Assessing *Camelina sativa* as a fallow replacement crop in wheat production systems

by

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B.S., Kwame Nkrumah University of Science and Technology, Ghana, 2006
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
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Abstract

Emerging sustainability issues with summer-fallow period has prompted producers to identify fallow replacement crops in wheat (*Triticum aestivum*) production systems. Camelina [*Camelina sativa* (L.) Crantz] has been identified as a potential fallow replacement crop in the semiarid Great Plains. Camelina has uses in animal and human nutrition, biofuel production, and bio-based products.

Three field experiments were conducted to develop production recommendations for camelina in wheat production systems in the semiarid Great Plains. In the first study, three camelina cultivars were evaluated in mid-March (March 17, 2014; March 18, 2015), early-April (April 3, 2013; April 1, 2014 and 2015), and mid-April (April 16, 2013; April 15, 2014 and 2015) at Hays, KS. Findings from this study showed delaying camelina planting until early- or mid-April resulted in 34% increase in seed yield. Planting date affected oil concentration, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and linolenic acid concentration. The concentrations of SFA, MUFA, PUFA, linoleic acid, and linolenic acid were also different among cultivars.

A second study was conducted to evaluate the response of camelina to nitrogen (N), and sulfur (S) fertilizer application. Nitrogen rates (0, 22, 45 and 90 kg ha^{-1}), and S rates (0 and 20 kg ha^{-1}) were applied in a randomized complete block design with a split-plot arrangement. The main plots were S application rates and the subplot factor was N rates. Sulfur application did not affect seed yield, oil, protein, or seed nutrient concentration. The agronomic optimum N rate was 49 kg N ha^{-1}, however, the economic optimum N rate ranged from 25 to 31 kg N ha^{-1} based on current N fertilizer cost, and camelina seed price. Nitrogen application had no effect on SFA, MUFA, and PUFA. Moderate N application increased seed calcium (Ca) concentration, whereas higher N rate

increased zinc (Zn), and manganese (Mn) concentration in the seed. There was a general negative relation between N application with copper (Cu), and molybdenum (Mo) in camelina seed. Our study shows that camelina needed to be applied with a minimum of 25 kg N ha^{-1} for optimum production.

A third study investigated effects of crop rotation on crop yield, soil water, soil CO_2 flux, and soil health in wheat-camelina rotation systems. Rotation systems in this study were wheat-fallow (W-F), wheat-sorghum (*Sorghum bicolor*) -fallow (W-S-F), wheat-spring camelina (W-SC), and wheat-sorghum-spring camelina (W-S-SC). Crop rotation had no effect on sorghum grain yield. However, winter wheat yield decreased by 15% when fallow was replaced by camelina in the rotation system. Camelina yield in W-SC was 2-fold greater than that in W-S-SC. Soil water content in the more intensified rotations were less than rotations with fallow, irrespective of sampling period. Soil pH, phosphorus (P), and total nitrogen (TN) were not different among rotation systems. Nonetheless, soil profile N, soil organic carbon (SOC), microbial biomass carbon and N (MBC and MBN), and potentially mineralizable nitrogen (PMN) were different among rotation systems. Soil particle aggregation increased with increasing cropping intensity. This suggests improved soil structure with cropping intensification.

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Dr. Nathan Nelson

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This dissertation is dedicated to my parents, Mr. Richard Obeng and Josephine Agyarko, my sister Vera Obeng, my brother Dominic Obeng (of blessed memory), and everybody who has contributed to my educational success.

Chapter 1 - Introduction and Literature Review

1.1 Introduction

Winter wheat (*Triticum aestivum*) is the most widely grown crop in the semiarid regions of the United States Great Plains. Wheat production in the region date back to the 19th century, when German Mennonite immigrants introduced "Turkey red" wheat to Kansas in the late 1870s, after which it spread throughout the southern Great Plains states (Travis and Robb, 2009). Wheat-fallow (W-F) or wheat-summer crop-fallow are the dominant wheat production systems in the Great Plains (Anderson, 2005; Croissant et al., 2008). The fallow phase of the production system was introduced to conserve soil water in semiarid regions, which stabilize wheat yields and prevent crop failure, particularly in drier years (Saseendran et al., 2009; Nielsen and Vigil, 2010). However, use of conventional tillage operations for weed control during the fallow period was fraught with challenges which includes: (1) insufficient crop residue return; (2) soil organic matter depletion; (3) soil erosion; (4) declining soil fertility; and (5) inefficiency in soil water storage (Bowman et al., 1999). In recent times, introduction and adoption of conservation tillage practices such as reduced till (RT), and no-till (NT) has helped to curtail these problems, increased soil water storage, and allowed for cropping intensification (Smika, 1990; Halvorson and Reule, 1994; Anderson, 2005; Nielsen et al., 2011; Hansen et al., 2012).

Identifying crops that are adapted to dryland environments of the Great Plains has been a major challenge for producers and researchers. Potential crops considered as good fit should provide ground cover, protect the soil and its resources, early maturing, and should be economically profitable to the grower. Some of the crops that have been evaluated as fallow replacement crops in the Great Plains includes grain crops [e.g. corn (*Zea mays*), sorghum (*Sorghum bicolor*) (Bowman and Halvorson, 1998; Norwood and Currie, 1998; Tarkalson et al.,

2006)], legumes [e.g. soybean (*Glycine max*) (Merrill et al., 2004)], and oilseed crops [e.g. canola (*Brassica napus*), and sunflower (*Heliantus annuus*) (Merrill et al., 2004)]. Oilseed crops have been identified by the United States Department of Agriculture (USDA), and Department of Energy as one of the seven bioenergy feedstocks. It is anticipated that biofuel crops will help in efforts to mitigate global climate change that results from greenhouse gas emission from fossil fuels (Cole et al., 1997; Schneider and McCarl, 2003; Searchinger et al., 2008). In addition, biofuel crops can provide energy security, due to the finiteness of fossil fuels. The world fossil fuel reserves are expected to diminish by 2050 (Singh and Singh, 2012). Biofuels can contribute significantly to reduce our dependency on fossil fuels, and lower greenhouse gas emissions, through carbon sequestration and less carbon dioxide emissions during production and transformation of plant biomass (Bessou et al., 2011).

Oilseed camelina [*Camelina sativa* (L.) Crantz] has emerged as a potential biofuel crop for water limited environments (Hergert et al., 2011). Camelina is early maturing, and requires less inputs such as fertilizer, and water (Kagale et al. 2014). Biodiesel produced from camelina met aviation standards when it was tested in commercial airline and military jet fighters (Agusdinata et al., 2011). Other uses of camelina include adhesives, animal nutrition, and as a food processing agent (Berti et al., 2016). Studies in Montana and Wyoming showed camelina can replace fallow in W-F systems (Krall et al., 2011). Camelina production on underutilized fallow strips avoids direct competition for land use with food crops, resulting in integrated camelina production.

Although camelina has been evaluated as a potential fallow replacement crop in cropping system in the northern Great Plains and Pacific Northwest (Pavlista et al., 2016; Sintim et al., 2016; Wysocki et al., 2013; Schillinger et al., 2012), limited studies have been conducted in the central Great Plains region of Kansas. Growing camelina can provide ground cover during the fallow

period, thereby suppressing weeds, improve soil health, and diversify the wheat-based crop production system. Camelina production can increase cropping systems diversification, increase precipitation use efficiency, improve farm income, profitability, and long-term sustainability of agriculture in the region.

1.2 Wheat production in the Great Plains

The Great Plains region in the United States span from the middle of the continental USA about the 100th meridian westward to the Rocky Mountains, and from Texas north to the Canadian border (Unger and Baumhardt, 2001). The Great Plains covers several states in the central part of the US including Colorado (CO), Kansas (KS), Montana (MT), North Dakota (ND), Nebraska (NE), New Mexico (NM), Oklahoma (OK), South Dakota (SD), Texas (TX), and Wyoming (WY). This region experiences high variability in rainfall distribution, both over space and time. Scanty rainfall amounts result in drought in most seasons, with few wet years that have excess rainfall (Rosenberg, 1987). The region produces more than 60% of total wheat produced in the USA (Paulsen and Shroyer, 2008). Due to water limitations, the predominant wheat production system in the region has been W-F since the 1930s. Neither crops or weeds are allowed to grow on the field during fallow period, with the aim of enhancing precipitation storage for next wheat crop.

1.2.1 Wheat-fallow cropping system

In the W-F system, wheat is planted in September or October, and harvesting is done in June of the following year, preceded by a 14-month fallow period (Obour et al., 2015). This rotation system was developed by producers due to the semiarid climatic conditions, where annual precipitation range from 350 mm to 500 mm (Anderson, 2005). The fallow phase allows for soil water storage and recharge for the subsequent wheat crop and help prevent yield loses in drier years.

However, use of CT operations for weed control during fallow had resulted in insufficient crop residue return to the soil, depletion of soil organic matter (SOM), declining soil fertility, soil erosion and inefficient water storage (Bowman et al., 1999). Up to 60% loss of soil SOM in W-F system was reported by Bowman et al. (1990). Previous research showed less than half of the total precipitation received in the 2-year W-F system is available for wheat growth (Anderson 1998; Farahani et al. 1998), and the rest are subjected to losses through evaporation, runoff, and leaching during fallow (Anderson, 2005).

In the 1970s, advancement in the herbicide industry led to the development of broad spectrum herbicides like glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine]. Its proven efficacy simplified weed management and propelled rapid adoption of herbicides as a means of weed control (Blackshaw and Harker, 2002; Gianessi, 2005; Duke and Powles, 2008). This paradigm shift in the 1980s resulted in less tillage operations for weed control in W-F production system. The introduction of glyphosate is credited with increased adoption of conservation tillage practices (NT or RT) in wheat production systems in the Great Plains (Givens et al., 2009). Benefits of eliminating tillage include reduced soil erosion, increased soil organic matter content, less soil compaction, cool soil temperature, improved soil structure, and enhanced water infiltration, (McGregor et al., 1975; Halvorson et al., 2002; Lankoski et al., 2006). Smika (1990) found that precipitation storage increased by 20%, and wheat yields increased 14% when herbicides were used for weed control during fallow compared to tillage. The increase in soil water storage associated with NT had allowed for growing wheat in rotation with row crops like corn, sunflower, sorghum, and proso millet (*Panicum miliaceum*) (Peterson et al., 1996).

1.2.2 Intensified wheat- row crop- fallow cropping system

This system is characterized by replacing the long fallow period with a spring or summer crop which provides two crops in 3 years, instead of one crop in 2 years, and 14-months fallow period. A typical 3-yr crop rotation system starts with winter wheat planted in September through October and harvested the following June or July depending on the location in the Great Plains. Rotation schemes with cereal grain crops (corn-; proso millet-; sorghum-), legumes (as forages and grains) and oilseed crops have been evaluated in 3-yr and 4-yr cropping systems for potential replacement of the traditional W-F. These crops are harvested in late October and the land remains fallow until the following September when it is planted to winter wheat again. This cropping system allows production of two crops in three years with a 10 to 11-month fallow period (Hansen et al., 2012).

Intensified cropping increases crop yield and economic return compared to W-F system (Aase and Schafer, 1996; Dhuyvetter et al., 1996). For instance, in Akron, CO, wheat yield in W-F was 890 kg ha^{-1}, whereas land productivity in wheat-corn-proso millet-fallow was 2030 kg ha^{-1} (Anderson, 2005). Previous studies comparing W-F to a 3-yr wheat–grain sorghum–fallow (W-S-F), continuous wheat and continuous grain sorghum (S-F) using CT, RT, and NT systems in western Kansas concluded that soil water and yield in W-F and S-F are not necessarily higher than W-S-F (Norwood et al., 1990). McGee et al. (1997) showed that NT wheat-corn-fallow (W-C-F; with 10- months fallow period) rotation was equally effective in water storage as the W-F (with a 14-months fallow period) rotation in a 3-year multi-location study conducted at Sterling, Stratton, and Walsh, Colorado. Similarly, annualized grain yield and crop residue yield in a 3-yr W-S-F or W-C-F- rotation was 75% and 100% greater than W-F cropping system (Peterson and Westfall,

2004). Peterson et al. (1993) found that wheat-corn-proso millet -fallow increased net return up to 25%, compared to W-F.

Water storage and precipitation use efficiency (PUE) of the 3-yr rotation system is improved over the W-F system. Reported PUE for intensified crop production systems in the Great Plains ranged from 17 to 45% (Peterson and Westfall, 2004). The <50% PUE indicate inefficient water storage in the fallow period associated with these 3-yr cropping systems. The intensity of this cropping system could further be increased by incorporating a short-season crop in the fallow period between the time when the summer crop is harvested and planting of the next wheat crop. As suggested by Peterson and Westfall (2004), intensifying the cropping system by growing a crop to use the summer precipitation at the time it is received will increase overall crop and soil productivity of the dryland agroecosystem through improved PUE and added residue to the soil. Benefits of intensified cropping system includes soil structure improvement (Bowman et al., 1999; Wright and Anderson, 2000), increased nutrient cycling (Bowman and Halvorson, 1997; Anderson, 2005; Chen et al., 2012), breaking disease cycle (Cook and Veseth, 1991; Anderson, 1998; Krupinsky et al. 2007; Lessen et al., 2013), and weed management (Froud-Williams, 1988; Anderson, 2003; Anderson, 2008; Lessen et al., 2013) and profitability of the crop production system (Peterson et al., 1993; Dhuyvetter et al., 1996; Chen et al., 2012; Lessen et al., 2013).

1.3 Selecting potential crops for fallow replacement

The expected characteristics of crops adopted as fallow replacement crops in wheat production systems include easy management, early maturity, high resistance to disease and pest, compatible with existing farm machinery, and ability to improve farm revenue (Obour et al., 2015). Incorporating oilseeds into cereal-based rotational systems can promote crop diversity and increase profitability of dryland crop production in the Great Plains (Johnston et al., 2002). Over the years,

several oilseed crops have been evaluated in the Great Plains as fallow replacement crops and they include camelina, canola, indian brown mustard (*Brassica juncea*), safflower (*Carthamus tinctorius*), sunflower, and soybean. However, not all of the crops are good candidates for incorporation into the wheat production system in the Great Plains due to agronomic issues such as cold tolerance, water use, disease and pest resistance (Obour et al., 2015).

Growing deep rooted oilseed crops can extract water deep from the soil profile, which can affect soil water availability for the subsequent crop. In Kansas, Jaafar et al. (1993) found that 87 to 96% of sunflower roots were in the surface 165 cm, although some roots were found up to 269 cm deep. Similarly, a study in northeastern Colorado showed that sunflower can extract water to a depth of 165 cm (Nielsen, 1999), which can deplete soil water. Johnston et al. (2002) demonstrated that canola is capable of extracting water from 114 to 165 cm deep. Growing these deep-rooted oilseed crops can deplete soil water, which can pose as a limiting growth factor for the succeeding crop, especially in areas of the Great Plains that experiences low annual rainfall, and irregular distribution. For example, wheat yields following sunflower at Akron, CO were significantly reduced compared to that following fallow (Nielsen, 1999).

Growing early maturing crop as a fallow replacement crop is vital, to provide enough time for soil water recharge. Canola is very attractive to growers, and there is a readily available market in the Great Plains compared to other oilseed crops. This stems from continuous breeding efforts, varietal developments, improved oil quality and numerous options for weed control (Obour et al., 2015). Also, canola oil is considered the third most important edible oil due to reduced erucic acid content (Downey and Rimer, 1993). Disease, pest, and bird damage can cause significant yield loss in canola, and their control adds to the production cost (Obour et al., 2015).

Compared to sunflower, and canola, camelina is shallow rooted (60 cm deep), early maturing (85 to 100 days), has low nutrient and water requirements (Kagale et al. 2014). In addition, camelina is easier to manage with minimum pest and disease infestations, and is compatible with existing farm machinery. These attributes give camelina a comparative advantage as a fallow replacement crop in water-limited environments in the Great Plains.

1.4 Background of camelina

Camelina is an indigenous crop found in Northern Europe (Hulbert et al., 2012). It is also known as gold-of-pleasure or false flax, and belongs to the mustard family. Popularity of camelina waned after World War II when it was displaced by commodity grain and other oilseed crops due to lack of farm subsidies (Ehrensing and Guy, 2008). It has been reported that some plant breeding and germplasm screening occurred in the US and Canada in the 1980's (McVay and lamb, 2008). In recent years, there has been increase interest in camelina as an oilseed crop in the Great Plains region and western U.S. because of its fatty acids profile, which makes it suitable for bioenergy production (Pilgeram et al., 2007; Shonnard, 2010; Pavlista et al., 2011; Obour et al., 2015). Camelina has low input requirements (e.g. water, fertilizer and pesticide) in comparison with other biofuel feedstocks (Putnam et al., 1993), has the potential to improve sustainability and productivity of cereal-based dryland cropping systems. In addition, the unique fatty acid profile of camelina makes it a good candidate for nutritional and industrial applications (Hulbert et al., 2012). It has longer shelf life compared to other high omega-3 fatty acid oils (Eidhin et al., 2003). The seeds can be used to generate straight vegetable oil (SVO) which is less costly compared to biodiesel (Paulsen et al., 2011). Compared with combustion of diesel fuel, the SVO derived from camelina has shown to reduce net greenhouse gases by two thirds (Shonnard et al., 2010).

1.5 Camelina production in the Great Plains

In the USA, camelina production is mostly in few states in the Great Plains (North Dakota, South Dakota, Colorado, Montana, Idaho, Wyoming) and the Pacific Northwest (Oregon and Washington). Among these states, Montana has the largest acreage with 9,712 and 32,375 hectares of camelina planted in 2007 and 2011, respectively (McVay and Lamb, 2008). The large scale production in Montana was due to research efforts that were targeted at addressing camelina adaptation, agronomic management, and to supply seeds for emerging biodiesel markets. Camelina is planted between 4 and 6 kg ha^{-1} (Berti et al., 2016), and yields between 350 and 3,000 kg ha^{-1} depending on environment. Winter and spring varieties are available to growers in the Great Plains, however spring types are more popular. Spring planting is done in late February to early June, whereas winter varieties are planted in the fall from September through October (Obour et al., 2015). Winter varieties germinate, and survive the winter by going into rosette stage, and resumes growth in the spring when conditions are favorable. As at now, there are no labeled herbicides for broadleaf weed control, hence fall planted camelina can be advantageous as they emerge early in the spring, and improved plant stand can suppress weeds. In addition, fall planted varieties mature early before the onset of greater summer temperatures, which can negatively affect seed yield (Obour et al., 2015). Other challenges associated with camelina production include Downy Mildew disease, post-emergence damping off, and shattering (Hulbert et al. 2012).

1.6 Camelina growth habit

Camelina is usually grown as a summer annual, but in milder climates it can also be grown as a winter annual. The crop is shallow-rooted, about 60 cm deep (Gesch, 2013), and thrives well in water-limited environments (Putnam et al., 1993). The plant become woody upon maturity and can grow to between 30 to 80 cm tall (McVay and Lamb, 2008). The leaves are 5 to 9 cm long,

arrow-shaped, and pointed with smooth edges. It is well adapted to cooler climates, and matures between 85-100 days. The pods are teardrop-shaped with small seeds, and depending on variety and growing conditions, it can produce approximately 350,000 seeds per pound (Hulbert et al., 2012). Camelina 1,000 seed weight is between 0.8 to 2.0 grams (Ehrensing and Guy, 2008). It has better spring freezing tolerance (up to -2^{o}C) and drought tolerance compared to canola (McVay and Lamb, 2008). A controlled environment study showed camelina can emerge at 0^{o}C (Allen et al., 2014).

1.7 Camelina oil and fatty acid composition

Camelina oil content ranges between 29 and 41% (Ehrensing and Guy, 2008). The oil has a longer shelf life because of the presence of γ- tocopherol (vitamin E), which acts as an antioxidant and increases the oil's stability (Abramovic, 2007; Salminen et al., 2006). Camelina oil is low in saturated fatty acids, and high in omega-3 fatty acids, thus it is considered as a potential high-quality edible oil (Ehrensing and Guy, 2008). The fatty acids profile of the oil contains eicosenoic (20:1, 12–17%), linoleic (18:2, 15–23%), linolenic (18:3, 31–40%), and oleic, (18:1, 12–19%) acids as the major fatty acids. It contains other minor fatty acids including behenic (22:0), eicosadienoic (20:2), eicosatrienoic (20:3), erucic (22:1), palmitic (16:0), and stearic (18:0) acids (Putnam et al., 1993; Zubr, 1997; Moser, 2010; Singh et al., 2014; Berti et al., 2016). These fatty acids can be grouped into three, namely saturated fatty acids (SFA; C16:0, C18:0, and C20:0), monounsaturated fatty acids (MUFA; C18:1, C20:1, and C22:1), and polyunsaturated fatty acids (PUFA; C18:2, C18:3, C20:2, and C20:3) (Jiang et al., 2013). Variation in oil content and fatty acid composition are due to genotypic differences and environment. Obour et al. (2017) demonstrated that camelina oil and linolenic acid content were greater in cooler production environment like Montana compared to Kansas which is relatively warmer. The authors also,

found that camelina SFAs and MUFAs increased at the warm environment, when camelina experienced air temperatures above 25^{o}C during most part of flowering and grain filling period. Reasons being that heat stress during flowering and filling period negatively affects the enzymes that synthesize PUFAs, but increased SFAs and MUFAs (Singer et al., 2016).

1.8 Uses of camelina

The composition of camelina makes it suitable for diverse uses such as animal feed, seed coating, biofuel, and industrial products (biolubricants, resins, adhesives). The uses of camelina varieties can differ depending on the proportion of MUFAs to PUFAs in the oil. For example, a high ratio of MUFAs to PUFAs is desirable for biofuel production (Moser and Vaughn 2010), and varieties with erucic acid content of <2% to 5% are suitable for food purposes (EC, 1976; Food Standards Australia New Zealand, 2003).

1.8.1 Camelina use in biofuel production

Biodiesel production from feedstocks varies by location. For e.g. sunflower and canola are popular biodiesel feedstocks in Europe and Canada respectively, whereas palm and coconut oils are primarily used in tropical countries, and in the US, soybean oil and animal fats are primarily used (Moser, 2009; Moser, 2012, Moser, 2016). Studies showed that a very small proportion of the domestic diesel fuel demand at these locations can be met using these lipids. In the US, it is estimated that only 6% of biodiesel demand can be supplied if all the soybean harvested was dedicated to biodiesel production (Hill et al., 2006). The cost of refining these commodity oils can account for up to 80% of biodiesel expenses (Haas et al., 2006). Employing low cost feed stocks like camelina is an alternative to lower the cost, and increase biodiesel supply (Moser, 2016).

Camelina fatty acid composition can differ with environment, and this can result in differences in physical properties of the biodiesel produced (Moser, 2016; Berti et al., 2016; Yang

et al., 2016). Evaluation of biodiesel is based on three properties, namely: cetane number; cloud point (CP), which is used to assess cold flow; and oxidative stability. Unformulated camelina B100 fails to meet the standards for CP and oxidative stability, although it meets the standards for CP (Yang et al., 2016, Berti et al., 2016). However, this can be corrected by adding antioxidants to meet current standards, and still keep low production cost.

Camelina-based jet fuel can be produced through the standard two-step processes used in the production of renewable jet fuels. The steps are initial hydrodeoxygenation or hydrotreatment, then selective catalytic cracking or hydrocracking and isomerization, which is followed by product separation and formulation (Moser and Vaughn, 2010; Berti et al., 2016). Linear alkanes are produced after the first step (i.e. hydrodeoxygenation) and this can be used in renewable diesel mixture (Moser and Vaughn, 2010; Berti et al., 2016).

Previous research on camelina hydro-processed renewal jet (HRJ) fuel and Jet Propellant-8 (JP-8) (typical jet fuel) showed camelina HRJ has superior thermal oxidative stability than that of JP-8 (Corporan et al., 2011). The authors also reported engine operation did not experience any anomaly with the camelina HRJ, but carbon monoxide emissions were lower compared to the JP-8. Nonetheless, the camelina HRJ contained an elastomer sealing swelling which was lower than that for JP-8, and could lead to fuel leak in aircrafts (Corporan et al., 2011). In general, studies have demonstrated that camelina HRJ has similar attributes to conventional fuels use in turbine engines (Corporan et al., 2011; Sivakumar et al., 2015). Camelina-based jet fuels blended with JP have been successfully tested in fighter jets, private jets, and commercial airlines (Agusdinata et al., 2011; Berti et al., 2016).

Patil and Deng (2009) characterized camelina oil using four heterogeneous metal oxide catalysts of relatively different order of effectiveness (i.e., BaO > SrO > CaO > MgO). They found

that although BaO catalyst produced the highest biodiesel, it has limited use because of its noxious and toxic properties. The SrO catalyst (the 2nd most effective catalyst) was found to be a better catalyst, because the camelina biodiesel produced with this catalyst had properties closer to the American Society and Testing Methods (ASTM) biodiesel standards. Similarly, Ciubota-Rosie et al. (2013) evaluated camelina biodiesel with the ASTM D6751 (USA) and EN 14214 (European Union) testing standards showed that camelina-derived biodiesel does not meet all the quality specifications set by ASTM D6751 and EN 14214. However, the specifications which were not met (including cetane number and oxidation stability) could be corrected using suitable additives.

Camelina oil, *Jatropha curcas* oil, and waste cooking oil were compared as a potential for large-scale biodiesel production (Patil et al., 2009). The researchers found that there is no difference in the viscosity of the three oils compared to regular diesel, although their calorific value is lower than regular diesel because of their oxygen content. Environmentally, life cycle analysis showed that camelina-based jet fuel and biodiesel can reduce carbon emissions by 75 and 80% respectively when compared to petroleum-based fuels (Shonnard et al., 2010).

1.8.2 Camelina in animal nutrition

The cold-pressed meal left after camelina oil extraction contains 10 to 15% oil, 40% protein, 10-15% crude fiber, 23-44 moles g^{-1}glucosinolates, and 1-6% phytate, which makes it desirable for animal feed (Zubr, 2003; Singh et al., 2014; Berti et al., 2016). It also contains 15-20 % linolenic acid (an essential unsaturated fatty acid) and high percentage of omega 3 fatty acid (~36%), which is desirable in livestock diet. Camelina meal contains traces of anti-nutritive compounds such as erucic acid, sinapine, and glucocinolates (Salminen et al., 2006; Russo and Reggiani, 2012; Colombini et al., 2014; Obour et al., 2015). High erucic acid in feed meal can result in fat deposition in the heart muscle and myocardial lesions of farm animals (Morris, 1980).

Glucocinolates in animal feed can cause fertility and growth impairment, in addition to irritation in the gastro-intestinal mucosa, and local necrosis in livestock (Russo and Reggiani, 2012). Because of these reasons, the U. S. Food and Drug Administration (FDA) permits a maximum of 10% camelina meal in animal rations (Moriel et al., 2011).

Cow performance, reproductive ability, calf birth weight, weaning weight, immune health, and calf metabolism were similar among pregnant cows fed with camelina meal compared to corn or soybean meal (Loehr et al., 2009). Cherian et al. (2009) studied effect of camelina meal on egg production, egg quality, and yolk lipid content. Brown Leghorn layers were fed with corn- and soybean meal-based diet with added camelina meal at 0, 5, 10, and 15%. The authors found camelina meal at low levels (5 and 10%) did not result in changes in egg production and quality, however camelina meal >15% resulted in reduced egg production, yolk size, and yolk fat, without affecting egg weight. Evaluation of camelina meal and distiller's dry grain with solubles (DDGS) in the diets for replacement heifers showed that the reproductive performance of heifers fed on diets containing camelina meal and DDGS did not differ (Grings et al., 2015).

Pekel et al. (2009) carried out a study to compare the effect of dietary camelina meal and flaxseed on broiler chick performance, and to test whether prophylactic levels of Cu can normalize any detrimental effects of flaxseed or camelina meal. Results from that study showed adding Cu to camelina meal had only a numerical improvement on breast weight, legs, wings, and breast yield, however flaxseed meal did not have any benefits. Aziza et al. (2010) found that the inclusion of camelina meal in chicken diets led to an increase in phenolic compounds, antioxidant capacity, γ-tocopherol, and α-linolenic acid content found in chicken breast and thighs.

1.8.3 Camelina in human nutrition

The antioxidative properties of camelina has been found to be useful in the food industry to control food quality loss processes such as oxidation (Salminen et al., 2006; Eidhin and O'Beirne, 2010). Camelina antioxidation property is due to the high levels of tocopherol (Abramovic, 2007). Food oxidation leads to discoloration, flavor loss, and formation of toxic compounds. For instance, camelina oil and rapeseed meal were effectively used to inhibit oxidation of cook pork meat (Salminen et al. 2006). The authors reported that the antioxidative activity of camelina oil and rapeseed were due to the presence of sinapic acid, and its derivative, sinapine. Assessment of the oxidative stability of camelina oil and sunflower oil as components in salad dressings, and mayonnaises, showed that camelina oil was superior (Eidhin and O'Beirne, 2010). In terms of deep frying however, camelina oil was less stable compared to sunflower oil.

Camelina oil products are sold on the Canadian market, and it includes: original, roasted onion and basil, and roasted garlic and chili (Santoro, 2014). Camelina contains high content (~36%) of omega 3 fatty acids. The consumption of food high in omega 3 fatty acids help to fight inflammation, heart disease prevention, brain health, etc. (Santoro, 2014). Karvonen et al. (2002) reports that ability of camelina oil to lower serum cholesterol in hypercholestrolemic people is comparable to rapeseed and olive oils.

1.8.4 Industrial uses of camelina

Camelina oil has high amounts of unsaturated fatty acids (~90%), which makes the oil fast drying, and useful for making cosmetics, dermatological products, polymers, paints, and vanishes (Zaleckas et al., 2012; Kasetaite et al., 2014; Obour et al., 2015). Vegetable oils with high unsaturated fatty acids can be epoxidized and used in the manufacture of adhesives, coatings,

lubricants, and resins. Kim et al. (2015) showed that epoxidized camelina oil has the potential to be used for making pressure-sensitive adhesives, coatings and resins.

Camelina meal has potential in the paper industry. Paper-reinforced camelina was studied by Kim and Netravali (2012). They showed sieving camelina meal increased protein content about 4.7%, and decreased fat content, and the processing and addition of camelina meal to recycled newspaper was able to produce sustainable and bio-degradable green composite sheets and fibers. Mechanical strength and water resistance of camelina sheet and fiber also increased with an increase in recycled paper content.

1.9 Camelina agronomic research in the Great Plains

Choosing the best planting date for camelina is critical for successful production, especially in the Great Plains where uneven rainfall distribution occurs regularly. Winter camelina in the US is planted in early Fall, from September to early October (Gesch and Cermak, 2011; Gesch and Archer, 2013; Berti et al., 2015), and harvested earlier than spring type. Therefore, the winter variety stand a better chance of avoiding high summer temperatures that reduces seed yield and oil content. Studies in the Pacific Northwest and northern Great Plains of the US showed that planting between April-May (early spring) results in greater yield compared to planting in June (Gesch, 2014; Sintim et al., 2016). Reasons being that, the reproductive phase of early planted camelina coincides with favorable growth conditions. Greater air temperature at flowering and seed set can result in lower camelina yield, low oil content and low PUFAs (Obour et al., 2017). This occurs because greater air temperature during flowering shortens grain filling, and also reduces the activity of the enzymes that synthesizes camelina seed oil and PUFAs (Singer et al., 2016).

Nutrients such as nitrogen and sulfur have been identified to be critical in oilseed crop production. Putnam et al. (1993) reported that camelina has a lower nitrogen requirement compared to other oilseed crops such as canola and sunflower. Camelina produces small and greenish-yellow leaves, and matures early under conditions of N deficiency (Solis et al., 2013). Studies across the Great Plains showed that camelina N requirement in the region can range from 28 to 90 kg ha^{-1}. Nitrogen applied at 28 kg ha^{-1} was found to be optimum for camelina production in Wyoming under conditions of less rainfall (Sintim et al., 2015), whereas 90 kg ha^{-1} was the optimum in Corvallis, Oregon (Wysocki et al., 2013). This wide variation can be attributed to differences in production environment, soil type, and camelina genotype. Studies elsewhere showed that camelina yield increased with N rates up to 200 kg ha^{-1} (Jiang and Caldwell, 2016; Solis et al., 2013). Greater response to N fertilizer application in the above studies negates the notion of camelina as low input crop. Nitrogen application has been found to be positively correlated with protein content, and negatively correlated with oil content in the Great Plains (Sintim et al., 2015). Unlike N, most studies reported limited camelina response to sulfur application (Solis et al., 2013; Wysocki et al., 2013; Sintim et al., 2015; Mohammed et al., 2017). This may be due to medium to high levels of S already in the soil, and moderate soil organic matter content in the native soils, which is a good supply of plant nutrients including sulfur. However, Jiang et al. (2013) reported S application increased seed yield and oil content, and may be due to the fact that S is a co-enzyme in the synthesis of some vitamins needed for plant growth, yield, seed oil and protein synthesis.

Camelina grown in rotation or intercropped with other crops was popular back in the iron age (Larsson, 2013). In the past few years, there has been field evaluations of camelina as rotation crop with wheat (Chen et al., 2015), corn and soybean production (Gesch et al, 2014; Dobre et al.,

2014; Berti et al., 2015). Crop yields following camelina are usually unaffected, with enhanced yield reported in crops such as corn, soybean and wheat (Gesch et al., 2015). Nonetheless, in Montana, wheat yield following camelina reduced in comparison to a W-F system (Chen et al., 2015), due to less soil water availability for the subsequent wheat crop.

Other studies in the Great Plains have addressed planting method, and harvesting time. Planting at 20 mm depth with a no-till drill results in better emergence, and earlier flowering compared with seed broadcasting (Aiken et al., 2015). Sintim et al., (2016) demonstrated that delaying harvesting to 90% seed ripening results in greater seed loss compared to 50% seed ripening. Developing production recommendation for camelina in the Great Plains will provide farmers with more options for fallow replacement crops and increase revenue.

1.10 Camelina research needs in central Great Plains

Research on agronomic requirements of camelina has been carried out extensively in parts of the northern Great Plains and Pacific Northwest, however, there is limited information for the central Great Plains. These studies have covered production issues such as genotype selection (Guy et al., 2014), planting method (Pavlista et al., 2011; Schillinger et al., 2012; Aiken et al., 2015), planting date (Pavlista et al., 2011; Sintim et al. 2016, Schillinger et al. 2012), and fertility requirements (Wysocki et al., 2013; Sintim et al., 2015; Afshar et al., 2016; Mohammed et al., 2017). New environments where camelina is being introduced may still require agronomic evaluation in order to identify potential adaptation issues. Reasons being that there have been disparities in agronomic response to various recommendations. For e.g. mid-April has been reported to be the best planting date in Wyoming (Sintim et al., 2016), whereas mid-April to mid-May has been reported by Gesch (2014) in Minnesota. Optimum yield was achieved in Montana when N was applied at 60 kg N ha^{-1}. Wysocki et al. (2013) found that 17, 60, and 90 kg ha^{-1} was

the optimum N rate for Pendleton, Moscow/Pullman, and Corvallis respectively. Some of the factors accounting for these differences may be due to differences in production conditions such as temperature, rainfall, and soil type. Hence, site-specific research is needed to address production issues in camelina in new environments where camelina has not been grown previously.

Most of the fertility studies has been conducted on nitrogen and sulfur. Studies on other nutrients such as phosphorus and micronutrients are either lacking or inconclusive. Weed pressure, especially broadleaves can pose as a big challenge in camelina production, since there are no registered herbicides to control broadleaves in camelina. In addition, the critical time for weed competition is not well understood. Considerable research is needed to understand how camelina production affects soil health. Recently, the use of camelina as cover crop (Eberle et al., 2015) and relay-cropping (Berti et al., 2015; Berti et al., 2017) are in the spotlight, and research to understand its management such as inter-seeding, water use, and termination are needed. Plant breeding tools should be used to develop high-yielding cultivars, with high oil quality. Although camelina is mesophilic crop (Moser and Vaughn, 2010), developing cultivars that can tolerate warmer temperatures can expand production to southern regions of the Great Plains.

This dissertation research will develop agronomic production recommendations for camelina by testing superior camelina germplasm, determine optimum planting dates, and nitrogen and sulfur fertility requirements of camelina grown in the central Great Plains. The study will also investigate impacts of incorporating camelina in wheat-based systems on wheat yields, camelina water use and soil health.

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Chapter 2 - Seed yield and quality response to camelina cultivar and planting date

Abstract

Camelina (*Camelina sativa* (L.) Crantz*)* has been identified as a potential fallow replacement crop in non-irrigated cropping systems in US Great Plains. This study investigated the effects of planting date and cultivar on spring camelina seed yield and oil quality under non-irrigated conditions. Three spring camelina cultivars (Blaine Creek, Pronghorn, and Shoshone) were planted at three planting dates: mid-March (March 17, 2014; March 18, 2015), early-April (April 3, 2013; April 1, 2014 and 2015), and mid-April (April 16, 2013; April 15, 2014 and 2015) at Hays (KS, US). A delay in planting date until early-April or mid-April increased seed yield up to 34%. Blaine Creek (503 kg ha^{-1}) produced greater seed yield than Shoshone (356 kg ha^{-1}), however, it was not different from Pronghorn (422 kg ha^{-1}). Mid-March planting increased polyunsaturated fatty acids (PUFA), oil and linolenic acid concentrations compared to April planting dates. Whereas April plantings increased saturated fatty acid (SFA) concentration. Pronghorn had greater concentration of MUFA but less PUFA. Heat stress during the growing season reduced seed yield, oil, PUFA, linoleic acid, and linolenic acid concentrations. However, increase in precipitation amounts increased seed yield, oil, PUFA, and linolenic acid concentrations. Our findings showed planting window for spring camelina in western Kansas was early April to mid-April for optimum growth and seed yield, but depends on available soil water at planting.

2.1 Introduction

Identifying alternative crops adapted to the semiarid US Great Plains is crucial to the sustainability of wheat (*Triticum aestivum*)-based cropping systems in the region. Camelina (*Camelina sativa* (L.) Crantz) is a cold and drought tolerant oilseed with potential as a fallow replacement crop in semiarid regions of the US Great Plains (Obour et al., 2015). Integrating biofuel feedstock such as camelina into cereal-based crop production has the potential to diversify the systems in the Great Plains. Camelina is grown in many parts of the world for uses in animal (Loehr, 2009; Cherian, 2012; Grings et al., 2015) and human nutrition (Abramovic et al., 2007; Eidhin and O'Beirne, 2010), biofuel production (Patil et al., 2009; Patil and Deng, 2009), and for bio-based products (Li et al., 2014; 2015). Camelina oil consists of approximately 90% unsaturated fatty acids (Budin et al., 1995; Gugel and Falk, 2006). Greater unsaturated fatty acid concentration is critical for biofuel uses such as jet fuel and biodiesel, and bio-based products including adhesives, coatings, resins, and vanishes (Kong et al., 2013; Kim et al. 2015). In addition, the antioxidant properties of camelina have been utilized in the food industry to prevent food oxidation, which causes discoloration, flavor loss, and formation of toxic compounds (Abramovic et al., 2007).

In the US, research efforts aimed at characterizing the agronomic potential of camelina have been undertaken over the years, mostly in the northern Great Plains states such as Minnesota (Gesch, 2014), Montana (Pilgeram et al., 2007; McVay and Khan, 2011; Chen et al., 2015), North Dakota (Gilbertson et al., 2007), Wyoming (Sintim et al., 2016), and the Pacific North West (Schillinger et al., 2012). Among these locations, Montana has the largest production acreage of camelina (Pilgeram et al. 2007; NASS, 2016). In contrast, there is a paucity of camelina research in the central Great Plains. Recent studies conducted under both irrigated and non-irrigated

conditions in the central Great Plains showed camelina could be grown in this region with comparable yields to those produced in cooler environments in the northern Great Plains (Aiken et al., 2015; Pavlista et al., 2011; 2016).

Planting date is an important consideration in camelina production due to its influence on conditions of the growing environment. Temperature and soil water are important environmental conditions affecting camelina seed yield and quality. In general, high temperature can result in plant sterility, seed abortion, reduced seed number, and shortened grain filling (Hatfield and Prueger, 2011; 2015). Morrison et al. (2002) reports that heat stress reduced seed yield in *Brassica* cultivars. Other studies showed camelina oil concentration is high under low temperature conditions (Kirkhus et al., 2013; Obour et al., 2017). Obour et al. (2017) also reported that the proportion of PUFAs decreased when camelina experienced temperatures above 25^{o}C during seed development. Therefore, timing camelina planting dates to ensure flowering and seed filling coincide with periods of adequate soil water availability and favorable growing temperature is critical for camelina production.

Previous research in the Great Plains showed camelina planting date varies by location. For example, planting camelina in late February or early March resulted in superior seed yield compared to mid-April or a later planting date in Montana (McVay and Lamb, 2008). Gesch (2014) showed the optimal planting window for camelina in Minnesota ranged from mid-April to mid-May. Sintim et al. (2016) demonstrated that mid-April planting date increased seed yield and oil concentration in Wyoming. In another study in western Nebraska, Pavlista et al. (2011) reported that camelina yield was consistent when planted within late March to end of April.

There is greater variation in weather conditions in the Great Plains. Average annual precipitation in the US Great Plains ranges from <381 mm in some parts of Montana, Wyoming

and west Texas to >1270 mm in eastern Texas and Oklahoma (Shafer et al., 2014). Similarly, annual average temperature in US Great Plains ranges from 4^{o}C in the mountains of Wyoming and Montana, greater than 21^{o}C in South Texas, with extremes ranging from -56^{o}C in Montana to 49^{o}C in North Dakota and Kansas (Shafer et al., 2014). Hence, the optimum camelina planting date for the northern Great Plains may be different from central Great Plains. The central Great Plains has relatively early springs and warmer summer temperatures compared to northern Great Plains. Therefore, spring camelina planting in the central Great Plains should occur earlier to avoid greater summer temperatures during seed development since camelina is a mesophilic crop. Identifying the best planting time is critical for camelina production in the central Great Plains. We hypothesized that planting camelina in March will result in more yield, greater oil concentration and increase fatty acid composition, compared to a later planting date. The objectives of this study were to determine the effect of planting date and cultivar on (1) growth components and seed yield, and (2) protein, oil concentration, and fatty acid composition of camelina grown in the US central Great Plains.

Materials and Methods

2.1.1 Site description

This study was conducted at Kansas State University Agricultural Research Center near Hays, KS (38°86′ N, 99°27′ W, and 609 m elevation) from 2013 to 2015. The soil at the study location was mapped as a Crete silt loam (Fine, smectitic, mesic Pachic Udertic Argiustolls) formed from loess material. Before planting in each year, four composite soil samples were collected at 0-15 cm soil depth, air-dried, ground to pass through a 2-mm mesh sieve, and analyzed for soil chemical properties at the Kansas State University soil testing laboratory following standard soil test procedures (Table 2.1).

2.1.2 Study description and plot management

The experiment was arranged in a randomized complete block design with a split-plot arrangement and four replications. Planting date was assigned to the main plots and camelina cultivar as the sub-plot factor. The planting dates were mid-March (March 17, 2014; March 18, 2015), early-April (April 3, 2013; April 1, 2014 and 2015), and mid-April (April 16, 2013; April 15, 2014 and 2015). We chose these planting dates because the long-term average temperature in February at Hays, KS is below the base temperature of 5^{o}C for camelina emergence (George et al., 2015). Whereas, planting in May will prolong the growth cycle of camelina into the relatively warm summer months that will negatively affect camelina yields and oil concentration. Hence, we selected planting dates that fell between March and April. Planting in mid-March of 2013 was not possible due to inclement weather conditions.

The sub-plot treatments were spring camelina cultivars Blaine Creek, Shoshone, and Pronghorn. These camelina cultivars were chosen because they are commercially available and are well adapted to the US Great Plains. Pronghorn and Shoshone are early, and medium maturing

cultivars respectively, released in Wyoming. Whereas Blaine Creek is a medium maturing, and high yielding variety released by Montana State University. Camelina was planted at a seeding rate of 5.6 kg seeds ha^{-1} using a Great Plains no-till drill (Great Plains Manufacturing, Inc. Salina, KS) at 1.9-cm depth and with a 19.1-cm row spacing. Individual plot sizes were 9.1 × 3.0 m. The seeding rate was adjusted to percent germination percentage for each camelina cultivar in each year of the study. The study was conducted under rain-fed conditions. Urea was surface broadcast after planting at 50 kg N ha^{-1}. The entire plot area was sprayed with glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine] and Prowl H_2O [N-(1-ethylpropyl)-3, 4-dimethyl-2,6-dinitrobenzenamine] to provide pre-emergent weed control before planting camelina. Over the 3yr study period, post emergence weed control was by hand, and no pests or disease incidence occurred to warrant control measures. For both 2013 and 2015, the study was conducted on a no-till field where winter wheat was grown the previous year. The study in 2014 was established in no-till sorghum (*Sorghum bicolor*) stubble. The daily weather data, including temperature, and rainfall (Table 2.2), were obtained from the Kansas State University Mesonet (http://mesonet.k-state.edu) weather station located within 100 m of the experimental site. To examine heat stress during the growing season, we calculated heat stress index (H_i), as described by Morrison and Stewart (2002) as follows:

$$\underset{j=1}{\overset{n}{H_i}} = \sum (T_{\max} - T_{\mathrm{F}})\, \Delta t \qquad [1]$$

where $T_{\max}$ is the maximum daily temperature, T_{F} is threshold heat stress temperature (i.e. 29.5^{o}C) which results in seed yield losses for *Brassica species* (Morrison and Stewart, 2002), $(T_{\max} - T_{\mathrm{F}}) \geq 0$, Δt is a time (day) step and n is the number of days during growth stage.

2.1.3 Data collection

Data were collected on stand count at maturity, above ground biomass at maturity, 1000 seed weight, and seed yield. Time of physiological maturity was recorded when the siliques were ripened for harvest (Martinelli and Galasso, 2010). Stand count at maturity was recorded as the average value obtained by counting the number of plants within three quadrats (1 m × 0.4 m) placed randomly in each plot. Total aboveground biomass was determined by harvesting whole plants (stalk, branches, leaves, and seeds) from ground level within two quadrats (1 m × 0.4 m) taken from each plot. The samples were weighed fresh, oven-dried at 50˚C for 2 weeks and weighed again for dry matter determination. Combine yield was determined by harvesting 1.5 m × 9 m area from each plot. Seed yield was adjusted to 80 g kg^{-1} seed moisture content. Two samples of 250 seeds each were counted and weighed for each plot, and averaged for determination of 1000-seed weight.

2.1.4 Protein, oil concentration and fatty acid analysis

The oil concentration and fatty acid composition of the combined harvested seeds were quantified as described previously by Obour et al. (2017). Nine major camelina fatty acids were identified in this study, and were classified into three main groups, namely, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The SFA comprised of palmitic acid (C16:0), stearic acid (C18:0), and arachidic acid (C20:0). The MUFA category contained oleic acid (C18:1), erucic acid (C22:1), and gondoic acid (C20:1). The PUFAs consisted of linoleic acid (C18:2), linolenic acid (C18:3), and eicosadienoic acid (C20:2). In addition, the proportion of the two main PUFA, linoleic and linoleic acid were reported due to their significance in human nutrition. Seed protein concentration was analyzed using Fourier transform near-infrared spectroscopy and a specific calibration derived for a scanning

monochromater (PertenDA-7200, Perten Instruments, Hägersten, Sweden) similar to Sintim et al. (2016).

An estimate for biodiesel that can be produced from the seed yield was obtained by the procedure described by Kemp (2006). The procedure uses mechanical pressing, and assumes 80% extraction rate, which leaves oil in the by-products, and makes it suitable to be processed into livestock feed (Sintim et al., 2015). Ninety percent (90%) of the seed yield was used for the biodiesel estimation, with an assumption that 10% was lost due to post-harvest losses. Oil yield was calculated based on oil content, and converted to volume of biodiesel at 1 kg ha^{-1} to 0.439L (Kemp, 2006; Sintim et al., 2015).

2.1.5 Statistical analysis

All data were subjected to an analysis of variance (ANOVA) using the PROC Mixed procedure in the Statistical Analysis System (SAS) 9.4 software package (SAS Institute Inc., Cary, NC). Planting date and camelina cultivar were treated as fixed effects, year and block were considered as random effect. The LSMEANS procedure and associated PDIFF were used for mean comparisons. Interaction and treatment effects were considered significant when *F*-test *P*-values were < 0.05 (or < 0.1 where specified). Pearson correlation was carried out to find relationships between precipitation, heat stress and seed yield and oil quality.

2.2 Results

2.2.1 Weather conditions

Mean air temperature in March over the three years (2013 to 2015) was less than the long-term average (Table 2.2). However mean air temperature in April, May, June, and July over the three years were greater than the long-term average. In general, heat stress index increased with increasing monthly mean air temperatures. The monthly and cumulated heat stress index over the three growing seasons were greater than the long-term average (Table 2.2). Precipitation in March, April, and May over the three years was less than the long-term average (Table 2.2). Notwithstanding, precipitation in June and July over the three years were greater than the long-term average (Table 2.2).

2.2.2 Stand count, camelina seed yield, 1000-seed weight, and aboveground biomass

Planting date had significant effect on stand count at harvesting ($P < 0.0001$; Table 2.3). Stand count at harvest for early-April and mid-April planting dates were 58% greater than a mid-March planting date (Table 2.4). Stand count for Blaine Creek was 11 and 37% greater than Pronghorn and Shoshone respectively (Table 2.5). Time of planting affected camelina seed yield ($P = 0.02$; Table 2.3). Seed yield was 34% greater when camelina was planted in early- and mid-April compared to when planting was done in mid-March (Table 2.4). Seed yield for Blaine Creek was 16 and 29% greater than Pronghorn and Shoshone respectively (Table 2.5).

Planting date had no effect on 1000 seed weight. However, 1000 seed weight was different among camelina cultivars ($P = 0.01$; Table 2.3). Pronghorn and Shoshone did not show difference in 1000 seed weight, however, 1000 seed weight for Blaine Creek was 6 and 7% greater than Pronghorn and Shoshone, respectively (Table 2.5). As related to total plant biomass, time of planting affected total biomass production ($P < 0.0001$; Table 2.3). Total biomass for an early-

April and a mid-April planting date were 44% greater than a mid-March planting date (Table 2.4). Total plant biomass for Blaine Creek was 11 and 19% greater than Shoshone and Pronghorn respectively (Table 2.5).

2.2.3 Protein, oil, and fatty acid composition

Protein concentration differed among the camelina cultivars (Table 2.5). Protein concentration for Blaine Creek and Pronghorn were not different, however, protein concentration for both (Blaine Creek and Pronghorn) were 1% greater than Shoshone (Table 2.5). Time of planting had a significant ($P = 0.06$) effect on camelina oil concentration. Oil concentration was 2% greater when camelina was planted in mid-March compared to when it was either planted in early-April or mid-April (Table 2.4).

Planting date had a significant ($P < 0.05$) effect on SFA, MUFA, PUFA, and linolenic acid constituents (Table 2.3). The proportion of SFA ranged from 11.3% when planted in mid-March to 11.8% when planted in mid-April. The concentration of PUFA for early-April and mid-April planted camelina were not different, notwithstanding the early-April and mid-April planted camelina were 2% less than mid-March planted camelina respectively (Table 2.4). There were differences in SFA among cultivar ($P < 0.0001$; Table 2.3). The proportion of SFA was greater in Shoshone and Pronghorn, which were not different, but they were greater than Blaine Creek (Table 2.5).

The proportion of MUFA ranged from 34.7% when planted in mid-March to 35.8% when planted in mid-April. The concentration of MUFA for early-April and mid-April planted camelina were not different, however the early-April and mid-April planted camelina were 1% greater than mid-March planted camelina (Table 2.4). There was difference in MUFA among cultivars ($P <$

0.0001; Table 2.3). The proportion of MUFA in Pronghorn was 1% greater than Blaine Creek and Shoshone (Table 2.5).

The proportion of PUFA ranged from 51% when planted in mid-April to 53% when planted in mid-March. The concentration of PUFA for camelina planted in mid-March was 2% greater than camelina planted in both early-April and mid-April (Table 2.4). There were differences in PUFA among cultivar ($P < 0.0001$; Table 2.3). The proportion of PUFA in Blaine Creek and Shoshone were 1% greater than in Pronghorn (Table 2.5).

Linoleic acid concentration ranged from 21 to 23%, and was greatest in Shoshone, which was 1 and 2% greater than in Blaine Creek and Pronghorn (Table 2.5) respectively. Linolenic acid ranged from 26.9 to 28.7%. The concentration of linolenic acid for camelina planted in mid-March was 1 and 2% greater than early-April and late-April planted camelina (Table 2.4). Blaine Creek had the greatest linolenic acid concentration and was 1 and 2% greater than Pronghorn and Shoshone, respectively (Table 2.5).

2.3.4. Protein yield, oil yield, and estimated biodiesel production

Protein yield was different among planting dates (Table 2.3; $P = 0.02$). Protein yield was 1.5-fold greater when camelina was planted in early- and mid-April than when it was planted in mid-March (Table 2.4). Similarly, oil yield when camelina was planted in early-April and mid-April was 1.4-fold greater than when planted in mid-March (Table 2.4; $P = 0.07$). Oil yield differed among camelina cultivars (Table 2.3; $P = 0.09$). Oil yield for Blaine Creek was 17 and 29% greater than Pronghorn and Shoshone respectively (Table 2.5). Estimated biodiesel produced from camelina seed was 1.4-fold greater when camelina was planted in early-April and mid-April than when it was planted in mid-March (Table 2.4). Estimated biodiesel was different among cultivars

(Table 2.3; P = 0.09). The estimated biodiesel for Blaine Creek was 1.2 and 1.4-fold greater than Pronghorn and Shoshone, respectively (Table 2.5).

2.3 Discussion

2.3.1 Growth components and yield responses

Total precipitation in March (Table 2.2) may have influenced plant establishment when camelina was planted early. Mean precipitation across the three years in March was less compared to that in April (Table 2.2), and this accounted for the lesser plant stand when camelina was planted in March compared to April (Table 2.4). Camelina biomass production increased with increasing precipitation from planting to maturity, however total biomass reduced significantly with heat stress from planting to flowering. In our present study, precipitation from planting to maturity and total biomass were positively correlated with an r^2 of 0.20 (P = 0.07; Table 2.6), and suggests increase in precipitation accounted for 20% of the increase in total biomass (Table 2.6). On the other hand, heat stress from planting to maturity correlated negatively with total camelina biomass (P = 0.05; Table 2.6). Therefore, an increase in heat stress caused a reduction in total biomass. The lesser biomass produced with mid-March planting was mostly due to reduced soil moisture availability when camelina was planted in March compared to April planting dates (Table 2.2).

Seed yield of oilseed crops including camelina grown under non-irrigated conditions in the central Great Plains were limited by precipitation and heat stress during flowering and seed formation (Aiken et al., 2015). Reduced seed yield for camelina planted in mid-March compared to April plantings was due in part to less precipitation when camelina was planted in March (Table 2.2), that affected plant stand count and subsequent yield (Table 2.4). Precipitation from planting to maturity was positively correlated to seed yield accounting for 47% of camelina yield increase (Table 2.6). Likewise, heat stress from planting to maturity negatively correlated to seed yield with

r^2 of 0.21 (P = 0.05; Table 2.6), suggesting increase in heat stress accounted for 21% of yield loss (Table 2.6). Heat and moisture stresses at the later part of the growing season have been reported to cause premature senescence and hasten maturity in rape (*Brassica napus and Brassica rapus*) and crambe (*Crambe abyssinica*) (Adamsen et al., 2005), and in canola (Clayton et al., 2004, Chen et al., 2005). Heat stress shortens the grain filling period resulting in yield loss (Hatfield and Prueger, 2011; 2015). Previous research reported heat stress and drought reduced camelina seed yield (Berti et al., 2011; Aiken et al., 2015; Obour et al., 2017).

In our study, Blaine Creek produced greater seed yield than Pronghorn and Shoshone. Differences in seed yield among camelina cultivars has been widely reported by others (Vollmann et al., 2007; Urbaniak et al., 2008; Sintim et al., 2016). Results of our present study agrees with the findings of Sintim et al. (2016), who found seed yield of Blaine Creek and Pronghorn were greater than that of Shoshone when camelina was planted in Wyoming. Yields reported in that study ranged from 720 to 1018 kg ha^{-1}, greater than the yield range of 356 to 503 kg ha^{-1} reported in the present study. The lesser seed yield observed may be due to warmer air temperatures and uneven distribution of rainfall over the 3-yr. Guy et al. (2014) reported camelina seed yield ranging from 127 kg ha^{-1} at Lind, WA in a dry year with 174 mm seasonal precipitation to 3302 kg ha^{-1} at Pullman, WA with 587 mm seasonal precipitation.

The 1000 seed weight in the present study ranged between 1.09 and 1.16 g, which is within 1000-seed weight range (0.96 to 1.81) reported for camelina (Vollmann et al., 2007). The authors reported a decrease seed yield associated with camelina cultivars that had 1000-seed weights above 1.5 g, suggesting significantly larger seed size had limited agronomic value in camelina production. In our present study, seed yield for Pronghorn and Blaine Creek were similar, however

Blaine Creek had greater seed weight. Therefore, greater seed weight did not translate into seed yield in our study.

2.3.2 Oil concentration and other seed quality attributes

Oil concentration was superior when camelina was planted early in mid-March compared to early-April and mid-April. Camelina planted in early and mid-April experienced warmer air temperatures from 1 week pre-flowering through to 3 weeks post-flowering, compared to mid-March planted camelina (Table 2.2). The warmer air temperature during flowering resulted in lower oil concentration for camelina planted in early and mid-April (Table 2.4). This is similar to findings by other researchers who have reported a negative relationship between camelina oil concentration and high temperatures (Pavlista et al., 2011; Kirkhus et al., 2013; Obour et al., 2017). Greater oil concentration for camelina planted in mid-March did not translate into oil yield because of less seed yield compared to camelina planted in early-April and mid-April. As stated earlier, the lesser seed yield with March planting was partly due to low soil water availability that resulted in poor plant establishment. Similarly, estimated biodiesel production was greater when camelina was planted in early-April and mid-April.

The average oil concentration observed in our study is less than 32 to 33% reported in other studies in the Great Plains (Pavlista et al., 2011; Sintim et al., 2016), but are similar to the 27 to 29% reported by Pavlista et al. (2016) when camelina was rain-fed, or provided with little irrigation. Though the protein differences among planting dates in the current study were small, our results confirmed previous findings of an inverse relationship between oil and protein concentration in camelina seed (Sintim et al., 2016; Obour et al., 2017).

Fatty acid composition observed in the present study is consistent with findings by other researchers (Zubr and Matthaus, 2002; Vollmann et al., 2007; Kirkhus et al., 2013). However, the

concentrations of linoleic acid in the present study were greater than previously reported. For instance, linolenic concentration ranged from 12.6% to 16.6% in central and northern Europe (Zubr and Matthaus, 2002), 15.4 to 19.3% in Chile (Berti et al., 2011), and 19 to 20% in western Nebraska (Pavlista et al., 2016), smaller than the 21.4 to 23.4% found in the present study. However, the proportion of linolenic acid in our study (ranged from 23.2 to 30.4%) was smaller than the 36.3 to 39.7% reported by Zubr and Matthaus (2002), or 32.1 to 35.0% found in western Nebraska (Pavlista et al., 2016). In the current study, Shoshone contained more linoleic acid but produced less linolenic acid than Blaine Creek or Pronghorn cultivars, suggesting a negative association between the two fatty acids. In our study, we found that linoleic acid and linolenic acid had a negative relationship with an r^2 of 0.79 ($P < 0.0001$; Table 2.6). Other researchers confirmed an inverse relationship between linolenic and linoleic acid concentration in oilseed crops including camelina (Obour et al., 2017) and flax (Zhang et at., 2016). These results are consistent with the relative order of synthesis of these fatty acids in a developing seed, linolenic acid is formed by the desaturation of linoleic acid (Singer et al., 2016).

In the present study, Blaine Creek had the greatest proportion of PUFAs but tended to have smaller SFA and MUFAs; in agreement with previous findings reported by Obour et al. (2017). In our study, we found that PUFA and SFA had a negative relationship with an r^2 of 0.71 ($P < 0.0001$; Table 2.6). Similarly, we found that PUFA and MUFA had a negative relationship with an r^2 of 0.91 ($P < 0.0001$; Table 2.6). The proportions of PUFA, MUFA, and SFA provide useful information on the suitability of an oilseed crop for bio-based industrial and human nutrition applications (Jiang et al. 2014).

2.4 Summary and Conclusions

Delaying the time of planting until the first or second week in April when there was adequate precipitation enhanced plant establishment, biomass production, seed yield, oil yield and protein yield. Blaine Creek produced more yield, whereas Shoshone was the least yielding cultivar. Protein concentration was more in Blaine Creek and Pronghorn than Shoshone. There was variation in oil concentration when camelina was planted at different times, however protein concentration did not change. Blaine Creek and Pronghorn contained more linolenic acid than Shoshone. However, linoleic acid concentration was greater in Shoshone than Blaine Creek or Pronghorn. Planting in early or mid-April increased MUFA and SFA, however, it reduced PUFA concentration. We fail to accept the hypothesis that planting camelina in March will increase seed yield, protein yield, oil yield, SFA and MUFA. We accept the hypothesis that planting camelina in March will increase oil concentration, linoleic acid, and PUFA. Future research should be focused on investigating soil and environmental factors interacting with yield, oil and protein accumulation in camelina.

2.5 References

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Table 2.1 Chemical composition of the soil samples taken at 0-15 cm at the experimental site over the three growing seasons.

Year	pH	Organic matter† (g kg^{-1})	Phosphorous	Potassium	Calcium	Magnesium	Nitrate-N
			(mg kg^{-1})				
2013	6.7	19	62.0	704	3777	498	18.0
2014	7.1	18	20.0	502	3272	589	3.6
2015	6.4	23	17.4	632	3110	631	14.5

Soil was sampled from 0-15 cm depth and soil analysis performed using standard procedures.

†Organic matter by dry combustion using Leco C/N analyzer; pH was determined potentiometrically by an electrode (Thomas, 1996); available P by Mehilich-3 extraction method (Mehlich, 1984) and P concentration following extraction was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES);

‡Exchangeable Ca, Mg, and K concentration were determined on an ICP-OES after NH_4OAc extraction (Knudsen et al, 1982); and NO_3-N by 2 M KCl extraction procedure and N concentration determined colorimetrically by cadmium reduction (Keeney and Nelson. 1982).

Table 2.2 Climatic conditions over the three camelina growing seasons at Hays, KS.

	Mean air temperature (°C)					Heat stress index					Precipitation (mm)				
Month	2013	2014	2015	3-yr av.	Normal	2013	2014	2015	3-yr av.	Normal	2013	2014	2015	3-yr av.	Normal
January	-0.9	-2.0	-0.8	-1.2	-1.9	0	0	0.5	0.2	0	19.3	4.1	11.7	11.7	11.7
February	0.2	-3.1	-0.6	-1.2	0.4	0	0	0	0.0	0	30.2	23.4	18.0	23.9	18.8
March	4.6	3.9	7.8	5.4	5.6	0.5	0	3.8	1.4	0	19.8	4.3	2.3	8.8	32.3
April	9.3	11.8	13.1	11.4	11.8	7.1	7.6	1.1	5.3	0	26.9	23.1	24.4	24.8	53.8
May	18.2	18.3	16.3	17.6	17.2	41.2	47.1	6.0	31.4	0	54.9	20.8	163.6	79.8	82.8
June	24.7	23.3	24.6	24.2	23.0	129.9	61.2	98.9	96.7	33.5	69.3	240.0	19.3	109.5	86.9
July	33.3	24.3	26.6	28.1	25.8	135.9	98.3	144.7	126.3	125.8	179.8	59.9	104.4	114.7	84.6
Total						314.6	214.2	225	251.3	159.3	400.3	375.7	343.7	373.2	370.9

	Mean air temperature (°C)			
Planting date	1 week pre-flowering	1 week post-flowering	2 weeks post-flowering	3 weeks post-flowering
Mid-March	13.8	15.3	17.1	18.8
Early-April	15.4	18.6	19.0	21.8
Mid-April	18.9	19.4	21.5	23.0
	Mean precipitation (mm)			
Mid-March	14.7	20.7	48.9	53.2
Early-April	15.4	24.9	34.1	53.1
Mid-April	59.9	23.8	45.5	64.8

3-yr av. = Average of the three growing seasons.

Normal = 30-yr average at Hays, KS.

Table 2.3 Analysis of variance summary of the effects of cultivar and planting date on stand count, total biomass, and camelina seed yield and quality traits over three growing seasons at Hays, KS.

Effect	Stand count (plants m^{-2})	Yield (kg ha^{-1})	1000 seed weight (g)	Biomass (kg ha^{-1})	Protein (%)	Oil (%)	SFA (%)
Date (D)	<.0001	0.0217	0.7096	<.0001	0.4292	0.0615	0.0194
Cultivar (C)	0.0199	0.0671	0.0101	0.097	0.0206	0.8826	<.0001
D × C	0.2076	0.5735	0.6619	0.2749	0.5751	0.2847	0.2273

Effect	MUFA	PUFA	Linoleic acid	Linolenic acid	Protein yield	Oil yield	Biodiesel (L ha^{-1})
	%				kg ha^{-1}		
Date (D)	0.0421	<.0001	0.4603	0.0188	0.0305	0.0767	0.0767
Cultivar (C)	<.0001	<.0001	<.0001	0.0134	0.1200	0.0918	0.0918
D × C	0.1599	0.1295	0.7134	0.2932	0.4418	0.7145	0.7145

Date = Planting date; Protein = protein concentration; Oil = oil concentration; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acids.

Table 2.4 Stand count, oil concentration, linolenic acid, protein yield, oil yield, and estimated biodiesel as affected by planting date over three growing seasons at Hays, KS.

Planting date	Stand count (plants m^{-2})	Yield (kg ha^{-1})	Biomass (kg ha^{-1})	Oil (%)	SFA (%)	MUFA (%)
Mid-March	27b	317b	1628b	29a	11b	35b
Early-April	64a	481a	2895a	27b	12a	36a
Mid-April	68a	483a	2900a	27b	12a	36a
SEM	5.1	45.6	161.3	0.6	0.1	0.2
P-value	<.0001	0.0217	<.0001	0.0615*	0.0194	0.0421

Planting date	PUFA (%)	Linolenic acid (%)	Protein yield (kg ha^{-1})	Oil yield (kg ha^{-1})	Biodiesel (L ha^{-1})
Mid-March	53a	29a	97b	83b	36b
Early-April	51b	28b	144a	118a	52a
Mid-April	51b	27c	147a	119a	52a
SEM	0.3	0.4	13.7	11.9	5.2
P-value	<.0001	0.0188	0.0305	0.0767*	0.0767*

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acids. Means within column followed by the same letter are not significantly different using the least squares means (LSMEANS) multiple comparison procedure ($P < 0.05$). * = significant at $P < 0.1$. SEM = standard error of the mean.

Table 2.5 Stand count, 1000 seed weight, protein concentration, linoleic acid, linolenic acid, oil yield, and estimated biodiesel as affected by cultivar selection over three growing seasons at Hays, KS.

Cultivar	Stand count (plants m^{-2})	Yield (kg ha^{-1})	1000 seed weight (g)	Biomass (kg ha^{-1})	Protein (%)	SFA (%)
Blaine Creek	63a	503a	1.16a	2758a	30a	11b
Pronghorn	56b	422b	1.09b	2219c	30a	12a
Shoshone	40c	356c	1.08b	2446b	29b	12a
SEM	5.4	48.3	0.02	175.4	0.2	0.1
P-value	<.0001	0.0671*	0.0101	0.097*	0.0206	<.0001
Cultivar	MUFA (%)	PUFA (%)	Linoleic acid (%)	Linolenic acid (%)	Oil yield (kg ha^{-1})	Biodiesel (L ha^{-1})
Blaine Creek	35b	52a	22b	29a	126a	55a
Pronghorn	36a	51b	21c	28b	104b	46b
Shoshone	35b	52a	23a	27c	89c	39b
SEM	0.2	0.3	0.2	0.5	13.0	5.6
P-value	<.0001	<.0001	<.0001	0.0134	0.0918*	0.0918*

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acids. Means within column followed by the same letter are not significantly different using the least squares means (LSMEANS) multiple comparison procedure ($P < 0.05$). SEM = standard error of the mean. * = significant at $P < 0.1$.

Table 2.6 Pearson correlation analysis of precipitation, heat stress, camelina yield and quality traits over three growing seasons at Hays, KS.

	Precipitation from planting to maturity	HSI from planting to maturity	Biomass (kg ha^{-1})	1000 seed weight (g)	Yield (kg ha^{-1})	Protein	Oil	SFA	MUFA	PUFA	Lino	Linoln
						%						
Precipitation from planting to maturity	1	-0.32789	0.19583	0.25662	0.46703	-0.21925	0.45442	-0.51297	-0.36408	0.50471	-0.39406	0.56414
		(0.0019)	(0.0725)	(0.0562)	(<.0001)	(0.0492)	(<.0001)	(<.0001)	(0.0005)	(<.0001)	(0.0002)	(<.0001)
HSI from planting to maturity		1	-0.20876	0.22608	-0.21237	0.31386	-0.04562	0.07348	0.14859	-0.15261	-0.12387	-0.02241
			(0.0552)	(0.0938)	(0.051)	(0.0043)	(0.6748)	(0.4988)	(0.1696)	(0.1582)	(0.253)	(0.8367)
Biomass (kg ha^{-1})			1	-0.02378	0.75925	-0.03546	-0.14664	0.11845	0.13821	-0.16572	0.14956	-0.20838
				(0.8619)	(<.0001)	(0.7549)	(0.1805)	(0.2803)	(0.2071)	(0.1296)	(0.1719)	(0.0556)
1000 seed weight (g)				1	0.05016	0.48598	0.13893	-0.43761	-0.06998	0.21988	-0.19378	0.2669
					(0.7135)	(0.0002)	(0.3072)	(0.0007)	(0.6083)	(0.1035)	(0.1524)	(0.0468)
Yield (kg ha^{-1})					1	-0.07859	0.18772	-0.2108	0.02074	0.06802	-0.21483	0.1713
						(0.4884)	(0.0854)	(0.0528)	(0.8506)	(0.5362)	(0.0483)	(0.117)
Protein (%)						1	0.17916	-0.15357	0.04622	0.03292	-0.21165	0.15793
							(0.1095)	(0.1711)	(0.682)	(0.7705)	(0.0579)	(0.1591)
Oil (%)							1	-0.74992	-0.36915	0.6238	-0.59959	0.77946
								(<.0001)	(0.0004)	(<.0001)	(<.0001)	(<.0001)
SFA (%)								1	0.35367	-0.70912	0.68607	-0.87924
									(0.0008)	(<.0001)	(<.0001)	(<.0001)
MUFA (%)									1	-0.9084	-0.06768	-0.52429
										(<.0001)	(0.5334)	(<.0001)
PUFA (%)										1	-0.24891	0.78398
											(0.0201)	(<.0001)
Lino (%)											1	-0.79528
												(<.0001)

HSI = heat stress index, Protein = protein concentration; Oil = oil concentration; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acids, Lino = linoleic acid; Linoln = linolenic acid. The values in the table are Pearson correlation coefficients, and the values in bracket shows the corresponding *P*-values.

Chapter 3 - Nitrogen and sulfur application effects on camelina seed yield and fatty acid composition

Abstract

Camelina (*Camelina sativa* L. Crantz) is a short-season oilseed crop, with low nutrient requirements compared to other oilseed crops. A 3-yr experiment (2013 to 2015) was conducted to study the effects of nitrogen (N) and sulfur (S) application on camelina yield, and seed quality under non-irrigated conditions. Treatments were arranged in a randomized complete block design with a split-plot arrangement. Two S rates (0 and 20 kg ha^{-1}) were assigned as the main plot factor and four N rates (0, 22, 45 and 90 kg ha^{-1}) as sub-plots. Sulfur application had no effect on seed yield or oil concentration. Branches $plant^{-1}$ and seed pod^{-1} increased with N application, however stand count reduced with increasing N rate. A quadratic response model described the relationship between N rate and seed yield, with maximum yield occurring at 49 kg N ha^{-1}. However, the economic optimum N rate ranged from 25 to 31 kg N ha^{-1} and depended on camelina seed price and N fertilizer costs. Oil and protein concentration did not differ with N and S application. Average oil and protein concentrations for the 3 years were 26% and 31%, respectively. Nitrogen application had no effect on monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), linoleic acid and linolenic acid. Moderate N application increased calcium (Ca) concentration in camelina seed, whereas higher N rate increased zinc (Zn), and manganese (Mn). There was a general negative relation between N application with copper (Cu), and molybdenum (Mo) in camelina seed. Based on our results, camelina required a minimum rate of 25 kg N ha^{-1} for optimum production.

3.1 Introduction

Emerging sustainability issues with summer fallow in wheat production systems in the semiarid Great Plains has prompted studies to find fallow replacement crops. Cropping intensification can help producers overcome some of the problems associated with fallow, such as loss of soil organic matter, lack of residue return, soil erosion, and improve precipitation storage efficiency (Obour et al, 2015). Camelina has been identified as a potential fallow replacement crop in wheat production systems in the Great Plains (Obour et al., 2015). Typically, camelina protein concentration ranges from 45 to 47%, and oil concentration ranges from 29 to 41% (Ehrensing and Guy, 2008). The oil contains about 30% monounsaturated, 64% polyunsaturated, and 6% saturated fatty acids (Fleenor, 2011). Camelina fatty acid profile show proportions of oleic, linoleic, and linolenic acids, and other traceable fatty acids which makes it desirable for applications in feed processing, edible oil, and biofuel production (Patil et al., 2009; Patil and Deng, 2009; Jiang et al. 2014; Li et al., 2015).

Nitrogen (N) and sulfur (S) are the two most important nutrients in oilseed crop production (Nad et al., 2001). Studies to understand camelina fertility requirements have been undertaken in U.S., mostly in the northern Great Plains, where it is winter and spring planted as a rotational crop. In a study by Mohammed et al. (2017), they found that 60 kg N ha^{-1} was the optimum for seed yield, and S applied at 11 kg ha^{-1} increased yield compared to the control. Afshar et al. (2016) reports that irrespective of tillage (conventional till or no till) or N fertilizer source, N applied at 90 kg ha^{-1} produced the highest camelina yield, although there was no significant difference between this fertilizer N rate and 45 kg N ha^{-1}. Depending on the time of planting and soil water availability, 28 and 56 kg N ha^{-1} was required for camelina production in Sheridan, Wyoming

(Sintim et al., 2016). In a multilocation study by Wysocki et al. (2013) they found that optimum camelina N rate varied by location, and was 17, 60, and 90 kg ha^{-1} for Pendleton, Moscow/Pullman, and Corvallis respectively. The above authors reported no response to S application. However, in Alberta (Canada), Jiang et al. (2013) reported significant increase in seed yield due to S fertilization when N application ranged from 120 to 200 kg ha^{-1}.

The effect of N and S nutrients on seed quality, and fatty acids composition has also been reported. Jiang et al. (2013), found that N application increased protein concentration, oil yield, protein yield, and polyunsaturated fatty acids (PUFA), but decreased monounsaturated fatty acids (MUFA). In the previous study at both 0 and 25 kg S ha^{-1} rates, protein yield increased with increasing N rate, but oil concentration decreased (Jiang et al., 2013). An increase in protein concentration with increasing N rate, with a corresponding decrease in oil concentration has also been reported in Wyoming (Sintim et al., 2015) and Montana (Afshar et al., 2016), however Sintim et al (2015) did not report an effect of S on oil content. Mohammed et al. (2017) found that N application did not affect oil concentration, however the highest oil yield was achieved when S was applied at 28 kg ha^{-1}. Other studies did not detect any effect of S on oil content (Solis et al., 2013; Wysocki et al., 2013).

The differences in N and S responses in the above studies may be due to variation in crop production environment, primary related to difference in soil type, residual N, air temperature and precipitation. Apart from seed yield, camelina quality traits (protein and oil concentration, and fatty acids composition) are also influenced by air temperature and precipitation (Berti et al., 2011; Kirkhus et al., 2013). Under water stress conditions, activity of the enzymes responsible for seed oil production are hampered (Singer et al., 2016) resulting in greater protein accumulation at the expense of oil.

The central Great Plains region experience earlier springs and warmer summers than the northern Great Plains, which could affect camelina responses to N and S fertilizer application. To our knowledge, little or no studies have been conducted to investigate camelina N and S requirement in the central Great Plains. Because of greater climate variability and precipitation distribution across the Great Plains, site specific research is needed to identify the optimum N and S requirement for camelina in the central Great Plains. In addition, the effect of N and S on camelina fatty acids composition has not been studied extensively. Thus, our hypothesis was that increasing nitrogen and sulfur application would increase camelina yield, oil, protein, and fatty acids concentration. The objectives of this study were to determine N and S requirements for optimum camelina yield, and then evaluate N and S application effects on oil concentration, fatty acids and nutrient composition.

3.2 Materials and Methods

3.2.1 Site description

The experiment was conducted at Kansas State University Agricultural Research Center near Hays, KS (38°86′ N, 99°27′ W, and 609 m elevation) for three growing seasons, spring 2013 through summer 2015. The soil at the study location was Crete silt loam (Fine, smectitic, mesic Pachic Udertic Argiustolls) formed from loess material. Before planting in each year, four composite soil samples were taken at 0-60 cm depth (for nitrate-N analysis) and 0-15 cm (for other nutrient analysis), air-dried and ground to pass through a 2-mm mesh sieve and analyzed for soil chemical properties at the Kansas State University soil testing laboratory following standard soil test procedures. Soil chemical properties for the 3-yr and details of soil test procedures are summarized in Table 3.1.

3.2.2 Study design and plot management

The experiment was a randomized complete block-design with four replications in a split plot arrangement. Two S application rates (0 and 20 kg S ha^{-1}) were assigned to the main plots and four N application rates (0, 22, 45 and 90 kg N ha^{-1}) were the sub-plot factor. Blaine creek, a commercial spring camelina cultivar was used in the study. Camelina was planted at a seeding rate of 5.6 kg ha^{-1} with a no-till drill (Great Plains Manufacturing, Inc. Salina, KS) at 1.9 cm deep and 19.1 cm between rows into wheat stubble in April 17, 2013 and April 15, 2015, and sorghum stubble in April 15, 2014. Sub-plots were 9.1 m × 3.0 m. The studies were conducted under rain-fed conditions for the 3-yr. The entire plot area was sprayed with glyphosate [isopropylamine salt of *N*-(phosphonomethyl) glycine] and Prowl H_2O [N-(1-ethylpropyl)-3,4-dimethyl-2,6 dinitrobenzenamine] to provide pre-emergent weed control before planting camelina. Post

emergence weed control during the 3-yr was by hand, and no pests or disease incidence occurred to warrant control measures.

The sulfur form used in this study was disintegrating sulfur granules, S-bentonite (90% S) derived from elemental S, and the entire S rate was surface-broadcast applied to plots immediately after planting. The S fertilizer had a particle size smaller than 5 mesh (4 mm opening) and larger than 9 mesh (2 mm opening). Elemental S was used to avoid confounding effects of ammonium-based S materials with the N fertilizer treatments. Nitrogen source was urea, half-doses of the N fertilizer treatments were surface-broadcast applied at the time of planting, and the other half applied two weeks after emergence. Weather condition during the 3-yr is summarized in Table 3.2. The daily weather data including temperature, and precipitation were obtained from the Kansas State University Mesonet weather station located in the vicinity of the experimental site (~150 m north of the plots).

3.2.3 Data collection procedure

Data collected included flowering date (50% plants blooming), stand count at maturity, number of branches plant^{-1}, pods plant^{-1}, number of seeds pod^{-1}, total aboveground biomass and seed yield. Seed yield component data (stand count, branches plant^{-1}, pod plant^{-1}, seed plant^{-1} and total biomass) were recorded only for 2014 and 2015 growing seasons. Stand count at maturity was recorded by counting the number of plants within three quadrats placed randomly in each plot. The number of plants within the three quadrats were then averaged for the plot. The number of branches plant^{-1} was determined by carefully cutting five whole plants (stalk, branches, leaves, and seeds) from ground level randomly from each plot at physiological maturity (when 90% of the matured seeds were dried, and had turned brown) and then the number of branches in each plant

was counted and averaged. The number of pods plant^{-1} was recorded by carefully counting the number of pods in the 5 randomly selected plants used to determine the number of branches plant^{-1}. Ten pods were randomly collected from the above plants and seeds counted to determine the number of seed pod^{-1}. Total aboveground biomass was determined by harvesting whole plants from ground level within two quadrats taken from each plot. Samples were weighed fresh and oven-dried at 50^{o}C for one week and weighed again for dry matter determination. Camelina seed yield was determined by harvesting 1.5 m × 9 m area from each plot using a plot combine (Hege 125 plot combine, Wintersteiger Inc., Salt Lake City, UT). Seed moisture content was determined using a DICKEY-john grain moisture tester (DICKEY-john Inc., Auburn, IL), and seed yield adjusted to 8% moisture content. Two samples of 250 seeds each were counted and weighed for each plot, and averaged for 1000-seed weight determination.

3.2.4 Seed protein, oil concentration, fatty acids, and nutrient analysis

Camelina seed nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), boron (B), and molybdenum (Mo) were determined according to AOAC (AOAC, 1990) methods. Oil and fatty acid composition was analyzed by a procedure described by Miquel and Browse (1992) with minor modifications as described by Obour et al. (2017).

Nine major camelina fatty acids were identified in this study, and were classified into three main groups, namely, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). The SFA comprised of palmitic acid (C16:0), stearic acid (C18:0), and arachidic acid (C20:0). The MUFAs proportion comprised of oleic acid (C18:1), erucic acid (C22:1), and gondoic acid (C20:1). The PUFAs consisted of linoleic acid (C18:2),

linolenic acid (C18:3), and eicosadienoic acid (C20:2). In addition, the proportion of the two main PUFA fatty acids, linoleic and linoleic acid were reported due to their significance in human nutrition. Seed samples collected at harvest from each plot were analyzed for N concentration by dry combustion using a LECO CN analyzer (LECO Corporation, St. Joseph, MI). Then, the protein concentration was calculated by multiplying the N content by 6.25.

3.2.5 Economic analysis

A quadratic response curve was fitted to describe camelina yield response to N applied. The three years were analyzed together since we were interested in the general response of camelina to N application. Using the current N fertilizer price of \$0.72 kg^{-1} N and current camelina seed price range of \$0.20 to \$0.35 kg^{-1} (Chen et al., 2015), the economic optimum N rate ($EONR$) was calculated with equation 1 and $Nmax$ was calculated using equation 2.

$$EONR = PR - \beta_1/(2 * \beta_2) \quad [1]$$

$$Nmax = -\beta_1/2\beta_2 \quad [2]$$

where parameters β_1 and β_2 are coefficients of the quadratic response function (i.e. $y = \beta_0 + \beta_1 X - \beta_2 X^2$); price ratio ($PR$) = price of N fertilizer/camelina seed value; and $Nmax$ = agronomic maximum N rate.

3.2.6 Statistical analysis

All data were subjected to ANOVA analysis using the Proc Mixed procedure in the SAS 9.4 software package (SAS Institute Inc., Cary, NC). Sulfur rate and N application rate were treated as fixed effects, and year and block were considered as random effect. The LSMEANS procedure and associated PDIFF were used for mean comparisons. Interaction and treatment effects were considered significant when *F* test *P* values were ≤ 0.05. The response model of yield components,

seed yield, and quality traits to N application was determined using a contrast statement (i.e. polynomial contrast). Linear and quadratic models were fitted to the variables. In cases where both linear and quadratic models were significant ($P < 0.05$), the General Linear F-test was performed to identify if the linear or quadratic model was significant at $P < 0.05$. The reduced model (i.e. linear model) was selected as the significant model when the null hypothesis was accepted (F calculated < F critical), whereas the full model (i.e. quadratic model) was selected as the significant model when we failed to accept the null hypothesis (F calculated > F critical). Principal component analysis (PCA), was performed to investigate relationships between seed yield and quality traits, and to find climatic variables that could best predict variations in camelina seed yield, oil concentration and fatty acid composition.

3.3 Results and discussion

3.3.1 Weather conditions

In general, growing season precipitation was not uniformly distributed with most of the recorded precipitation amounts below the long-term average (Table 3.2). For instance, precipitation distribution in the 2013 season was very poor with 4-out of 5 months been drier than long-term average. Similarly, precipitation amounts in June 2014 was 2.8-fold greater than the long-term average but April and May were drier that the long-term average. In 2015, about 85% of the growing season precipitation occurred in the months of May and July. Mean growing season air temperature during flowering and seed set were relatively less in 2014 compared to that recorded in 2013 and 2015 growing seasons (Table 3.2). The combination of greater air temperatures and less precipitation resulted in relatively less than ideal growing conditions for camelina production particularly in the 2013 growing season.

3.3.2 Yield components

Stand count at maturity showed a linear response to nitrogen application. Application of N fertilizer decreased plant stand, with stand at maturity ranging from 100 plants m^{-2} when no N fertilizer was applied to 88 plants m^{-2} with 90 kg N (Table 3.4). The decline in plant density with N application is possibly due to increased plant competition, thus increased vegetative growth earlier in the growing season which caused the plants to till out. Plant density at harvest decreased with increasing N application rates (Jiang and Caldwell, 2016), consistent with the results of the present study. Our findings are in contrast to others (Malhi et al., 2014; Solis et al., 2013) who found N application had no detrimental effects on plant stand. Camelina standard population density is 190 plants m^{-2}, but 50 to 70% reduction in stand density had no effect on seed yield

(McVay and Khan, 2011). Due to the greater plasticity to compensate for stand loss, reduced plant density with N application may not cause a significant decrease in grain yield (Table 3.4).

There were linear and quadratic effects on total biomass as a function of N application (Table 3.4). However, based on the general linear *F*-test the full model did not provide any better fit than the reduced model. Application of N fertilizer had a significant effect (P = 0.03) on aboveground biomass produced. Averaged across year and S rate, total aboveground biomass ranged from 3259 kg ha^{-1} for the control to 3800 kg ha^{-1} when N fertilizer was applied at 45 kg N ha^{-1} (Table 3.4). There were linear and quadratic effects on number of branches $plant^{-1}$ as a function of N fertilizer application (Table 3.4). The general linear *F*-test showed that the full model did not provide any better fit than the reduced model. However, the number of pods were not affected by either N or S fertilizer application. The number of pods $plant^{-1}$ averaged 217 pods $plant^{-1}$ over the study period. Seeds pod^{-1} showed both linear and quadratic response to nitrogen application. Nonetheless, based on the general linear *F*-test the full model did not proved any better fit than the reduced model. The application of N fertilizer had an effect on the number of seeds pod^{-1} (Table 3.4). Averaged across year and S rate, the number of seeds ranged from 7 seeds pod^{-1} with the control to 9 seed pod^{-1} when N was applied at 90 kg N ha^{-1} (Table 3.4). Branches $plant^{-1}$ showed both linear and quadratic response to nitrogen application (Table 3.4). The general linear *F*-test showed that the full model did not provide any better fit than the reduced model. The increase in the branches $plant^{-1}$ and seed pod^{-1} with N application in camelina is consistent with other studies (Urbaniak et al., 2008; Solis et al., 2013; Jiang and Caldwell, 2016).

3.3.3 Seed yield

Sulfur × N rate interaction had no effect on camelina seed yield. Similarly, S application had no effect on camelina seed yield (Table 3.3). The contrast test showed that were linear and quadratic effects on seed yield as a function of N application. However, based on the general linear *F*-test, the full model did not provide any better fit than the reduced model. In our current study, residual S was two to three times greater than S applied (Table 3.1). However, we did not see S response, and this could be due to the following reasons. First, although elemental sulfur has a high S concentration (i.e. 90% S), S applied must undergo oxidation through the action of *Thiobacillus* bacteria to convert it to available from i.e. sulfur sulfate form (SO_4). Environmental conditions affecting the conversion process includes temperature, moisture, aeration, soil pH, and soil fertility status. Unfavorable environmental conditions such as excessively dry conditions, waterlogged conditions, alkaline soils, temperatures below 13 to 15^oC, and low fertility soils can slow down the availability of elemental sulfur. In our study, spring camelina was planted in spring when temperature and precipitation were low, and was harvested before the warm summer temperatures. These factors and the short life cycle of the crop could have resulted in low S availability during camelina growth, hence the no yield response to S application. Our findings agreed with previous studies that showed S application had no effect on camelina seed yield (Solis et al., 2013; Wysocki et al., 2013; Sintim et al., 2015). The forms of sulfur used in studies by Soils et al. (2013), Wysocki et al. (2013), and Sintim et al. (2015) were gypsum, ammonium sulfate/ammonium thiosulfate, and elemental sulfur respectively.

Nitrogen application affected camelina seed yield ($P < 0.001$). Averaged across year and S rate, seed yield ranged from 559 kg ha^{-1} with no N to 738 kg ha^{-1} with 45 kg N ha^{-1} (Table 3.4).

A quadratic function described the relationship between camelina seed yield and N applied (Fig. 3.1). Applying N beyond 22 kg N ha^{-1} resulted in no significant yield benefit (Fig. 3.1). Based on the quadratic curve, the maximum N required to maximize camelina yield was 49 kg N ha^{-1} (i.e. agronomic optimum N rate). However, the economic optimum N rate ranged from 6 to 40 kg N ha^{-1} based on N fertilizer costs and camelina grain price (Table 3.7). Changes in relative fertilizer N and camelina seed price influenced the economic optimum N rate. In our current study, economic optimum N rate was relatively sensitive to changes in price ratio (i.e. N fertilizer/ camelina seed value). For instance, when price ratio increased from 2 to 6, economic optimum N rate decreased by 34 kg N ha^{-1} from 40 to 6 N ha^{-1} (Fig. 3.3). In general, at greater camelina seed price, N fertilizer price had less effect on the economic optimum N fertilizer rate. However, at a lower seed price of \$0.20 kg^{-1}, economic optimum N rate varied significantly with N fertilizer price, ranging from 6 kg N ha^{-1}, when N fertilizer costs was \$1.20 to 25 kg N ha^{-1} when N fertilizer cost was set at \$0.72 kg^{-1} (Table 3.7). At a current N fertilizer price of \$ 0.72 kg^{-1} N and current camelina seed price range of \$0.20 to \$0.28 kg^{-1} (Chen et al., 2015), the optimum N fertilizer application rate ranges from 25 to 31 kg N ha^{-1} for our environment.

Nitrogen rate of 56 kg N ha^{-1} was found to maximize camelina seed yield in year one of a study in Wyoming (Sintim et al., 2015). However, the authors found only 28 kg N ha^{-1} was needed to maximize yields when camelina was planted late in year two with limited growing season soil water availability. This suggest camelina response to N fertilizer is highly dependent on growing season precipitation. Nitrogen fertilizers for e.g. urea must undergo hydrolysis and requires the action of urease enzyme before it is converted to available N form i.e. ammonium-N (Havlin et al., 2005). Hence there will be less N available for plant uptake when moisture is lacking in the soil, and this can affect seed yield. This is supported by the PCA in our current study which showed

cumulative precipitation from planting to flowering is the dominant factor that explained most of the variation in camelina seed yield (Fig. 3.2). In a multi-location study in the Pacific Northwest, optimum N rates for spring camelina ranged from 0 to 90 kg N ha^{-1} depending on annual precipitation and available soil N (Wysocki et al., 2013). In a recent study in Montana, camelina seed yield with only 45 kg N ha^{-1} was not statistically different from that obtained with a combination of 134-22-22-28 kg ha^{-1} N-P_2O_5-K_2O-S (Mohammed et al., 2017). The agronomic optimum N rate obtained in that study was 60 kg N ha^{-1}. The N rates from the above studies are within the range of optimum N rates obtained in the present study for western Kansas. Nonetheless, camelina response to greater N application rates has been reported in higher yielding environments. For instance, maximum camelina seed yields were achieved with N rates ranging from 170 to 200 kg N ha^{-1} in Canada (Malhi et al., 2014; Jiang and Caldwell, 2016), and 185 kg N ha^{-1} in Chile (Solis et al., 2013).

Nitrogen and sulfur application did not affect 1000-seed weight. Average 1000-seed weight was 1.2 g. The 1000-seed weights reported in the current study is consistent to the range of 0.98 to 1.8 reported by other researchers (Vollmann et al., 2007; Obour et al., 2017). The supply of assimilates to the seed to a large extent determines seed weight (Egli and Bruening, 2001) and this occurs between anthesis and maturity (i.e. seed filling). Therefore, in our study perhaps, the supply of nutrients to the seeds during this period was not significant enough to cause differences in 1000-seed weight among the treatments. The plausible reason may be, for e.g. although there was a 0 kg N ha^{-1} treatment, residual nutrients in the soil at the beginning was adequate to supply the nutrients needed for seed formation.

3.3.4 Seed protein, oil, fatty acids, and nutrient composition

Application of N and S had no significant effect on protein concentration ($P > 0.05$; Table 3.3), although protein showed a linear response to N application (Table 3.4). These findings are in contrast with previous studies that showed increase protein concentration with N and S fertilization (Jiang et al., 2013). In that study, the increase in protein with no S fertilizer ranged from 24% with 20 kg N ha^{-1} to 26% with 120 kg N ha^{-1}, and with 25 kg ha^{-1} of S, protein concentration was 24 and 27% for the same N rates respectively. Similarly, Sintim et al. (2015) showed N rate had an effect on camelina protein concentration with values ranging from 29.3% for the unfertilized check treatment to 30.4% for the treatment that had 112 kg N ha^{-1}. Though not statistically significant, the difference in protein concentration in the above study was < 1%, which is similar to the response observed in the present study (31.8% for the control and 32.3% with 90 kg N ha^{-1}).

Similar to protein, neither N nor S application significantly affected oil concentration (Table 3.4). This agrees with the findings of Sintim et al. (2015) and Mohammed et al. (2017) who found neither N or S fertilizer application had a positive effect on oil concentration in camelina grown in Wyoming and Montana. Nitrogen fertilizer application had been reported to decrease oil concentration in camelina (Jiang et al., 2013; Solis et al., 2013; Sintim et al., 2015; Mohammed et al., 2017). This decrease in oil content is possibly due to increase in N availability that results in increased synthesis of proteins at the expense of fatty acids due to competition for carbon skeletons during carbohydrate metabolism (Rathke et al., 2005).

There were linear and quadratic effects on protein yield and estimated biodiesel, as a function of N application. However, based on general linear *F*-test, the linear model was significant at $P < 0.05$ for protein yield and estimated biodiesel (Table 3.5). Protein yield and estimated

biodiesel produced increased with N application, possibly due greater seed yield response to N application (Table 3.5). Similar results were reported in other studies conducted in the US Great Plains (Sintim et al., 2015; Mohammed et al., 2017). In the present study, protein yield produced with 45 kg N ha^{-1} increased 25% over the control. Similarly, estimated biodiesel produced with 45 kg N ha^{-1} increased 1.3-fold above the unfertilized check (Table 3.5).

Application of N had no significant ($P > 0.05$) effect on the proportion of SFA, MUFA and PUFAs (Table 3.5). This is in contrast with Jiang et al. (2013) who showed a decrease in proportion of MUFA with increasing N rates, however, PUFAs content did increase when N rates increased irrespective of S application level. This disparity could be due to differences in environment and camelina cultivar used. The proportion of MUFA ranged from 31.0 % to 32.7 % and that of SFAs ranged from 9.2 to 9.9% (Jiang et al., 2013), which were less than 34.8 to 35.2% and 11.8 to 11.9% respectively found in the present study. However, PUFAs content is the present study were less than the range of 56.2 to 57.7% observed in the above study.

The proportion of linolenic acid in the present study (ranged from 26.9 to 27.3%) was smaller than 36 to 40% reported by Zubr and Matthaus (2002), 36 to 39 by Kirkhus et al. (2013) or 32 to 35% found in western Nebraska (Pavlista et al., 2016). Nevertheless, the concentration of linoleic acid in the present exceeded those reported in the above studies. For example, linoleic content ranged from 12.6% to 16.6% in the study by Zubr and Matthaus (2002), 15.3 to 16.5 % in Kirkhus et al. (2013) and 19 to 20% in western Nebraska (Pavlista et al., 2016), which were less than 22.8 to 23% found in the present study (Table 3.5). Linoleic acid is an important fatty acid for brain and eye development, and the prevention of heart diseases (Jiang et al., 2013).

Sulfur application did not affect all the measured nutrient concentrations of camelina seeds (Table 3.6). Moderate N application increased Ca concentration, whereas higher N application rate

increased Zn and Mn contents. Also, there was a general decrease in Cu and Mo content with N application. There were no significant effects of N application on N, P, K, S, Mg, Fe, and B of camelina seeds (Table 3.6). Studies indicate that deficiency in S can inhibit plants' use of N, whereas high N can create S deficiency (Jamal et al., 2010). The S fertilizer source and slow S mineralization may be the reason why we did not see S fertilizer response in some of the measure parameters. This is evident in the lack of S application effect on S concentration in the seeds (Table 3.6).

3.4 Summary and conclusion

Our study shows that N application had an effect on stand count, number of branches, seeds pod^{-1}, seed yield, plant biomass, protein yield, and estimated biodiesel production. However, we did not see an effect of N application on protein concentration, oil concentration, fatty acids and nutrient composition. Sulfur application did not affect growth components, seed yield, or quality traits. Our findings suggest that the optimum N rate for camelina production in our study location is 22 kg N ha^{-1}.

Our results support our hypothesis that increasing N application would increase seed yield, oil, and protein concentration. We reject the hypothesis that increasing N and S application would increase fatty acids concentration. The results show that camelina is a rich source of nutrients, and unsaturated fatty acids which makes it suitable for industrial application such as animal feed, adhesives, and biodiesel production. Future studies can be conducted to test higher S rates and different S sources to see if there will be any response.

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Table 3.1 Pre-planting soil chemical properties at the experimental site, Hays, KS in 2013, 2014 and 2015.

Year	pH	Organic matter	NO_3-N	SO_4-S	P	K
		g kg^{-1}	kg ha^{-1}		mg kg^{-1}	
2013	6.4	20	34.0	76	56	670
2014	7.3	10	26.5	67	22	439
2015	7.1	20	26.7	44	37	582
	Ca	Mg	Zn	Fe	Mn	Cu
	mg kg^{-1}					
2013	2598	471	1.1	29	143	2.1
2014	4364	732	0.4	10	44	1.3
2015	4496	997	0.8	23	79	1.6

Soil was sampled from 0-60 cm for nitrate and sulfate analysis, and 0-15 cm depth for the other nutrient analysis. Assumed bulk density is 1.33. Soil analysis performed using standard procedures.

Organic matter by dry combustion using Leco C/N analyzer; pH was determined potentiometrically by an electrode (Thomas, 1996); available P by Mehilich-3 extraction method (Mehlich, 1984) and P concentration following extraction was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES);

‡Exchangeable Ca, Mg, K, Zn, Fe, Mn, and Cu concentration were determined on an ICP-OES after NH_4OAc extraction (Knudsen et al, 1982); and NO_3-N by 2 M KCl extraction procedure and N concentration determined colorimetrically by cadmium reduction (Keeney and Nelson. 1982).

Table 3.2 Mean air temperature, and total precipitation monthly precipitation in Hays, KS, in 2013, 2014, and 2015.

	Mean air temperature				Precipitation			
Month	2013	2014	2015	30-yr av.	2013	2014	2015	30-yr av.
	ºC				mm			
January	-0.9	-2.0	-0.8	-1.9	19.3	4.1	11.7	11.7
February	0.2	-3.1	-0.6	0.4	30.2	23.4	18.0	18.8
March	4.6	3.9	7.8	5.6	19.8	4.3	2.3	32.3
April	9.3	11.8	13.1	11.8	26.9	23.1	24.4	53.8
May	18.2	18.3	16.3	17.2	54.9	20.8	163.6	82.8
June	24.7	23.3	24.6	23.0	69.3	240.0	19.3	86.9
July	33.3	24.3	26.6	25.8	179.8	59.9	104.4	84.6
Total					400.3	375.7	343.7	370.9

30-yr av. = 30-year average

Table 3.3 Analysis of variance of the effects of nitrogen and sulfur on camelina growth and yield components, seed quality traits, and seed mineral concentration.

Effect	Stand count	Branches plant^{-1}	Seeds pod^{-1}	1000 seed weight (g)	Yield (kg ha^{-1})	Biomass (kg ha^{-1})	Oil (%)	Protein (%)	Protein yield (kg ha^{-1})	Biodiesel (L ha^{-1})	LIN (%)	LINO (%)	SFA (%)	MUFA (%)
Srate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Nrate	*	***	**	NS	***	**	NS	NS	***	***	NS	NS	NS	NS
Srate × Nrate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	PUFA (%)	N	P	K	S	Ca	Mg	Zn	Fe	Mn	Cu	B	Mo	
		g kg^{-1}						mg kg^{-1}						
Srate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
Nrate	NS	NS	NS	NS	NS	*	NS	**	NS	**	*	NS	**	
Srate × Nrate	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

Srate = Sulfur rate; Nrate = Nitrogen rate; LIN = Linoleic acid; LINO = Linolenic acid; SFA = Saturated fatty acids; MUFA = Monounsaturated fatty acids; PUFA = Polyunsaturated fatty acids; N = seed nitrogen concentration; P = seed phosphorus concentration; K = seed potassium concentration; S = seed sulfur concentration; Ca = seed calcium concentration; Mg = seed magnesium concentration; Zn = seed zinc concentration; Fe = seed iron concentration; Mn = seed manganese concentration; Cu = seed copper concentration; B = seed boron concentration; Mo = seed molybdenum concentration.

NS (not significant) at $P > 0.05$; * Significant at $P< 0.05$; ** Significant at $P< 0.01$; *** Significant at $P< 0.001$.

Table 3.4 Effect of nitrogen application on stand count, branches per plant, seeds per pod, 1000-seed weight, biomass, seed yield oil and protein concentration over 2-years (2014 and 2015).

Nitrogen rate (kg ha^{-1})	Stand count (plants m^{-2})	Branches $plant^{-1}$	Seeds pod^{-1}	1000 seed weight (g)	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Oil (%)	Protein (%)
0	100a	34b	7b	1.18a	3259b	559b	25.1a	31.8a
22	88b	33b	9a	1.20a	3678b	669ab	25.3a	32.0a
45	92b	32b	9a	1.19a	3800a	738a	25.6a	32.1a
90	88b	41a	9a	1.18a	3714b	659ab	26.0a	32.3a
SE	5.9	1.7	0.03	0.03	463	62.5	1.4	0.80
L (*P*-value)	0.041	0.005*	0.001*	0.698	0.029*	0.025*	0.06	0.022
Q (*P*-value)	0.247	0.009	0.024	0.338	0.022	0.001	0.981	0.872
			General linear *F*-test					
F-calculated		3.9	0.6		0.4	2.5		
F-critical		4.0	4.0		3.9	3.9		

SE = Standard error; Polynomial contrast: L = Linear; Q = Quadratic; * = Significant model at $P < 0.05$ based on general linear *F*-test.

† Seed yield, oil and protein concentration were taken over three years.

Table 3.5 Effect of nitrogen application on protein yield, biodiesel, oil concentration and fatty acid composition over three growing seasons at Hays, KS.

Nitrogen rate (kg ha^{-1})	Protein yield (kg ha^{-1})	Biodiesel (L ha^{-1})	LIN (%)	LINO (%)	SFA (%)	MUFA (%)	PUFA (%)
0	182c	62c	23.0a	26.9a	11.8a	35.2a	51.5a
22	218b	76a	22.9a	27.3a	11.8a	35.0b	51.7a
45	243a	85a	22.8a	27.3a	11.9a	34.8b	51.7a
90	218b	76a	23.0a	27.1a	11.8a	35.0b	51.7a
SE	24.0	8.8	0.6	1.1	0.3	0.3	0.5
L (*P*-value)	0.018*	0.004*	0.700	0.907	0.786	0.576	0.576
Q (*P*-value)	0.001	<.001	0.406	0.325	0.642	0.180	0.443
			General linear *F*-test				
F-calculated	1.9	2.1					
F-critical	3.9	3.9					

LIN = Linoleic acid; LINO = Linolenic acid; SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty acids; PUFA = Polyunsaturated fatty acids; SE = Standard error; Polynomial contrast: L = Linear; Q = Quadratic; * = Significant model at $P < 0.05$ based on general linear *F*-test.

Table 3.6 Sulfur and nitrogen effects on the nutrient concentration of camelina seeds.

S rates (kg ha^{-1})	N	P	K	S	Ca	Mg	Zn	Fe	Mn	Cu	B	Mo
	g kg^{-1}						mg kg^{-1}					
0	53.9	8.81	10.9	8.0	2.77	2.96	51.9	137	24.1	6.13b	8.88	0.48
20	54.5	8.8	10.9	8.04	2.77	2.96	51.7	121	23.9	6.24a	9.00	0.51
SE	0.26	0.08	0.09	0.09	0.04	0.02	0.67	5.89	0.31	0.08	0.28	0.02
P-value	0.160	0.919	0.833	0.621	0.954	0.988	0.809	0.086	0.635	0.365	0.753	0.192
N rates (kg ha^{-1})												
0	54.0	8.9	10.9	8.0	2.78ab	2.9	50.6b	126	23.4b	6.35b	9.04	0.56a
22	53.8	8.8	11.1	7.9	2.87a	3.0	51.1ab	123	23.5b	6.18ab	8.84	0.53ab
45	54.3	8.8	10.8	8.0	2.73b	2.9	52.6a	122	23.9ab	6.18ab	8.97	0.45b
90	54.6	8.6	11.0	8.0	2.7b	2.9	52.9a	145	25.3a	6.03a	8.91	0.44b
SE	0.27	0.10	0.10	0.10	0.05	0.02	0.71	8.25	0.42	0.08	0.29	0.03
P-value	0.056	0.197	0.237	0.565	0.017	0.097	0.007	0.185	0.006	0.023	0.931	0.006

Means within treatment followed by same letter are not significantly different using the least squares means (LSMEANS) at $P < 0.05$.

Table 3.7 Economic optimum nitrogen rate as a function of N fertilizer price and camelina seed value.

	Economic optimum N rate					
	Camelina value (US$ kg^{-1} seed)					
N price	0.20	0.22	0.25	0.28	0.30	0.35
US $ kg^{-1} N	kg N ha^{-1}					
0.72	25	28	31	31	35	40
0.84	21	24	27	27	32	38
0.96	16	19	24	24	29	35
1.08	11	15	20	20	25	33
1.20	6	10	16	16	22	30

Table 3.8 Price ratio as a function of N fertilizer price and camelina seed value.

N price	Price ratio					
	Camelina value (US$ kg^{-1} seed)					
(US $ kg^{-1} N)	0.20	0.22	0.25	0.28	0.30	0.35
0.72	4	3	3	3	2	2
0.84	4	4	3	3	3	2
0.96	5	4	4	3	3	3
1.08	5	5	4	4	4	3
1.20	6	5	5	4	4	3

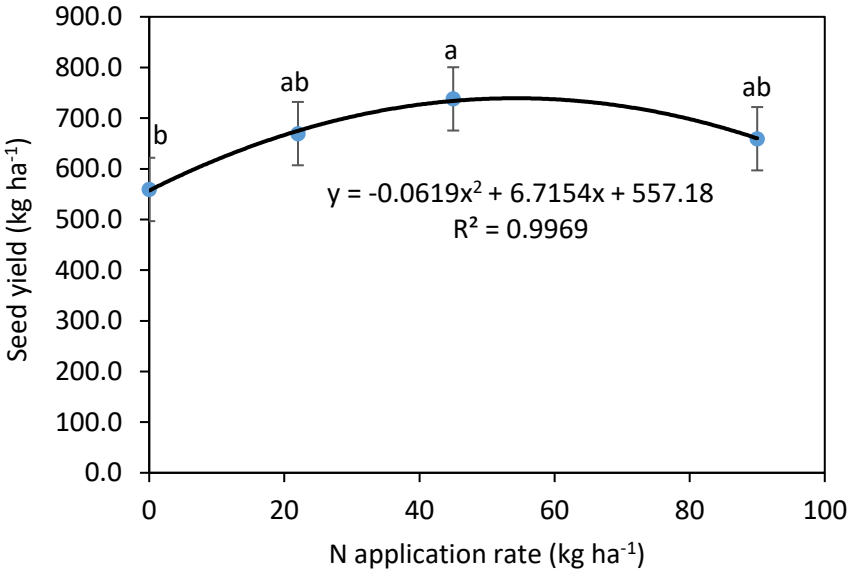

Figure 3.1 Camelina seed yield response to N rate over three growing seasons.

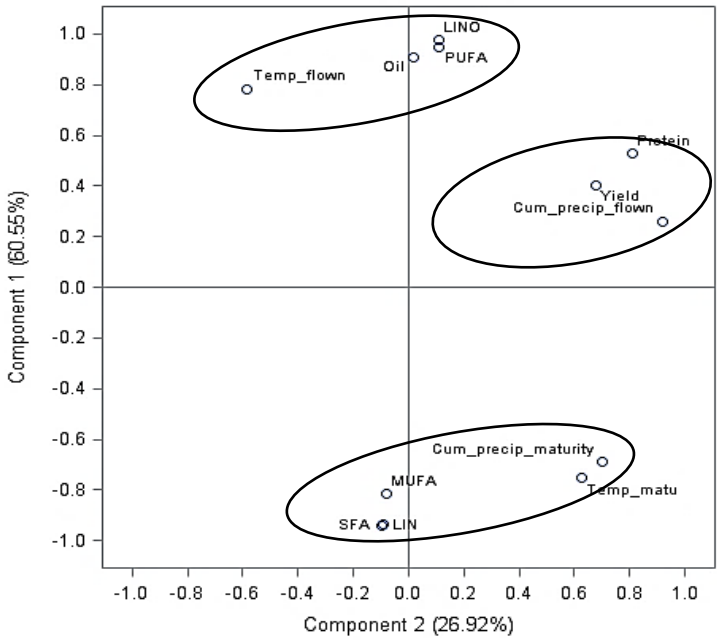

Figure 3.2 Principal component analysis of weather conditions (temperature and precipitation from planting to maturity), yield and seed quality traits.

Cum_precip_flown = Cumulative precipitation from planting to flowering (mm); Cum_precip_maturity = Cumulative precipitation from planting to maturity (mm); Temp_flown = Mean air temperature at flowering (ºC); Temp_matu = mean air temperature at maturity (ºC); LIN = Linoleic acid (%); LINO = Linolenic acid (%); MUFA=Monounsaturated fatty acids (%); PUFA = Polyunsaturated fatty acids (%); SFA = Saturated fatty acids (%); Oil = Oil concentration (%); Protein = Protein concentration (%); Yield = Seed yield (kg ha^{-1}).

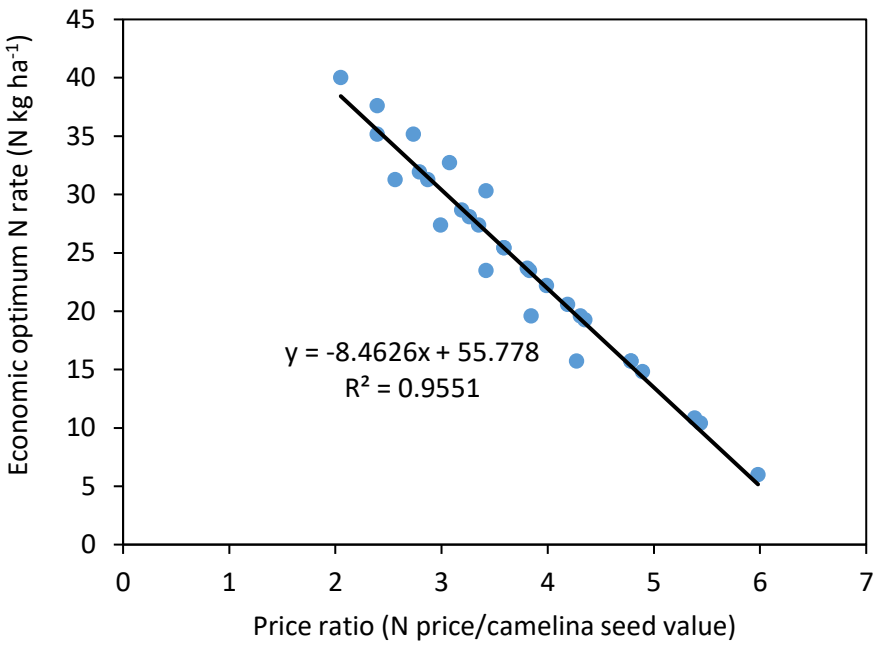

Figure 3.3 Relationship of fertilizer N: camelina seed price ratio on economic optimum N rate.

Chapter 4 - Cropping sequence influenced crop yield, soil water, and nutrient cycling in wheat-camelina cropping system

Abstract

Integrating camelina (*Camelina sativa* L. Crantz) into wheat (*Triticum aestivum* L) -based cropping systems can potentially increase land productivity and farmer income. However, there is limited information on how this can affect soil resources. This study investigated the effect of replacing fallow with camelina on crop yield, soil water content, soil CO_2 flux, and soil health. Treatments were four crop rotation systems, namely wheat-fallow (W-F), wheat-sorghum (*Sorghum bicolor* (L) Moench) -fallow (W-S-F), wheat-spring camelina (W-SC), and wheat-sorghum-spring camelina (W-S-SC). Crop rotation had no effect on sorghum grain yield. There was a 15% reduction in wheat yield when camelina replaced fallow in the rotation. Camelina yield was two-fold greater when planted after wheat (W-SC) compared to that after sorghum in the 3-yr rotation system. Soil water content was less in the more intensified crop rotations compared to systems with fallow regardless of sampling time. Soil biological activity in the rotations increased from spring to summer when the soil had more moisture and high temperatures, and was least in W-S-SC in the summer of 2016 compared to the other rotation systems. Soil profile N (measured at 0-60 cm) ranged from 6 to 15 kg N ha^{-1}, and was greatest in the W-F system. Soil pH, phosphorus (P), and total nitrogen (TN) averaged 6.2, 38 kg ha^{-1}, and 0.14% respectively, and were not different among the crop rotation systems. Soil organic carbon varied among crop rotations, and was least in W-F (1.4%). Soil microbial biomass carbon (MBC) was greatest in W-S-SC, whereas W-SC had less microbial biomass nitrogen (MBN) irrespective of sampling time. Rotations with camelina (W-SC and W-S-SC) had greater potentially mineralizable nitrogen (PMN). Increasing cropping intensity increased the proportion of larger water stable soil aggregates, while the less

intensified system (W-F) had greater proportion of smaller aggregates. This suggests improved soil structure with cropping intensification. Although the inclusion of camelina in the rotation system added soil health benefits to the system, wheat yield was reduced.

4.1 Introduction

Wheat production in the Great Plains goes back as far as the 19th century (Travis and Robb, 2009), and has contributed significantly to the socio-economic development of the region. The fallow phase in wheat production was introduced to stabilize wheat yields by helping recharge and soil water storage (Saseendran et al., 2009; Nielsen and Vigil, 2010). In the wheat-fallow (W-F) system, winter wheat is planted in September or October and harvested in June of the following year. This is then proceeded by 14-months of fallow (Obour et al., 2015). Conventional tillage used for weed control during the fallow period destroy crop residue and results in loss of soil organic matter, soil erosion, and inefficient soil water storage (Bowman et al., 1990; Anderson 1998; Farahani et al. 1998). In recent years, the use of herbicides for weed control during fallow, and the development of conservation tillage practices (e.g. no till and reduced till) has supported more frequent cropping due to increased soil water storage.

Intensified cropping system can utilize the soil water that is lost during the fallow period. Furthermore, cropping intensification can help reduce soil erosion by providing ground cover, improved soil quality through residue return and nutrient cycling (Andersen, 1999). In the intensified 3-yr wheat rotation system, there is 10 to 12-month fallow period. Crops that have been evaluated in 3-yr and 4-yr rotations in wheat production systems in the Great Plains include corn [*Zea mays*] (Bowman and Halvorson, 1998; Norwood and Currie, 1998; Tarkalson et al., 2006), grain sorghum [*Sorghum bicolor* (L.) Moench] (Norwood et al., 1990; Norwood, 1994) legumes [e.g. soybean (*Glycine max*) (Merrill et al., 2004)], and oilseed crops [e.g. canola (*Brassica napus*), and sunflower (*Heliantus annuus*) (Merrill et al., 2004)]. Recently, camelina has been identified as a potential oilseed crop that can be incorporated into the wheat production system (McVay and Lamb, 2008; Chen et al., 2015; Obour et al., 2015).

Camelina is a short-seasoned oilseed crop which is believed to have originated from Northern Europe (Hulbert et al., 2012). Production requirements for nutrients (nitrogen and sulfur), water, equipment, and pesticides are less demanding compared to other oilseed crops (Putnam et al., 1993). Camelina can be used in the manufacture of adhesives, animal feed, biodiesel, vanishes, and food processing agent (Salminen et al., 2006; Li et al., 2015; Yang et al., 2016). Due to the short life cycle (85 to 90 days), camelina can replace portions of the fallow period allowing ample time for soil water recharge before planting the subsequent wheat crop. Despite camelina's potential as a fallow replacement crop, very limited cropping system studies have been conducted to investigate camelina as a fallow replacement crop in the Great Plains.

A study by Chen et al. (2015), shows that winter wheat yield in drier years was 13% greater in W-F, than when winter wheat was planted after spring camelina. Similar trend of reduced winter wheat yield after spring camelina was reported by Hess et al. (2011). Reasons for the reduced yield could be due to less soil water availability for the subsequent crop (Nielsen and Vigil, 2005; Aiken et al., 2015). Aside soil water availability, cropping sequence can affect quality and quantity of crop residue and subsequent changes in soil organic matter, microbial processes and nutrient cycling (Bockus and Shroyer, 1998; Andersen, 1999). Anderson (2005), reports that N measured at 1.8 m depth was greater in W-F compared to continuous cropping. Bowman and Halvorson (1998) showed that phosphorus in winter wheat in continuous cropping was 13 to 30% greater than when fallow was in the rotation. Soil organic matter can increase water stable aggregation by slowing down rapidly moving water entering soil aggregates (Tisdall and Oades, 1982; Mrabet et al., 2001).

There is limited information on soil health, and the environmental impact of wheat-camelina rotation systems, and these need to be addressed. Producers, researchers, and decision

makers require quantified information on cropping system to assess the risk level of incorporating new crops into existing cropping systems. Rotation effects may include and not limited to disease incidence, insects, pathogen, weeds, nutrient cycling, and soil water use. This information can provide the basis of the need for amendments such as fertilizer, irrigation, pesticides, and herbicides. We hypothesized that crop yield will not reduce with cropping intensification, and soil health benefits will increase with increasing cropping intensity. The objective of this study was to investigate the effect of replacing fallow with camelina on crop yield, soil water, soil CO_2 flux, and soil health indicators.

4.2 Materials and Methods

4.2.1 Site description and study design

Field experiments were conducted at Kansas State University Agricultural Research Center near Hays, KS (38°86′ N, 99°27′ W, and 609 m elevation) to investigate the potential of incorporating camelina into non-irrigated cropping systems in the central Great Plains. The study was conducted under no-till conditions with four crop rotation treatments, namely; W-F, wheat-sorghum-fallow (W-S-F), wheat-spring camelina (W-SC), and wheat-sorghum-spring camelina (W-S-SC). The crop rotation treatments were replicated four times in a randomized complete block design. All phases of each of the crop rotation schemes were present in each block in each year of the study. Individual plot size was 10.7 m × 6.1 m. The soil at study location was mapped as Crete silt loam (Fine, smectitic, mesic Pachic Udertic Argiustolls) formed from loess material. Soil samples were taken from 0-15 cm depth before starting the experiment in fall 2013. The soil samples were air-dried, and sieved through a 2-mm mesh sieve, and analyzed for soil chemical properties at the Kansas State University Soil Testing lab. Briefly, soil organic matter was determined by dry combustion using Leco CN analyzer (LECO Corporation, St. Joseph, MI). Soil pH was determined potentiometrically by an electrode (Thomas, 1996). Soil nitrate-N was extracted using 2 M KCl extraction procedure, and N concentration determined colorimetrically by cadmium reduction (Keeney and Nelson. 1982). Available phosphorus (P) was determined by Mehlich-3 extraction method (Mehlich, 1984). Exchangeable calcium (Ca), magnesium (Mg), and potassium (K) concentration were determined on an ICP-OES after NH_4OAc extraction (Knudsen et al, 1982). Soil pH (6.7); organic matter (15 g kg^{-1}); nitrate N (2.2 kg ha^{-1}); P (20 mg kg^{-1}); K (528 mg kg^{-1}); Ca (3376 mg kg^{-1}); and Mg (677 mg kg^{-1}).

4.2.2 Plot management

Winter wheat variety 'Danby' was planted October of each year at 67 kg ha^{-1} in 19-cm row spacing. Spring camelina (cv. Blaine Creek) was usually planted in April in 19-cm row spacing at 5.6 kg ha^{-1} seeding rate. Grain sorghum (Sorghum Partners hybrid NK5418) was planted the first week in June in 38-cm row spacing at 65,000 seeds ha^{-1}. Planting of all three crops was done using a Great Plains 3P100GNT drill (Great Plains Manufacturing, Inc. Salina, KS).

Pre-emergent weed control in camelina was done using glyphosate [isopropylamine salt of *N*-(phosphonomethyl) glycine] and Prowl H_2O [N-(1-ethylpropyl)-3,4-dimethyl-2,6 dinitrobenzenamine]. In season weed control in wheat was done using 2, 4-dichlorophenoxyacetic acid and Methyl 3-[[[[(4-methoxy-6-methyl-1, 3, 5- triazin-2-yl) amino] carbonyl] amino] sulfonyl]-2-thiophenecarboxylate when needed. Herbicides for weed control in grain sorghum were a pre-mixture of 25.3% of [alachlor, 2-chloro-2',6'-diethyl-N-(methoxymethyl) acetanilide] and 15.3% of [atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino) s-triazine]. Before the study began, P was applied to the entire study area at a rate of 60 kg P_2O_5 ha^{-1} in fall 2013. Nitrogen fertilizer in urea form was applied at 56 kg N ha^{-1} to both grain sorghum and winter wheat before planting, and 45 kg N ha^{-1} to spring camelina two weeks after emergence.

4.2.3 Harvesting and seed quality analysis

Determination of grain yield was done by harvesting 1.5 m × 11 m area from the middle section of each plot using a small combine harvester. Spring camelina was usually harvested by mid-July, whereas winter wheat and sorghum were harvested in June and October respectively. Camelina, sorghum, and wheat yields were adjusted to 8%, 13.5% and 13.5% grain moisture content respectively. After harvesting, camelina seed oil and protein content was determined using Fourier transform near-infrared spectroscopy (Antaris II FT-NIR Spectrophotometer Analyzer) as

described by McVay and Khan (2011). Crop yield on annual basis (i.e. annualized yield) was calculated by summing yields for all the crops in the rotation system, and dividing by the total number of years in the system cycle (Peterson and Westfall, 2004).

4.2.4 Crop water use and residue measurement

Water use by each rotation system was determined using a neutron attenuation probe (Gardner, 1986). Aluminum access tubes were installed in each plot to a depth of 1.2 m for soil water monitoring. Neutron attenuation were lowered into the access tubes to 100 cm in 25 cm depth increments i.e. 0 to 25 cm, 25 to 50 cm, 50 to 75 and 75 to 100 cm. Readings were taken prior to planting, and every 3 weeks during the growing season. The neutron probe was calibrated against soil gravimetric water content (GWC) from soil samples taken in the plot area. The soil GWC was converted to volumetric water content (VWC) by multiplying the GWC by bulk density (Bd) at each depth. Soil water in the profile was estimated by summing soil water content in all the depths.

$$Gravimetric\ water\ content\ (gg^{-1}) = \frac{Wet\ \ soil - oven\ dried\ soil}{oven\ dried\ soil} \quad [1]$$

$$Bulk\ density\ (Bd;\ g\ cm^{-3}) = \frac{mass\ of\ dried\ soil}{volume\ of\ dried\ soil} \quad [2]$$

$$Volumetric\ water\ content\ (VWC) = GWC\ \times \text{Bd} \quad [3]$$

$$Soil\ water\ (by\ depth) = VWC\ \times \text{depth} \quad [4]$$

Water use by each crop rotation system was determined by adding stored water (i.e. soil water in profile at planting minus soil water in profile at harvesting) and effective rainfall, with an assumption that deep percolation, ground water use, and surface runoff were negligible. Water use and water use efficiency (WUE) was then calculated using the following formula:

$$Water\ use = (Soil\ water\ at\ harvesting + Precipitation) - soil\ water\ at\ planting \quad [5]$$

$$\text{Water Use Efficiency (WUE)} = \frac{Yield}{Water\ use} \quad [6]$$

Surface residue return and ground cover was measured after camelina and winter wheat harvest in August 2015 and 2016 on each plot of the rotation system. Residue samples were randomly collected from two (1 m × 0.4 m) quadrats in the central rows and composited for each plot. Residue samples were washed with deionized water to remove soil, and oven dried at 65 ^{O}C until constant weight for dry matter determination. Ground cover was taken using the meter stick method (Laflen et al., 1981). Briefly, a meter stick was placed on each plot, and each cm point intercepting with residue was counted, and the percentage residue estimated.

4.2.5 Soil health assessment

4.2.5.1 Soil sampling and processing

Four batches of soil samples were taken for soil health assessment. Briefly, first batch of soil samples were taken in August 2016 from fallow plots at 0-7.5 cm, 7.5-15 cm, 15-30 cm, and 30-60 cm, and air dried. The soil was ground through 2-mm sieves, and analyzed for soil inorganic N. Second batch of soil samples was taken in August 2016 at 0-5cm from both fallow and wheat stubble plots. The samples were air dried, ground through a 2-mm sieve and analyzed for soil phosphorous, pH, soil organic carbon (SOC), and total nitrogen (TN). Third batch of soil samples were taken in August 2016 at 0-5 cm, sieved through a 6-mm mesh screen to remove large plant materials and stored at 4^{O}C until they were analyzed for microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and potentially mineralizable nitrogen (PMN). Fourth batch of undisturbed soils was taken in two groups: one in August 2016 with soil bulk density sampler at 0-5 cm for bulk density determination. The other was in September 2017 with a shovel at 0-5 cm and used for soil aggregate size determination. The 0-5 cm was selected because short-term changes in soil properties will most likely occur in the upper soil surface.

4.2.5.2 Soil chemical properties

Soil inorganic N

The air-dried soil samples taken at 0-7.5 cm, 7.5-15 cm, 15-30 cm, and 30-60 cm, were analyzed for soil inorganic N. Briefly, soil inorganic N was extracted by adding 30 mL of 2 M KCl to 3 g of soil sample. The soil samples were shaken for 1 h on an orbital shaker set at 200 rpm, and filtered through Whatman no. 42 filter paper. The collected extract was analyzed calorimetrically for NO_3-N using AQ2 Discrete Analyzer (Seal Analytical, Inc., Mequon, Wisconsin).

Soil extractable P

Soil P was extracted using Mehlich 3 extract (Mehlich, 1984) for soil samples taken at 0-5 cm soil depth because P is relatively immobile in the soil. About 20 mL of Mehlich 3 solution was added to 2 g of air dried soil. The soil samples were shaken for 5 mins on an orbital shaker set at 200 rpm, and filtered through Whatman no. 42 filter paper. The collected extract was analyzed calorimetrically for extractable P using AQ2 Discrete Analyzer (Seal Analytical, Inc., Mequon, Wisconsin).

Soil pH, soil organic carbon (SOC) and total nitrogen (TN)

Soil pH within 0-5 cm depth was determined using 1:2 soil to water volume ratio. The mixture was stirred and allowed to stand for 30 mins, after which soil pH was measured using a pH meter (Thomas, 1996). Portions of the air-dried soil samples at 0-5 cm depth were finely ground with a mortar and piston and screen through 1-mm sieves. The sieved samples were then analyzed for SOC and TN by combustion using a C N Analyzer (EA 112) (Mikha and Rice, 2004).

4.2.5.3 Soil biological properties

Soil microbial biomass C and N

Soil samples were collected in August 2016 from both fallow, and wheat stubble plots. Four composite samples were taken at 0-5 cm from each plot using a sampler of 7.5 cm in diameter, and 5 cm in height. The field moist soils were passed through 6-mm mesh sieve to remove large plant materials. Soil samples were stored in zip lock bags at 4^{o}C until analysis. Before microbial analysis, soil water content for each sample was determined, and adjusted to 0.28 kg kg^{-1} (i.e. 60% water filled pore space). Water was added to soil samples with gravimetric water content less than 0.28 kg kg^{-1} to adjust it back to this level.

The field moist soils were analyzed for microbial biomass C and N using the fumigation-incubation method (Jenkinson and Powlson, 1976). Two 25 g soil samples were weighed from each plot into two 125 mL Erlenmeyer flasks. One set of the flasks were fumigated with chloroform in a vacuum desiccator which contained a beaker with 70 mL of ethanol-free chloroform with a boiling chip and wet paper towels. The desiccator was sealed with vacuum grease, and evacuated under a laboratory hood for 2 minutes to allow chloroform to boil for each round of evacuation. Evacuation was done 3 times. At the end of the third evacuation, the desiccator was closed tightly to allow chloroform to diffuse through the soil. Both the fumigated and non-fumigated samples were placed in a dark at room temperature for 24 h. After 24 h, the beaker and paper towels were removed, and the desiccator was evacuated 3 minutes each time for 10 times. Both the fumigated and non-fumigated samples were placed in 940 mL mason jars containing water (~50 mL), and tightly closed to maintain a humidified atmosphere. The samples were incubated at 25^{o}C for 10 days. After the 10 days' incubation, the headspace of the jars of both samples were sampled for evolved CO_2-C i.e. soil microbial respiration using a syringe. The CO_2-C collected from both the fumigated and non-fumigated samples were analyzed using a gas

chromatograph (Shimadzu GC-8A, Shimadzu Scientific Instruments, and Columbia, MD) (Gajda and Martyniuk, 2005). The difference between evolved CO_2 from fumigated and non-fumigated soils, divided by a conversion factor (Kc) of 0.45 (fraction of biomass C mineralized into to CO_2) was calculated to give MBC (Jenkinson and Powlson, 1976). Microbial biomass N (MBN) was determined by adding 100 mL of 1 M KCl to both fumigated and non-fumigated soil. The samples were shaken for 1 h on an orbital shaker set at 300 rpm, and filtered through Whatman no. 42 filter paper. The collected extract was analyzed calorimetrically for inorganic N (NH_4-N and NO_3-N) (Gelderman and Beegle, 1998; Maynard et al., 2006) using AQ2 Discrete Analyzer (Seal Analytical, Inc., Mequon, Wisconsin).

The difference between inorganic N measured in fumigated and non-fumigated soil, divided by a conversion factor (Kc) of 0.54, gave the MBN (Jenkinson and Powlson, 1976). Potentially mineralizable N (PMN) was estimated as the difference between inorganic N (NH_4-N and NO_3-N) in field moist soil sample, and inorganic N (NH_4-N and NO_3-N) in non-fumigated incubated soil samples (Maynard et al., 2006; Gugino et al., 2009).

Soil biological activity assessment

Soil biological activity was quantified by field soil CO_2 flux measurements following the method described by Parking and Doran (1996) with slight modifications. The in-situ soil CO_2 flux measurements were done using an automated chamber system (LI-8100, LICOR Biosciences, Lincoln, NE, USA). Measurements were made in stationary chambers made from PVC tubing (10 cm inner diameter, 5 cm in height) which were inserted randomly in each plot at a depth of 2.5 cm, from 9 a.m. to 12 p.m. CST each sampling period. Plant biomass were clipped from the interior collar of the chamber a day before each sampling period. This was to ensure that there was minimal soil disturbance during the sampling period, and to ensure accuracy. Soil CO_2 flux measurements

were made at a 45-second dead band interval, and 1-minute sampling period. The LI-8100 sampling flow rate was set to the lowest setting available as suggested by the user manual. Around the same time, soil temperature measurements were taken and soil samples collected at 0-10 cm using a soil probe to determine gravimetric soil water content. Individual sampling periods were July 2015 (camelina harvest), October 2015 (sorghum harvest), March 2016 (camelina planting), June 2016 (wheat harvest), August 2016 (after camelina harvest), and in November 2016 (after wheat planting).

4.2.5.4 Soil physical properties

Soil bulk density, and porosity

Soil bulk density was determined by the core method (Blake and Harge, 1986). Briefly, two undisturbed soil samples were randomly taken at 0-5 cm soil depth from each plot using a steel cylinder of 221 cm^3 volume (7.5 cm in diameter, and 5 cm in height). The samples were then dried at 105 ^{O}C to determine oven dry weight, and bulk density computed as dry weight of soil divided by the sample volume. Soil porosity and water filled air space were calculated as described by Danielson and Sutherland (1986). Calculations were done as follows:

$$Bulk\ density\ (Bd;\ g\ cm^{-3}) = \frac{mass\ of\ dried\ soil}{volume\ of\ dried\ soil} \quad [7]$$

$$Total\ Porosity\ (TP;\%) = \left(1 - \frac{Bd}{Pd}\right) \times 100 \quad Pd = \text{particle density; assume 2.65 g cm}^{-3} \quad [8]$$

$$Gravimetric\ water\ content\ (\Theta g;\%\ or\ g g^{-1}) = \frac{wet\ soil - oven\ dried\ soil}{oven\ dried\ soil} \times 100 \quad [9]$$

$$Volumetric\ water\ content\ (\Theta v;\%\ or\ cm^3 cm^{-3}) = Bd \times \Theta g \text{ or } \frac{water\ by\ volume}{volume\ of\ soil} \quad [10]$$

$$Air\ filled\ porosity\ (AFP;\%\ or\ cm^3 cm^{-3}) = TP - \Theta v \quad [11]$$

$$Water\ filled\ pores\ space\ (WFPS;\%\ or\ cm^3 cm^{-3}) = \Theta v / TP \quad [12]$$

Wet aggregate stability (WAS)

The method of wet stable aggregates assessment was similar to that described by Nimmo and Perkins (2002) and Kemper and Rosenau (1986), with slight modifications. Three undisturbed composite soil samples weighing a total of about 1 kg, were collected randomly from each plot at 0-5 cm depths using a spade. The soil samples were placed in paper bags. Samples were transported to the lab and air dried for 1 week. Samples were sieved through 8 mm mesh to obtain starting aggregate size of > 8 mm which was then used for the WAS analysis. A sub sample of about 40 g of > 8 mm aggregates was oven dried at 105ºC for 48 hours for gravimetric water content (GWC) determination. Particle size distribution of WSA, and mean weight (MWD), were determined using 50 g soil placed on a nest of sieves with 4750, 2000, 1000, 500, and 250 μm connected to a motor. The samples were immersed into a bucket containing deionized water. The bucket was topped to bring water level to the base of the top sieve, and made to stand for 10 minutes. Afterwards, the samples were sieved for 10 minutes by a vertical displacement of 35 mm at 30 oscillations mm^{-1}, by aid of an oscillating mechanical motor. The soil collected on each sieve was washed into pre-weighed mason jars, and oven dried at 105ºC for 48 h to obtain oven dry soil mass. After obtaining the dry weight, 13.9 g L^{-1} sodium hexametaphosphate solution was added and made to stand for 24 h to disperse soil aggregates, and then washed for sand correction. Wet stable aggregates, and sample mean weight diameter (MWD) was calculated as follows (Kemper and Chepil, 1965):

$$\text{WSA} = \frac{(Mm - Mf)}{(Mt - Mf)} \quad [13]$$

where *Mm* is the oven-dry mass of material left on sieve after sieving, *Mf* is dry mass of fragments on the same sieve after dispersion, and *Mt* is total sample dry mass, and:

$$MWD = \sum_{i=1}^{n} \bar{x}_i w_i \quad [14]$$

where *MWD* is the sum of products of (1) the mean diameter, $\bar{x}_i$ (mm), of each size fraction and (2) the proportion of the total sample mass, w_i (g), occurring in the corresponding size fraction, where the summation is carried out over all *n* size fractions, including the one that passes through the finest sieve.

4.2.6 Statistical analysis

Data was analyzed with Proc GLM procedure in SAS 9.3 software package (SAS Institute, Inc. Cary, NC). Data collected over the two years were analyzed together. Rotation scheme were considered fixed effects in the model. Least significant difference (LSD) was used for mean comparison. Orthogonal contrast was used to compared selected treatments of interest (e.g. fallow vs camelina; W-S-F vs W-S-SC; and W-F vs all other treatments). Treatment effects were considered significant when *P* values were ≤ 0.05.

4.3 Results

4.3.1 Weather conditions

Active growth period of our study crops overlaps from April-September. Monthly precipitation and temperatures at Hays (KS) during the growing season of camelina, sorghum, and wheat are summarized in Table 4.1. Camelina had a short growth period compared to wheat and sorghum, and was highly impacted by weather conditions. The 2015 growing season was dry compared to the other years (Table 4.1). Precipitation at camelina planting in April 2015 was less than April 2016, which was greater than the long-term average. Precipitation in May and July of 2015 was greater than 2016 (Table 4.1). In June however, precipitation in 2016 was greater than 2015, but less than the long-term average.

Mean air temperature in April and May of 2015 were greater than April and May of 2016. Mean air temperatures of 2015 and 2016 were greater and less than the long-term average in April and May respectively (Table 4.1). Mean air temperature in June and July 2015 were less than June and July 2016 respectively. The mean air temperature of June and July 2015 and 2016 were greater than the long-term average in April and May respectively (Table 4.1).

4.3.2 Crop yield, oil and protein concentration

Winter wheat yield averaged 2286 kg ha^{-1} with fallow (W-F and W-S-F), which was greater than that obtained by replacing fallow with camelina (W-SC and W-S-SC) in the rotation (Table 4.2). Wheat yields with grain sorghum but not camelina in the rotation was greater than wheat yield when both sorghum and camelina were in the rotation (Table 4.2). However, wheat yield in W-F was not different from average wheat yield from the more intensified rotation systems (Table 4.2). Spring camelina seed yield when planted after wheat (W-SC) was two-fold greater than that after sorghum (Table 4.2). Camelina oil and protein concentrations were not different when planted

after wheat (W-SC), or after sorghum in a W-S-SC rotation system. Average camelina oil and protein concentration was 28, and 29% respectively (Table 4.2). Grain yield calculated on annual basis was not different with fallow or when fallow was replaced with camelina (Table 2). Similarly, annualized grain yield between W-S-F and W-S-SC were not different. Nonetheless, annualized grain yield in W-F was less than average annualized grain yield from the more intensified rotation systems (Table 4.2).

4.3.3 Soil water content and crop water use

Soil water content measured at wheat planting within 0-100 cm was 30% less in the continuous cropping systems (W-SC, and W-S-SC) compared to rotations that had fallow (W-S-F and W-F) (Table 4.3). Volumetric water content in November was similar in W-SC, W-S-F, and W-S-SC (Fig. 4.1a). However, volumetric soil water content was greater at all the sampling depths in W-F than the other rotations systems, except at 25 to 50 cm depth (Fig. 4.1a).

At camelina planting in March, soil water content measured at 0 to 25cm, and 25 to 50 cm depth was similar for all the rotation systems. (Fig. 4.1b). Notwithstanding, volumetric soil water content at 50 to 75 cm and 75 to 100 cm was greater in W-F than W-S-SC. The soil water content at wheat harvest in summer measured at upper soil depths was similar for all the rotation systems (Fig. 4.1c). However, soil water at the lower depth (i.e. 75 to 100 cm) was greater in W-F than W-S-SC (Fig. 4.1c).

Winter wheat water use efficiency (WUE) was not different among the crop rotation systems (Table 4.3). However, WUE in sorghum differed between W-S-F and W-S-SC. Sorghum WUE with W-S-SC was 30% greater than when grown after wheat in a W-S-F rotation (Table 4.3). Similarly, camelina WUE was significantly affected by crop rotation treatments. Camelina WUE was 40% greater when planted after wheat in W-SC, than when grown after sorghum (Table

4.3). Crop water use was greatest in sorghum, followed by wheat, and then camelina. Average water use was 408 mm, 495 mm, and 534 mm for camelina, wheat, and sorghum respectively.

Crop residue amounts measured before wheat planting in 2015 and 2016 growing seasons was greater in the 3-yr rotation systems (W-S-F, and W-S-SC) compared to W-F or W-SC (Table 4.3). Compared to W-F, increasing cropping intensity increased the percent ground cover measured at wheat planting (Table 4.3).

4.3.4 Soil biological activity

Soil biological activity measured in July 2015 was greater in the intensified crop rotation systems (W-S-SC>W-S-F=W-SC) compared to W-F. Biological activity was greatest in W-SC, and W-F at sorghum harvest (October) and spring camelina planting (March), respectively (Fig. 4.2a). June sampling after wheat harvest in 2016 showed greater biological activity in W-S-F, W-SC, and W-F, compared to W-S-SC (Fig. 4.2a). August sampling after camelina harvest, showed biological activity was not different between rotation systems. After wheat planting in November, biological activity was greater in W-S-SC than the other rotation systems (Fig. 4.2a).

Soil temperature taken simultaneously during the CO_2 flux sampling times were similar among the crop rotation systems (Fig. 4.2b). Gravimetric soil water content (GWC) measured during the CO_2 flux sampling time at camelina harvest was similar between rotation systems (Fig. 4.2c). However, soil GWC was greater in W-F than W-S-SC, and W-SC, but it was not different from W-S-F during sorghum harvest in October 2015. There were no differences in GWC between rotation systems during camelina planting in March 2016 (Fig 4.3c). During wheat harvest in June 2016, GWC was greater in W-F and W-S-F, than W-SC and W-S-SC. After camelina harvest in August, GWC was greater in W-F, and W-SC, than W-S-F, and W-S-SC. November GWC after wheat planting was greater in W-F, and W-S-F compared to W-SC, and W-S-SC (Fig. 4.2c).

4.3.5 Soil microbial biomass C and N, and potentially mineralizable N

Microbial biomass carbon (MBC) was not different between fallow and when camelina was in the rotation, irrespective of time of soil sampling (Table 4.4). Microbial biomass C between W-S-F and W-S-SC was not different in soil sampled in the fallow phase. Notwithstanding, MBC between W-S-F and W-S-SC were different when soil were sampled in wheat stubble (Table 4.4). Comparing W-F to the more intensified rotation systems, MBC was not different when soil was sampled before wheat planting and after wheat harvest (Table 4.4).

Microbial biomass N was greater in fallow than when camelina was in the rotation, when soil was sampled in the fallow phase (Table 4.4). However, there were no differences in MBN when soil sampling occurred in wheat stubble. Microbial biomass N was greater in W-F than the other intensified rotation when soil was sampled in the fallow phase. Nonetheless, when soil was sampled in wheat stubble, there were no differences in MBN between W-F and the intensified cropping systems (Table 4.4).

Potentially mineralizable N was not different between fallow and when camelina was in the rotation, irrespective of time of soil sampling (Table 4.4). Similarly, PMN between W-S-F and W-S-SC were not different at the two sampling times (Table 4.4). Comparing W-F to the more intensified rotation systems, PMN was not different when soil was sampled before wheat planting or after wheat harvest (Table 4.4).

4.3.6 Soil nitrogen, phosphorus, pH, total nitrogen and organic carbon

Soil profile N (within 0-60 cm depth) measured in the fallow phase before wheat planting was greatest with W-F (Fig. 4.3a). In general, soil N with W-F was 38, 42, and 55% greater than that of W-SC, W-S-F, and W-S-SC, respectively. However, soil P measured at 0-5 cm depth was not different among the crop rotation treatments regardless of the crop phase sampled (wheat

stubble or fallow phase before wheat planting) (Fig. 4.3b). Soil organic carbon (SOC) was not different when soil was sampled before wheat planting (Fig. 4.4a). Nonetheless, SOC differed among the crop rotation systems when soil sampling was done in wheat stubble after wheat harvest in August. At that sampling period, SOC in W-S-F was 12% greater than that in W-F rotation system (Fig. 4.4a). Total nitrogen (TN) was not significantly different among the crop rotations regardless of sampling time (Fig. 4.4b). Similarly, soil pH was not different among crop rotations irrespective of sampling time (Fig. 4.5a).

4.3.7 Soil bulk density, porosity, and wet aggregate

Soil bulk density at 0-5 cm depth after three seasons was significantly ($P<0.05$) affected by crop rotation. Bulk density ranged from 1.1 with W-F to 1.3 g cm^{-3} with W-SC (Table 4.5). Soil bulk density for W-SC was greater than W-F, but bulk density was not different among W-S-F, W-SC, and W-S-SC rotation systems (Table 4.5). Total porosity with W-F was 10% greater than W-SC but similar among W-F, W-S-F, and W-S-SC (Table 4.5). Water and air-filled pore space were not different among crop rotation systems. Air filled porosity ranged from 32 to 38%, whereas water filled pore space ranged from 27 to 35% (Table 4.5).

Soil aggregate size fractions measured at 0-5 cm in August 2017 after four growing seasons showed differences among rotation systems in particle sizes >4.75 and <0.25 cm (Fig. 4.5b).
In general, increasing cropping intensity increased the proportion of soil aggregates >4.75 mm, which was greatest in the more intensified W-S-SC rotation system (Fig. 4.5b). However, aggregate size fractions <0.25 mm was greatest in the less intensified W-F cropping system (Fig. 4.5b). Mean weight diameter was between 3 and 4 mm and was not different among crop rotation treatments.

4.4 Discussion

4.4.1 Crop yield and water use

Wheat yield reduced when it was planted after camelina. The reduction in wheat yields may be due to less available soil water for wheat production, mostly due to camelina water use. In the present study, soil water measured at wheat planting in W-F was 211 mm, significantly greater than 148 mm measured in the W-SC or 127 mm in W-S-SC rotation treatment (Table 4.3). Seasonal average water use by camelina was around 408 mm mostly in the top 100 cm of the soil, which is within the range of camelina water use reported in Nebraska (Hergert et al., 2016). Unless this soil profile water depleted by camelina is adequately replenished from rainfall, the subsequent crop yields could be affected as reported in the present study.

The decrease in winter wheat yields following camelina has been reported by other researchers (Lessen et al., 2012; Chen et al., 2015; Obour et al., 2018). The reduction in wheat yields following camelina were 13% in Moccasin, MT (Chen et al., 2015), 18% across the Great Plains (Obour et al., 2018) and 31% in Culbertson, MT (Lenssen et al., 2012) compared to yields after fallow. In our current study however, the decrease in wheat yield after camelina was 15% less than that after fallow.

Although we did not see any difference in sorghum grain yield between W-S-F and W-S-SC rotation systems, sorghum production per unit of water used (WUE) was improved in W-S-SC than in W-S-F. The inclusion of camelina in the rotation increased residue return and may have reduced evapotranspiration, thereby making more water available for sorghum production. On the other hand, camelina showed greater production per unit of water used when it was planted after wheat in W-SC, than W-S-SC, due to greater water used by sorghum. Though wheat yields of W-F and W-SC are similar, including sorghum in the rotation (W-S-F and W-S-SC) increased total

annualized yield. Therefore, a producer may gain more income by annual cropping than W-F if there is market for the additional crop in the rotation. Other researchers report of an increase in land productivity when the rotation system comprised of a diversity of crops compared to W-F. For example, Anderson (2005) reported total yield of 890 kg ha^{-1} for W-F compared to 2030 kg ha^{-1} for wheat-corn-proso millet-fallow. Similarly, Peterson et al. (1993) reported a 25% increase in net return for wheat-corn-proso millet-fallow compared to W-F. Annualized yield difference between W-F and W-SC was not prominent, and this may be due to the smaller camelina yields compared to sorghum.

Spring camelina yield in W-SC was greater than that of W-S-SC. The difference in yield between the two rotation systems could be due to less soil moisture availability when camelina was planted after sorghum compared to wheat. In the current study, soil water content in W-S-SC after sorghum harvest was less than that measured in W-SC (Fig. 4.1a). In addition, soil profile water content at camelina planting in W-S-SC was less than that in W-SC (Fig. 4.1b), possibly due to greater sorghum water use compared to camelina or wheat. Greater demand for soil water by sorghum negatively affected soil water availability to camelina when it was planted after sorghum. On the other hand, camelina following wheat (W-SC) had more soil water for camelina growth due to the relatively less demand for water by wheat, and the longer fallow period between wheat harvest and when camelina was planted.

4.4.2 Crop rotation and soil microbial activity

In our current study, biological activity tends to be greater from spring (March) through to summer sampling periods (July and August). Soil water content and temperatures were generally greater during summer months (Fig.4.2 b and c) and this contributed to the higher microbial activity as captured in the CO_2 flux measurements. Previous research showed increase in

temperature increases soil microbial activity and CO_2 flux (Kirschbaum, 1995; Zak et al., 1999; Teramoto et al., 2017), similar to findings in the present study. Other researchers report that decreased soil water potential decreases the metabolic activity of most microbial species, which results in lowered respiration and nutrient mineralization (Griffin 1981, Schimel et al. 2007; Manzoni et al., 2012).

Soil biological activity was generally greater in W-S-SC than the other rotation systems. This could be due to quality and quantity of residue from W-S-SC, which provided more carbon substrate for microbial activity. The proportions of organic compounds (e.g. cellulose, hemicellulose, starch, protein, lipids, and polyphenols) present in plants can influence the degree and rate of decomposition (Kononova, 1966; Martens, 2000). The quality of residue in W-S-SC suggests faster decomposition compared to the other rotation systems. Sorghum stem and bark contains sucrose, cellulose, hemicellulose and phenolic acids (Billa et al., 1997). Similarly, camelina contains substantial amounts of phenolic compounds (Matthäus, 2002; Abramovič et al., 2007; Terpinc et al., 2012). Hence, the greater biological activity in W-S-SC compared to W-SC was due to the presence of sorghum which supplied more organic compounds in the residue.

4.4.3 Crop rotation and nutrient cycling

Providing more residue cover through cropping intensification can increase SOC (Bowman et al., 1999; Blanco-Canqui et al., 2013). Soil organic carbon (SOC) correlates well with soil biological, chemical, and physical properties. In the current study, residue biomass and SOC were generally greater in the more intensified crop rotations (W-S-F, W-SC, and W-S-SC) compared to W-F (Table 4.3 and Fig. 4.4a). This is consistent with findings that the quality and quantity of organic input determines the extent of SOC accretion (Lal, 2004; Allen et al., 2011; Deb et al., 2015).

High MBC contribution was present in W-S-SC than the other rotation systems with fallow when soil was sampled after wheat harvest (i.e. in wheat stubble). This could be attributed to high residue (Table 4.3), and the quality of residue contributed by W-S-SC. Although residue biomass in W-S-F was similar to W-S-SC, MBC from soil sampled from wheat stubble was greater in W-S-SC than in W-S-F possibly due to differences in residue quality. Previous research showed that the origin and composition of plant residue can affect their C and N mineralization rates (Heal et al., 1997; Nicolardot et al., 2001). This finding suggest camelina potentially contributes more labile C to the organic pool when it is present in the rotation system. Microbial biomass N was greater in the rotations with fallow than that with camelina. Reason may be due to greater demand for nitrogen when fallow was replaced with camelina and crops were growing annually. This indicates that the fallow period enhanced the accumulation of MBN. Studies show that MBN in less cropped soils is greater than in continuously cropped soils (Ayanaba et al. 1976; Adams and Laughlin, 1981).

In general, potentially mineralizable N was greater in the soils sampled after wheat harvest, than before wheat planting. This may be due to the presence of more plant residue available for N mineralization in the samples taken in wheat stubble than in fallow plots. The presence of camelina in the rotation added plant residue with low C/N ratio that contributed to N mineralization. This was evident in W-SC, and W-S-SC rotations in soil samples after wheat harvest.

Greater profile inorganic N in W-F may be due to more N mineralization during the long fallow period. Wienhold and Halvorson (1999) suggested the long fallow period between residue addition after harvest and planting increases soil N mineralization, whereas less decomposition/mineralization occurs in short fallow periods because of high C/N ratio in wheat residue, hence N immobilization. Similar findings of greater inorganic N in soils under W-F

compared to continuous cropping have also been reported by other researchers (Hurisso et al., 2013; Thomas et al., 2016). In our study it was observed that W-F had greater residual inorganic N concentration hence the low concentration of PMN. Unlike N, P use by the crops in rotation was not significant enough to cause differences in soil available P.

4.4.4 Crop rotation and soil water movement

Greater soil bulk density after three seasons was recorded in the intensified cropping systems compared to W-F. This could be due to frequent exposure of the soil to farm machinery for cropping activities such as spraying, planting, and harvesting. Previous research showed that the use of farm machinery can increase soil bulk density (Gameda et al., 1987; Ngunjiri and Siemens, 1993). The effect of less frequent cropping was apparent with numerically lower soil bulk density, and greater total soil porosity in W-F, compared to the more intensified cropping systems. Reeves et al. (1984) reports that root growth of spring wheat was less when soil bulk density was 1.5 g cm^{-3} in the 0-20 cm depth, compared to when soil bulk density was 1.32 g cm^{-3}. Hence bulk densities measured in the current study were not high enough to cause production issues such as poor aeration, poor infiltration, and restricted root development.

In our current study, we observed a faster turn over for soil aggregation in W-S-SC than W-F. The observed differences may be due to increase crop residue (Table 4.3) and SOC after wheat harvesting (Fig. 4.4a), which was the binding factor for the soil aggregates in the intensified crop rotations. This increase in water stable aggregates suggests potentially low erodibility in the intensified crop rotation compared to W-F. This result is consistent with previous studies that showed increase in soil SOC promotes macroaggregation, and increase aggregate stability through biological and physicochemical bonding mechanisms (Tisdall and Oades, 1982; Bowman et al., 1999; Mrabet et al., 2001; Blanco-Canqui et al., 2013).

4.5 Conclusion

Understanding the effect of wheat-camelina cropping systems on wheat yield, soil water availability, water use, and soil properties can provide useful information to growers interested in adopting camelina in wheat-based cropping systems in the Great Plains. Our results showed wheat yields were reduced by approximately 15% when camelina replaced fallow in the crop rotation. However, sorghum yields were not affected by including camelina in the crop rotation. Greater soil water demand in the more intensified system was the reason for lower wheat yields, which also caused lower camelina yields in W-S-SC compared to W-SC. Crop rotations with fallow had greater residual soil profile N and more soil water storage that resulted in greater wheat and sorghum yields.

Our study showed replacing fallow with camelina improved soil particle aggregation, soil microbial activity, and increased MBC, PMN, and SOC. Crop rotation had no effect on soil pH, P or TN concentration and this may be due to the 2 years duration of this study, which was not long enough to cause changes in soil pH, P, or TN. We accept our hypothesis that sorghum yield was not affected by increasing cropping intensity, however, we reject our hypothesis that wheat and camelina yields will not be affected by cropping intensification. We accept the hypothesis that increasing cropping intensity improved soil particle aggregation, increased MBC, PMN, and SOC. Nonetheless, we reject the hypothesis that increasing cropping intensity will increase TN, P, and reduce soil pH. Overall, including camelina in wheat-based cropping system added soil health benefits to the soil.

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Table 4.1 Monthly mean air temperature and precipitation at Kansas State University - Western Kansas Agriculture Research Center, Hays, KS from 2013 to 2016.

	Precipitation						Mean air temperature					
Month	2013	2014	2015	2016	Average (2013-2016)	Average (147-years)	2013	2014	2015	2016	Average (2013-2016)	Average (116-years)
	mm						°C					
January	19.3	4.1	11.7	17.3	13.1	11.7	-0.9	-1.7	-0.8	-0.6	-1.0	-1.8
February	30.2	23.4	18.0	17.8	22.4	18.5	0.2	-3.4	-0.6	3.4	-0.1	0.5
March	19.8	4.3	2.3	14.2	10.2	32.0	4.6	3.9	7.8	9.2	6.3	5.7
April	26.9	23.1	24.4	189.5	66.0	55.1	9.3	11.9	13.1	12.9	11.8	11.8
May	54.9	20.8	163.6	77.2	79.1	83.1	18.2	18.0	16.3	16.0	17.1	17.2
June	69.3	240.0	19.3	87.4	104.0	87.6	24.6	23.3	24.6	25.3	24.4	23.0
July	179.8	59.9	104.4	87.6	108.0	84.3	25.5	24.3	26.5	26.6	25.7	26.2
August	15.0	41.7	11.7	96.0	41.1	74.7	25.0	25.9	24.7	24.3	25.0	25.3
September	75.7	150.9	10.7	52.8	72.5	56.1	22.3	20.1	24.3	21.4	22.0	20.3
October	25.1	54.6	44.5	16.8	35.2	36.3	12.2	13.9	14.8	15.9	14.2	13.4
November	29.5	1.3	46.5	30.0	26.8	21.6	4.4	3.3	6.8	8.7	5.8	5.3
December	1.3	18.5	45.0	14.5	19.8	17.0	-2.0	0.4	2.0	-3.0	-0.6	-0.5
Total	546.9	642.6	501.9	701.0	598.1	569.4						
April-September	421.6	536.4	334.0	590.6	470.6							

Table 4.2 Wheat, grain sorghum and camelina seed yields averaged over two growing seasons (2015 and 2016) as affected by crop rotation.

					Camelina	
Crop rotation	Wheat yield	Grain sorghum	Camelina yield	Annualized yield	Oil concentration	Protein concentration
	kg ha^{-1}				%	
W-F	2258a	-	-	1129b	-	-
W-S-F	2314a	3734a	-	2016a	-	-
W-SC	1953a	-	844a	1399b	28.0a	29.6a
W-S-SC	1914a	3694a	380b	1996a	28.3a	29.5a
Mean yield	2109	3714	612	1635	28.1	29.5
LSD (*P<0.05*)	404	1826	225	435	1.3	1.5
Contrast				*P > F*		
Fallow vs camelina	*			NS		
W-S-F vs W-S-SC	*			NS		
W-F vs. all others	NS			***		

W-F = Wheat-fallow; W-S-F = Wheat-sorghum fallow; W-SC = Wheat-spring camelina; W-S-SC = Wheat-sorghum-spring camelina; all others = W-S-F, W-SC, and W-S-SC together. LSD = least significant difference. Means within column followed by same letter (s) are not significantly different (*P<0.05*). Data are averaged over two growing seasons (2015 and 2016) and four replicates (n = 8 for wheat yield and n = 4 for all other parameters). NS = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

Table 4.3 Soil water content at wheat planting, water use efficiency from November 2015 to October 2016 during wheat, camelina, and sorghum growing seasons, and crop residue, and ground cover after camelina harvest as affected by rotation system.

	Water use			Water use efficiency					SW at wheat planting at 0-100 cm depth
Crop rotation	Wheat	Sorghum	Camelina	Wheat	Sorghum	Camelina	Residue biomass (kg ha^{-1})	Ground cover (%)	
	mm^{-1}			kg ha^{-1} mm^{-1}					
W-F	518a	-	-	5.3a	-	-	1503c	67.1b	21.1
W-S-F	510a	536a	-	5.7a	6.7b	-	3784a	82.5ab	21.7
W-SC	421a	-	407b	5.3a	-	2.0a	2194b	82.5ab	14.8
W-S-SC	426a	442a	493a	5.2a	9.5a	1.2b	3316a	92.3a	12.7
LSD (*P<0.05*)	130	413	0.1	0.6	1.3	0.2	591.0	15.5	

Means within column followed by same letters (s) are not significantly different (P<0.05). SW = Soil water at wheat planting measured from 0-100 cm depth, W-F = Wheat- fallow, W-S-F = Wheat-sorghum-fallow, W-SC = Wheat -spring camelina, W-S-SC = Wheat -sorghum-spring camelina. Means are averaged across four replications (n = 3).

Table 4.4 Soil microbial biomass carbon & nitrogen (MBC &MBN), potentially mineralizable N (PMN) at 0-5 cm depth measured in August 2016 after 3 growing seasons (2014 to 2016) as affected by crop rotation system.

	Before wheat planting			After wheat harvest		
Crop rotation	MBC (µg C g^{-1} soil)	MBN (µg N g^{-1} soil)	PMN (µg N g^{-1} soil)	MBC (µg C g^{-1} soil)	MBN (µg N g^{-1} soil)	PMN (µg N g^{-1} soil)
W-F	166a	150a	2b	74b	69a	12b
W-S-F	157a	90ab	14a	26.6b	87a	22ab
W-SC	238a	26b	14a	103ab	44a	45a
W-S-SC	168a	55b	25a	169a	90a	28ab
LSD *(P<0.05)*	100	68	20	88	80	30
Contrast			*P > F*			
Fallow vs camelina	NS	*	NS	NS	NS	NS
W-S-F vs W-S-SC	NS	NS	NS	*	NS	NS
W-F vs. all others	NS	*	NS	NS	NS	NS

MBC = microbial biomass carbon; MBN = microbial biomass nitrogen; PMN = potentially mineralizable nitrogen; W-F = Wheat-fallow; W-S-F = Wheat-sorghum fallow; W-SC = Wheat-spring camelina; W-S-SC = Wheat-sorghum-spring camelina; all others = W-S-F, W-SC, and W-S-SC together. LSD = least significant difference. Means within column followed by same letter (s) are not significantly different (*P<0.05*). Means are averaged across four replications (n = 4). NS = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

Table 4.5 Soil bulk density, volumetric water content, total porosity, air filled porosity, and water filled pore space at 0-5 cm depth measure in August 2017, as affected by rotation system after four seasons.

Crop rotation	Soil bulk density (g cm^{-3})	Total porosity (%)	Air filled porosity (%)	Water filled pore space (%)
Wheat-fallow	1.1b	53.8a	36.3a	33.0a
Wheat-sorghum-fallow	1.2ab	52.1ab	37.6a	28.4a
Wheat-spring camelina	1.3a	48.6b	31.6a	35.1a
Wheat-sorghum-spring camelina	1.2ab	51.6ab	37.7a	27.3a
LSD *(P<0.05)*	0.1	4.3	8.3	10.4
Contrast		*P > F*		
Fallow vs camelina	NS	NS	NS	NS
W-S-F vs W-S-SC	NS	NS	NS	NS
W-F vs. all others	NS	NS	NS	NS

LSD = least significant difference. Means within column followed by same letter (s) are not significantly different (*P<0.05*). Means are averaged across four replications (n = 4). NS = not significant; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

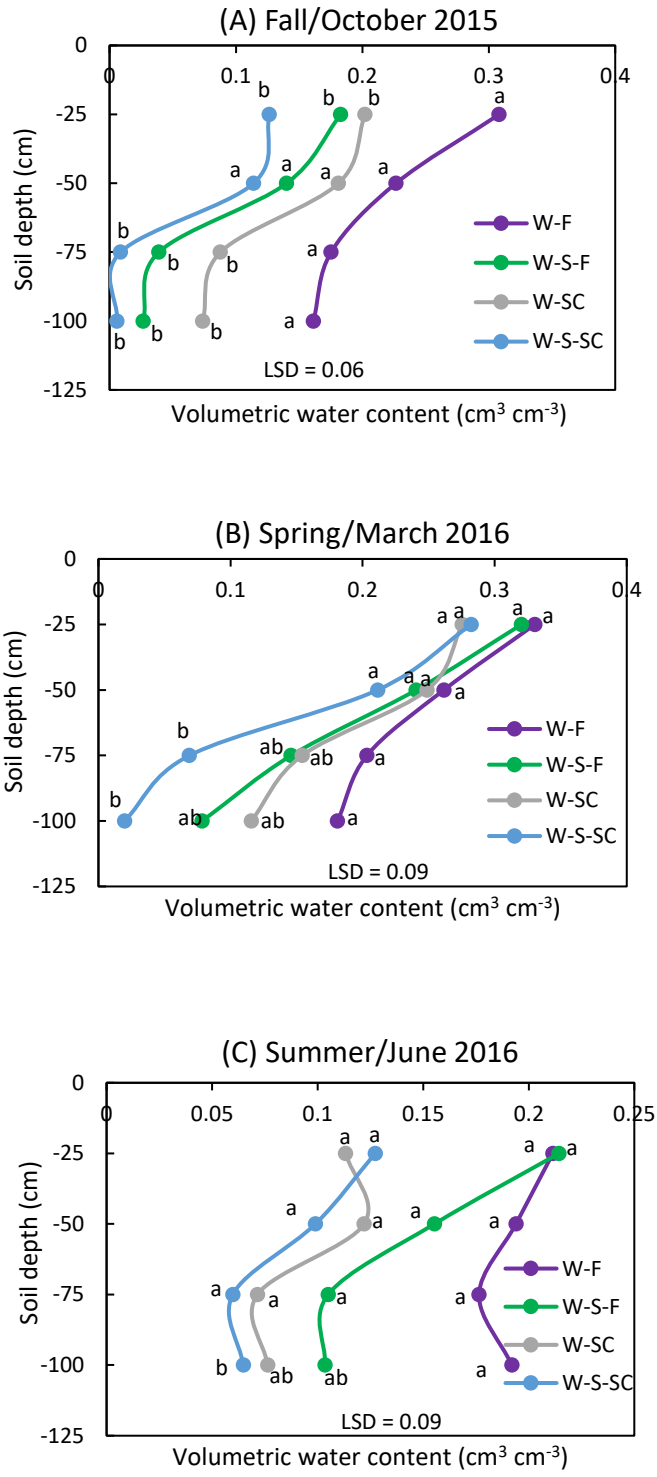


Figure 4.1 Volumetric soil water content measured at wheat planting (October 2015), camelina planting (March 2016) and wheat harvest (June 2016) as affected crop rotation.

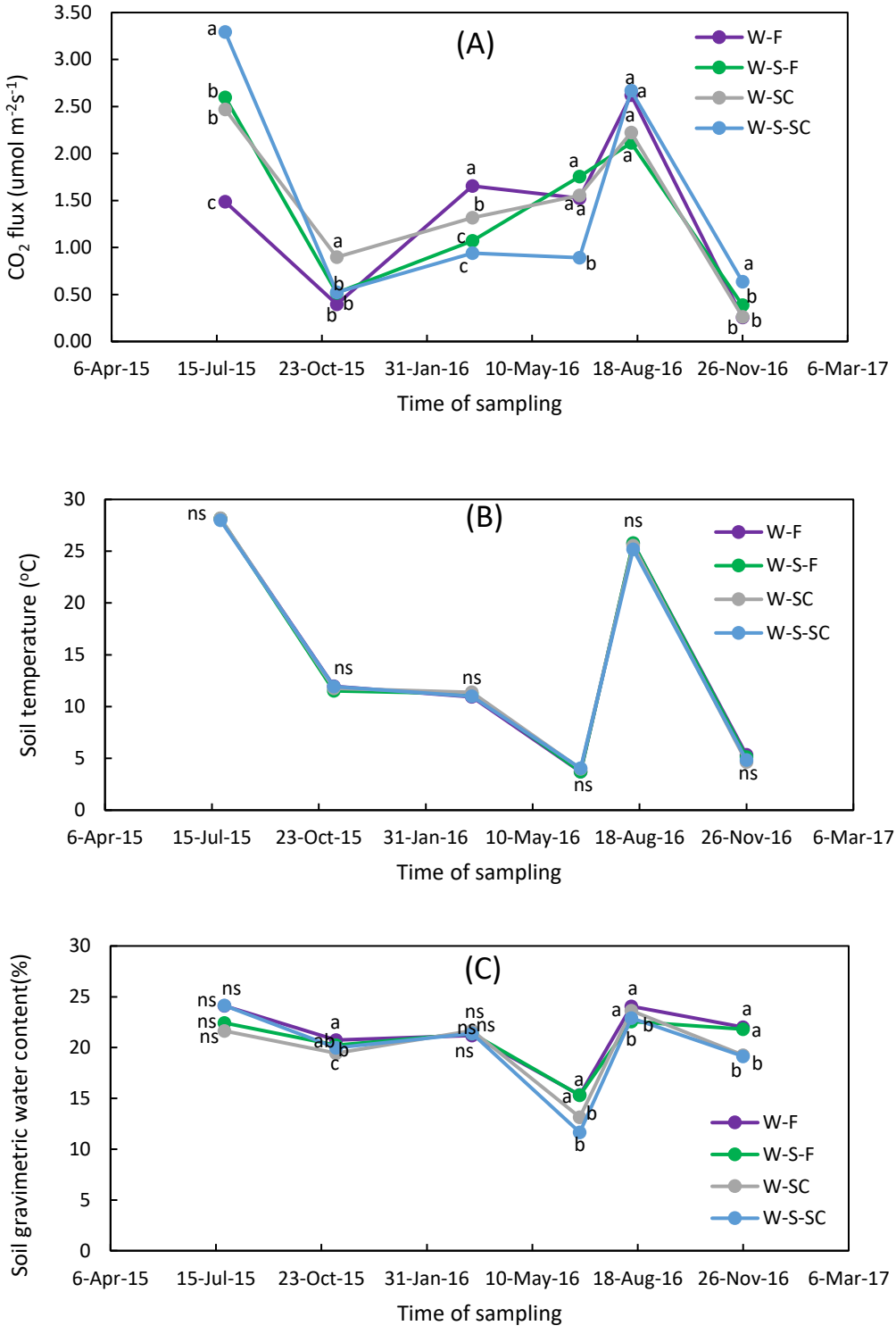


Figure 4.2 Soil CO_2 flux, gravimetric soil water content, and soil temperature sampled from wheat harvesting in 2015 to wheat planting in 2016 as affected by crop rotation system.

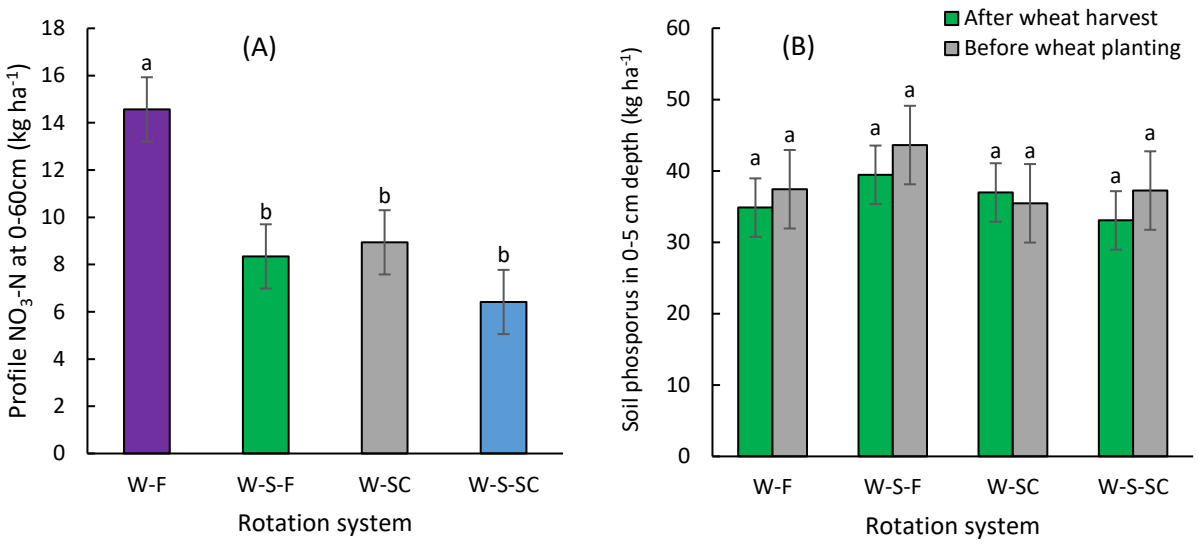


Figure 4.3 Soil nitrate-N (a) and phosphorus content (b) measured in August 2016 after three growing seasons as affected by crop rotation system.

Mean comparison is between rotation systems with the same sampling time. Means with same letter(s) are not significantly different ($P<0.05$).

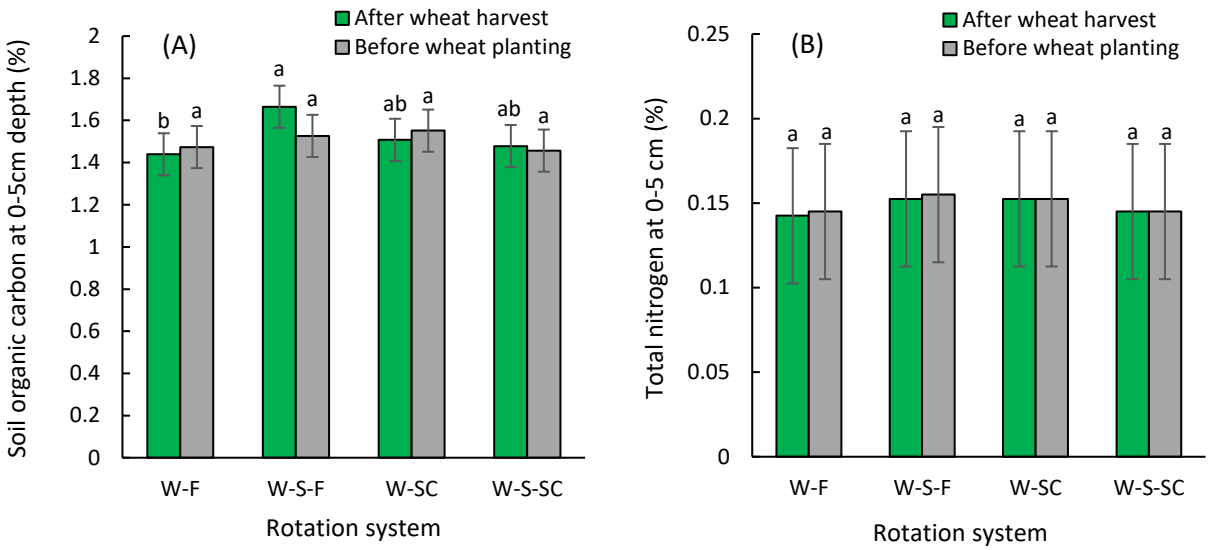


Figure 4.4 Soil organic carbon (a) and total nitrogen (b) content measured in August 2016 after 3 seasons as affected by crop rotation system.

Mean comparison is between rotation systems with the same sampling time. Means with same letter(s) are not significantly different ($P<0.05$).

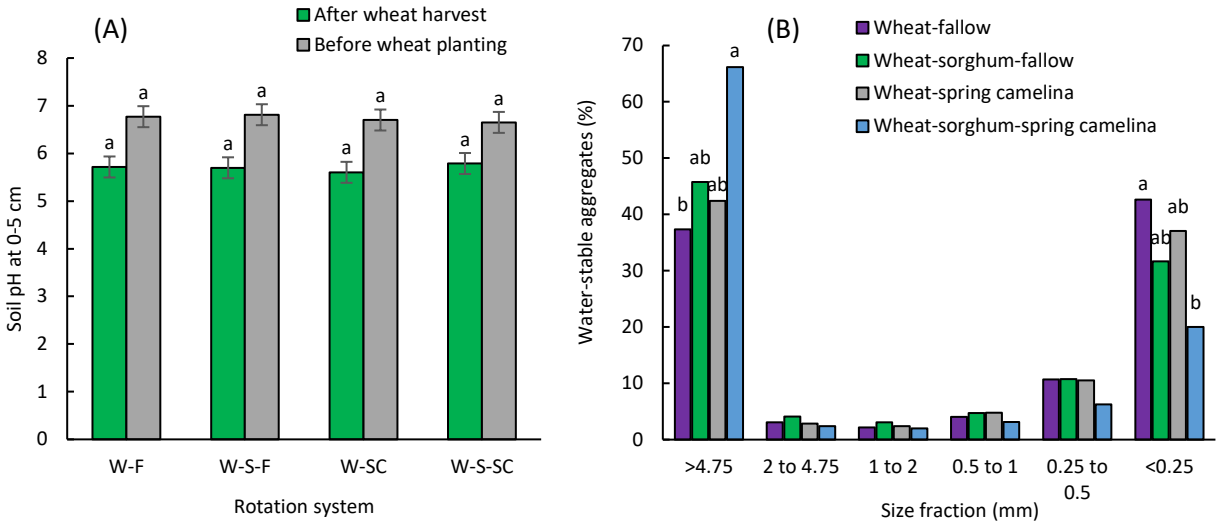
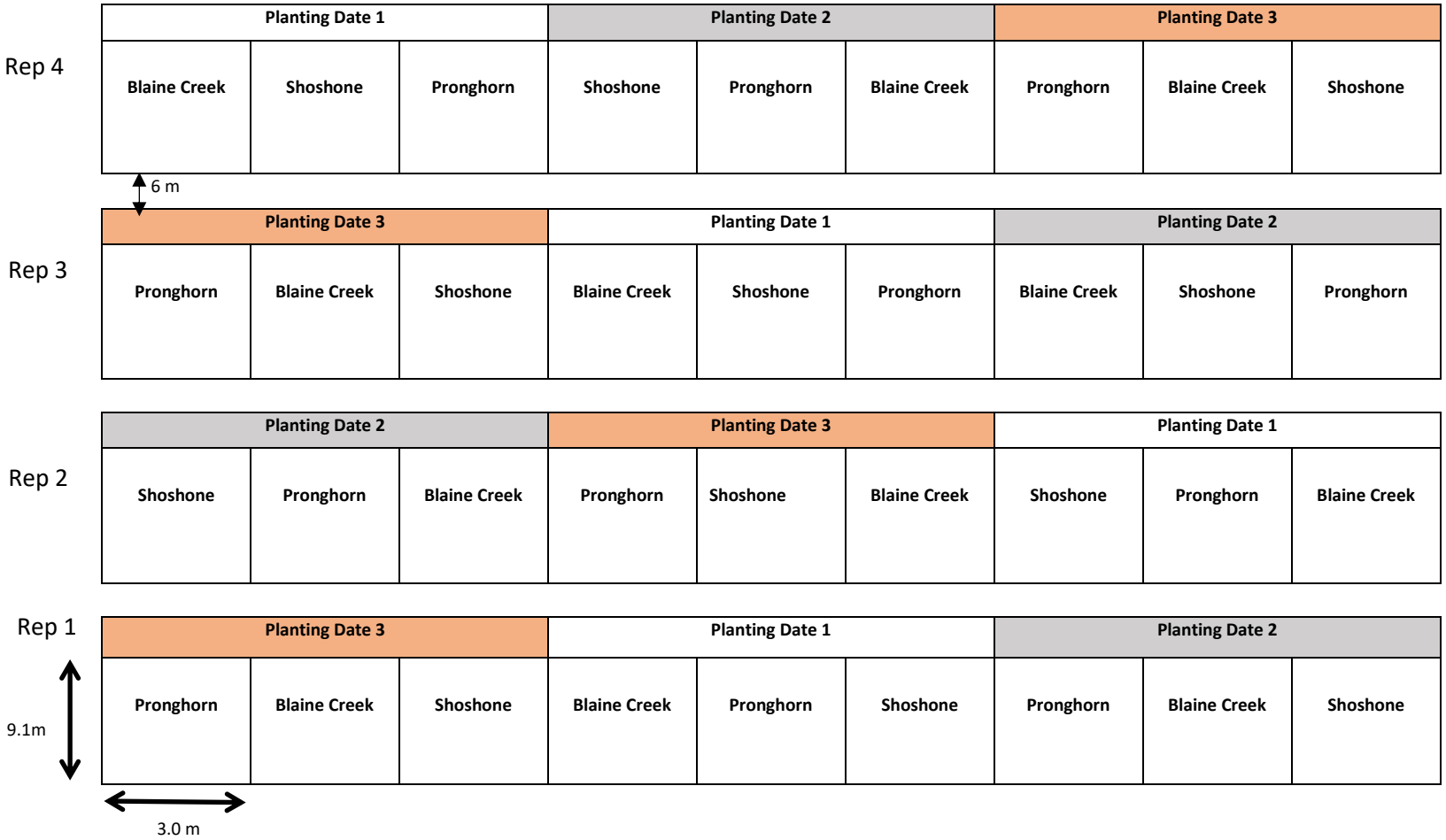


Figure 4.5 Soil pH measured in August 2016 after 3 seasons as affected rotation system; (b) soil aggregate classes at 0-5 cm depth measured in August 2017 as affected by crop rotation system.

Mean comparison is (a) between rotation systems with the same sampling time, and (b) within size fractions. Means with same letter(s) are not significantly different ($P<0.05$).

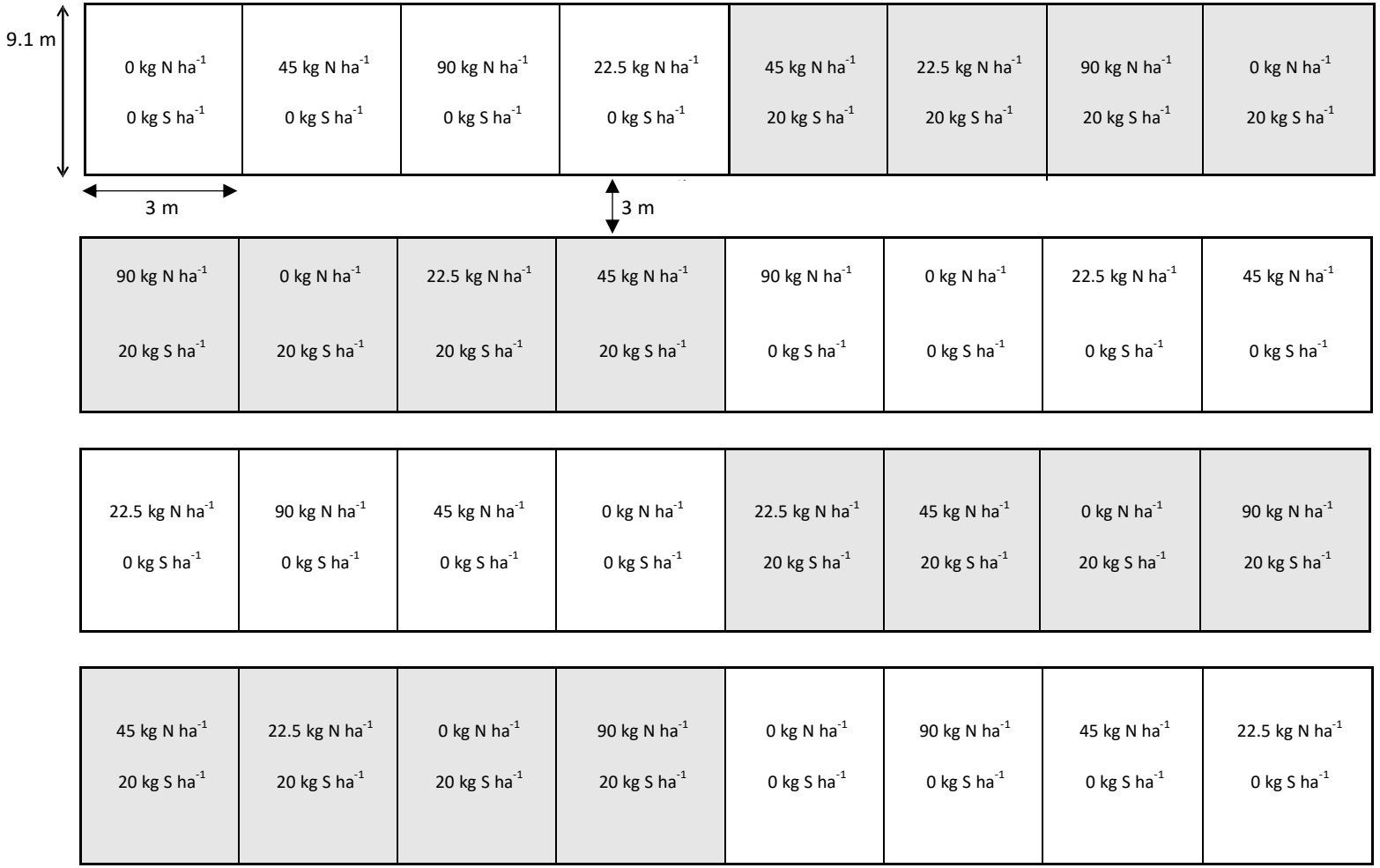
Appendix A - Field layout of studies

Appendix A.1 Layout of spring camelina by planting date study.



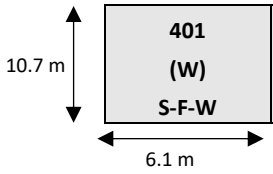
Main plot = Planting date Sub-plot = Cultivars

Appendix A.2 Layout of camelina nitrogen and sulfur requirement study.



N = nitrogen; S = sulfur.

Appendix A.3 Layout of winter wheat-camelina rotation study.

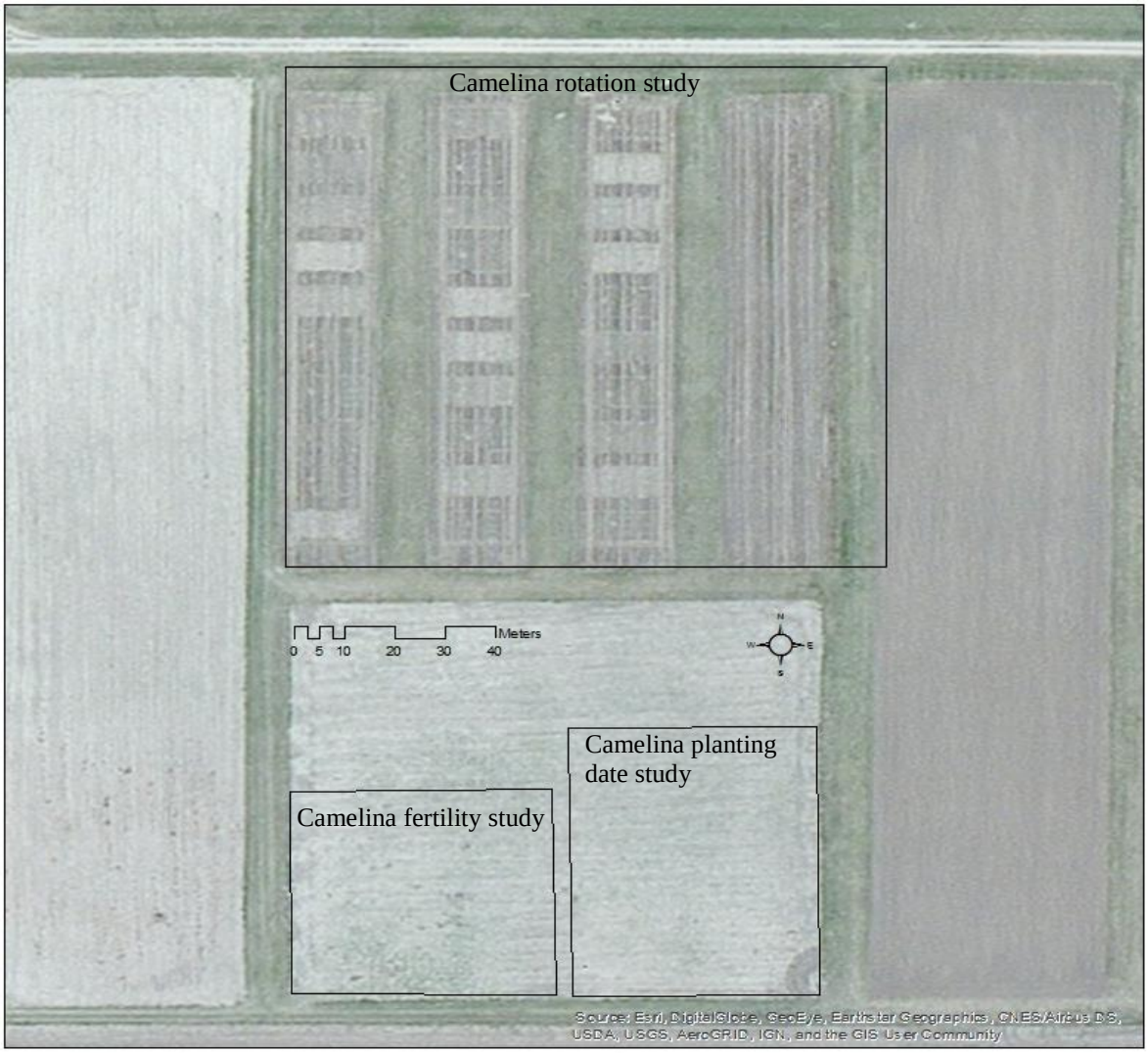


401 (W) S-F-W	402 (W) W-SC	403 (S) SC-W-S	404 (W) W-F	405 (S) F-W-S	406 (W) S-SC-W	407 (SC) SC-W	408 (S) SC-W	409 (SC) W-S-SC	410 (F) F-W
301 (F) F-W	302 (S) F-W-S	303 (S) SC-W	304 (SC) SC-W	305 (SC) W-S-SC	306 (W) S-F-W	307 (W) W-F	308 (S) SC-W-S	309 (W) W-SC	310 (W) S-SC-W
201 (SC) W-S-SC	202 (W) W-F	203 (S) F-W-S	204 (SC) SC-W	205 (W) S-F-W	206 (W) S-SC-W	207 (W) W-SC	208 (S) SC-W-S	209 (F) W-S-F	210 (F) F-W
101 (W) W-SC	102 (W) S-SC-W	103 (W) W-F	104 (S) F-W-S	105 (S) SC-W-S	106 (W) S-F-W	107 (W) F-W	108 (SC) SC-W	109 (W) W-S-SC	110 (F) W-S-F

9.1 m

W = Winter wheat
F = Fallow
S = Grain sorghum
SC = Spring camelina
*( ) = crop planted

Appendix A.4 Map of study location during 2014 studies in Hays, KS.



Appendix B - Field and soil analysis pictures

Appendix B. 1 Camelina growth approaching maturity.

Appendix B. 2 Harvesting camelina using a combine harvestor.

Appendix B. 3 Soil extraction before chemical analysis.

Appendix B. 4 AQ2 Discrete Analyzer for soil nitrate-N and available phosphorous analysis.

Appendix B. 5 Soil water measurement using a Neutron Attenuation Probe.

Appendix B. 6 Soil CO_2 flux measurement using an automated CO_2 chamber system.