peroxides and other factors all can facilitate oxidation, the compound structure of fatty esters have been shown to have the greatest catalyzing effect. Oleic acid, a monounsaturated fatty acid, has the lower crystallization temperatures seen in polyunsaturated fatty acids, but still has a relatively low oxidation rate

(Knothe, 2005; Dunn, 2005). However, no studies have shown what the addition of natural antioxidants can have on oleic acid levels in soybean. While there were significant differences among the genotypes ($P<0.0001$) for oleic acid concentration, there were no differences among Se treatments within each genotype or averaged over all genotypes ($P>0.05$) for oleic acid concentration (Table 3.2). TN07-93RR averaged 40.4 % oleic acid and 5.1 % linolenic acid, while AG3906 averaged 25.1 % oleic acid and 8.6 % linolenic acid. TN07-93RR averaged a 61 % increase in oleic acid and 41 % decrease in linolenic acid, compared with AG3906.

The 68 % increase in oleic acid of TN07-93RR when compared to a traditional soybean of 24% oleic acid, is a significant breeding accomplishment. To develop soybeans lines with 40 % oleic acid with a Roundup Ready® background with high yield could have significant impacts on the soybean market and the biodiesel industry. Genetic gains represent more than half of all improvements combined for U.S. crop productivity. In 2008, total value of soybean production in Tennessee was 434 million dollars, over 100 million dollars higher than the past ten years, due in part to Tennessee genetics (NASS, 2009).

Since there were no significant differences in oleic acid content when different Se treatments were applied, this method may not work for increasing oleic acid content in soybeans. However, this data does support the hypothesis of adding Se to increased oleic acid soybeans for added health benefits, without decreasing the oleic acid content. Oleic acid, also known as Omega 9 fatty acid, has been shown to lower the risk of a heart attack, arteriosclerosis, and aid in cancer prevention. This in combination with the antioxidant role of Se could improve the overall health benefits of consuming soy products. Future research is needed to more thoroughly study the effects of Se on increased oleic acid soybean lines.

Table 3.2 Descriptive statistics of mean seed yield, maturity, lodging, height, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid content of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under four sodium selenate treatments: 0 mg L^{-1} (control), 3 mg L^{-1} (low), 6 mg L^{-1} (medium) and 12 mg L^{-1} (high).

		Agronomic Traits				**Fatty Acid Profile**				
		Seed Yield	**Maturity**	**Lodging**	**Height**	**Palmitic**	**Stearic**	**Oleic**	**Linoleic**	**Linolenic**
Genotype	**Selenium Treatment**	**kg ha^{-1}**			**cm**	**% Total Lipid**				
AG3906	control	3982	260	1.4	79	10.4	5.1	25.4	50.9	8.3
AG3906	high	3885	259	1.1	75	10.4	4.9	25.3	50.6	8.7
AG3906	med	4023	259	1.0	72	10.4	4.9	25.2	51.0	8.6
AG3906	low	3900	259	1.1	71	10.4	4.8	24.6	51.4	8.8
TN07-93RR	control	3653	258	2.2	83	9.6	4.4	41.7	39.0	5.2
TN07-93RR	high	3744	259	2.1	85	9.9	4.6	39.7	40.9	5.0
TN07-93RR	med	3604	259	2.0	84	9.6	4.5	42.2	38.8	4.9
TN07-93RR	low	3586	259	1.9	87	10.0	4.8	37.9	42.3	5.1
$LSD_{(0.05)}$ genotype		183.89	0.48	0.17	3.25	0.14	0.13	1.11	1.00	0.29
$LSD_{(0.05)}$ genotype x treatment		NS	NS	NS	NS	NS	NS	NS	NS	NS

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
NS, non-significance.

Agronomic Traits Analysis of Variance

Here again there were differences among the genotypes ($P<0.0001$), but no differences among Se treatments within each genotype or averaged over all genotypes ($P>0.05$) for any agronomic trait measured in this study (Table 3.2). TN07-93RR averaged 3647 kg ha^{-1} seed yield, while AG3906 averaged 3948 kg ha^{-1} seed yield. The yield of TN07-93RR averaged 92 % of the yield of AG3906. Also, TN07-93RR (85 cm) was significantly taller than AG3906 (74 cm).

Scherder et al. (2006) reported the mean tocopherol of reduced palmitic acid soybean lines was 15 % greater than soybean lines with normal palmitic acid levels and reduced palmitic acid lines yielded significantly less than lines with normal palmitic acid levels. These results were in agreement with Ndzana et al. (1994) and Rebetzke et al. (1998). Scherder et al. (2006) also reported an overlap in differences in agronomic traits between the normal and reduced palmitic acid lines in the three populations tested.

Tocopherols, natural antioxidants, are known for inhibiting lipid peroxidation by quenching lipid peroxyl radical, adding to oxidaitive stability and increasing vitamin E in the human diet (Stone and Papa, 2003). Se is a natural antioxidant, along with vitamin E, known for producing glutahionine peroxidase. The similarities between Se and tocopherol suggest that although higher levels of an antioxidant may be present within a soybean line with modified fatty acid content, there are no differences in agronomic traits due to the increased antioxidant levels.

Correlation Coefficients among Agronomic Traits and Fatty Acid Content

Relationships among yield, lodging, maturity, height and fatty acid content of soybean are important, in addition to effects the Se treatments may have had on these traits. Therefore,

correlation analysis was conducted (Table 3.3).

Simple correlation analysis on TN07-93RR in the Se study showed maturity and stearic acid had a strong negative relationship (r=-0.89, P<.0001), height and stearic acid had a strong negative relationship (r=-0.84, P<0.0001), lodging and stearic acid had a moderate negative relationship (r=-0.55, P<0.001), yield and stearic acid had a strong negative relationship (r=-0.85, P<0.0001). Oleic acid and palmitic acid had a strong relationship (r=-0.85, P<0.0001), linoleic acid and palmitic acid had a strong positive relationship (r=0.89, P<0.0001), linoleic and oleic acid had a strong negative relationship (r=-0.98360, P<0.0001), linolenic acid and stearic acid had a moderate negative relationship (r=-0.60, P<0.0001). These simple correlation results suggest stearic acid had a strong impact on maturity and height as stearic acid increases maturity or height decreases. Stearic acid also had a strong negative impact on yield, as stearic acid increases yield decreases. This data supports the results obtained by Hartmann et al. (1997) and Lundeen et al. (1987) who found the soybean lines with the most stearic acid had significantly lower yields and shorter plant heights than normal stearic acid soybean lines. Hartmann et al (1997) also reported the maturity of the elevated stearic acid soybean lines were significantly earlier and significantly later maturing than the normal stearic acid soybean lines.

Oleic acid had a strong impact on palmitic acid, as oleic acid increases palmitic acid decreases. Oleic acid also had a strong impact on linoleic acid, as oleic acid increases linoleic acid decreases, which explains why linoleic and palmitic acid have a strong positive relationship, as linoleic acid increases palmitic decreases. Similar correlated changes have been observed in other studies. Carver et al. (1986) showed a gradual decrease in palmitic acid after recurrent selection for increased oleic acid contents. Negative associations between palmitic and oleic acid content have also been reported in oats (*Avena sativa* L.; Forsberg et al., 1974) and rapeseed

Table 3.3 Phenotypic correlations coefficients among agronomic traits of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A grown in Milan, TN, Springfield, TN, and Knoxville, in 2008 under four sodium selenate treatments: 0 mg L^{-1} (control), 3 mg L^{-1} (low), 6 mg L^{-1} (medium) and 12 mg L^{-1} (high).

Trait	Maturity	Height	Lodging	Yield	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Height	****0.94								
Lodging	****0.73	****0.79							
Yield	****0.96	****0.94	****0.80						
Palmitic	ns	ns	ns	ns					
Stearic	****-0.89	****-0.84	***-0.55	****-0.85	ns				
Oleic	ns	*0.35	**0.43	*0.33364	****-0.85	*-0.38			
Linoleic	ns	ns	ns	ns	****0.89	ns	****-0.98		
Linolenic	**0.48	*0.37	ns	*0.36	ns	****-0.60	ns	ns	**-0.31
Treatment	ns	ns	ns	ns	ns	ns	ns	ns	ns

*, **,***,**** Significant at P=0.05, P=0.01, P=0.001, P=0.0001, ns=non-significance

(*Brassica napus L.;* Auld et al., 1992). Hartmann et al. (1997) and Lundeen et al. (1987) found soybean lines with elevated stearic acid exhibited a significant reduction in oleic acid.

Although, there were correlations between agronomic traits and fatty acid content, Se treatments had no impact on yield, maturity, height, lodging or fatty acid content. As reported earlier, a study conducted by Scherder et al. (2006) reported that reduced palmitic acid soybean lines showed a significant increase in tocoperol, a natural antioxidant. So, while adding increasing levels of Se, a natural antioxidant, may not have an effect on fatty acid content, future studies may prove increased oleic levels may increase natural antioxidant levels within the soybean.

Essential and Non-Essential Amino Acid and Total Protein and Oil Content Analysis of Variance

There were differences among the genotypes ($P<0.0001$) for amino acid, total protein and oil content, but no differences among Se treatments within each genotype or averaged over both genotypes ($P>0.05$) for amino acid, total protein and oil content (Table 3.4). TN07-93RR averaged 41.6 % protein content, while AG3906 averaged 40.2 % protein content. TN07-93RR averaged 21.9 % oil content, while AG3906 averaged 24.3 % oil content. TN07-93RR averaged a 3 % increase in protein content and 10 % decrease in oil content over AG3906. In addition, there were significant differences ($p < 0.05$) among all essential and non-essential amino acids in the 6 $F_{6:9}$ increased oleic acid soybeans.

Studies on other modified fatty acid soybeans have had similar results. Ndzana et al. (1994) showed reduced palmitic acid soybean populations consistently produce less oil content than normal palmitic soybean lines. Lundeen et al. (1987) observed soybean lines with elevated stearic acid content had significantly greater protein content than those with normal stearic acid.

Table 3.4 Descriptive statistics of essential and non-essential amino acid and total protein and oil concentration of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under four sodium selenate treatments: 0 mg L^{-1} (control), 3 mg L^{-1} (low), 6 mg L^{-1} (medium) and 12 mg L^{-1} (high).

LINE	PRO[1]	OIL	ALA[a]	ARG[c]	ASP[a]	CYS[c]	GLU[a]	GLY[a]	HIS[c]	ILE[b]	LEU[b]	LYS[b]	MET[b]	PHE[b]	PRO[a]	SER[a]	THR[b]	TRP[b]	TYR[c]	VAL[b]
AG3906[d]	40.37	24.23	2.44	3.57	5.18	0.70	6.97	3.07	2.06	2.17	3.27	1.77	0.72	2.27	2.68	2.73	2.11	0.52	2.01	2.86
AG3906[e]	40.19	24.40	2.44	3.58	5.17	0.68	6.79	3.12	2.08	2.15	3.23	1.75	0.71	2.28	2.68	2.72	2.11	0.53	2.03	2.87
AG3906[f]	40.18	24.39	2.41	3.56	5.16	0.68	7.00	3.01	2.05	2.15	3.28	1.79	0.71	2.27	2.66	2.68	2.09	0.52	2.00	2.83
AG3906[g]	40.39	24.23	2.45	3.57	5.16	0.70	6.84	3.12	2.05	2.16	3.23	1.79	0.72	2.26	2.69	2.75	2.12	0.53	2.03	2.87
TN07-93RR[d]	41.50	21.93	2.43	3.78	5.29	0.62	7.59	2.82	1.92	2.27	3.49	1.99	0.70	2.32	2.70	2.52	1.99	0.46	1.93	2.87
TN07-93RR[e]	41.68	21.93	2.44	3.81	5.30	0.60	7.60	2.88	1.93	2.27	3.49	1.98	0.70	2.33	2.71	2.55	2.00	0.46	1.93	2.87
TN07-93RR[f]	41.53	22.03	2.44	3.78	5.27	0.62	7.53	2.86	1.90	2.27	3.47	2.01	0.71	2.32	2.71	2.54	2.00	0.46	1.94	2.88
TN07-93RR[g]	41.80	21.93	2.46	3.83	5.33	0.61	7.56	2.94	1.90	2.28	3.49	2.05	0.71	2.36	2.73	2.58	2.02	0.46	1.97	2.92
LSD (0.05) genotype	0.2	0.13	0.02	0.02	0.03	0.01	0.09	0.05	0.03	0.01	0.03	0.04	0.01	0.02	0.02	0.04	0.02	0.01	0.02	0.02
LSD (0.05) genotype x treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

PRO[1]: protein

[a]Non-essential amino acids.

[b]Essential amino acids.

[c]Essential as well as non-esstial, depending upon classification based on animal requirement.

[d]Control selenium treatment.

[e]High selenium treatment

[f]Medium selenium treatment

[g]Low selenium treatment

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level.

It has been previously reported; however, that soybean lines with elevated stearic acid had no effect on protein content and elevated stearic acid content had significantly greater oil content (Hartmann et al., 1997) and genetic correlations between palmitic acid content and oil and protein content were typically nonsignificant ($P>0.05$). Therefore, it may be hard to determine exactly what effects modified fatty acid concentrations may have on soybean protein and oil content from this study of two genotypes.

Scherder et al. (2006) reported reduced palmitic lines had greater protein and lower oil than normal palmitic acid soybean lines in three populations, but differences between the means between reduced and normal palmitic acid lines were not always significant. The reduced palmitic lines used in that study also had higher tocopherol, a natural antioxidant, which along with this study points to natural antioxidants having no effect on protein and oil content. However, Tapiero et al. (2003) reported plants convert Se mainly into Se-methioine (Se-Met) and incorporate it into protein in place of methionine (Met), and Se-Met can account for >50 % of the total Se content of the plant. In addition, Wang et al. (1996) reported significantly higher protein contents for soybeans grown on soils with Se concentrations of 2.3 mg L^{-1} over soybeans grown on soils with Se concentrations of 0.1-0.26 mg L^{-1}. Yin et al. (1986) reported similar correlations between environmental Se and protein contents. So, selecting for altered protein and oil content among increased oleic acid soybean lines under Se applications may be possible, but needs further research. The number of populations used for such a study should be as large as possible for identifying increased oleic acid lines with increased protein or oil content from Se applications. It may also be advisable to increase Se applications beyond those used in the current study.

Soil Accumulation of Selenium

In the United States, the total Se in soils varies from 0.005 mg L^{-1} to 4.0 mg L^{-1}. Soils are considered seleniferous when Se levels exceed 5.0 mg L^{-1} (Mayland, 1994). In this study, three soil samples were taken from inside the two rows treated with Se to confirm no Se accumulated in the soil from Se applications. There were no differences ($P>0.05$) among sampling 1 and 2 for each genotype under each treatment for Se content or any other element tested, except there were differences ($P<0.01$) among boron levels (Table 3.5). The differences in boron levels cannot be linked to Se applications because boron levels increase or decrease with sampling 1 and 2 and not with Se treatments. Therefore, it can be concluded that foliar Se applications may be made with little to no effect on soil Se levels.

Selenium Accumulation in the Meal vs. the Oil

To determine the ability to accumulate seed Se, both meal and oil components were analyzed (SAS, ver. 9.1.3). In the meal there were significant differences among the genotypes ($P<0.0001$) for Se concentrations among Se treatments (Table 3.6). AG3906 had significant differences between all Se treatments: control (4.50 mg L^{-1}), low (5.81 mg L^{-1}), med (8.05 mg L^{-1}) and high (9.86 mg L^{-1}). AG3906 had an average Se increase of 1.79 mg L^{-1} between each treatment. The greatest Se accumulation occurred between the low (5.81 mg L^{-1}) and med (8.05 mg L^{-1}) treatments with a 2.24 mg L^{-1} increase in Se. TN07-93RR had significant differences between Se treatments low, med and high: control (4.56 mg L^{-1}), low (5.67 mg L^{-1}), med (7.01 mg L^{-1}) and high (9.83 mg L^{-1}). TN07-93RR had an average Se increase of 1.76 mg L^{-1} between each treatment. The greatest Se accumulation occurred between the med (7.01 mg L^{-1}) and high (9.83 mg L^{-1}) treatments with a 2.82 mg L^{-1} increase in Se. These results show it is possible to increase Se levels in soybean meal using foliar Se applications. In the increased oleic acid

Table 3.5 Descriptive statistics of elemental analysis of two soil samplings: one taken before and one taken after selenium applications to a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under four sodium selenate treatments: 0 mg L^{-1} (control), 3 mg L^{-1} (low), 6 mg L^{-1} (medium) and 12 mg L^{-1} (high).

Line	Treatment	Sampling	Boron	Magnesium	Phosphorus	Potassium	Calcium	Manganese	Copper	Zinc	Selenium
AG3906	control	1	5.05	3003.33	1584.13	6181.33	1664.53	1131.60	15.22	61.70	2.10
AG3906	control	2	5.95	2685.33	734.07	4761.33	1727.40	1324.07	17.03	71.53	2.34
AG3906	low	1	4.56	2509.53	733.47	4378.67	1600.53	1162.27	15.09	65.60	2.20
AG3906	low	2	5.58	2605.13	1024.67	5322.67	1666.73	995.53	16.60	66.83	1.87
AG3906	med	1	4.98	2510.00	779.53	4372.67	1501.67	1364.93	14.97	63.53	2.42
AG3906	med	2	8.61	3346.67	1825.00	7161.33	1739.80	1369.20	19.83	71.71	2.55
AG3906	high	1	5.19	2797.33	1299.47	5554.00	1476.13	1180.80	16.80	61.56	2.28
AG3906	high	2	5.60	2816.00	1330.00	5962.00	1614.33	1184.00	17.01	63.88	2.52
TN07-93RR	control	1	3.88	2247.07	744.93	4194.00	1297.80	1008.93	12.73	51.06	1.89
TN07-93RR	control	2	8.81	3259.40	1938.60	7418.00	1648.27	1274.13	21.89	68.51	2.38
TN07-93RR	low	1	5.63	3131.80	1858.60	6788.67	1731.67	1229.67	15.61	57.75	2.37
TN07-93RR	low	2	6.19	2691.33	631.27	4644.00	1679.93	1270.07	17.29	66.47	2.34
TN07-93RR	med	1	4.41	2354.93	595.00	4020.67	1407.80	1075.07	13.88	58.09	2.00
TN07-93RR	med	2	5.25	2646.67	564.40	4628.67	1547.00	1012.53	16.77	62.95	2.16
TN07-93RR	high	1	7.24	3634.60	2852.67	8514.67	1430.53	1288.87	20.30	65.25	2.68
TN07-93RR	high	2	5.77	2877.47	1062.67	5417.33	1528.20	1158.33	17.09	64.68	2.32
LSD (0.05)			2.40	NS	NS	NS	NS	NS	NS	NS	NS

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
NS, non-significance.

Table 3.6 Descriptive statistics of selenium uptake in the meal by genotype and treatment of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under four sodium selenate treatments: 0 mg L^{-1} (control), 3 mg L^{-1} (low), 6 mg L^{-1} (medium) and 12 mg L^{-1} (high).

Genotype	Treatment	Boron	Magnesium	Phosphorus	Potassium	Calcium	Manganese	Copper	Zinc	Selenium
AG3906	control	28.30	5935.56	11118.52	42174.07	7078.89	103.14	44.43	128.01	4.50
AG3906	low	39.20	8570.37	15999.63	53214.81	7047.41	154.40	57.77	145.18	5.81
AG3906	med	29.93	5886.30	11092.22	42018.52	6928.89	109.58	43.48	129.93	8.05
AG3906	high	34.02	7582.59	13945.93	47829.63	6492.96	140.67	46.38	130.68	9.86
TN07-93RR	control	25.53	6047.41	10703.70	37104.07	6732.96	88.56	44.40	125.95	4.56
TN07-93RR	low	24.92	5947.41	10358.89	36023.33	6784.81	84.66	43.38	118.49	5.67
TN07-93RR	med	26.30	5935.56	10452.59	36356.67	6446.30	83.00	44.32	122.86	7.01
TN07-93RR	high	26.08	5956.67	10484.81	36332.59	6422.59	84.14	44.63	123.66	9.83
LSD (0.05)		NS	NS	NS	NS	NS	NS	NS	NS	1.25

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
NS, non-significance.

soybean, the greatest increase in Se comes from the highest foliar Se treatment, suggesting, higher Se treatments will result in greater increases in Se.

In the oil, there were only significant differences among TN07-93RR ($P<0.0001$) for Se concentrations among Se treatments (Table 3.7). TN07-93RR showed an increase of 0.15 mg L^{-1} between the control (0.03) and the high (0.28) Se treatment.
In a study by Kopsell et al. (2003) plants of rapid-cycling *Brassica oleracea* population were grown in nutrient solutions with sodium selenate (Na_2SeO_4) concentrations: 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 mg Na_2SeO_4 L^{-1}. Se concentration in the seeds and in the leaf tissue increased linearly with Na_2SeO_4 concentrations. However, the Se level in the leaf tissue was higher than in the seed tissue at each Se treatment level. This confirms Se is not evenly distributed in plant tissues and plant species differ greatly in their ability to accumulate Se.

The data also supports the results obtained from Zhang et al. (2003) who found the greater Se level in seed grains comes from not the forms of Se supplied, but from genotype differences. This should be taken into account for selecting high Se-accumulating plants and plants that are able to accumulate high levels of Se into the oil to improve Se benefits to the oil industry.

Biodiesel Properties

Oxidative stability and low temperature operability of biodiesel are normally inversely related: structural factors that improve oxidative stability adversely influence low temperature operability and vice versa. In general, polyunsaturated FAME are more desirable if solely taking oxidative stability into consideration. Fatty acid methyl esters that are most appropriate for providing both acceptable oxidative stability and low

Table 3.7 Descriptive statistics of selenium uptake in the oil by genotype and treatment of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under two sodium selenate treatments: 0 mg L^{-1} (control) and 12 mg L^{-1} (high).

Genotype	Treatment	Boron	Magnesium	Phosphorus	Potassium	Calcium	Manganese	Copper	Zinc	Selenium
AG3906	control	4.94	9.03	248.07	81.42	214.98	0.11	0.50	0.61	0.08
AG3906	high	4.78	11.67	263.53	87.41	251.03	0.13	0.51	0.65	0.21
TN07-93RR	control	4.64	11.87	244.27	73.16	258.40	0.14	0.74	0.68	0.03
TN07-93RR	high	5.04	9.15	265.60	67.67	245.00	0.10	0.53	0.49	0.28
LSD (0.05)		NS	NS	NS	NS	NS	NS	NS	NS	0.15

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
NS, non-significance

temperature operability are the 16 and 18 carbon monounsaturated methyl palmitoleate (C16:1) and methyl oleate (C18:1) (Knothe, 2005); (Dunn, 2005).

Taking into consideration the more stringent EN 14214 biodiesel standard when evaluating TN07-93RR and AG3906, specifications such as IV (< 120) and IP (> 6 h) can be used to eliminate AG3906 from consideration as having the best fuel properties. AG3906 exceeds the maximum limit for IV (128.2) and does not meet minimum IP limit (5.3 h). However, TN07-93RR was able to meet these requirements with a 113.8 IV and a 7.4 IP (Table 3.8). It should be noted that the USA ASTM D6751 does not specify limits for IV and contains a less stringent minimum limit for IP (>3h). As a consequence, both AG3906 and TN07-93RR were satisfactory according to the less stringent ASTM D6751 biodiesel fuel standard.

Higher IP values are considered more desirable, as they are indicative of superior oxidative stability. AG3906 is characterized as having the highest contents of polyunsaturated FAME content (58 wt %; Table 3.9), which is simultaneously responsible for high IV and low IP. In addition, AG3906 is plagued with the highest abundance of methyl linolenate (C18:3; 7.2 wt %; Table 3.8), which simultaneously causes disproportionate increases in IV and decreases in IP versus other FAME. The deleterious relationship between polyunsaturated FAME content and IP is further demonstrated by Figure 3.1, which indicates an inverse relationship between these parameters with an R^2 value of 0.722.

As previously mentioned, monounsaturated FAME are considered the most desirable for overall fuel properties of biodiesel. As seen in Table 3.8, AG3906 contains the lowest percentage of monounsaturates (25 wt %) and TN07-93RR contained the

Table 3.8 Descriptive statistics from the USDA National Center for Agricultural Utilization Research in Peoria, IL of mean fatty acid composition by genotype and treatment of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under two sodium selenate treatments: 0 mg L^{-1} (control) and 12 mg L^{-1} (high).

Treatment	Genotype	Myristic Acid	Palmitic Acid	Palmitoleic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	Arachidic Acid	Gadoleic Acid	Behenic Acid	Σ sat	Σ monounsat	Σ polyunsat
control	AG3906	0.1	10.9	0.1	5.0	23.5	51.0	7.2	0.3	0.1	0.3	16.6	25.2	58.2
high	AG3906	0.1	10.9	0.1	5.0	23.5	50.9	7.2	0.4	0.1	0.3	16.7	25.2	58.1
control	TN07-93RR	0.1	10.9	0.1	4.7	36.2	42.1	3.2	0.5	0.2	0.4	16.6	38.2	45.2
high	TN07-93RR	0.1	10.7	0.1	4.7	35.9	42.6	3.1	0.5	0.2	0.4	16.4	37.9	45.7
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
ns, non-significance.

Table 3.9 Descriptive statistics from the USDA National Center for Agricultural Utilization Research in Peoria, IL of mean fatty acid composition by genotype of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under two sodium selenate treatments: 0 mg L^{-1} (control) and 12 mg L^{-1} (high).

Genotype	Myristic Acid	Palmitic Acid	Palmitoleic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	Arachidic Acid	Gadoleic Acid	Behenic Acid	Σ sat	Σ monounsat	Σ polyunsat
AG3906	0.1	10.9	0.1	5.0	23.5	51.0	7.2	0.4	0.1	0.3	16.7	25.2	58.2
TN07-93RR	0.1	10.8	0.1	4.7	36.1	42.3	3.2	0.5	0.2	0.4	16.5	38.0	45.5
LSD (0.05)	ns	ns	ns	ns	0.8	0.7	0.1	ns	ns	0.1	ns	0.7	0.8

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
ns, non-significance.

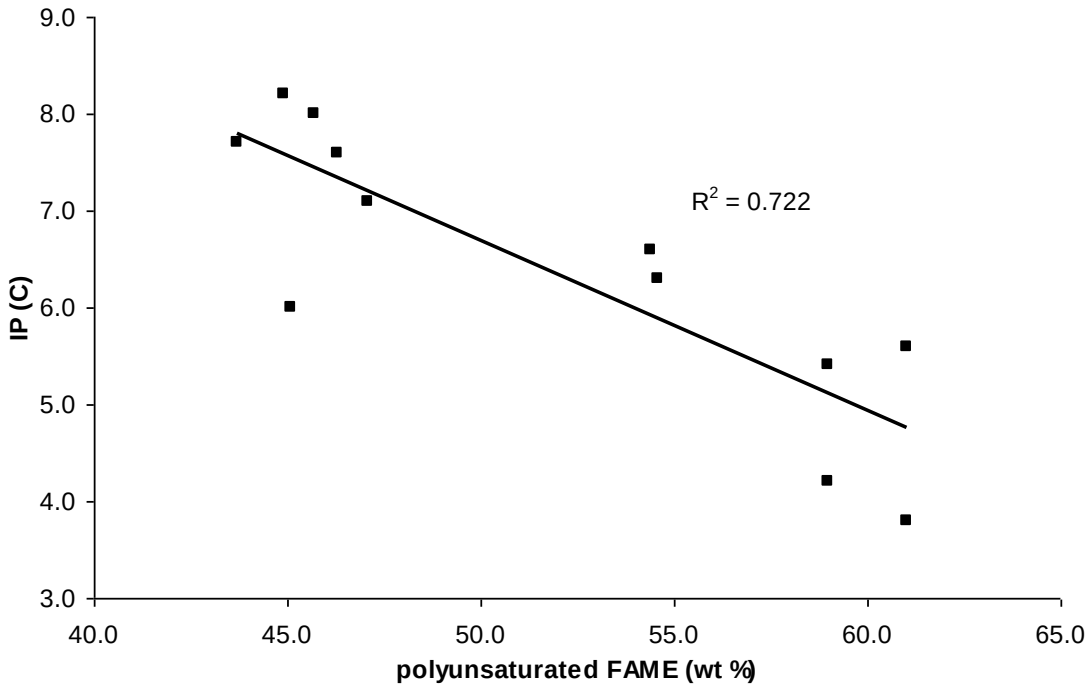

Figure 3.1. Relationship between polyunsaturated fatty acid methyl ester (FAME) content (wt %) and induction point (IP) (R^2 0.722).

highest percentage of monounsaturated FAME (~ 38 wt %). This explains the difference between the PV of these samples: higher PV are indicative of greater amounts of oxidation.

The highest content of oleic acid that has been achieved by conventional breeding is >70 % (Alt et al., 2005). However, the stability of the oleic content across environments has been a major consideration and the influence of the mid-oleic trait on agronomic and seed traits has yet to be determined. A soybean oil with >80 % oleic acid has been developed by the DuPont Company (Kinney et al., 1998), but the genetic modification involved the use of the *bla* gene for ampicillin resistance as a selectable marker. Although the likelihood of transferring the gene from the high-oleic soybean was small, the soybean was pulled off the market. Recently, Pioneer HiBred Int'l has developed a new high oleic soybean event that is targeted for commercialization.

Generally CP and PP values of less than 0 ^{o}C are desired. Preferably, these parameters should be as low as possible. Lower values for CP and PP indicate superior low temperature performance. Utilizing this arbitrary guideline, AG3906 and TN07-93RR both have CP values that are equal to or greater than 1 ^{o}C (Table 3.10). Conspicuous among these samples is the comparatively elevated levels of saturated FAME content in Knoxville, TN versus the other two locations (Table 3.11). AG3906 and TN07-93RR in Knoxville, TN contain greater than 17 wt % saturated FAME; whereas, AG3906 and TN07-93RR in the other two locations contain less than 16.5 wt % of these constituents. In this study, no significant differences between palmitic acid and only slight differences among stearic acid levels between genotypes. The significant difference comes from the variation in stearic acid levels between locations. Although this study was not designed to test the influence of environments on saturated fatty acid

Table 3.10 Descriptive statistics from the USDA National Center for Agricultural Utilization Research in Peoria, IL of mean biodiesel properties by genotype of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under two sodium selenate treatments: 0 mg L^{-1} (control) and 12 mg L^{-1} (high).

		Cold Flow		Oxidative Stability				
Genotype	**AV (mg KOH/g)**	**CP (°C)**	**PP (°C)**	**IP (110°C,h)**	**OT (°C)**	**u, 40 oC, mm2/s**	**PV (meq./kg)**	**IV (g I2 / 100 g)**
AG3906	0.0	1.2	-0.5	5.3	179.0	4.2	13.2	128.2
TN07-93RR	0.0	1.3	-0.5	7.4	184.1	4.3	7.6	113.8
LSD (0.05)	ns	ns	ns	1.3	1.9	0.1	4.8	0.9

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
ns, non-significance.

Table 3.11 Descriptive statistics from the USDA National Center for Agricultural Utilization Research in Peoria, IL of mean biodiesel properties by genotype and location of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under two sodium selenate treatments: 0 mg L^{-1} (control) and 12 mg L^{-1} (high).

			Cold Flow		Oxidative Stability				
Location	**Genotype**	**AV (mg KOH/g)**	**CP (°C)**	**PP (°C)**	**IP (110°C,h)**	**OT (°C)**	**u, 40 oC, mm2/s**	**PV (meq./kg)**	**IV (g I2 / 100 g)**
ETREC	AG3906	0.0	2.6	1.0	6.5	179.6	4.2	16.8	124.0
ETREC	TN07-93RR	0.0	3.2	1.7	8.1	184.1	4.3	8.6	112.5
HREC	AG 3906	0.0	0.8	-1.0	4.7	178.0	4.2	12.4	131.5
HREC	TN07-93RR	0.1	0.6	-1.0	7.4	183.9	4.3	7.0	115.5
MREC	AG3906	0.0	0.3	-1.5	4.8	179.5	4.1	10.4	129.0
MREC	TN07-93RR	0.0	0.2	-2.2	6.9	184.3	4.4	7.2	113.5
LSD (0.05)		ns	0.3	0.5	ns	ns	ns	ns	ns

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
ns, non-significance.

stability, comparisons can be made between saturated fatty acid levels and cold flow properties CP and PP.

Saturated FAME have high melting points versus unsaturated FAME, which result in poor low temperature properties. Figure 3.2 demonstrates the strong relationship between saturated FAME content and CP (R^2 0.9197) and PP (R^2 0.9511): higher saturated FAME content resulted in higher (less desirable) CP and PP values.

Clemente et al. (2007) produced a soybean with high oleic acid (>85 %) and low saturated fatty acid (<6 %) by down regulating two genes which increased oleic acid and decreased palmitic acid. It was developed from A3237 and the overall agronomics were able to demonstrate an improvement in cold flow properties compared to conventional soybean oil. They reported conventional soybean oil had a CP of -1 ^{o}C and a PP of 0 ^{o}C and the high oleic/low saturated fatty acid soybean had a CP of -5 ^{o}C and a PP of -9 ^{o}C. This data supports the results found in this study that increases in oleic acid and lower saturated fatty acid content can lead to better cold flow performance.

Lastly, all samples were satisfactory with respect to kinematic viscosity, as all values were within the limits prescribed in ASTM D6751 and EN 14214. In addition, Se treatments had no effect on the biodiesel properties tested in this study (Table 3.12), which may suggest that there were insufficient concentrations of Se in the oil to affect biodiesel properties.

Conclusions

In conclusion, this study has provided an evaluation of the environmental and agronomic performance, seed quality, and biodiesel properties of a $F_{6:9}$ increased oleic acid soybean line with the foliar application of the antioxidant, selenium. Selenium concentrations were measured

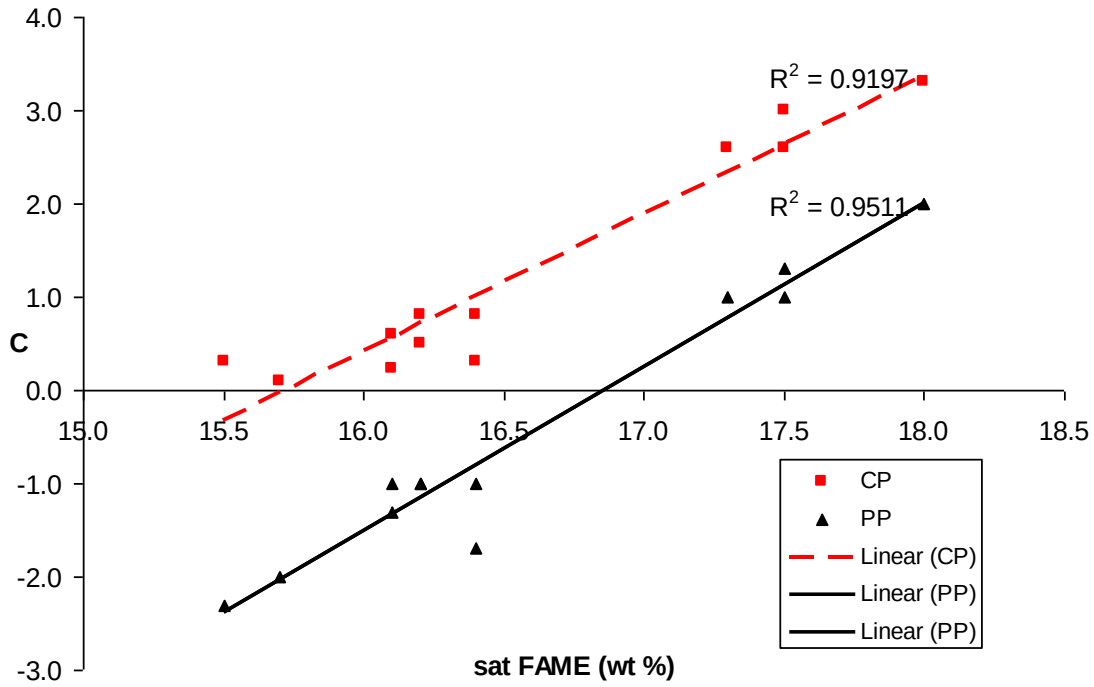

Figure 3.2. Relationship between saturated fatty acid methyl ester (FAME) content wt %) and cloud point (CP) (R^2 0.9197) and pour point (PP) (R^2 0.9511).

Table 3.12 Descriptive statistics from the USDA National Center for Agricultural Utilization Research in Peoria, IL of mean biodiesel properties by genotype and treatment of a $F_{6:9}$ increased oleic acid soybean from 'Allen' x N98-4445A and a commercial check grown in Milan, TN, Springfield, TN, and Knoxville, TN in 2008 under two sodium selenate treatments: 0 mg L^{-1} (control) and 12 mg L^{-1} (high).

			Cold Flow		Oxidative Stability				
Treatment	**Genotype**	**AV (mg KOH/g)**	**CP (°C)**	**PP (°C)**	**IP (110°C,h)**	**OT (°C)**	**u, 40 oC, mm2/s**	**PV (meq./kg)**	**IV (g I2 / 100 g)**
control	AG3906	0.0	1.2	-0.4	5.2	178.8	4.2	15.1	128.0
high	AG3906	0.0	1.2	-0.6	5.5	179.2	4.2	11.2	128.3
control	TN07-93RR	0.0	1.3	-0.3	7.7	183.6	4.4	6.9	113.7
high	TN07-93RR	0.1	1.3	-0.7	7.2	184.6	4.3	8.3	114.0
LSD (0.05)		ns	ns	ns	ns	ns	ns	ns	ns

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
ns, non-significance.

in both the meal and oil, to determine if any selenium had been metabolized by the plant and what effects the selenium would have on the biodiesel properties. It was found that while the $F_{6:9}$ derived increased oleic acid soybean line did have significantly higher oleic acid and lower linolenic acid concentrations than the commercial check used in this study, yield was not affected. Selenium applications had no effect on the fatty acid profile, or on yield; which, suggests the upper limit for selenium treatments was not reached. Se did significantly accumulate in the meal and in the oil. However, no changes were seen across Se treatments when the samples where tested for biodiesel properties: acid value, cloud point, iodine value, pour point, peroxide value, induction period, onset temperature, and kinematic viscosity. We were able to show by testing the $F_{6:9}$ derived increased oleic acid soybean line that increased oleic acid concentrations can improve biodiesel performance, while increase in polyunsaturated and saturated fatty acids can decrease biodiesel performance of soybean based biofuels. We were able to demonstrate higher polyunsaturated content lead to lower IP values, lower PV values were indicative of increased monounsaturated FAME content and elevated levels of saturated FAME content resulted in higher CP and PP values.

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Appendices

Appendix A

Tables

Table A.1 Descriptive statistics of mean oleic acid and linolenic acid content and seed yield of 15 $F_{6:8}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 3 commercial checks grown in a late maturity group III Roundup® Ready Preliminary Yield Trial in Knoxville, TN in 2007.

PYT3LRR 2007			
	Oleic (18:1)	**Linolenic (18:3)**	**Seed Yield**
Genotype	**% Total Lipid**		**kg ha^{-1}**
*HBK3824	35.4	3.8	1750.05
†TN07-022RR[a]	45.8	2.5	1402.65
†TN07-021RR	40.5	2.6	1350.13
†TN07-015RR[a]	48.0	3.8	1326.66
*AG4103	28.0	4.2	1248.16
†TN07-024RR	48.9	3.5	1219.76
TN07-029RR	48.2	2.4	1156.00
TN07-017RR	46.7	2.9	1150.42
TN07-028RR	43.2	2.4	1140.61
TN07-016RR	47.6	3.5	1117.48
TN07-019RR	45.5	2.7	1055.27
TN07-018RR	44.6	2.6	1013.77
TN07-023RR	53.4	2.6	1001.58
TN07-020RR	48.0	2.5	998.22
TN07-027RR	47.6	2.4	912.25
TN07-026RR	55.2	2.2	885.50
TN07-025RR	49.9	3.4	807.12
*AG3906	27.6	4.7	781.89
LSD (0.05)	9.50	NS	329.91

* commercial checks.
$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
NS, non-significance.
† selected to be sent to FL to be increased for the 2008 growing season
[a] were among the six final lines selected to be tested over multiple environments in 2008

Table A.2 Descriptive statistics of mean oleic acid and linolenic acid content and seed yield of 3 $F_{6:8}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 3 commercial checks grown in a late maturity group III Roundup® Ready Preliminary Yield Trial in Knoxville, TN in 2007.

PYT3L2RR 2007			
	Oleic (18:1)	**Linolenic (18:3)**	**Seed Yield**
Genotype	**% Total Lipid**		**kg ha^{-1}**
*HBK3824	28.8	5.0	1384.84
*AG4103	27.9	4.3	1301.22
†TN07-186RR	55.3	2.0	959.63
TN07-185RR	45.9	3.0	740.62
TN07-184RR	44.0	3.7	628.85
*AG3906	28.8	5.1	315.67
LSD (0.05)	9.02	1.15	649.18

* commercial checks

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level.

† selected to be sent to be increased for the 2008 growing season

Table A.3 Descriptive statistics of mean oleic acid and linolenic acid content and seed yield of 45 $F_{6:8}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in a maturity group III Roundup® Ready Preliminary Yield Trial in Knoxville, TN in 2007.

PYTOle3RR 2007			
	Oleic (18:1)	Linolenic (18:3)	Seed Yield
Genotype	% Total Lipid		kg ha^{-1}
*HBKR3824	32.7	3.9	1650.31
†TN07-96RR[a]	54.3	2.2	1488.84
†TN07-93RR[a]	59.1	2.0	1284.36
†TN07-102RR	50.7	3.3	1160.59
†TN07-95RR	52.3	2.3	1129.45
†TN07-130RR	52.7	2.2	1124.31
*AG3906	31.1	5.4	1100.45
†TN07-98RR	57.1	3.2	1090.44
TN07-92RR	51.0	4.6	1072.78
TN07-114RR	47.5	2.1	1061.27
†TN07-106RR	56.6	1.9	1050.87
TN07-99RR	49.2	2.9	1027.78
TN07-101RR	54.0	2.9	1025.59
TN07-105RR	50.4	2.3	1021.04
TN07-109RR	51.8	2.1	1003.49
TN07-120RR	52.3	2.3	998.63
TN07-110RR	52.7	2.1	993.41
TN07-113RR	52.1	2.1	982.33
TN07-104RR	45.4	3.7	976.81
TN07-107RR	47.6	2.2	976.34
TN07-103RR	52.7	3.3	969.26
TN07-111RR	49.2	2.4	963.81
TN07-137RR	48.9	2.1	944.15
TN07-129RR	45.3	2.4	937.09
TN07-100RR	57.6	3.0	931.50
TN07-97RR	55.7	3.7	929.11
TN07-134RR	48.9	3.8	927.46
TN07-135RR	55.4	1.9	911.64
TN07-124RR	48.4	2.1	907.41
TN07-122RR	55.0	2.1	891.16
TN07-131RR	54.5	2.0	874.04
TN07-126RR	54.3	2.8	866.69
TN07-117RR	51.8	2.2	863.69
TN07-123RR	47.5	2.2	855.86
TN07-136RR	53.6	2.0	853.76
TN07-115RR	49.4	2.1	850.93
TN07-128RR	53.6	2.7	849.43
TN07-121RR	52.1	2.0	848.60
TN07-133RR	48.8	4.2	829.54
TN07-118RR	52.6	2.4	828.19
TN07-125RR	50.7	2.1	823.92
TN07-108RR	51.4	4.1	806.20
TN07-132RR	55.8	1.9	803.47
TN07-116RR	50.9	1.9	768.91
TN07-112RR	48.9	2.3	745.10
TN07-127RR	53.7	2.9	734.29
LSD (0.05)	8.58	NS	511.95

* commercial checks

$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;

NS, non-significance

† selected to be sent to FL, to be increased for the 2008 growing season

[a] were among the six final lines selected to be tested over multiple environments in 2008

Table A.4 Descriptive statistics of mean oleic acid and linolenic acid content and seed yield of 5 $F_{6:8}$ increased oleic acid soybeans from 'Allen' x N98-4445A and 2 commercial checks grown in a maturity group IV early Roundup® Ready Preliminary Yield Trial in Knoxville, TN in 2007.

PYT4e2RR 2007			
	Oleic (18:1)	**Linolenic (18:3)**	**Seed Yield**
Genotype	**% Total Lipid**		**kg ha^{-1}**
*AG4103	25.9	6.7	2796.30
†TN07-236RR[a]	40.3	4.6	2730.13
†TN07-238RR[a]	42.1	5.7	2626.30
†TN07-239RR	43.4	2.6	2236.03
*AG4403	23.2	7.5	1961.10
TN07-237RR	40.5	4.6	1908.24
TN07-235RR	40.1	5.4	1895.28
LSD (0.05)	9.02	1.25	350.51

* commercial checks
$LSD_{0.05}$, Least Significance Difference at the 0.05 probability level;
NS, non-significance.
† selected to be sent to FL, to be increased for the 2008 growing season
[a] were among the six final lines selected to be tested over multiple environments in 2008

Table A.5 Descriptive statistics of mean oleic acid and linolenic acid content and seed yield of 15 $F_{6:8}$ increased oleic acid soybeans from 'Allen' x N98-4445A selected from Preliminary Yield Trials in Knoxville, TN in 2007.

		Oleic (18:1)	**Linolenic (18:3)**	**Seed Yield**
Test	**Genotype**	**% Total Lipid**		**kg ha^{-1}**
PYT4e2RR	TN07-236RR	40.3	4.6	2730.13
PYT4e2RR	TN07-238RR	42.1	5.7	2626.30
PYT4e2RR	TN07-239RR	43.4	2.6	2236.03
PYTOle3RR	TN07-96RR	54.3	2.2	1488.84
PYT3LRR	TN07-022RR	45.8	2.5	1402.65
PYT3LRR	TN07-021RR	40.5	2.6	1350.13
PYT3LRR	TN07-015RR	48.0	3.8	1326.66
PYTOle3RR	TN07-93RR	59.1	2.0	1284.36
PYT3LRR	TN07-024RR	48.9	3.5	1219.76
PYTOle3RR	TN07-102RR	50.7	3.3	1160.59
PYTOle3RR	TN07-95RR	52.3	2.3	1129.45
PYTOle3RR	TN07-130RR	52.7	2.2	1124.31
PYTOle3RR	TN07-98RR	57.1	3.2	1090.44
PYTOle3RR	TN07-106RR	56.6	1.9	1050.87
PYT3L2RR	TN07-186RR	55.3	2.0	959.63

Table A.6 Descriptive statistics of mean oleic acid and linolenic acid content and seed yield of 6 $F_{6:8}$ increased oleic acid soybeans from 'Allen' x N98-4445A selected from Preliminary Yield Trials in Knoxville, TN in 2007 to be grown in 2008 yield trials in Milan, TN, Springfield, TN, Knoxville, TN and Warsaw, VA.

		Oleic (18:1)	**Linolenic (18:3)**	**Seed Yield**
Test	**Genotype**	**% Total Lipid**		**kg ha^{-1}**
PYT4e2RR	TN07-236RR	40.3	4.6	2730.13
PYT4e2RR	TN07-238RR	42.1	5.7	2626.30
PYTOle3RR	TN07-96RR	54.3	2.2	1488.84
PYT3LRR	TN07-022RR	45.8	2.5	1402.65
PYT3LRR	TN07-015RR	48.0	3.8	1326.66
PYTOle3RR	TN07-93RR	59.1	2.0	1284.36

Appendix A

Figures

Figure A.1 Pedigree Selection Method (Poelhman and Sleper, 2006)

Initial Cross/F1 generation: In **2001 ETREC**, **cross 01-12D** formed the basis of this experiment. The **female parent** was **TN96-58RRF2** grown from seed harvested in 2001 winter greenhouse. The **male parent** was **increased oleic acid germplasm** gathered from random pollen among plant rows 2001-**40,002**, **40,007**, **40,024**, and **40,025** each of which were $F_{3:4}$ rows derived from F_3 single plant fatty acid selections. The increased oleic acid pollen donor was early generation material from the same population that ultimately gave rise to germplasm line N98-4445A, whose complex pedigree is described by Burton et al. (2006).

F2 generation: In **2002 ETREC**, seed harvested from F_1 hybrid plants grown at Costa Rica Seeds Co. in a lighted winter nursery in Costa Rica during winter 2001-2002 was used to grow random F_2 population rows 2002-**20,447-20,466.**

F3 generation: In **2003 ETREC**, seed source obtained from bulk threshing random pod-picks (one pod per plant) collected from F_2 plant rows 2002-**20,447-20,466** was used to grow the random F_3 population plant row designated as 2003-**35,026.**

F4 generation: In **2004 ETREC**, a single, undesignated F_3 plant harvested from within plant row 2003-**35,026** was used to grow the $\mathbf{F_{3:4}}$ row 2004-**45,071** from which the F_4 plant designated as 2004-**45,071** and several other agronomically desirable, early maturing plants were selected.

F5 generation: In **2005 ETREC**, a single F_4 plant designated as 2004-**45,071-4** (which had 52.4% 18:1 and 3.2% 18:3) was used to grow the $\mathbf{F_{4:5}}$ row 2005-**55,062** from which the F_5 plant designated as 2005-**55,062** and several other agronomically desirable, early maturing plants were selected.

F6 generation: In **2006 ETREC**, a single F_5 plant designated as 2005-**55,062-1** (which had 50.4% 18:1 and 3.1% 18:3) was used to grow the $\mathbf{F_{5:6}}$ row 2006-**67,014** from which the F_6 plant designated as 2006-**67,014** and several other agronomically desirable, early maturing plants were selected.

F7 generation: In **2006/2007 Homestead, FL** winter nursery, a single F_6 plant designated as 2006-**67,014-16** (which had 56.6% 18:1 and 2.6% 18:3) was used to grow the $\mathbf{F_{6:7}}$ plant row **FL07-5051.**

F8 generation: In **2007 ETREC**, new $\mathbf{F_{6:8}}$ soybean lines with increased oleic acid concentrations (and which also carried the gene for resistance to Roundup® herbicide were planted in four different breeder Preliminary Yield Trials (PYTs) at Knoxville, TN (**PYTOle3RR**, **PYT3LRR**, **PYT3L2RR**, and **PYT4e2RR**) in 2-row plots, 20' length, 30" row spacing, 3 reps. Each new line was given a permanent TN07- line designation, which will be used to track its pedigree for ultimate release of a new cultivar. For example, line **TN07-130RR** was planted from seed source grown in 2006/2007 Homestead, FL winter nursery designated as row **FL07-5051**.

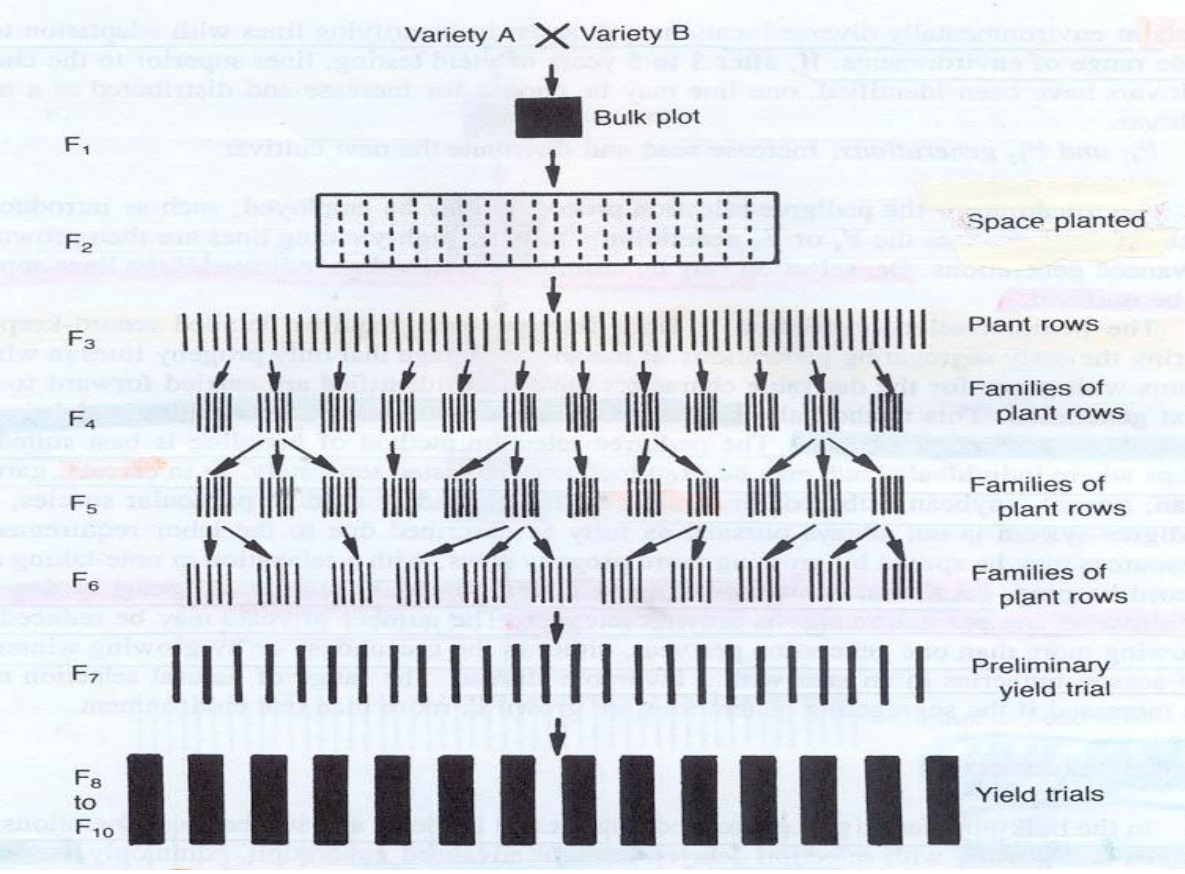

Figure A.1, Continued.

Vita

Benjamin D. Fallen is originally from Stovall, Virginia. He graduated from Halifax County High School, South Boston, VA, in 2002. He then went to Virginia Polytechnic Institute and Sate University, Blacksburg, VA and earned a Bachelor of Science in Crop and Soil Environmental Sciences in 2006. From April 2006 to July 2007 he worked as an agricultural specialist for the Soybean Breeding and Genetics program at Virginia Tech.

In July of 2007 he enrolled at the University of Tennessee and began soybean breeding research under Dr. Vincent Pantalone. Benjamin hopes to continue his education at the University of Tennessee pursuing a PhD. in Plant Science with a concentration in soybean breeding.