2013

Production Of Canola As A Biofuel Feedstock In The Piedmont Region Of North Carolina

Matthew Rhyan Miller

North Carolina Agricultural and Technical State University

Production of Canola as a Biofuel Feedstock in the Piedmont Region of North Carolina

Matthew Rhyan Miller

North Carolina A&T State University

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department: Natural Resources

Major: Plant, Soil and Environmental Science

Major Professor: Dr. M.R. Reddy

Greensboro, North Carolina

2013

School of Graduate Studies
North Carolina Agricultural and Technical State University
This is to certify that the Master's Thesis of

Matthew Rhyan Miller

has met the Thesis requirements of
North Carolina Agricultural and Technical State University

Greensboro, North Carolina
2013

Approved by:

Dr. M.R. Reddy Major Professor	Dr. Ron Gehl Committee Member
Dr. Abolghasem Shahbazi Committee Member	Dr. Louis Jackai Department Chair

Dr. Sanjiv Sarin
Dean, The Graduate School

Biographical Sketch

Matthew Rhyan Miller was born in Morganton, NC in 1985. He graduated from East Burke High School in 2003. In the summer of 2009 at the University Of North Carolina Of Pembroke he received a B.S in Biology. In 2010 he began his studies in Plant, Soil and Environmental Science at NC A&TSU.

Dedication

To my family and friends for their much needed love and support.

Acknowledgements

My thanks and appreciation to my advisor and committee members, Dr. M.R. Reddy, Dr. Ramesh Ravella, Dr. Abolghasem Shahbazi and Dr. Ron Gehl for persevering with me throughout the time it took me to complete this research. I am grateful to many persons, who shared their memories and experiences, and contributed to this research work, Bryce Holmes, Ashwin Devudigari, Marvin Clarke, Amal Elamin, Dietrich Blum, Emmanual Hayden, Chenna Reddy, Mamadou Diallo, and Audra Lampley. Finally I would like to thank my family Rebecca Miller Vance, Randy Vance, Katie Neely and Dennis J. Miller (RIP) for pushing me from the very beginning of my life to pursue graduate studies.

Table of Contents

List of Figures

List of Tables

Abstract

Canola in the past and present has been evaluated as a domestic fuel source and a means to stimulate rural economic development. In order to ease the transition of the economy in North Carolina new rotational crops can be looked upon as sources of additional revenue. Canola *Brassica napus* (L.) production was evaluated for cultivation in a Piedmont soil (Mecklenburg Sandy Clay Loam) at NC A&T State University research farm located in Greensboro, NC (Guilford County). The experiment was conducted using a split plot design with main plot factor cultivar (Virginia and DKW 46-15) and subplot factor fertilizer: (N-P_2O_5-K_2O) in ($kgha^{-1}$) 0-0-0, 70-28-84, 70-28-864 + Soysoap, 140-56-168 and 140-56-168 + Soysoap. Soysoap™ was applied as a foliar spray to evaluate its effectiveness in enhanced nutrient absorption. Canola was planted in October and harvested in May in all three years (2009-2012). Analysis from 3 consecutive years revealed that plots that received the 140-56-168 ($kgha^{-1}$) fertilizer treatment produced significantly higher seed yields than the control. Canola seed was mechanically extracted in 2011 and 2012. Neither canola cultivar nor fertilizer treatment affected mechanically extracted oil percentages in 2011 or 2012. Cultivar selection in 2010 had a significant effect ($p < 0.001$) on hexane extracted oil percentages in which the Virginia cultivar produced a significantly higher oil percentage than DKW 46-15. After evaluating cultivars oil yield potential, the Virginia cultivar would be more suitable towards biofuel production in NC versus DKW 46-15.

Chapter 1

Introduction

Mass biodiesel production requires a stable availability of fat and oil resources. Almost 65% of the world's available rapeseed oil is dedicated to biodiesel production. Theoretically, biofuel can replace up to 13% of petroleum-based fuels (Best, 2006). Both rural and urban areas in North Carolina have experienced tobacco-related job loss and income, for which there are few economic alternatives (Gale Jr, Foreman, & Capehart Jr, 2000). Competition from foreign textile producers caused an estimated loss of 82,000 jobs from 1977-1997 in North Carolina (Conway, Connolly, Field, & Longman, 2003).

Alternative crops provide supplemental income for farmers and novel opportunities for the expansion of NC's rural economy. Annual energy crops promote farm diversification and renewable energy production. Canola production provides a domestic and renewable source of energy as well as additional income, improving rural economic development. Canola yields double the amount of oil per acre as compared to soybeans; therefore, canola is a good alternative for local oil production (Atkinson et al., 2011). Biodiesel produced from canola oil also creates lower CO_2 emission levels, as compared to biodiesel produced from soybeans (Kim, Kim, Kim, & Lee, 2013). Canola is an ideal feedstock for biodiesel because of its combustion characteristics, cold temperature behavior, and oxidative stability (González-García, García-Rey, & Hospido, 2012).

The sustainable production of biofuel feedstock relies on efficient use of initial inputs (or fertilizer nutrients) during cultivation. There are 16 essential nutrients required for canola's growth and development. Of these nutrients, canola yield potential is most affected by the supply of Nitrogen (N), Phosphorus (P), and Sulfur (S) (Ahmad, et al., 2006; Franzaring, et al., 2012;

Siddiqui, et al., 2008). The supply and mobility of nutrients are dependent on several environmental factors, such as temperature, precipitation, and soil chemical characteristics (CEC, pH, organic matter). Plant and soil analysis can help estimate the efficiency of fertilizer usage.

Lacking knowledge about how to optimize nutrient efficiency can lead to the misuse of agricultural chemicals. A misappropriation of fertilizers and pesticides can pollute the environment, increase carbon emissions and contaminate the food supply (Mousavi-Avval, Rafiee, Jafari, & Mohammadi, 2011).

1.1 Objectives

This study was conducted to elucidate the following objectives:

1. Evaluate yield potential of two winter canola cultivars grown in NC Piedmont soil and climatic conditions.
2. Determine if optimization of nutrient management could be achieved through the use of varying (N-P-K) rates with soysoap application.

CHAPTER 2

Literature Review

2.1 Biofuel & Energy Expenditures

The world has depended on fossil fuels (coal, oil and natural gas) as a primary energy source since the Industrial Revolution. In 1973, about 71% of global energy sources were from fossil fuels; and in 2009 fossil fuels contributed to 60% of total energy use (Zellou & Cuddington, 2012). In 2012 both the United States and Europe spent around $1 billion per day on oil imports (Murray & King, 2012). Heavy duty diesel vehicles consume (30%) (11 Mbpd worldwide of the liquid transportation fuel with an expected 2.5% annual increase until 2020 (Radinko & Jeremija, 2012). United States oil production is steadily decreasing. In 1980, crude oil reserve-to-production ratio was estimated at 9%, but are now reduced to 6%, while worldwide production has declined at rates of 4.5% to 6.7% per year (Murray & King, 2012).

Crude oil is limited and difficult to obtain, which creates an unpredictable market with fluctuating prices. In 2007 the spot price of West Texas intermediate crude oil was $50 per barrel, in July 2008 it rose to $145 per barrel, and within only 5 months it dropped to $30 per barrel (Casassus, Liu, & Tang, 2012). The price of petrol from 2010-2011 in the United States experienced an increase of 77 cents per gallon (Murray & King, 2012).

Energy security, or the availability of resources for energy consumption in a given period of time, is gaining worldwide attention. It involves elements such as: availability of energy to an economy, spatial distance of resources between production and point of consumption, cost of research and exploration, geopolitical implications, and environmental sustainability (Kruyt, Van Vuuren, De Vries, & Groenenberg, 2009). Globally, governments are seeking alternative sources

of energy, such as bioenergy (hydroelectric, solar photovoltaic, geothermal, wind etc.) and bio-fuels (bioethanol and biodiesel), to help alleviate fossil fuel dependency.

Theoretically global energy consumption is estimated around 15×10^{12} Watts (W). The Earth receives 120×10^{15} W of light energy from the sun; only 6% is used for biomass production (Radinko & Jeremija, 2012). There is an abundance of untapped energy potential supplied through solar radiation; therefore, it is resourceful to use energy sources derived from photosynthesis and other such processes.

In 2008 only 2% (1,200 billion liters of gasoline equivalents) of the global annual energy consumption was supplied by biodiesel and bioethanol (De Fraiture, Giordano, & Liao, 2008). U.S. Department of Agriculture and Department of Energy has estimated that close to 30% of fossil fuel displacement (80 billon gal) is achievable by utilizing biomass crops to supplement our energy needs (Gray, Zhao, & Emptage, 2006).

Biodiesel is a non-petroleum-based diesel fuel derived from a renewable feedstock, such as vegetable oils or animal fats. It can function (alone, or blended with conventional petro-diesel) in unmodified diesel-engine vehicles. In 1938, Chavanne obtained the first patent for biodiesel production, which utilized palm oil and ethanol (Guzatto, Defferrari, Reiznautt, Cadore, & Samios, 2012).

Biodiesel is produced through a transesterification reaction in which oils or fats react with an alcohol (chiefly methanol) in the presence of a catalyst (acid, base or lipase). This reaction produces monoalkyl esters of long chain fatty acids and a glycerol byproduct (Meher, Vidya Sagar, & Naik, 2006). Mass transfer, kinetic, and equilibrium reactions are the three main mechanisms that facilitate the transesterification process (Nigam & Singh, 2011). Other methods for the production of biodiesel include: direct use (straight vegetable oil), blending,

microemulsions, and pyrolysis (thermal cracking) (Ma & Hanna, 1999). During the refinement of oil for the production of biodiesel, free fatty acids are separated to produce an acid value of less than 0.2 mg KOH/g (Borugadda & Goud, 2012).

The two primary expenses in biodiesel production are the cost of processing and the raw materials used for production. Acquiring raw feedstock makes up an estimated 60–75% of the total cost of biodiesel fuel (Barua, 2011). During the production of biodiesel from oilseed crops there are several byproducts, including straw, protein meal, and glycerine, that have added value for on- and off- farm practices (Smith, Janzen, & Newlands, 2007). Byproducts, such as glycerol, could help offset the costs of biodiesel production and conversion. It has been estimated that 9,071 kilograms of biodiesel produces an estimated 907 kilograms of glycerol (Lin, Gaustad, & Trabold, 2013). Glycerol is used in cosmetics and pharmaceuticals, as well as in several biotechnological applications.

A byproduct of biodiesel production with canola is the meal produced from pressing, which contains about 37-38% crude protein after oil extraction. By comparison, soybean meal contains around 44% crude protein. It takes 110,000-140,000 seeds to make 0.45 kg of canola seed meal. Canola seed meal is used as livestock fodder supplement due to the balanced amino-acid composition. However, canola is not a complete source of protein.

Energy efficiency is an important consideration in all aspects of biofuel production. Conversion of biomass to liquid fuel is 2-3 times less efficient than conversion of crude oil to liquid fuel (Howarth et al., 2008). However, when biofuels are utilized for heat and electricity, their efficiency almost equals that of crude oil. Compared with petro-diesels, biodiesel has several advantages, including: the reduction of carcinogenic emissions by 94%, up to 4 times

greater degradability, extended engine life, higher lubricity, and improved fuel economy (González-García, García-Rey, & Hospido, 2012).

Biodiesel has a lower toxicity, and reduced sulfur dioxide (SO_2) emissions compared with conventional diesel fuel (Romano & Scandurra, 2011). However, biodiesel has greater nitrous oxide emissions (NO_x) and a lower calorific capacity; this leads to higher fuel consumption, lower freezing point, and decreased shelf life. In external combustors (boilers or heating devices) that use biodiesel blends, there is an estimated 1% reduction in NO_x emissions for every 1% of biodiesel added (Lin, 2013).

Worldwide, governments are showing interest in biofuels for several reasons including: energy security, trade balance, GHG emission reduction, and rural income generation (De Fraiture, et al., 2008). Brazil was the first to adopt policies regarding the production of biofuels, followed by the US and the EU (Firbank, 2012). In 1975, Brazil's Proálcool promoted the production of ethanol from sugarcane (Cavalcanti & Jalles, 2013). In 2007, the United States Energy Independence and Security Act mandated the annual production of 36 billion gallons by 2022 (Lin, et al., 2013). The American Taxpayer Relief Act of 2012 (ATRA) made permanent energy tax extenders, including: credits for non-business energy property and renewable electricity production, and tax credits for alternative fuels produced in the US (Nunns, Rohaly, & Center, 2013). Table 1 shows international interest in renewable fuels for use in the transportation sector (Murphy, Woods, Black, & McManus, 2011).

The global production of biofuels such as biodiesel, accounted for 2 billion liters in 2005, whereas bioethanol accounted for 32 billion liters in 2006 (De Fraiture, et al., 2008). Coyle (2007) provided a summary of the different feedstock’s utilized for global biofuel production. Corn is the primary feedstock used in the US; wheat and sugar beets are used in the European

Union. Sugarcane, soybeans and palm oil serve Brazil's bioethanol production needs. Animal fats, recycled oil and soybeans are used for biodiesel in the US, while soybean and sunflower oil are used in the European Union. Castor seed oil is the dominant feedstock for biodiesel used in Brazil.

Table 1.

Examples of Recent Policies Influencing the Use and Production of Biofuels

Year	Policy	Summary
2006	Biofuels Research Advisory Council EU Vision	25% of transport fuel in EU by 2030
2009	EC – Renewable Energy Directive	10% of transport energy as renewable by 2020
2009	EC – Fuel Quality Directive	Increases biofuel to 15% of transport energy by 2020
2007	Energy Independence & Security Act 2007	7% gasoline & diesel consumption in USA in 2022
2008	Gallagher Review	5 to 8% of transport energy recommended
2009	UK Renewable Energy Strategy	10% transport energy by 2020a
2008	IEA, 2008 Energy Technology Perspective	26% of total transport fuel demand in 2050
2010	IEA, 2010 Energy Technology Perspective	20% of total liquid fuel demand in 2050

There are some issues that need to be addressed in order to promote sustainable biofuel production systems. These include: food vs. fuel paradigm pollution from agricultural activities, and fuel consumption during the cultivation and conversion of biofuel crops (Langeveld, Dixon, & Jaworski, 2010). In the fuel vs. food paradigm, arable land that is used for edible crop production transitions into use for fuel production (Hao, Colson, Karali, & Wetzstein, 2013). Land requirement projections estimate an additional 100 Mha land is needed for biofuel feedstocks in order to produce substantial reductions in greenhouse gases (GHG) by 2050 (Murphy, et al., 2011). The change in land use dynamic is also monetarily driven, due to institutional level tax incentives and subsidies for using oilseed crops in biofuel production (Rathmann, Szklo, & Schaeffer, 2010).

In North America from 2005-2007, there was an increase of 8.11% of land used for biofuel production, including an increase of 2.43% in oil seed crop production, with an increase of 212.79% in oil seed crops focused towards biodiesel production (Rathmann, et al., 2010). Comparatively, other countries are dedicating more land toward the production of oilseed crops. Land percentage changes for oilseed crops used as biodiesel feedstocks are as follows: Africa (+112.32%), Asia (+423.32%), Europe (+75.89%) and South America (+978.42%) (Rathmann, et al., 2010).

Dedicating more land towards biofuel crop production places a higher demand on resources, such as water and fertilizer that are also used by edible crops. The increased demand for water in biofuel feedstock production could affect water supply and decrease water quality. There is also the risk of water pollution from agricultural chemical runoff and salinization from fertilizer application.

There is also a higher demand in energy required for the production of biofuels. Biodiesel life cycle energy use includes agricultural production, oil extraction, and transesterification reactions (Janulis, 2004). During the production of biofuel there is a high demand on inputs, including energy requirements and fuel consumption (Mousavi-Avval, Rafiee, Jafari, & Mohammadi, 2011).

A sustainable energy sector would have: low resource (land and water) requirements, high adaptability, high energy output per hour of labor, and negligible environmental damage (Giampietro & Ulgiati, 2005). Some ways to reduce environmental damage during the production of biofuel crops involve: using livestock manure as organic fertilizer, using non-arable degraded grassland, and using non-edible forest residues to aid in the production of biofuels (Iriarte, Rieradevall, & Gabarrell, 2012).

2.2 Canola Benefits and Fertilizer Requirements

Oil seed crops are of particular interest due to their valued use in a variety of products and by-products. Global oil seed *Brassica* production has increased from 2.7 million hm^2 to over 30 million hm^2 since the 20^{th} century (Zhu, Liu, Shao, & He, 2011). Advancements in breeding have increased *Brassica* oilseed crop production. Fifty years of plant breeding techniques have altered fatty acid composition of *Brassica napus* (L.), *Brassica campestris* (L.) and *Brassica rapa* (L.) to create the canola "Canadian Oil, Low Acid" cultivar. Alterations in fatty acid composition changes the nutritional quality of canola, yielding one of the healthiest edible oils on the market (McInnis, Larson, & Miller, 1993). Canola produces seed oil with less than 2% erucic acid and cake meals with less than 30 μmol of aliphatic glucosinolates per gram (Raymer, 2002).

Canola belongs to the *Brassicacae* (mustard) family which includes *Brassica rapa* (L.), *Brassica napus* (L.) and *Brassica juncea* (L.) species. Around 1,000 years ago *Brassica oleracea* (L.) (Chromosomes n=9) and *Brassica rapa* (L.) (n=10) cross-pollinated and produced *Brassica napus* (L.) (n=19), one of the largest oil producing species of the *Brassica* family (Thomas, 2003). Thirteen percent of the world's edible oil supply comes from *Brassica* related species, primarily *Brassica napus* (L.) and *Brassica rapa* (L.), which typically contain (40%) or more oil with 35-40% protein in the cake press.

The disadvantages of monoculture include soil degradation, intense use of agricultural chemicals, nutrient loss through leaching, and loss of biodiversity (Zegada-Lizarazu & Monti, 2011). Crop rotation helps reduce the pressure from pests and diseases, improves water use efficiency (WUE) and provides market flexibility (Paulitz, Schroeder, & Schillinger, 2010). Including canola into crop rotations has several benefits over monoculture systems.

Another benefit of canola crop rotation is the use of canola crop to combat crop-specific pathogens (Kirkegaard, Christen, Krupinsky, & Layzell, 2008). Canola can serve as a break crop for such disease cycles as *Rhizoctonia solani* (L.) (Paulitz, et al., 2010). Soil-borne and leaf diseases are reduced when canola is included in wheat cropping systems. Reports indicate up to an 85% increase in wheat yield when canola was included in the rotation, compared to continuous wheat rotation systems (Angus et al., 2011).

Canola production can affect several aspects of soil structure by releasing chemicals through root exudation, thereby producing stable biopores (Kirkegaard, et al., 2008). *Brassica* root tissues can promote the immobilization and release of mineral nitrogen into the soil (Ryan, Kirkegaard, & Angus, 2006).

Brassicas are a well-known biofumagant, able to eliminate harmful pathogenic microflora. Biofumagants are plants that, when damaged, produce volatile chemicals that hinder pest growth and development (Karavina & Mandumbu, 2012). *Brassica* tissue contains high levels of glucosinolates, which, when hydrolyzed, release isothiocyanates, nitriles, oxazolidinethione ionic thiocyanate (SCN^-) and organic cyanides. These products have biocideal properties to several plant species (Morra & Kirkegaard, 2002). *Brassica* root tissues also suppress mycorrhizal fungi (Mozafar, Anken, Ruh, & Frossard, 2000).

Annual *Brassica napus* (L.) production in the United States is estimated at over 499,787 ha, with most in North Dakota for spring canola (George, Tungate, Beeck, & Stamm, 2012). As with most crops, canola yields vary year to year. During 2010 winter canola trials conducted in Williamsdale, NC using a split fertilizer application of (46-0-0 N-P-K fall: 80-0-0-26 N-P-K-S spring) for the following cultivars in the same location (Hybristar, Kadore and Dimension)

produced the respective crop seed yields: (1701, 1636, 2295 lbs/ac). In 2011 the same cultivars experienced a percentage difference of (+113.22), (+90.71) and (-6.06%) (Stamm, 2011).

FAO (2008) stated that global demands for fertilizer nitrogen increased 1.4% (7.3 million tons), potassium increased 2% (4.2 million tons) and phosphorus increased 2.4% (3.6 million tons) in 2008. These increases will directly impact environmental health. To lessen environmental consequences, there is a need to optimize nutrient use efficiency and prevent fertilizer leeching and run-off. Nutrient efficiency of plants can be characterized by production response per unit of nutrient applied and absorbed, under similar environmental conditions (Fageria, Baligar, & Li, 2008).

Breeding highly efficient cultivars and using management practices will maximize crop N use efficiency (Barraclough et al., 2010). Management practices focusing on fertilizer type and rate, seeding depth, tillage system, and irrigation practices all influence canola seed yield and quality (Hamzei, 2011; Mohammadi, Eskandari, Rokhzadi, & Heidari, 2012; Rathke, Christen, & Diepenbrock, 2005).

An important consideration is fertilizer consumption that will be required to produce a crop such as *B. napus* (L.). Agronomic practices can reduce cultivation cost, and optimize production through improvement of seed yield, oil content, and nitrogen use efficiency (NUE). Canola yield potential is directly proportional to nutrient availability. Nitrogen (N), Phosphorus (P), and Sulfur (S) are the most influential nutrients for canola seed and oil yields (Ahmad, Jan, & Arif, 2006; Franzaring, Gensheimer, Weller, Schmid, & Fangmeier, 2012; Siddiqui, Mohammad, Khan, & Khan, 2008). Various physiological processes affect the movement of plant nutrients via absorption and translocation. Nitrogen is considered one of the most crucial nutrients for oilseed crop production. Nitrogen serves as a component for enzymes and provides

the structural framework to amino acids, proteins, nucleotides, chlorophyll, chromosomes, genes, and ribosomes (Anjum et al., 2012).

During the vegetative stage (between germination and flowering), inorganic N is collected in leaves for use in the nitrate assimilation pathway, and amino acids are synthesized and stored. Amino acids are later used for proteins and enzymes that are responsible for facilitating biochemical pathways, influencing plant growth and development. As canola pod development occurs, N is translocated from vegetative (leaf, stem and root) into the generative organs (pod and seed) (Anjum, et al., 2012). During the late flowering stage through the ripening phase, the majority of N is translocated and partitioned from canola leaves into seeds. Previous researchers have estimated N uptake when planting and harvesting canola. This data can elucidate nutrient cycling processes and help estimate field residuals left after canola harvest. For every 90 kg of seed produced, there should be at least 6 kg N ha^{-1} supplied as fertilizer (Rathke, Christen, & Diepenbrock, 2005). Scientific studies concluded, that from 1,960 $kgha^{-1}$ of canola seed yield produced; 76 kg N ha^{-1} grain and 49 kg N ha^{-1} straw while K_2O occurred in the above ground portion (grain, straw etc.) around 93 kg K_2O ha^{-1} (Holzapfel, 2007).

Nitrogen applications of (75, 150, and 225 $kgha^{-1}$) produced the respective values of seed N (50.7%), (44.5%) and (41%) (Franzaring, et al., 2012). The maximum canola seed yields for winter canola occur with fertilizer applications in the range of 180-220 kg N ha^{-1} (Jackson, 2000). Increasing N levels reduces oil percentage in canola seeds (Ahmad & Abdin, 2000). When treatments included a combination of both S and N fertilizers, the oleic and linoleic fatty acids increased the nutritional value of canola oil. Increasing fertilizer rates from 20 kg N ha^{-1} to 70 kg N ha^{-1} increased seeds per pod and pods per plant, while reducing oil percentages in *Brassica* crop (Siddiqui, et al., 2008).

Nitrogen fertilizer management practices can enhance economical and environmental outcomes. Improved N fertilizer efficiency is achieved with split applications of fertilizer throughout the growing season (Garnett, Conn, & Kaiser, 2009). It is an unrealistic expectation to reach (100%) efficiency in N use, which striving to do so is problematic for fertilizer application (Miller, 2010). The rate and extent to which N losses occur depends on many factors such as: amount and formulation of N, pH of the soil , soil moisture, soil temperature, and soil texture (Balasubramanian et al., 2004; Ghobadi et al., 2006; Pregitzer & King, 2005; Singh, Sanabria, Austin, & Agyin-Birikorang, 2012).

Methods for improving N use efficiency include utilizing precision agriculture focusing on the amount and placement of N, using slow release fertilizers, and proper timing of fertilizer application (Samborski, Tremblay, & Fallon, 2009). For example, solid fertilizer should be applied earlier in the season for optimal nitrogen uptake and utilization, which influences crop yield (Gianquinto et al., 2004). When nitrogen fertilizers are misused they can contribute to point- and non- point sources of pollution. Nonpoint source pollution from agricultural practices contributes significantly to water pollution in the United States (Rabotyagov, Valcu, & Kling, 2012). Within the last century agricultural and cultural activity has increased N emissions to such levels that many ecosystems experienced a shift, reflected by alterations of N cycling and biodiversity in diatom, lichen, mycorrhizal fungi, and terrestrial plant communities (Pardo et al., 2011).

Other nutrients important for a productive canola crop include phosphorus (P), potassium (K) and calcium (Ca). Phosphorus, found in nucleic acids and phospholipids of plant organs, is crucial for energy metabolism (Grant & Bailey, 1993). Phosphorus affects plant processes such

as root development, flowering, and ripening (Thomas, 2003). Grant & Bailey (1993) estimated that canola seed yields of 2,000 $kgha^{-1}$ contained 22 kg P ha^{-1} in the seed and straw.

Potassium is not quite as important for canola yield but still needed in large amounts to ensure proper physiological and morphological growth. Canola physiological functions impacted by potassium supply are: respiration for carbohydrate metabolism, formation of chlorophyll and proteins, water use efficiency, and assimilatory production and translocation mechanisms (Orlovius, 2003). After harvest, canola plots that were applied with 60 kg K ha^{-1} contained 5.8-7.7 g of K per kg of seed, and control plots contained between 5.5-7.3 g of K per kg of seed (Brennan & Bolland, 2005). This demonstrates how the addition of K fertilizer can alter seed composition. Canola plants that are deficient in K exhibit stunted growth, shorter internodes, leaf scorch, wilting, and necrosis (Orlovius, 2003).

Calcium is another important nutrient that helps maintain plant fertility. There is a relatively high demand for calcium in dicots such as canola. They depend on calcium for physiological processes such as cell elongation , cell division and detoxification of metal cations including Al^{3+}, Fe^{2+} and Mn^{2+} (Grant & Bailey, 1993).

The second most important nutrient for canola is sulfur (S). Sulphate is absorbed by plant roots and enters the leaf tissue through the xylem (Orlovius, 2003). Once absorbed, sulfur is assimilated into amino acids such as cysteine, which produces methionine or cysteine proteins (peptide and glutathione) (Rausch & Wachter, 2005). Sulfur is involved in chlorophyll synthesis, as well as oil synthesis, for members of the *Brassicacae* family. Proper S availability is vital for fatty acids synthesis and for metabolites, such as: coenzyme A, vitamin B, biotin, lipoic acid and sulpholipids (Malik, Aziz, & Wahid, 2004; Meher, et al., 2006). Sulfur is a critical component for nitrate-reductase, an enzyme responsible for the formation of amino acids and proteins from

nitrate (Ahmad & Abdin, 2000). Grant & Bailey (1993) recommended that, during flowering, canola leaf tissue concentration of S are normal at (.2-.25%), with suboptimal (≤.2%) and excessive (≥1.0%).

2.3 Water logging

Canola's physiological and morphological characteristics are disrupted when soil is exposed to prolonged periods of saturation. Water logging of soil causes: increased ethylene production, decreased superoxide dismutase enzymes (SOD) and Catalase (CAT), and accumulation of malondialdehyde (MDA). These factors decrease pod and leaf photosynthetic rates, which decreases yields of pods per plant and seeds per pod (Zhou & Lin, 1995). Reactive oxygen species (ROS) damage cells and organelles, contributing to enzyme inactivation, while degrading pigments, proteins, lipids, and nucleic acids (Habibzadeh, Sorooshzadeh, Pirdashti, & Modarres Sanavy, 2012; Karuppanapandian, Moon, Kim, Manoharan, & Kim, 2011). Water logged soil degrades plant's chlorophyll production by reducing stomata conductance, which limits water uptake and contributes to early leaf senescence (Parent, Capelli, Berger, Crèvecoeur, & Dat, 2008).

Plants use oxygen as a primary electron acceptor in aerobic respiration. Under waterlogged conditions, oxygen is limited and plants switch to anaerobic respiration, as microbes use Nitrate (NO_3^-), Iron (Fe), Manganese (Mn) and Sulfate (SO_4^-) for energy production (Hartman, 2011). Anaerobic respiration decreases stomata conductance, net CO_2 assimilation rate and root hydrolytic conductivity (Ashraf, 2012). These reductions deplete carbohydrate reserves and hinder translocations, which affects plant physiological and morphological functions (Parent, et al., 2008). Nutrient deficiency is common in water logging situations.

During spring water logging, there is a decrease in the uptake of N, P, K and Ca, which contributes to a reduced seed yield in *B. napus* (L.) (Boem, Lavado, & Porcelli, 1996).

2.4 Chlorophyll Sensor Based Technology

Leaf chlorophyll is in the visible electromagnetic radiation (EMR) spectrum 400-700 nm wavelength. The photosynthetic reactions that take place in plants only convert 8-10% of sunlight energy into sugars to drive metabolic functions (Kirschbaum, 2011). A plant's photosynthetic potential is directly proportional to the quantity of chlorophyll present in the leaf tissue. Plant sensor-based chlorophyll readings help determine the N status of a crop before symptoms of nutritional deficiencies manifest. Sensor-based technology could improve NUE by allowing for more timely and accurate fertilizer recommendations (Samborski, et al., 2009).

Plant species, leaf variegation, leaf thickness and leaf age affect the accuracy of chlorophyll meters. There is not always a strong correlation between photosynthetic activity and leaf N. This is due to the different allocation of leaf soluble proteins, such as those involved in the Calvin cycle, and the pigments, which are the protein/reaction centers for thylakoid complexes (Jifon, Syvertsen, & Whaley, 2005). Applying N fertilizer increases chlorophyll meter readings (Gianquinto, et al., 2004). With increasing N supply, the contents of leaf chlorophyll A, chlorophyll B, as well as the total chlorophyll increased, but there was only a slight reduction to chlorophyll A/B ratios (Anjum, et al., 2012).

Advanced calibration of sensor-based meters is limited by several factors, including: environmental conditions, soil properties, plant genotype, leaf structure variations, and plant developmental stage. An important consideration when using optical sensors are plant morphological and physiological characteristics which influence absorption and reflectance of light beams (Araus, Casadesus et al. 2001). Estimating N need with sensor-based meters, it is

crucial that readings are taken during the developmental stage that accurately reflects the crop's yield potential. Holzapfel (2007) suggests that this critical period for canola falls between the five-leaf and early flowering stage.

2.5 Oil Production, Extraction and Storage

Globally, 79% of the available vegetable oil comes from palm, soybean, rapeseed and sunflower seeds (Dyer, Stymne, Green, & Carlsson, 2008). The most critical factor in optimizing *Brassica napus* (L.) yield is the balance between crude protein synthesis and oil accumulation (Rathke, et al., 2005). When the availability of soil N increases, protein synthesis increases, thereby reducing carbon availability for fatty acid synthesis (Ghasemnezhad & Honermeier, 2008). Increasing the availability of N increases seed yield, energy production, CO_2 storage, and crude protein; however, the oil content of canola decreases (Rathke, et al., 2005).

Prior to flowering, up to 76% of the plant's N is located in the leaves, while the remaining N is partitioned in the stems. During pod formation, plants reallocate N from leaves to pod walls, where N will mobilize into the seed (Holzapfel, 2007). Canola seed experiences rapid lipid accumulation 7-35 days after flowering (Anjum, et al., 2012).

After flowering canola begins three stages of pod development. The first stage occurs 0-20 days after anthesis (DAA), when siliques reach their maximum length; the second occurs 20-50 DAA, when the replum becomes lignified; the third occurs 50–70 DAA, when lignified cells undergo senescence (Roberts, Elliott, & Gonzalez-Carranza, 2002). The most sensitive stage of canola development is during seed filling, which high temperatures and drought stress can affect the chlorophyll content of the seeds, leading to poor seed quality (Onyilagha, Elliott, Buckner, Okiror, & Raney, 2011). During seed processing, seeds are crushed and the chlorophyll is

extracted. Untreated seed "green seed", (excessive amounts of chlorophyll) is less marketable due to the resulting oil's dark coloration.

After harvesting there are several energy-intensive steps required for processing and storing canola seed, including: seed cleaning, seed coat removal, chaff separation, cracking (heating) extraction, oil purification, and purified oil storage (Anitescu & Bruno, 2011). Different oil extraction techniques yield different quantities and qualities of oil. There is a trade-off in quantity and quality of oil produced during solvent extraction versus mechanical extraction (Santori, Di Nicola, Moglie, & Polonara, 2012). Solvent extraction leaves around 2% of oil in the meal. Mechanical extraction method leaves more oil in the meal, around 7-8%, but produces a higher quality meal, with a higher available energy content and increased palatability (Landero, Beltranena, Cervantes, Araiza, & Zijlstra, 2012; Seneviratne, Beltranena, Goonewardene, & Zijlstra, 2011).

Whether the goal is edible food oil or biofuel, several steps in post-oil extraction techniques improve storage stability and the oil's taste, smell and appearance. Oil is refined by: degumming (removes substances that would separate during storage), neutralization (reduces FFA and oxidation products), and deodorization (reduces FFA, removes odors and volatile compounds) (McKevith, 2005). Canola possesses a few undesirable compounds, such as lignin, cellulose, and glucosinolate, which are difficult for animals to digest. Additional refinement is needed, which is another step that produces a high amount of energy during the processing and refinement of canola oil.

2.6 Canola Yield Potential Factors

Canola seed is released when the pod's two silique valves split, separating the replum from the pericarp edge. This process is known as dehiscence, or "pod shatter" (Wang, Ripley, &

Rakow, 2007). Pod shatter, influenced by cultivar selection and environmental conditions (such as dry conditions during growth and heavy snowfall after maturity), causes significant seed loss in canola (Lewis, Leslie, & Liljegren, 2006; Wang, et al., 2007).

Pod shatter increases with insect infestation from *Dasineura brassicae* (L.), bacterial infection from *Alternaria*, and adverse weather conditions (Child & Evans, 1989; Roberts, et al., 2002; Thomas, 2003). Manual harvest operations are linked to an increased persistence of feral canola and increased seed loss (Zhu et al., 2012). Manual harvesting results in an estimated yield loss of 50% (Zhang et al., 2012). Conventional agricultural practices, using combines, results in a yield loss averaging only 5.9% (Gulden, Shirtliffe, & Thomas, 2009). Using combines instead of manual harvesting also reduces costs up to 30% (Zhang et al., 2012). In order to obtain a higher seed yield, harvest operations should occur during early morning or evening times, when moisture levels of seed are between 10-15%. The same researcher recommended storing seeds at a moisture level of 8.3% (Hall 2011).

2.7 Seed Storage and Moisture Content

Canola seed is highly susceptible to mold, micoflora and mite infestation when seed moisture is above 10% (Sathya, Jayas, & White, 2009). Seeds should be dried at no more than 43^{o}C, and stored at 10^{o}C with 10% or less moisture and 65% relative humidity (Thomas, 2003). Canola stored at a temperature of -5^{o}C can experience fungal infestations when seed moisture content is as low as 7% (Humboldt, 2012). Surprisingly, pockets of high temperature and moisture occur and spoil seed quality even when seeds are stored in cool and dry conditions, at a temperature of 18^{o}C (Applewhite, 1993).

Field fungi such as *Cladosporium*, *Alternaria*, *Rhizoctonia solani*, *Fusarium* spp., *and Pythium* spp. contribute to *Brassica* seed spoilage (Garg, Li, Sivasithamparam, Kuo, & Barbetti,

2010; Meena, Awasthi, Chattopadhyay, Kolte, & Kumar, 2010; Saharan & Mehta, 2006). During storage, fungi such as *Penicillium* spp. and *Aspergillus* spp. also cause disease (Tańska, Konopka, Korzeniewska, & Rotkiewicz, 2011; Wallace & Sinha, 1962). Fungal infestations can lead to slow seed deterioration and mycotoxin production (Arora, 2003).

High seed moisture content and the presence of fungal and bacterial infections contribute to fermentation, oil oxidation, and grain germination (Humboldt, 2012). These factors affect the overall value, quality and viability of the canola seed (Tańska, Konopka, Korzeniewska, & Rotkiewicz, 2011). Improperly stored seeds show increased lipolytic enzymatic activity, which increases levels of mono- and diacylglycerols (Scrimgeour, 2005). Mono- and diacylglycerols are considered impurities in biodiesel (Pauls, 2011). Typically, lipolytic activity is inhibited in non-germinated seeds (Barros, Fleuri, & Macedo, 2010).

2.8 Fatty Acids

After oilseeds are harvested and oil is extracted, the most important factor affecting oil quality is fatty acid composition. Fatty acid composition determines the end use (edible or industrial) for the oil (Thomas, 2003). Genetic factors have the strongest influence on fatty acids for members of the *Brassica* family. The most common fatty acids in plants are palmitic, stearic, oleic, linoleic and linolenic acids; these account for 95-98% of the plant's fatty acids (Sheikh, Shahnawaz, & Baloch, 2010). The saturated fatty acids in canola are palmitic and stearic acids; the unsaturated fatty acids include palmitoleic, oleic, linoleic, and linolenic acids. The quality of oil is determined by oleic, linoleic and erucic acid contents and is highly affected by the cultivar selection (Aslam et al., 2009; Rad & Zandi, 2012; Schierholt & Becker, 2011).

In both corn and canola oil, oleic acid concentrations are typically greater than 60% (Shin et al., 2012). Linoleic and linolenic acids are desirable fatty acids, while palmitic and erucic are

undesirable fatty acids in terms of edibility (Joshi, Mali, & Saxena, 1998). Commodity canola oil has a fatty acid composition of 5-8% erucic acid, 60- 65% monounsaturated fats and 30- 35% polyunsaturated fats (Raymer, 2002). Fatty acid compositions reported for winter and spring rapeseed cultivars contain ranges of: 63.62-67.38% oleic, 18.87-19.06% linoleic, and 7.55-9.76% linolenic, with concentrations less than 1% for erucic, and palmitic acid (Rad & Zandi, 2012). The human body can't independently produce linoleic acid, which is one of the most nutritionally important unsaturated fatty acids, attributed to reducing cholesterol and preventing arteriosclerosis (Rad & Zandi, 2012).

Fatty acids behave inconsistently, due to a wide array of molecular species possessing various melting points (Scrimgeour, 2005). Oils with similar fatty acid composition may have different polymorphic forms and differences in solid fat content (crystal amount) (Karabulut & Turan, 2006; Ribeiro et al., 2009; Scrimgeour, 2005). The differences between the various fatty acids physical properties are attributed to structural differences, such as actyl chain length and the number of double bonds (Durrett, Benning, & Ohlrogge, 2008). Triacylglycerols (TAG) containing a long chain with saturated acids, possess higher melting points due to increasing chain length. However, melting points decrease with increasing unsaturation, such as with polyunsaturated fatty acids (Scrimgeour, 2005).

Environmental conditions, including heat and salinity, as well as management practices, including planting date and fertilizer application, affect fatty acid metabolism. When sowing times were delayed researchers noted that palmitic, stearic, and linoleic acids increased (Turhan, Gul, Egesel, & Kahriman, 2011). Nitrogen significantly influences biochemical reactions that affect plant physiological development of fatty acids (DeBonte, Iassonova, Liu, & Loh, 2012).

Fatty acid composition undergoes different effects, depending on fertilizer combination. When S and N are applied together, oleic acid and linoleic acid increased, while eicosenoic acid and erucic acid decreased. However, when S and N are applied separately, this result does not occur (Ahmad & Abdin, 2000).

Compared to a control, an application of 100 or 150 kg N ha^{-1} increases concentrations of palmitic, stearic, oleic, linoleic, linolenic, arachidic, eicosenoic, behenic, erucic and nervonic acid (Zheljazkov, Vick, Ebelhar, Buehring, & Astatkie, 2012). Application of a combination of soil- and foliar- applied fertilizer (90-30-30-2 N-P-K-S) encouraged fatty acid unsaturation in erucic acid-free rapeseed genotypes (Siddiqui, et al., 2008).

An important consideration in cultivating canola is variability of seasonal weather, which affects the quality of the end product. Temperatures during crop production affect free fatty acid composition (Aksouh-Harradj, Campbell, & Mailer, 2006; Aslam, et al., 2009; Sheikh, et al., 2010). The canola flowering period lasts 2-5 weeks and is the most critical and sensitive time. During this period, the seed's cotyledons experience changes in fatty acid composition, accompanied by rapid oil accumulation (Aksouh-Harradj, et al., 2006).

Day duration and temperature during critical times such as flowering have a significant effect on seed weight and fatty acid composition. Researchers evaluated temperature changes 10 days after flowering using day time temperatures of 28°C and nighttime temperatures of 23°C for a duration period of either 5 or 10 days. Regardless of the duration of days , they reported an increase of 7.5-11% in oleic acid, a decrease of 15-13% in linoleic acid, and a decrease of 16-41% in linolenic acid (Aksouh-Harradj, et al., 2006). Increased temperature delays seed maturation and inhibits oleic acid desaturation (Deng & Scarth, 1998). Increasing temperature during canola growth has also been linked to increased oleic acid biosynthesis (Aslam, et al.,

2009). Salinity also causes alterations in canola's fatty acid composition. When NaCl is applied at 200 mM to *B. napus* (L.) seed, there is an increase in Peroxidase and Indole-3-Acetic Acid (IAA) oxidase, which affects physiological mechanisms (Bybordi, Tabatabaei, & Ahmadev, 2010).

Oil storage conditions, including duration of storage, affect oil quality standards such as oxidation stability. Storing canola for 90 days at a temperature of 25^{o}C resulted in an oil reduction of 2.3-3.1% and a peroxide increase of 60-70% (Suriyong, 2007). Oxidation stability depends on the amount and position of double bonds, which influence both nutritional and industrial applications (Knothe & Dunn, 2003). Oxidation stability increases with increasing concentrations of oleic acid. The stability of fatty acid compounds is affected when exposed to air, heat, light, and peroxides. High oleic fatty acid canola oils used for industrial operations require a long shelf life (9-12 months), which can be accomplished by reducing linolenic and linoleic acids, under ambient temperature (DeBonte, et al., 2012).

The structural properties of fatty acids determine the quality and quantity of biodiesel. Fatty acid composition impacts several characteristics of biodiesel production such as: flashpoint, viscosity, cetane number, cloud point, pour point, calorific value, acid value, ash content, and cold flow properties. Longer fatty acid carbon chains and an increasing molecular saturation generate a higher cetane number. A higher cetane number produces better fuel ignition characteristics. With increasing cetane number, cold flow properties of biodiesel improve and white smoke formation is minimized (Ramos, Fernández, Casas, Rodríguez, & Pérez, 2009). However, biodiesel with a higher cetane number is more likely to cause obstructions in fuel injectors (Knothe, 2008). A fatty acid composition of branched and aromatic compounds generates a lower cetane number. For example, methyl linolenate has a lower cetane number

compared to methyl palmitate and methyl stearate. In colder climates, crystallization, gelling, and increased viscosity limit biodiesel usage. If biofuel feedstock has a high percentage of unsaturated fatty acids, the cold flow properties (pour point, cloud point) will improve.

CHAPTER 3

Methodology

North Carolina Agricultural and Technical State University Research Farm is located 223m above sea level at coordinates: latitude: 36.06733°, longitude: -79.73447. The current climate hardiness zone is classified as zone 7A. The experimental site was conducted on a soil type classified as a Mecklenburg Sandy Clay Loam (Taxonomic class**:** Fine, mixed, active, Thermic Ultic Hapludalfs). The soil is well drained with 6-10% slopes, and moderately eroded.

3.1 Experimental Design

A three year long experiment was conducted from 2009-2012 in the piedmont region of North Carolina. Two cultivars of winter canola (Virginia and DKW 46-15) were grown during the three year study period. The experimental design was a split plot randomized complete block, with cultivar (2) as main plots and fertilizer rates (4) as sub plot, in 4 replications.

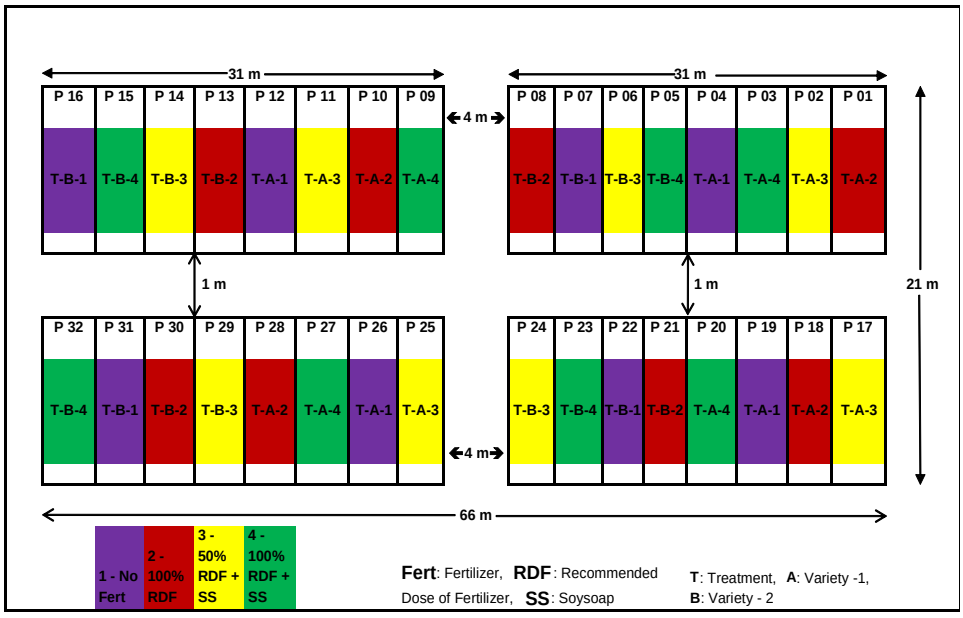

Figure 1. Canola plot randomization and experimental design during 2010-2011.

Fertilizer rates were calculated and adjusted from preplant soil analysis. The 100% recommended fertilizer rates were based on (N-P-K) rates suggested for *B. napus* (L.) cultivated in North Carolina. The 50% fertilizer rates were based on half of the full fertilizer rate recommendation. Fertilizer rates included: 140-56-168 (N-P-K) $kgha^{-1}$ (100% fertilizer recommendation +Soysoap), 140-56-168 (N-P-K) $kgha^{-1}$ (100% fertilizer recommendation), 70-28-84 $kgha^{-1}$ (N-P-K) + Soysoap (50% fertilizer recommendation + Soysoap) and 0-0-0 $kgha^{-1}$ (N-P-K) (control). In 2012 70-28-84 $kgha^{-1}$ (50% fertilizer recommendation) treatment was added. This treatment addition was presumed to help differentiate the effectives of soysoap application on plots that received lower fertilizer rates.

3.1.1 Soysoap. Soysoap is an agricultural surfactant made from biodegradable food waste. Surfactants in agricultural applications can help spread spray solutions, reduce retention and crystallization of spray droplets, and maximize penetration through leaf surface structures, such as trichomes and bud scales (Baseeth & Sebree, 2010). Surfactants may potentially aid in nutrient delivery to plant tissue by affecting absorption on stomata openings, cuticle hydrophilic pores, and through plasmodesmata (Li, Li, Xiao, Zhao, & Wang, 2009). This could potentially help minimize nutrient loss and increase crop oil and seed yields.

3.2 Land Preparation and Fertilizer Application

Plots were prepared at the beginning of planting by plowing then disking the field. The field was divided into (4) blocks with (8) plots per block. Each block represents a replication consisting of (4) plots for each of the (2) canola cultivars. Each subplot was 6.2 x $10m^2$. Seed planting was completed using a push seeder, which dug a furrow as it was pushed from one end of the plot to the other, allowing seeds to drop along the way. Eight rows of canola were planted with 15cm row spacing.

Fertilizer treatments were applied in split applications: pre-planting fertilizer was applied by broadcasting, and then at the 4-5 leaf stage fertilizer was banded. Banding was completed by using a warren hoe to dig furrows 1 inch away from the crop row with fertilizer placed by hand in the furrow which was then covered. Sources of fertilizer used in this study were 14-14-14 (N-P_2O-K_2O), NH_4NO_3 and K_2O Cl. Soysoap was mixed with water in a (0.12:30.28 soysoap:water ratio) and then applied using a backpack sprayer. Soysoap application was applied at the beginning of 50% flowering for 4 weeks. The planting dates for 2010, 2011 and 2012 were October 3, 2009, October 1, 2010, and October 7, 2011. Harvesting was done on May 30th in 2010, May 21st in 2011 and May 31st in 2012. After harvest in 2010 and 2011 a summer rotational crop was planted. In 2010 *Cucurbita pepo* (L.) was planted and in 2011 *Cucumis sativus* (L.).

3.3 Soil and Plant Sampling

Soil samples were collected using a soil auger from the top 6-8 inches of the soil. Samples were air dried for 24 hours then crushed using a wood mallet and sieved through a 2 mm sieve. Soil samples were analyzed for plant nutrients Ca, Cu, Fe, K, Mg, Mn, P, Zn using a modified Mehlich-3 procedure described by Sparks et al., (1996). Soil was measured 5 g of soil was measured into a 100mL plastic bottle. 25 mL of Mehlich extractant was added. Samples were then placed in a mechanical shaker for 5 minutes. Mixture was then filtered in Buchner funnels fitted with Whatman no. 42 porosity paper. Filtrate was analyzed on a Flow Injection Analyzer.

For the analysis of Ammonium and Nitrate, a 2M KCl modified method described by Sparks et al., (1996) was followed: Four grams of soil was placed with 40 mL 2M KCl extract into a centrifuge tube. Samples were disturbed using a mechanical shaker for 30 minutes. The

samples were removed and then centrifuged for 10 minutes at 2,500 RPM. Buchner funnels were fitted with Whatman no. 42 porosity paper. Supernatant was then poured into the prepared funnels into a 50ml plastic bottle. Samples were analyzed using Inductively Coupled Plasma Mass Spectrometry.

3.4 Chlorophyll Meter Readings

In 2012, chlorophyll meter readings were recorded a day after the canola crop was sprayed with soysoap. Using a Field Scout CM-1000 chlorophyll meter, (10) leafs from mature undamaged plants was selected. For each leaf the chlorophyll meter laser was focused in the middle of the leaf lamina between the midrib and edge recording (5) readings, which were averaged. Once readings were recorded the leaves were collected and removed from the field, washed, dried, and prepared in a grinder until particulate material was of a fine particle size.

3.5 Total Nitrogen of Leaf Tissue through Combustion

Total Leaf N was analyzed through a modified method described by Plank (1992), which used combustion analysis. A Leco (C-H-N Analyzer) was utilized for total leaf N analysis. Leaf material was weighed (2.5g) into a tin capsule which was folded and placed in the combustion machine. The samples were then oxidized with O_2 at 975^{o}C for 2 minutes and carbon, hydrogen, and nitrogen was calculated and recorded.

3.6 Canola Harvest

During 2010 and 2011 canola plots were harvested using hand loppers. Cut plots were then placed onto a tarp and manually threshed to extract seed. Seed was then sieved to remove excess particulate matter and then air dried. In 2012 canola harvest methods were changed in order to enhance harvesting of plots. Canola plants were harvested by cutting the plants at the base with a Stihl weed eater fitted with a trimmer attachment. Canola plants were placed onto a

plastic tarp and manually threshed. Seeds were cleaned with a canola sieve to remove chaff and air dried.

3.7 Oil Extraction

Oil was extracted from the seeds via two methods: Samples were taken from each plot and extracted in triplicate by hexane lipid extraction during 2010, 2011 and 2012. Mechanical seed oil extraction was completed in 2011 and 2012 due to the acquisition of an Okeotec CA 5963 seed press.

3.7.1 Soxhlet extraction. Lipid extraction was completed following a modification of AOAC Official Method 948.22 (Venkatachalam & Sathe, 2006). Forty grams of seed was weighed and then pulverized using a coffee grinder for 15 seconds. Prepared ground seed was then placed into a cellulose extraction thimble (43mm x 123mm). 400 ml of hexane was poured into the still pot of the soxhlet apparatus. The temperature was brought to 69ºC (boiling point of hexane). Once the proper temperature was achieved the extraction tube was inserted along with the condenser tube. The thimble was then placed into the condenser tube. Reflux took place for 6 hours. The still pot was removed, and contents poured into a 500ml beaker which was then air dried for 1 day under a fume hood to remove residual hexane. Samples were then weighed and lipid content was calculated.

3.7.2 Mechanical seed press. An Okeotec CA 5963 seed press was used to mechanically extract canola oil from seed. Seed from each plot was collected, weighed (2.2kg), and crushed. Samples were weighed and lipid percentage was calculated.

3.8 Fatty Acid Analysis

In 2011 and 2012 oil that was extracted through soxhlet lipid extraction had free fatty acid concentration calculated. Free fatty acids are considered impurities and affect the shelf life

of oil. During 2012 total fatty acid concentration was calculated for mechanically extracted oil. Fatty acid concentration helps define the overall quality of the biofuel feedstock (Van Gerpen 2005). To produce biodiesel from virgin feedstock it is important to determine if any differences exist between cultivars produced or from the fertilizer rates applied. Free- and total- fatty acid analysis was performed following methods developed by Bryce, (2000).

3.8.1 Free fatty acid analysis by solid phase extraction. Samples from soxhlet oil extraction were weighed between 0.23-0.27g and then placed into a 15ml vial. The addition of 10 ml of 2:1 chloroform:methanol was made. Samples were vortexted for 30 seconds. Samples were allowed to settle for 5 minutes. The samples were then centrifuged at 15^{o}C for 10 minutes at 3,000 rpm. From the bottom layer 3ml of sample was extracted and preconditioned on a supleco superclean LC-NH2 SPE cartridge by passing 6ml of hexane thru the column. Then the transfer of 3ml of 2:1 chloroform:methanol was made onto the column. Then 3ml of 2:1 chloroform/isopropanol was added thru the column to remove neutral lipids. All solvents were then discarded. The free fatty acids were eluted off the LC-NH2 column using 6 ml of 2% acetic acid in diethyl ether and collected in a 10ml sample vial. Samples were then analyzed on Gas Chromotgraph using nukol megabore column.

3.8.2 Total fatty acids transesterfication of fatty glycerides to fatty acid methyl esters. Samples from mechanical oil extraction were weighed between 0.23-0.27g of oil and then placed into a 10ml vial. The addition of 1.0ml of tetrahydrofuran at room temperature was added along with 1.0ml of 1M KOH/methanol and then vortexed for 30 seconds. Samples were then allowed to settle for 1 minute. The addition of 1ml of boron trifluoride/methanol was made and the sample was vortexed for 1 minute. The solution was then heated to 100^{o}C in a hot water bath for 15 minutes and then cooled to room temperature. The addition of 0.5ml NaCl [36g/100ml di

H_20) was made. Then 1ml of isooctane was added and the sample was vortexed for 10 seconds. Samples were then centrifuged for 15 minutes at 3,000 rpm at 15^oC and the top layer was removed and analyzed on a gas chromatograph using DB-23 capillary column.

CHAPTER 4

Results

Below are the results that were obtained for dry seed yield ($kgha^{-1}$), mechanical and solvent extraction oil percentage, chlorophyll meter readings (2012), total- and free- fatty acid concentrations. Statistical analysis was completed using SAS (9.2v, SAS Institute Inc, Cary, NC). Post hoc analysis was performed using Duncan Multiple Range Test (Duncan, 1955).

4.1 Dry Seed Yield ($kgha^{-1}$)

During the 2010 growing season statistical analysis was conducted on dry seed yield ($kgha^{-1}$) and revealed that the rate of fertilizer applied had a significant effect, ($p < 0.01$) on yield potential (Table 2 see Appendix). Post hoc analysis indicated that plots that received additional fertilizer produced significantly higher yields than the control (Table 3). There was a significant effect from cultivar, ($p = 0.04$) and fertilizer rate, ($p = 0.03$) on dry seed yield ($kgha^{-1}$) in 2011 (Table 2 see Appendix). Post hoc analysis did not show any statistical difference between cultivars Virginia and DKW 46-15 to produce significantly different seed yields. Post hoc analysis showed that plots applied with 140-56-168 ($kgha^{-1}$) with or without soysoap application produced significantly higher yields than the control (Table 3).

Table 3.

Post Hoc Duncan Multiple Range Test from Statistical Analysis During 2010 and 2011 Dry Seed Yield ($kgha^{-1}$)

Fertilizer Rate	2010	2011
0-0-0	334.9_{B}	283.3_{B}
70-28-84 + Soysoap	864.5_{A}	504.2_{BA}
140-56-168	929.7_{A}	654.2_{A}
140-56-168 + Soysoap	1013_{A}	789.2_{A}

Note. Fertilizer rates with a letter in common are not significantly different at ($\alpha = 0.05$) level of significance as indicated by Duncan's multiple range tests.

Table 4.

2010, 2011 and 2012 Mean Cultivar Fertilizer Efficiency

Year	DKW 46-15	Virginia
2010	3.42_A	2.22_B
2011	1.57	1.20
2012	3.71	3.15

Note. Fertilizer rates with a letter in common are not significantly different at ($\alpha = 0.05$) level of significance as indicated by Duncan's multiple range tests.

There was a significant interaction effect, ($p = 0.01$) found in 2012 dry seed yield ($kgha^{-1}$) (Table 2 see Appendix). Figure 2 shows Duncan groupings were significantly higher for DKW 46-15 (100%) and Virginia (100% + Soysoap) than DKW 46-15 (50% + Soysoap) and the control for both cultivars. Figure 2 also illustrates a large degree of variability found in the standard deviations. Table 4 shows the fertilizer use efficiency from each of the cultivars during 2010, 2011, and 2012. There was a significant cultivar effect, ($p = 0.01$) on fertilizer efficiency during 2010. DKW 46-15 made more efficient use of fertilizer supplied than the Virginia cultivar during 2010 (Table 4). During 2011-2012, there was no significant difference in fertilizer efficiency due to the effect of cultivar (Table 4), or from the fertilizer rate applied (Table 5).

Table 5.

2010, 2011 and 2012 Fertilizer Efficiency Calculated by Cultivars and Individual Fertilizer Rates

	DKW 46-15			Virginia		
Fertilizer Rate	2010	2011	2012	2010	2011	2012
70-28-84	†	†	1.47	†	†	1.40
70-28-84 + Soysoap	2.90	1.10	5.94	2.03	0.70	1.11
140-56-168	3.81	1.39	0.87	1.90	1.49	4.19
140-56-168 + Soysoap	3.54	1.10	3.06	2.73	2.53	5.64

Note. † Treatment not added until 2012

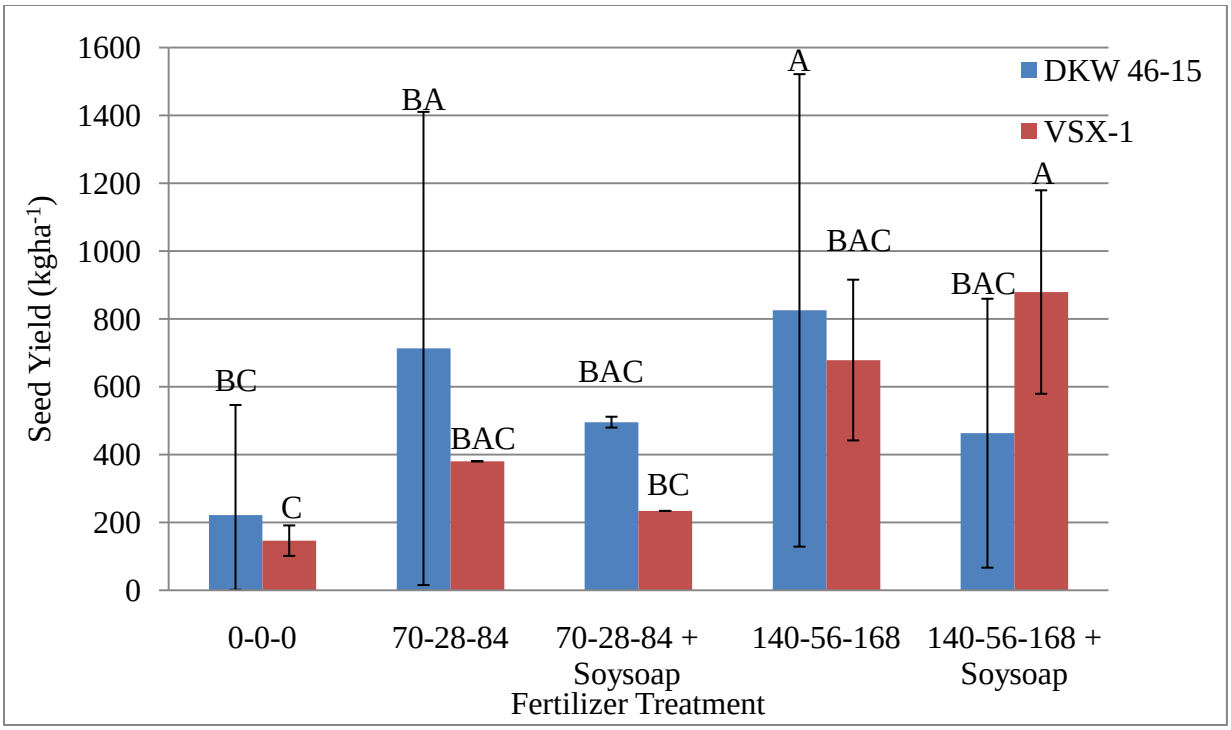

Figure 2. Duncan's multiple range test for the interaction effect from 2012 seed yield ($kgha^{-1}$) analysis.

4.2 Oil Percentage from Solvent and Mechanical Extraction

4.2.1 Mechanical extracted canola oil percentage during 2011 and 2012. Analysis of 2011 soxhlet extraction oil percentage revealed no significant difference in oil production due to the effects from cultivar, fertilizer rate, or from their interaction (Table 6). During the analysis of 2012 soxhlet extracted canola oil there was no significant difference in oil percentage due to the cultivar selected, fertilizer rate applied or from their interaction. However, it should be noted that there was a non-significant trend of 140-56-168 ($kgha^{-1}$) treatment producing the highest oil yields for DKW 46-15 in both 2011 and 2012. The Virginia cultivar did not display the same trend in 2011 or 2012. During 2011, although non-significant the Virginia cultivar highest yielding treatment was 0-0-0 ($kgha^{-1}$). During 2012, the highest oil yielding treatment for the Virginia cultivar was 70-28-84 + Soysoap.

Table 6.

Mechanically Extracted Oil Percentage From 2011 and 2012 Growing Seasons

	2011		2012	
Fertilizer Rate	DKW 46-15	Virginia	DKW 46-15	Virginia
0-0-0	20.28	22.01	19.25	20.95
70-28-84	†	†	16.38	19.37
70-28-84 + Soysoap	18.25	18.46	20.76	21.80
140-56-168	24.03	15.41	23.30	20.96
140-56-168 + Soysoap	18.25	19.47	21.46	20.94

Note. † Treatment not added until 2012

4.2.2 Soxhlet extracted canola oil percentage during 2010, 2011 and 2012. During the analysis of 2010 soxhlet extracted lipid percentage, a significant cultivar effect, ($p < 0.001$) was revealed (Table 7). Figure 3 illustrates a significantly higher oil percentage for the Virginia cultivar than DKW 46-15. During 2011, soxhlet extracted lipid percentages remained unaffected by the effects of cultivar, fertilizer rate, or from their interaction. Analysis of 2012 soxhlet extracted lipid percentage yielded similar results as in 2011.

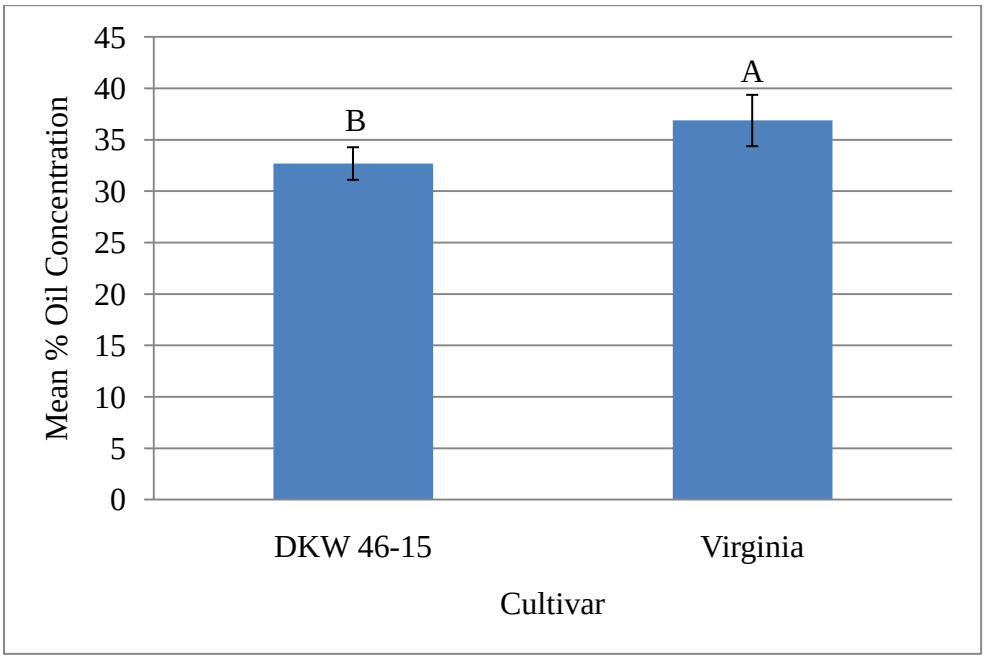

Figure 3. Canola oil percentage obtained through soxhlet extraction from 2010 seed harvest.

Table 7.

ANOVA Table for 2010 Solvent Extracted Oil Percentage

Source	*df*	Sum of Squares	Mean Square	*F*	*p*
Cultivar	1	122.73	122.73	34.78	0.001
Fertilizer Rate	3	24.27	8.09	2.29	0.12
Interaction	3	10.11	3.37	0.96	0.44
Corrected Total	28	257.85			
CV=5.4					

4.3 Chlorophyll Readings

Table 8 (see Appendix) shows the statistical analysis of chlorophyll meter readings taken on March 27th, April 10th and April 17th. Chlorophyll meter readings conducted on March 27th experienced a significant effect from the fertilizer rate, (p = 0.003) applied. Post hoc analysis revealed that plots which received additional fertilizer produced significantly higher chlorophyll meter readings than the control (Table 9). There was a correlation between March 27th chlorophyll meter readings and dry seed yield ($kgha^{-1}$) [r = 0.47, n = 26, p = 0.01]. During the chlorophyll meter readings conducted on April 10th there was a significant effect, (p = 0.03) from fertilizer rate (Table 8 see Appendix).

Table 9.

Fertilizer Rate Groupings for Sampling Dates During 2012 Chlorophyll Meter Readings

Fertilizer Rate	March 27th	April 10th	April 17th
0-0-0	206.60_{C}	152.39_{C}	143.67_{B}
70-28-84	262.93_{A}	209.78_{BA}	165.67_{A}
70-28-84 + Soysoap	252.90_{BA}	169.03_{BC}	166.55_{A}
140-56-168	274.46_{A}	201.31_{BAC}	167.52_{A}
140-56-168 + Soysoap	296.63_{A}	224.36_{A}	168.55_{A}

Note. Fertilizer rates with a letter in common are not significantly different at (α = 0.05) level of significance as indicated by Duncan's multiple range tests.

Post hoc analysis for chlorophyll readings showed fertilizer rates (100%+Soysoap) and (50%) producing significantly higher meter readings than the control (Table 9). There was a positive correlation found between April 10th chlorophyll meter readings and dry seed yield ($kgha^{-1}$) [$r = 0.73$, $n = 26$, $p = 0.001$]. Chlorophyll meter readings conducted on April 17th revealed a significant effect from the rate of fertilizer applied, ($p = 0.007$) (Table 8 see Appendix). Mean fertilizer rates for April 17th show significantly higher meter readings for plots applied with additional fertilizer (Table 9).

Table 11.

2012 Leaf Nitrogen Concentration (ppm) From Sampling Dates by Mean Fertilizer Rate

Fertilizer Rate	March 27th	April 10th	April 17th
0-0-0	4.19	2.57_{B}	2.74_{C}
70-28-84	4.68	3.38_{A}	3.16_{BC}
70-28-84 + Soysoap	3.98	3.02_{BA}	3.30_{B}
140-56-168	3.70	2.78_{BA}	3.47_{BA}
140-56-168 + Soysoap	3.97	2.81_{BA}	3.76_{A}

Note. Fertilizer rates with a letter in common are not significantly different at ($\alpha = 0.05$) level of significance as indicated by Duncan's multiple range tests.

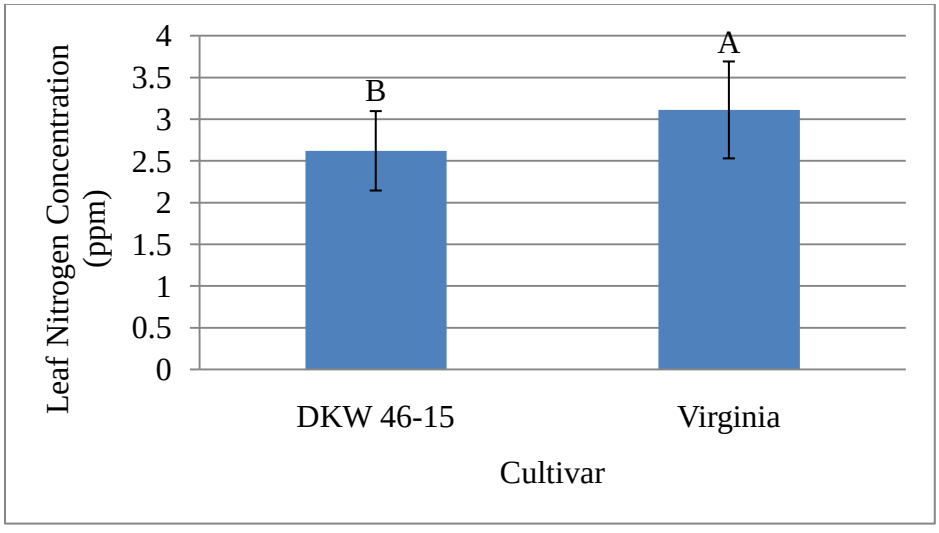

Figure 4. Cultivar post hoc analysis from April 10, 2012 chlorophyll meter readings.

There was a positive correlation found between April 17th chlorophyll meter readings and dry seed yield ($kgha^{-1}$) [$r = 0.63$, $n = 26$, $p = 0.001$]. On March 27th there was no significant effect found on leaf nitrogen concentration from the effects of cultivar, fertilizer rate, or from their interaction (Table 10 see Appendix). On April 10th there was a significant cultivar effect, ($p = 0.02$) on leaf N concentration (Table 10 see Appendix). Figure 4 shows the Virginia cultivar producing significantly higher concentrations of leaf N than DKW 46-15.

There was a positive correlation found between April 10th leaf N concentration and dry seed yield ($kgha^{-1}$) [$r = 0.50$, $n = 26$, $p = 0.008$]. There was a significant effect from fertilizer rate, ($p = 0.001$) found on leaf N concentration from samples collected on April 17th (Table 10 see Appendix). Post hoc analysis revealed that leaf N concentration was increased for plots that received additional fertilization (Table 11). There was a positive correlation found between April 17th leaf N concentration and dry seed yield ($kgha^{-1}$) [$r = 0.73$, $n = 25$, $p = 0.001$].

4.4 Free- and Total- Fatty Acid Concentrations

4.4.1 Analysis of free fatty acid concentration from soxhlet lipid extraction 2011-2012 .

2011 analysis of free fatty acids in soxhlet extracted oil revealed a significant effect, ($p = 0.03$) from fertilizer rate on the production of linoleic acid. However, post hoc analysis revealed no significant difference in the production of linoleic acid due to rate of fertilizer applied. Oleic, linolenic and palmitic acids did not reveal any statistical difference in free fatty acid production from the effects of cultivar selection, rate of fertilizer application, or from their interaction.

Results for the analysis of 2012 free fatty acid concentration were similar to 2011. Linoleic, linolenic, oleic, palmitic, eicosenoic, and erucic free fatty acids concentration during 2012 remained unaffected by the effects from cultivar selection, rate of fertilizer applied, or from their interaction.

4.4.2 Total fatty acid concentration from mechanically extracted lipids. During 2012, oil extracted by mechanical extraction was analyzed for total fatty acid concentration. As shown in Table 12 (see Appendix) there was a significant effect from cultivar on oleic acid content, (p = 0.001). Figure 5 illustrates the Virginia cultivar producing significantly lower concentrations of total oleic fatty acid than DKW 46-15. Table 12 (see Appendix) shows the ANOVA table for erucic acid and revealed a significant effect, (p = 0.001) from cultivar selection. Figure 6 illustrates the Virginia cultivar producing significantly higher total erucic fatty acid concentrations than DKW 46-15.

Analysis of linolenic acid revealed a significant cultivar effect, (p = 0.01) (Table 12 see Appendix). However, post hoc analysis revealed no significant difference on linolenic fatty acid production between Virginia and DKW 46-15. Linoleic acid showed a significant cultivar effect, (p = 0.001) (Table 12 see Appendix). The Virginia cultivar had a significantly higher linoleic fatty acid concentration than DKW 46-15 (Figure 7).

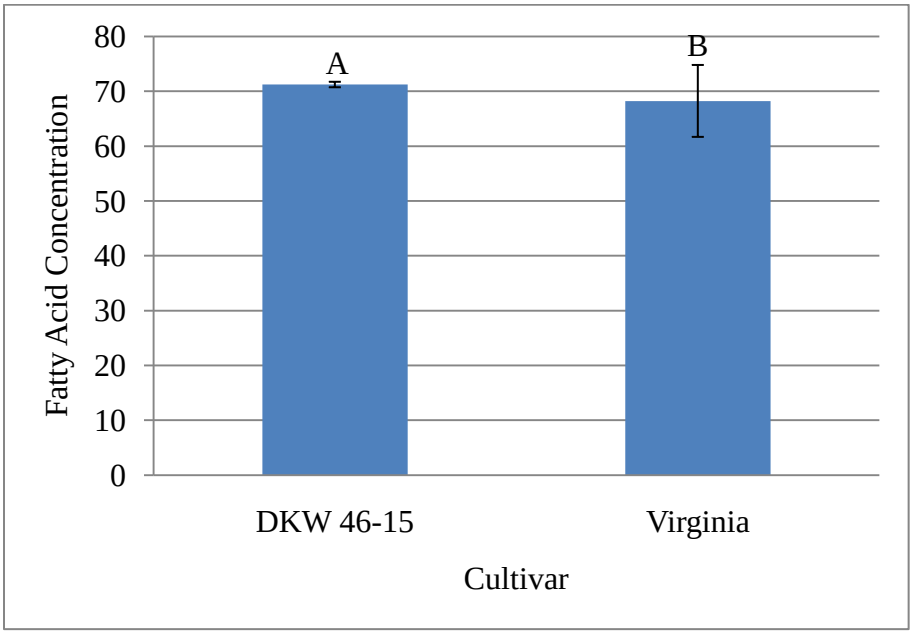

Figure 5. Oleic total fatty acid concentration from 2012 mechanically extracted oil.

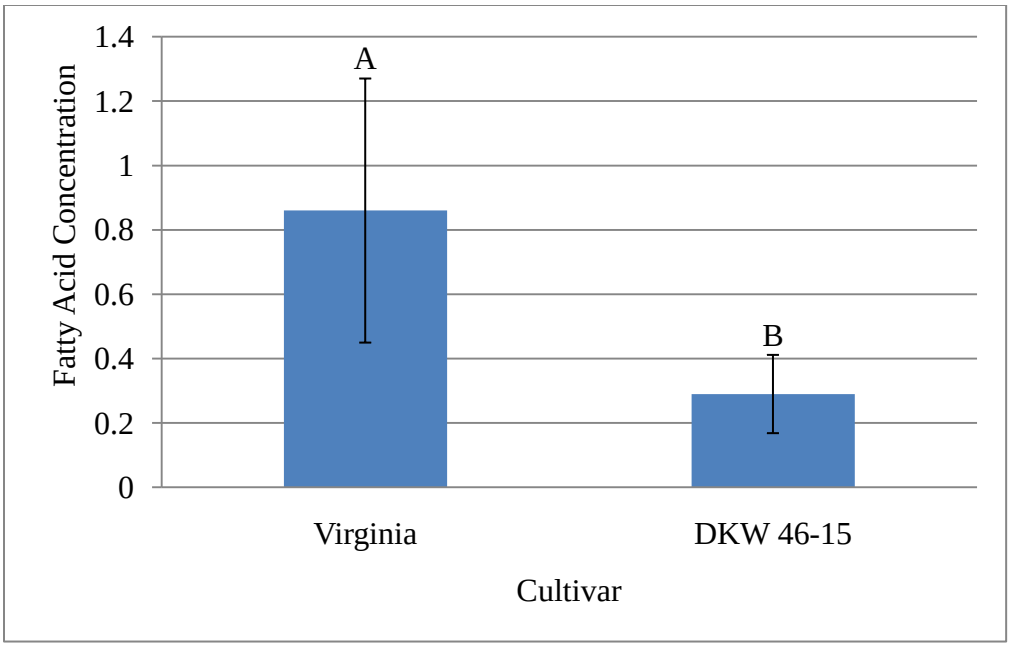

Figure 6. Erucic total fatty acid concentration from 2012 mechanically extracted oil.

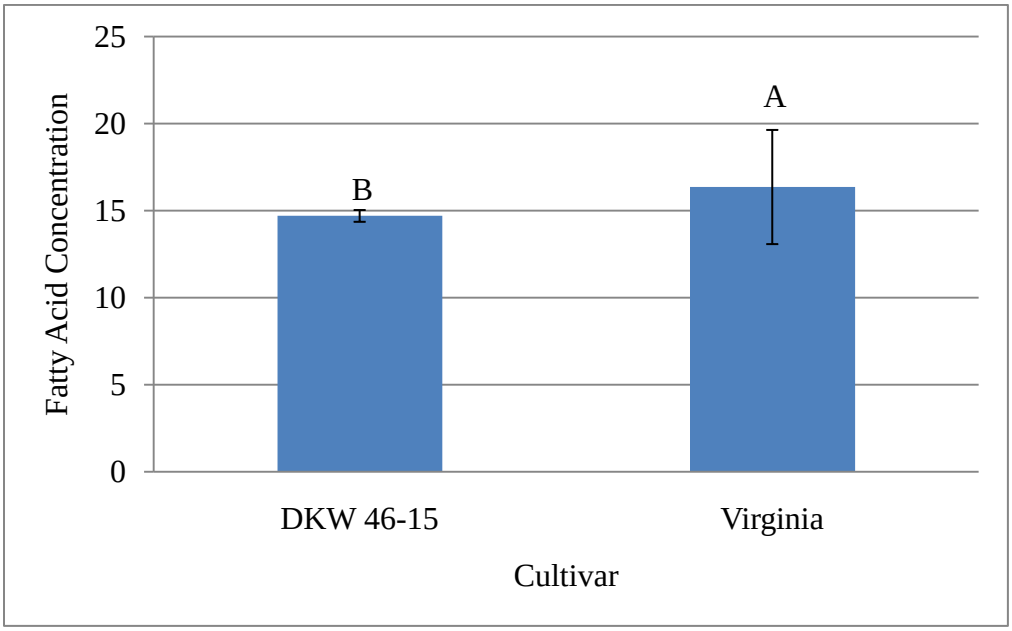

Figure 7. Linoleic total fatty acid concentration from 2012 mechanically extracted oil.

CHAPTER 5

Discussion

5.1 2010-2012 Seed and Oil Yield

No statistical difference was observed in soil samples collected that reflected changes in soil nutrients from fertilizer rate applied, cultivar selected, or from their interaction during 2009-2012 growing seasons. For 3 consecutive years fertilizer rate had a significant effect on dry seed yield ($kgha^{-1}$). During 2010, 2011, and 2012 fertilizer rates of (100%) with or without soysoap application produced significantly higher seed yields than the control. During the analysis of 2010 dry seed yield plots applied with 50% + Soysoap fertilizer rate produced significantly similar yields as 100% with or without soysoap fertilizer application. This result may be due to soysoap increasing nutrient efficiency which enables canola to produce higher seed yields with less fertilizer application. However, this trend was not observed in the following years.

The Virginia cultivar is a high yielding canola cultivar which was developed at Virginia State University and adapted to Virginia climatic conditions (Bhardwaj, 2007). The piedmont region of North Carolina shares similar climatic conditions as Virginia, so potentially the performance of the cultivar may be similar as well. Analysis of 2010 soxhlet extracted oil percentage revealed the Virginia (36.84%) cultivar producing significantly higher oil percentages than DKW 46-15 (32.69%). This trend was not observed during 2011 or 2012 however, climatic conditions were variable as illustrated in Figures 8-10.

Plant biological and chemical functions depend on temperature, which regulates processes such as, evapotranspiration, photosynthesis, and nutrient absorption (Thomas, 2003). Temperature stress prior to and during flowering directly affects canola seed yield (Faraji, Latifi, Soltani, & Rad, 2009). The photoperiod and temperature in which *Brassica napus* (L.) is exposed

to impacts the length of flowering, which can have an effect on seed yield (Tesfamariam, Annandale, & Steyn, 2010). Figure 8 illustrates the average monthly temperatures recorded in 2010, 2011, and 2012. During 2012 higher average monthly temperatures were recorded from November through January as compared to 2010. Increasing temperature during winter canola dormancy may have influenced the quantity and overall quality of both seed and oil yields during 2012.

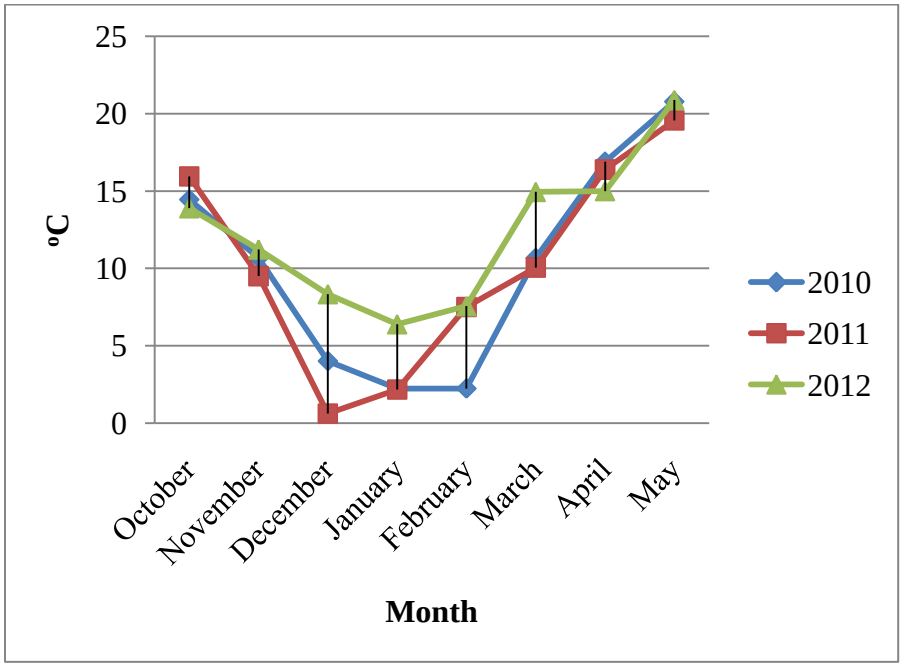

Figure 8. 2010-2012 Average monthly temperatures.

Growing degree days (GDD) is a calculation that estimates a crops daily heat value (Miller, Lanier, & Brandt, 2001). Global warming may be contributing to increasing growing degree days which alters the growing season for members of the *Brassica* family (Siebold and von Tiedemann 2013). During the experiment GDD were calculated from seeding to physiological maturity. Figure 9 shows the monthly GDD calculated for 2010, 2011, and 2012. GGD almost doubled in 2012 compared to previous years. A dramatic increase in GDD may have produced fluctuating seed and oil yields by contributing to uneven maturity times.

Wind and sources of mechanical stress may result in yield losses through pod shattering, leaf tearing and logging (Cipollini, 1999). Figure 10 illustrates wind speeds (mph) during the 2010, 2011, and 2012 growing seasons. Wind speeds were notably higher during 2010 and 2011 canola harvest times. The large losses that occurred in 2011 were due in part from wind damage that exacerbated pod shatter.

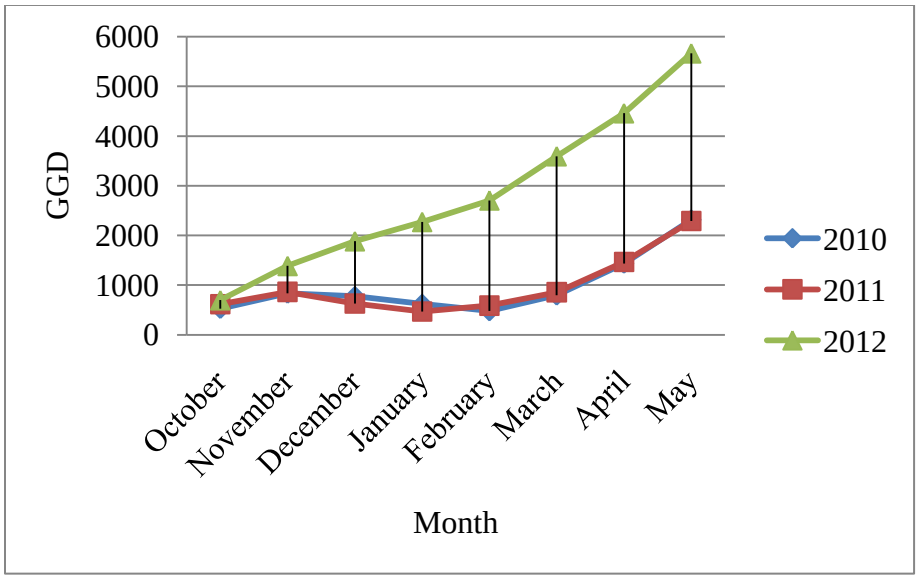

Figure 9. 2010-2012 Growing degree days during 2010-2012.

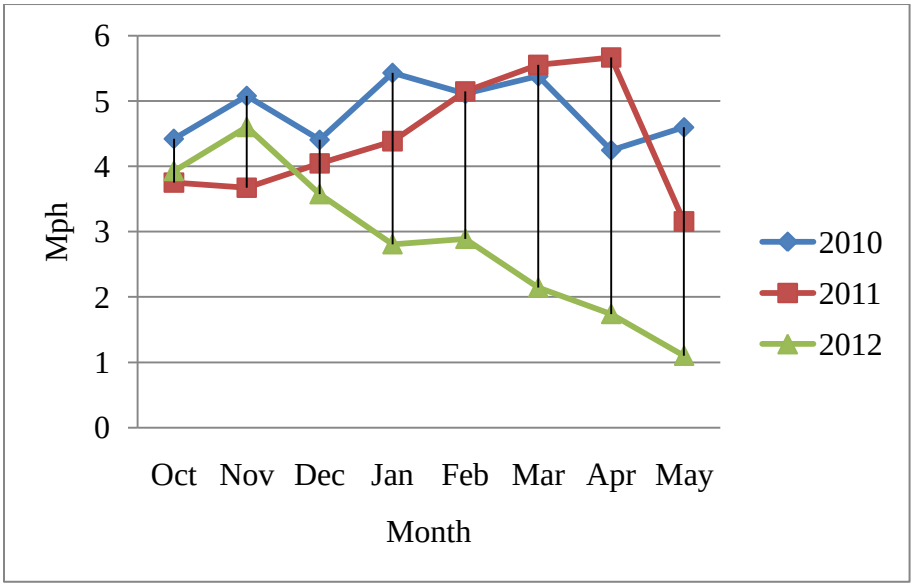

Figure 10. 2010-2012 Average monthly wind speeds (mph).

5.2 2012 Chlorophyll Readings

Hand held chlorophyll meters can be used to evaluate crop fertility using quick, nondestructive methods which measure chlorophyll content using absorbance and transmittance (Ronaghi & Ghasemi-Fasaei, 2013). Potentially chlorophyll meters could help evaluate the effectiveness of soysoap on leaf chlorophyll production. Chlorophyll readings were collected the day after the application of soysoap. Plots applied with additional fertilizer rates yielded significantly higher chlorophyll readings on March 27th and April 17th sampling dates. Overall, chlorophyll readings and leaf nitrogen concentrations were unaffected by the application of soysoap.

5.3 Free- and Total- Fatty Acid Concentrations

The use of a soxhlet apparatus for lipid extraction utilizes a reflux reaction which repeatedly washes canola seed with hexane. It has been reported that due to samples being extracted at the boiling point of solvents and refluxing for long periods of time that fatty acid thermal decomposition is inevitable (Xiao, 2010). The largest drawback of extracting canola oil with a soxhlet apparatus was the long time required (8hrs) accompanied by the large amount of waste produced when hexane was evaporated under the fume hood. When compared to mechanical extraction, the extraction time was quicker however; the amount of lipid extracted was much lower. Mechanical extraction does not expose lipids to hexane or long periods of heat potential. Therefore, differences in fatty acid concentrations may be revealed between cultivars or from the rate of fertilizer application.

In 2011 and 2012 soxhlet extracted oil showed no significant differences in free fatty acid concentration between cultivars, fertilizer rates applied or from their interaction. For mechanically extracted oil total fatty acid concentration was calculated in 2012. Significant

differences were found between cultivars for oleic, linoleic, linolenic and erucic total fatty acids. DKW 46-15 produced higher concentrations of oleic acid than the Virginia cultivar. However, the Virginia cultivar produced higher concentrations of linoleic, and erucic fatty acids.

5.4 Conclusion

For 3 consecutive years the effect from fertilizer rate had a significant influence on seed yield. Plots applied with 140-56-168 (N-P-K) fertilizer rate regardless of soysoap application produced significantly higher dry seed yields than the control. The analysis of 2010 soxhlet extracted oil percentages showed the Virginia cultivar being a superior oil yielder than DKW 46-15. Chlorophyll readings conducted supports the results of the dry seed yield analysis in that additional fertilizer applied increased dry seed yields. This is due to the assumption that increased chlorophyll production is synonymous with increased nitrogen activity. Increased nitrogen availability has been repeatedly shown to increase canola seed yield. Chlorophyll meter readings sampled on April 10th provided the strongest positive correlation with dry seed yield ($kgha^{-1}$). Chlorophyll meter readings conducted 3 weeks after 50% flowering may be an ideal time to estimate seed yield and leaf N concentration from meter readings. There was no significant increase found on canola dry seed yield ($kgha^{-1}$) or oil percentage using soysoap. However, further studies should be conducted to help further elucidate the effectiveness of soysoap as an agrichemical.

Fatty acid analysis showed that mechanically extracted oil shows significant differences in the production of oleic, linoleic, and erucic total fatty acid due to the cultivar selected. DKW 46-15 produced a higher level of oleic total fatty acid. High levels of oleic acid are favorable for the production of biofuel. The Virginia cultivar produced higher levels of linoleic and erucic total fatty acids. In terms of storability higher levels of linoleic acid can promote increased rates

of oxidation which can degrade the quality of fuel. Based on 2012 data, DKW 46-15 may possess more desirable fatty acid composition for the production of biodiesel.

References

Ahmad, A., & Abdin, M. (2000). Interactive effect of sulphur and nitrogen on the oil and protein contents and on the fatty acid profiles of oil in the seeds of rapeseed (*Brassica campestris* L.) and mustard (*Brassica juncea L*. Czern. and Coss.). *Journal of Agronomy and Crop Science, 185*(1), 49-54.

Ahmad, G., Jan, A., & Arif, M. (2006). Phenology and physiology of canola as affected by nitrogen and sulfur fertilization. *Journal of Agronomy, 5*(4), 555-562.

Aksouh-Harradj, N. M., Campbell, L. C., & Mailer, R. J. (2006). Canola response to high and moderately high temperature stresses during seed maturation. *Canadian Journal of Plant Science, 86*(4), 967-980. doi: 10.4141/p05-130

Angus, J., Kirkegaard, J., Peoples, M., Ryan, M., Ohlander, L., & Hufton, L. (2011). A review of break-crop benefits of brassicas. *17th Australian Research Assembly on Brassicas,* 15-17.

Anitescu, G., & Bruno, T. J. (2011). Liquid biofuels: Fluid properties to optimize feedstock selection, processing, refining/blending, storage/transportation, and combustion. *Energy & Fuels, 26*(1), 324-348.

Anjum, N. A., Gill, S. S., Umar, S., Ahmad, I., Duarte, A. C., & Pereira, E. (2012). Improving growth and productivity of oleiferous brassicas under changing environment: Significance of nitrogen and sulphur nutrition, and underlying mechanisms. *The Scientific World Journal, 2012*.

Applewhite, T. H. (Ed.). (1993). *Proceedings of the world conference on oilseed technology and utilization*. Amer Oil Chemists Society.

Araus, J., Casadesus, J., & Bort, J. (2001). Recent tools for the screening of physiological traits determining yield. *Application of physiology in wheat breeding,* 59-77.

Arora, D. K. (2003). *Fungal biotechnology in agricultural, food, and environmental applications*: CRC Press.

Ashraf, M. A. (2012). Waterlogging stress in plants: a review. *African Journal of Agricultural Research, 7*(13), 1976-1981.

Aslam, M. N., Nelson, M. N., Kailis, S. G., Bayliss, K. L., Speijers, J., & Cowling, W. A. (2009). Canola oil increases in polyunsaturated fatty acids and decreases in oleic acid in drought-stressed mediterranean-type environments. *Plant Breeding, 128*(4), 348-355. doi: 10.1111/j.1439-0523.2008.01577.x

Atkinson, D., Rich, B. A., Tungate, K. D., Creamer, K. S., Green, J. T., & Moore, A. D. (2011). *North Carolina Production Guide*. Retrieved from http://ncsc.ncsu.edu/wp

Balasubramanian, V., Alves, B., Aulakh, M., Bekunda, M., Cai, Z., Drinkwater, L., Oenema, O. (2004). Crop, environmental, and management factors affecting nitrogen use efficiency. *Agriculture and the Nitrogen Cycle, SCOPE, 65*, 19-33.

Barraclough, P. B., Howarth, J. R., Jones, J., Lopez-Bellido, R., Parmar, S., Shepherd, C. E., & Hawkesford, M. J. (2010). Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. *European Journal of Agronomy, 33*(1), 1-11.

Barros, M., Fleuri, L., & Macedo, G. (2010). Seed lipases: sources, applications and properties-a review. *Brazilian Journal of Chemical Engineering, 27*(1), 15-29.

Barua, P. K. (2011). Biodiesel from seeds of jatropha found in assam, India. *International Journal of Energy, Information and Communications, 2*, 53-65.

Baseeth, S. S., & Sebree, B. R. (2010). Renewable surfactants in spray adjuvants. *Lipid Technology, 22*(4), 79-82.

Best, G. (2006). Alternative Energy Crops for Agricultural Machinery Biofuels-Focus on Biodiesel.

Bhardwaj, H. L. (2007). Utilizing locally-produced canola to manufacture biodiesel. *Issues in New Crops and New Uses. ASHS Press, Alexandria, VA*, 43-46.

Boem, F. G., Lavado, R. S., & Porcelli, C. A. (1996). Note on the effects of winter and spring waterlogging on growth, chemical composition and yield of rapeseed. *Field Crops Research, 47*(2), 175-179.

Borugadda, V. B., & Goud, V. V. (2012). Biodiesel production from renewable feedstocks: Status and opportunities. *Renewable and Sustainable Energy Reviews, 16*(7), 4763-4784. doi: http://dx.doi.org/10.1016/j.rser.2012.04.010

Brennan, R., & Bolland, M. (2005). Tests to predict the potassium requirements of canola. *Crop Updates*, 16-17.

Bryce, H. (2000). *Fatty Acid Analysis*. Unpublished manuscript.

Bybordi, A., Tabatabaei, S. J., & Ahmadev, A. (2010). Effects of salinity on fatty acid composition of canola (*Brassica napus* L.). *Int J Food Agric Environ, 8*, 113-115.

Casassus, J., Liu, P., & Tang, K. (2012). Economic Linkages, Relative Scarcity, and Commodity Futures Returns. *Review of Financial Studies*.

Cavalcanti, T., & Jalles, J. T. (2013). Macroeconomic effects of oil price shocks in Brazil and in the United States. *Applied Energy, 104*, 475-486.

Child, R., & Evans, D. (1989). Improvement of recoverable yields in oilseed rape (Brassica napus) with growth retardants. *Aspects of Applied Biology*, *23*(1989), pp. 135–143.

Cipollini, D. F. (1999). Costs to flowering of the production of a mechanically hardened phenotype in Brassica napus L. *International Journal of Plant Sciences, 160*(4), 735-741.

Conway, P., Connolly, R., Field, A., & Longman, D. (2003). The north carolina textiles project: an initial report. *Journal of Textile and Apparel, Technology and Management, 3*(3), 1-12.

Coyle, B. T. (2007). "Aggregation of price risk over commodities: an economic index number approach." *American Journal of Agricultural Economics 89*(4): 1085-1097.

De Fraiture, C., Giordano, M., & Liao, Y. (2008). Biofuels and implications for agricultural water use: blue impacts of green energy. *Water Policy, 10*, 67.

DeBonte, L., Iassonova, D., Liu, L., & Loh, W. (2012). Commercialization of high oleic canola oils. *Lipid Technology, 24*(8), 175-177.

Deng, X., & Scarth, R. (1998). Temperature effects on fatty acid composition during development of low-linolenic oilseed rape (Brassica napus L.). *Journal of the American Oil Chemists' Society, 75*(7), 759-766. doi: 10.1007/s11746-998-0223-4

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, *11*(1), 1-42.

Durrett, T. P., Benning, C., & Ohlrogge, J. (2008). Plant triacylglycerols as feedstocks for the production of biofuels. *The Plant Journal, 54*(4), 593-607.

Dyer, J. M., Stymne, S., Green, A. G., & Carlsson, A. S. (2008). High-value oils from plants. *The Plant Journal, 54*(4), 640-655.

Fageria, N., Baligar, V., & Li, Y. (2008). The role of nutrient efficient plants in improving crop yields in the twenty first century. *Journal of Plant Nutrition, 31*(6), 1121-1157.

FAO (2008) *Current World Fertilizer Trends and Outlook to 2011/2012*. Retrieved from ftp://ftp.fao.org/agl/agll/docs/cwfto11.pdf (accessed September 2011)

Faraji, A., Latifi, N., Soltani, A., & Rad, A. H. S. (2009). Seed yield and water use efficiency of canola (Brassica napus L.) as affected by high temperature stress and supplemental irrigation. *Agricultural Water Management, 96*(1), 132-140. doi: http://dx.doi.org/10.1016/j.agwat.2008.07.014

Firbank, L. (2012). Assessing the environmental risks and opportunities of bioenergy cropping *Sustainable Bioenergy and Bioproducts* (pp. 189-212): Springer.

Franzaring, J., Gensheimer, G., Weller, S., Schmid, I., & Fangmeier, A. (2012). Allocation and remobilisation of nitrogen in spring oilseed rape (*Brassica napus* L. cv. Mozart) as affected by N supply and elevated CO_2. *Environmental and Experimental Botany*.

Gale Jr, H. F., Foreman, L. F., & Capehart Jr, T. C. (2000). *Tobacco and the economy: Farms, jobs, and communities* (No. 34007). United States Department of Agriculture, Economic Research Service.

Garg, H., Li, H., Sivasithamparam, K., Kuo, J., & Barbetti, M. J. (2010). The infection processes of Sclerotinia sclerotiorum in cotyledon tissue of a resistant and a susceptible genotype of Brassica napus. *Annals of botany, 106*(6), 897-908.

Garnett, T., Conn, V., & Kaiser, B. (2009). Root based approaches to improving nitrogen use efficiency in plants. *Plant, cell & environment, 32*(9), 1272-1283.

George, N., Tungate, K., Beeck, C., & Stamm, M. (2012). Exploring genotype by environment interaction in winter canola in North Carolina. *Journal of Agricultural Science, 4*(2), 237.

Gerpen, J. V. (2005). Biodiesel processing and production. *Fuel processing technology, 86*(10), 1097-1107.

Ghasemnezhad, A., & Honermeier, B. (2008). Yield, oil constituents, and protein content of evening primrose (Oenothera biennis L.) seeds depending on harvest time, harvest

method and nitrogen application. *Industrial Crops and Products, 28*(1), 17-23. doi: 10.1016/j.indcrop.2007.12.006

Ghobadi, M., Bakhshandeh, M., Fathi, G., Gharineh, M., Alami-Said, K., Naderi, A., & Ghobadi, M. (2006). Short and long periods of water stress during different growth stages of canola (Brassica napus L.): Effect on yield, yield components, seed oil and protein contents. *Journal of Agronomy, 5*(2), 336-341.

Giampietro, M., & Ulgiati, S. (2005). Integrated assessment of large-scale biofuel production. *BPTS, 24*(5-6), 365-384.

Gianquinto, G., Goffart, J., Olivier, M., Guarda, G., Colauzzi, M., Dalla Costa, L., MacKerron, D. (2004). The use of hand-held chlorophyll meters as a tool to assess the nitrogen status and to guide nitrogen fertilization of potato crop. *Potato Research, 47*(1-2), 35-80.

González-García, S., García-Rey, D., & Hospido, A. (2012). Environmental life cycle assessment for rapeseed-derived biodiesel. *The International Journal of Life Cycle Assessment,* 1-16.

Grant, C., & Bailey, L. (1993). Fertility management in canola production. *Canadian Journal of Plant Science, 73*(3), 651-670.

Gray, K. A., Zhao, L., & Emptage, M. (2006). Bioethanol. *Current Opinion in Chemical Biology, 10*(2), 141-146.

Gulden, R. H., Shirtliffe, S. J., & Thomas, A. G. (2009). Harvest losses of canola (*Brassica napus*) cause large seedbank inputs, *Weed Science, 51*(1), 83-86.

Guzatto, R., Defferrari, D., Reiznautt, Q. B., Cadore, Í. R., & Samios, D. (2012). Transesterification double step process modification for ethyl ester biodiesel production from vegetable and waste oils. *Fuel, 92*(1), 197-203. doi: 10.1016/j.fuel.2011.08.010

Habibzadeh, F., Sorooshzadeh, A., Pirdashti, H., & Modarres Sanavy, S. A. M. (2012). A comparison between foliar application and seed inoculation of biofertilizers on canola (Brassica napus L.) grown under waterlogged conditions. *Australian Journal of Crop Science, 6*(10), 1435.

Hall, B. (2011, August 26). *Minimizing Canola Harvest Losses*. Retrieved from http://www.ontariocanolagrowers.ca/linked/harvest-minimizing_losses_aug_26-11.pdf

Hamzei, J. (2011). Seed, oil, and protein yields of canola under combinations of irrigation and nitrogen application. *Agronomy Journal*, *103*(4), 1152-1158.

Hao, N., Colson, G., Karali, B., & Wetzstein, M. E. (2013). *Food before Biodiesel Fuel?* Paper presented at the 2013 Annual Meeting, February 2-5, 2013, Orlando, Florida.

Hartman, M. (2011, June 27). *How Waterlogging Hurts Canola*. Retrieved from http://www.canolawatch.org/2011/06/27/how-waterlogging-hurts-canola/

Holzapfel, C. B. (2007). *Estimating nitrogen fertilizer requirements of canola (Brassica napus L.) using sensor-based estimates of yield potential and crop response to nitrogen.* (Docteral dissertation). University of Manitoba.

Howarth, R., Bringezu, S., Bekunda, M., De Fraiture, C., Maene, L., Martinelli, L., & Sala, O. (2008). Rapid assessment on biofuels and environment: overview and key findings. *Biofuels: Environmental Consequences and Interactions with Changing Land Use*, 22-25.

Humboldt, S. Saskatchewan Ministry of Agriculture, (2012). *Long term storage of canola* (A3909). Retrieved from website: http://pami.ca/wp-content/uploads/2012/06/Report_A3909_canola_storage.pdf

Iriarte, A., Rieradevall, J., & Gabarrell, X. (2012). Transition towards a more environmentally sustainable biodiesel in South America: The case of Chile. *Applied Energy, 91*(1), 263-273. doi: http://dx.doi.org/10.1016/j.apenergy.2011.09.024

Jackson, G. D. (2000). Effects of nitrogen and sulfur on canola yield and nutrient uptake. *Agronomy Journal, 92*(4), 644-649.

Janulis, P. (2004). Reduction of energy consumption in biodiesel fuel life cycle. *Renewable Energy, 29*(6), 861-871.

Jifon, J. L., Syvertsen, J. P., & Whaley, E. (2005). Growth environment and leaf anatomy affect nondestructive estimates of chlorophyll and nitrogen in Citrus sp. leaves. *Journal of the American Society for Horticultural Science, 130*(2), 152-158.

Joshi, N., Mali, P., & Saxena, A. (1998). Effect of nitrogen and sulphur application on yield and fatty acid composition of mustard (Brassica juncea L.) oil. *Journal of Agronomy and Crop Science, 180*(1), 59-63.

Karabulut, I., & Turan, S. (2006). Some properties of margarines and shortenings marketed in Turkey. *Journal of Food Composition and Analysis, 19*(1), 55-58. doi: http://dx.doi.org/10.1016/j.jfca.2004.06.016

Karavina, C., & Mandumbu, R. (2012). Biofumigation for crop protection: potential for adoption in Zimbabwe. *Journal of Animal & Plant Sciences, 14*(3), 1996-2005.

Karuppanapandian, T., Moon, J. C., Kim, C., Manoharan, K., & Kim, W. (2011). Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. *Aust J Crop Sci, 5*, 709-725.

Kim, J. W., Kim, H. I., Kim, Y. J., & Lee, K. H. (2013). *An Experimental Study on Biodiesel Characteristics in a Common Rail Direct Injection Diesel Engine.* Paper presented at the Proceedings of the FISITA 2012 World Automotive Congress.

Kirkegaard, J., Christen, O., Krupinsky, J., & Layzell, D. (2008). Break crop benefits in temperate wheat production. *Field Crops Research, 107*(3), 185-195.

Kirschbaum, M. U. (2011). Does enhanced photosynthesis enhance growth? Lessons learned from CO2 enrichment studies. *Plant physiology, 155*(1), 117-124.

Knothe, G. (2008). Designer biodiesel: Optimizing fatty ester composition to improve fuel properties. *Energy & Fuels, 22*(2), 1358-1364. doi: 10.1021/ef700639e

Knothe, G., & Dunn, R. O. (2003). Dependence of oil stability index of fatty compounds on their structure and concentration and presence of metals. *Journal of the American Oil Chemist Society, 80*(10), 1021-1026.

Kruyt, B., Van Vuuren, D., De Vries, H., & Groenenberg, H. (2009). Indicators for energy security. *Energy Policy, 37*(6), 2166-2181.

Landero, J., Beltranena, E., Cervantes, M., Araiza, A., & Zijlstra, R. (2012). The effect of feeding expeller-pressed canola meal on growth performance and diet nutrient digestibility in weaned pigs. *Animal Feed Science and Technology, 171*(2), 240-245.

Langeveld, J., Dixon, J., & Jaworski, J. (2010). Development perspectives of the biobased economy: a review. *Crop Science, 50*(1), 142-151.

Lewis, M. W., Leslie, M. E., & Liljegren, S. J. (2006). Plant separation: 50 ways to leave your mother. *Current opinion in plant biology, 9*(1), 59-65.

Li, Y. T., Li, X. Y., Xiao, Y., Zhao, B.Q., & Wang, L.X. (2009). Advances in study on mechanism of foliar nutrition and development of foliar fertilizer application. *Scientia Agricultura Sinica, 1*, 022.

Lin, C. Y. (2013). Strategies for promoting biodiesel use in marine vessels. *Marine Policy, 40*(0), 84-90. doi: http://dx.doi.org/10.1016/j.marpol.2013.01.003

Lin, J., Gaustad, G., & Trabold, T. A. (2013). Profit and policy implications of producing biodiesel–ethanol–diesel fuel blends to specification. *Applied Energy, 104*, 936-944.

Ma, F., & Hanna, M. A. (1999). Biodiesel production: a review. *Bioresource Technology, 70*(1), 1-15.

Malik, M. A., Aziz, I., & Wahid, M. (2004). Growth, seed yield and oil content response of canola (Brassica napus L.) to varying levels of sulphur. *International Journal of Agricultural Biology, 6*(6), 1153-1155.

McInnis, M. L., Larson, L. L., & Miller, R. F. (1993). Nutrient composition of whitetop. *Journal of Range Management*, 227-231.

McKevith, B. (2005). Nutritional aspects of oilseeds. *Nutrition Bulletin, 30*(1), 13-26.

Meena, P., Awasthi, R., Chattopadhyay, C., Kolte, S., & Kumar, A. (2010). Alternaria blight: a chronic disease in rapeseed-mustard. *Journal of Oilseed Brassica, 1*(1), 1-11.

Meher, L. C., Vidya Sagar, D., & Naik, S. N. (2006). Technical aspects of biodiesel production by transesterification a review. *Renewable and Sustainable Energy Reviews, 10*(3), 248-268. doi: 10.1016/j.rser.2004.09.002

Miller, P., Lanier, W., & Brandt, S. (2001). Using growing degree days to predict plant stages. *Montana State University, USA. Extension Service*.

Miller, S. A. (2010). Minimizing land use and nitrogen intensity of bioenergy. *Environmental science & technology, 44*(10), 3932-3939.

Mohammadi, K., Eskandari, M., Rokhzadi, A., & Heidari, G. (2012). Canola traits and some soil biological indices in response to fertilizer and tillage management. *American-Eurasian J. Agric. & Environ. Sci*., *12*(3): 377-383

Morra, M. J., & Kirkegaard, J. A. (2002). Isothiocyanate release from soil-incorporated Brassica tissues. *Soil Biology and Biochemistry, 34*(11), 1683-1690. doi: http://dx.doi.org/10.1016/S0038-0717(02)00153-0

Mousavi-Avval, S. H., Rafiee, S., Jafari, A., & Mohammadi, A. (2011). Improving energy use efficiency of canola production using data envelopment analysis (DEA) approach. *Energy, 36*(5), 2765-2772.

Mozafar, A., Anken, T., Ruh, R., & Frossard, E. (2000). Tillage intensity, mycorrhizal and nonmycorrhizal fungi, and nutrient concentrations in maize, wheat, and canola. *Agronomy Journal, 92*(6), 1117-1124.

Murphy, R., Woods, J., Black, M., & McManus, M. (2011). Global developments in the competition for land from biofuels. *Food Policy, 36*, 52-61.

Murray, J., & King, D. (2012). Climate policy: Oil's tipping point has passed. *Nature, 481*(7382), 433-435.

Nigam, P. S., & Singh, A. (2011). Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science, 37*(1), 52-68. doi: 10.1016/j.pecs.2010.01.003

Nunns, J., Rohaly, J., & Center, U. (2013). Tax Provisions in the American Taxpayer Relief Act of 2012 (ATRA).

Onyilagha, J. C., Elliott, B. H., Buckner, E., Okiror, S. O., & Raney, P. J. (2011). Seed chlorophyll influences vigor in oilseed rape (*Brassica napus* L. var Excel.). *Journal of Agricultural Science, 3*(2), 73.

Orlovius, K. (2003). Oilseed rape. *Fertilizing for High Yield and Quality, Bulletin, 16*.

Pardo, L. H., Fenn, M. E., Goodale, C. L., Geiser, L. H., Driscoll, C. T., Allen, E. B., . . . Clark, C. M. (2011). Effects of nitrogen deposition and empirical nitrogen critical loads for ecoregions of the United States. *Ecological Applications, 21*(8), 3049-3082.

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M., & Dat, J. F. (2008). An overview of plant responses to soil waterlogging. *Plant Stress, 2*(1), 20-27.

Paulitz, T., Schroeder, K., & Schillinger, W. (2010). Soilborne pathogens of cereals in an irrigated cropping system: effects of tillage, residue management, and crop rotation. *Plant disease, 94*(1), 61-68.

Pauls, R. (2011). A review of chromatographic characterization techniques for biodiesel and biodiesel blends. *Journal of chromatographic science, 49*(5), 384-396.

Plank, C. O. (1992). Plant analysis reference procedures for the southern region of the United States. *Southern cooperative series bulletin*.

Pregitzer, K., & King, J. (2005). Effects of soil temperature on nutrient uptake *Nutrient Acquisition by Plants* (pp. 277-310): Springer.

Rabotyagov, S., Valcu, A., & Kling, C. L. (2012). *Reversing the Property Rights: Practice-Based Approaches for Controlling Agricultural Nonpoint-Source Water Pollution When Emissions Aggregate Nonlinearly*: Center for Agricultural and Rural Development, Iowa State University.

Rad, S., & Zandi, P. (2012). Comparison of winter and spring rapeseed cultivars considering their oil content and fatty acid composition. *American-Eurasian J. Agric. & Environ. Sci*. *12*(6): 775-780, doi: 10.5829/idosi.aejaes.2012.12.06.1750

Radinko, G., & Jeremija, J. (2012). Why biofuels. *International Journal of Research and Reviews in Applied Sciences, 13*(1), 126-132.

Ramos, M. J., Fernández, C. M., Casas, A., Rodríguez, L., & Pérez, Á. (2009). Influence of fatty acid composition of raw materials on biodiesel properties. *Bioresource Technology, 100*(1), 261-268. doi: 10.1016/j.biortech.2008.06.039

Rathke, G., Christen, O., & Diepenbrock, W. (2005). Effects of nitrogen source and rate on productivity and quality of winter oilseed rape (*Brassica napus* L.) grown in different crop rotations. *Field Crops Research, 94*(2), 103-113.

Rathmann, R., Szklo, A., & Schaeffer, R. (2010). Land use competition for production of food and liquid biofuels: An analysis of the arguments in the current debate. *Renewable Energy, 35*(1), 14-22.

Rausch, T., & Wachter, A. (2005). Sulfur metabolism: a versatile platform for launching defence operations. *Trends in plant science, 10*(10), 503-509.

Raymer, P. L. (2002). Canola: An emerging oilseed crop. *Trends in new crops and new uses*, 122-126.

Ribeiro, A. B., Basso, R. C., Grimaldi, R., Gioielli, L. A., Santos, A. O., Cardoso, L. P., & Gonçalves, L. A. (2009). Influence of chemical interesterification on thermal behavior, microstructure, polymorphism and crystallization properties of canola oil and fully hydrogenated cottonseed oil blends. *Food Research International, 42*(8), 1153-1162.

Roberts, J. A., Elliott, K. A., & Gonzalez, Z. H. (2002). Abscission, dehiscence, and other cell separation processes. *Annual Review of Plant Biology, 53*(1), 131-158.

Romano, A. A., & Scandurra, G. (2011). The investments in renewable energy sources: do low carbon economies better invest in green technologies? *International Journal of Energy Economics and Policy, 1*(4), 107-115.

Ronaghi, A., & Ghasemi, R. (2013). Soil-crop nitrogen relationships in maize grown on calcareous fields. *Journal of Plant Nutrition, 36*(7), 1120-1127. doi: 10.1080/01904167.2013.779704

Ryan, M., Kirkegaard, J., & Angus, J. (2006). Brassica crops stimulate soil mineral N accumulation. *Soil Research, 44*(4), 367-377.

Saharan, G., & Mehta, N. (2006). *Diseases of oil seed crops*: Indus Publishing.

Samborski, S. M., Tremblay, N., & Fallon, E. (2009). Strategies to make use of plant sensors-based diagnostic information for nitrogen recommendations. *Agronomy Journal, 101*(4), 800-816.

Santori, G., Di Nicola, G., Moglie, M., & Polonara, F. (2012). A review analyzing the industrial biodiesel production practice starting from vegetable oil refining. *Applied Energy, 92*(0), 109-132. doi: 10.1016/j.apenergy.2011.10.031

Sathya, G., Jayas, D., & White, N. (2009). Safe storage guidelines for canola as the seeds slowly dry. *Canadian Biosystems Engineering, 51*, 3-29.

Schierholt, A., & Becker, H. C. (2011). Influence of oleic acid content on yield in winter oilseed rape. *Crop Science, 51*(5), 1973-1979.

Scrimgeour, C. (2005). Chemistry of fatty acids. *Bailey's industrial oil and fat products*.

Seneviratne, R., Beltranena, E., Goonewardene, L., & Zijlstra, R. (2011). Effect of crude glycerol combined with solvent-extracted or expeller-pressed canola meal on growth performance and diet nutrient digestibility of weaned pigs. *Animal Feed Science and Technology, 170*(1), 105-110.

Sheikh, S., Shahnawaz, M., & Baloch, S. (2010). Effect of waterlogging on fatty acid composition of cotton (Gossipium hirsutum l.) seedling roots. *Pakistan Journal of Agriculture: Agricultural Engineering Veterinary Sciences, 26*.

Shin, J., Kim, H., Hong, S. G., Kwon, S., Na, Y. E., Bae, S. H., Kang, K. K. (2012). Effects of ultrasonification and mechanical stirring methods for the production of biodiesel from rapeseed oil. *Korean Journal of Chemical Engineering, 29*(4), 460-463.

Siddiqui, M. H., Mohammad, F., Khan, M. N., & Khan, M. A. (2008). Cumulative effect of soil and foliar application of nitrogen, phosphorus, and sulfur on growth, physico-biochemical parameters, yield attributes, and fatty acid composition in oil of erucic acid-free rapeseed-mustard genotypes. *Journal of Plant Nutrition, 31*(7), 1284-1298.

Siebold, M. and A. von Tiedemann (2013). Effects of experimental warming on fungal disease progress in oilseed rape. *Global Change Biology 19*(6), 1736-1747.

Singh, U., Sanabria, J., Austin, E., & Agyin-Birikorang, S. (2012). Nitrogen transformation, ammonia volatilization loss, and nitrate leaching in organically enhanced nitrogen fertilizers relative to urea. *Soil Science Society of America Journal, 76*(5), 1842-1854.

Smith, E. G., Janzen, H., & Newlands, N. K. (2007). Energy balances of biodiesel production from soybean and canola in Canada. *Canadian Journal of Plant Science, 87*(4), 793-801.

Stamm, M. J. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, (2011). *2011 National Winter Canola Variety Trail* (SRP 1062). Retrieved from website: http://www.agronomy.ksu.edu/extension/doc4157.ashx

Sparks, D. L., Page, A., Helmke, P., Loeppert, R., Soltanpour, P., Tabatabai, M., Sumner, M. (1996). *Methods of soil analysis part 3 chemical methods*: Soil Science Society of America Inc.

Suriyong, S. (2007). *Studies about mechanisms of oil seed deterioration under different storage conditions in oilseed rape (Brassica napus L.)*, Cuvillier Verlag.

Tańska, M., Konopka, I., Korzeniewska, E., & Rotkiewicz, D. (2011). Colour of rapeseed (Brassica napus) surface and contamination by fungi during storage of dry and wet seeds. *International Journal of Food Science & Technology, 46*(11), 2265-2273.

Tesfamariam, E. H., Annandale, J. G., & Steyn, J. M. (2010). Water stress effects on winter canola growth and yield. *Agronomy Journal, 102*(2), 658-666.

Thomas, P. (2003). *Canola Growers Manual*. Canada: Canola Council of Canada.

Turhan, H., Gul, M. K., Egesel, C. Ö., & Kahriman, F. (2011). Effect of sowing time on grain yield, oil content, and fatty acids in rapeseed (*Brassica napus* subsp. *oleifera*). *Turk. J. Agric. For, 35*, 225-234.

Venkatachalam, M., & Sathe, S. K. (2006). Chemical composition of selected edible nut seeds. *Journal of Agricultural and Food Chemistry, 54*(13), 4705-4714. doi: 10.1021/jf0606959

Wallace, H., & Sinha, R. (1962). Fungi associated with hot spots in farm stored grain. *Canadian Journal of Plant Science, 42*(1), 130-141.

Wang, R., Ripley, V., & Rakow, G. (2007). Pod shatter resistance evaluation in cultivars and breeding lines of Brassica napus, B. juncea and Sinapis alba. *Plant Breeding, 126*(6), 588-595.

Xiao, L. (2010). *Evaluation of extraction methods for recovery of fatty acids from marine products*. (Master thesis). University of Bergen, Norway.

Zegada-Lizarazu, W., & Monti, A. (2011). Energy crops in rotation. A review. *Biomass and Bioenergy, 35*(1), 12-25.

Zellou, A. M., & Cuddington, J. T. (2012). *Trends and Super Cycles in Crude Oil and Coal Prices*. (Working Paper No. 10). Retrieved from Colorado School of Mines website: http://econbus.mines.edu/working-papers/wp201210.pdf

Zhang, C. l., Li, J., Zhang, M. H., Cheng, Y. G., Li, G. M., & Zhang, S. J. (2012). Mechanical harvesting effects on seed yield loss, quality traits and profitability of winter oilseed rape (*Brassica napus* L.). *Journal of Integrative Agriculture, 11*(8), 1297-1304.

Zheljazkov, V. D., Vick, B., Ebelhar, M. W., Buehring, N., & Astatkie, T. (2012). Nitrogen applications modify seed and oil yields and fatty acid composition of winter mustard. *Industrial Crops and Products, 36*(1), 28-32.

Zhou, W., & Lin, X. (1995). Effects of waterlogging at different growth stages on physiological characteristics and seed yield of winter rape (*Brassica napus* L.). *Field Crops Research, 44*(2), 103-110.

Zhu, D., Liu, F., Shao, Y., & He, Y. (2011). Application of spad and vis/nir spectroscopy to estimation nitrogen status of oilseed rape plant. *Advances in Computer Science, Environment, Ecoinformatics, and Education* (pp. 341-347). Springer Berlin Heidelberg.

Zhu, Y., Li, Y., Colbach, N., Ma, K., Wei, W., & Mi, X. (2012). Seed losses at harvest and seed persistence of oilseed rape (*Brassica napus*) in different cultural conditions in Chinese farming systems. *Weed Research, 52*(4), 317-326.

Appendix

Table 2.

2010, 2011 and 2012 ANOVA Tables of Dry Seed Yield (kgha^{-1}) after Harvest

2010					
Source	*df*	Sum of Squares	Mean Square	*F*	*p*
Cultivar	1	54346.10	54346.10	1.31	0.27
Fertilizer Rate	3	1888472.50	629490.80	15.15	0.01
Interaction	3	75431.37	25143.79	0.60	0.62
Corrected Total	29	4779999.10			
CV = 24.99					
2011					
Cultivar	1	180276.97	180277	7.52	0.04
Fertilizer Rate	3	472446.61	157482.20	6.57	0.03
Interaction	3	185392.24	61797.41	2.58	0.16
Corrected Total	14	1019046.30			
CV = 26.87					
2012					
Cultivar	1	67306.08	67306.08	3.10	0.11
Fertilizer Rate	4	1032286.30	258071.60	11.89	0.001
Interaction	4	460562.63	115140.70	5.30	0.01
Corrected Total	25	3675608.40			
CV = 29.64					

Table 8.

2012 ANOVA Tables from Chlorophyll Meter Readings

Source	*df*	Sum of Squares	Mean Square	*F*	*p*
March 27th					
Fertilizer Rate	4	36709.32	9177.33	5.99	0.003
Cultivar	1	1471.90	1471.90	0.96	0.34
Interaction	4	6364.30	1591.07	1.04	0.41
Corrected Total	31	94965.66			
CV = 15.11					
April 10th					
Fertilizer Rate	4	21682.70	5420.68	3.27	0.03
Cultivar	1	118.03	118.03	0.07	0.79
Interaction	4	5360.47	1340.12	0.81	0.53
Corrected Total	31	161055.81			
CV = 21.21					
April 17th					
Fertilizer Rate	4	3510.32	877.58	5.10	0.01
Cultivar	1	95.16	95.16	0.55	0.46
Interaction	4	307.58	76.89	0.45	0.77
Corrected Total	31	25112.27			
CV = 8.12					

Table 10.

ANOVA Tables From the Analysis of 2012 Leaf Sample Nitrogen Concentrations

Source	*df*	Sum of Squares	Mean Square	*F*	*p*
March 27th					
Fertilizer Rate	4	2.76	0.69	0.52	0.72
Cultivar	1	0.01	0.01	0.01	0.91
Interaction	4	1.92	0.48	0.36	0.83
Corrected Total	30	30.15			
CV=28.58					
April 10th					
Fertilizer Rate	4	1.87	0.46	2.12	0.12
Cultivar	1	1.45	1.45	6.57	0.02
Interaction	4	0.32	0.08	0.37	0.82
Corrected Total	31	10.39			
CV=16.38					
April 17th					
Fertilizer Rate	4	3.69	0.92	8.2	0.001
Cultivar	1	0.07	0.07	0.68	0.42
Interaction	4	0.68	0.17	1.53	0.24
Corrected Total	30	12.87			
CV=10.19					

Table 12.

Total Fatty Acid Concentration from Mechanically Extracted Oil During 2012

Source	*df*	Sum of Squares	Mean Square	*F*	*p*
		Oleic			
Fertilizer Rate	4	2.76	0.69	0.52	0.72
Cultivar	1	92.69	92.69	67.92	0.001
Interaction	4	2.98	0.74	0.55	0.71
Corrected Total	19	473.05			
CV=1.68					
		Linolenic			
Fertilizer Rate	4	2.12	0.53	3.01	0.15
Cultivar	1	3.18	3.18	18.04	0.01
Interaction	4	0.17	0.04	0.25	0.89
Corrected Total	19	36.39			
CV = 5.78					
		Linoleic			
Fertilizer Rate	4	3.32	0.83	1.94	0.26
Cultivar	1	80.37	80.37	187.52	0.001
Interaction	4	0.45	0.11	0.27	0.88
Corrected Total	19	412.96			
CV= 2.63					
		Erucic			
Fertilizer Rate	4	0.26	0.06	0.96	0.46
Cultivar	1	2.44	2.44	34.80	0.001
Interaction	4	0.18	0.04	0.65	0.64
Corrected Total	25	4.35			
CV=45.74					