THESIS

CAMELINA VARIETY PERFORMANCE FOR YIELD, YIELD COMPONENTS AND OIL CHARACTERISTICS

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ABSTRACT

CAMELINA VARIETY PERFORMANCE FOR YIELD, YIELD COMPONENTS AND OIL CHARACTERISTICS

Oilseed crops have the potential to increase the stability and sustainability of American agriculture by replacing a portion of the fossil fuels consumed by this sector. There are several candidate oilseed species that have been identified as compatible with a dryland winter wheat-fallow rotation. Of these species, Camelina sativa has been previously identified as being a promising species for the High Plains region. This is due to its short growing season, drought tolerance, cold tolerance and resistance to many of the insect and pest species that cause yield reductions in other Brassica oilseed species. To evaluate the performance of this species in the Western United States, we carried out a two year variety trial in 2011 and 2012 to evaluate the performance of 15 varieties in two distinct geographical regions in the Western United States. Six of the varieties, Ligena, SSD10, SSD177, SSD87, SSD138, and Celine, were in the highest-yielding group of varieties in all of our combinations of environments, including irrigated environments. Five of the varieties have been identified as containing favorable alleles for yield and drought tolerance. These SSD varieties yielded well in our study but did not significantly outperform their parental varieties across all environments. The mean yield for the trial across all environments was 813 kg ha^{-1}. Lower-latitude environments in Colorado and Wyoming were not as high-yielding as higher-latitude environments in Montana and Washington State. Camelina did not perform as well at low latitudes even under irrigated conditions during the two years of our study. The low yields can be attributed to above-average, high temperatures. Decreasing the average maximum temperature during the growing season resulted in increased yield and was

positively correlated with an increase in the percent oil and percent of the oil profile comprised of polyunsaturated fatty acid and a decrease in the percent oil comprised of saturated fatty acids. From an agronomic perspective, the focus might be on reducing the number of warm days so that they comprise no more than 17% of the growing season.

In addition to yield, this study looked at the components of yield to see how they were affected by environmental conditions and how they contributed to yield. The number of plants per hectare had the largest effect on yield. This yield component showed significant genotype by environment (GxE) interaction. This yield component is strongly influenced by environmental conditions and not genotype. This suggests that the quickest and easiest way to increase yield is to increase the planting density of the field. In a dryland agricultural system, increased density may have a negative tradeoff in the form of increased water usage of the crop. If breeders are interested in choosing a variety for seed yield improvement, it would be beneficial to choose thousand seed weight, as this is highly heritable and related to genotype. The number of pods per plant has little relationship with the overall yields for camelina and showed significant GxE interaction.

In addition to the variety trial, we assessed the fall planting potential of 11 winter lines and three spring lines of camelina in Fort Collins, CO and Rocky Ford, CO from 2010 to 2011. We found significant differences between the dates of planting (p <0.001). The average yield of the fall seeded entries was 434 kg ha^{-1}, which was less than the average yield of 1033 kg ha^{-1} for a nearby spring seeded camelina variety trial. This showed that through fall seeding of camelina, it is possible to get a stand, but the yields are lower than spring seeded camelina. Our trial included an entry of pennycress (Thlaspi arvense), another oilseed species with potential for Colorado agricultural areas. This preliminary trial in 2010 to 2011 found that under irrigation, pennycress yielded 1392 kg ha^{-1}, which was much higher than the fall seeded camelina. In a follow up trial of the dryland potential of four lines of pennycress in Akron, CO in 2012, excessive drought conditions resulted in a failure of the plots.

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TABLE OF CONTENTS

CHAPTER ONE:

LITERATURE REVIEW

GLOBAL CLIMATE CHANGE

A major contributor to global climate change is carbon dioxide (CO_2) gas. Atmospheric concentrations of carbon dioxide CO_2 gas are correlated with the surface temperature of the earth (Lüthi et al., 2008; Tripati et al., 2009). In addition to directly increasing global temperatures, increased levels of CO_2 can cause dramatic changes in climate (Raymo et al., 1996). As a result of the anthropogenic emission of this gas, the global atmospheric CO_2 concentration has increased to a concentration of 385 parts per million (ppm) in 2010 from 280 ppm in the 18th century (Allison et al., 2010). This is higher than the atmospheric CO_2 concentrations that existed before the Industrial Revolution or any time in the past 800,000 years (Alison et al., 2010). The overall largest annual emitter of CO_2 is China, which emits 6,534 Tg of CO_2, followed by the United States, which emits 5,833 Tg annually (UCS Global Warming, 2012). Per capita, however, the United States is the largest emitter, at 19.18 tonnes person^{-1} year^{-1} (UCS Global Warming, 2012).

The increased concentration of CO_2 has tremendous implications for the environment. The International Panel on Climate Change (2007) has predicted that the unchecked increase in concentrations of greenhouse gasses will result in an annual temperature increase of ~0.2°C. Planetary warming between 1°C and 3°C is anticipated to cause ocean warming and acidification, rising sea levels, increased incidence of extreme weather phenomena and large-scale extinction events (Allison et al., 2010).

The combustion of fossil fuels releases CO_2 that was sequestered by plants and fossilized, beneath the earth's surface millions of years ago. The release of this fossilized carbon through the combustion of these fossil fuels raises the atmospheric CO_2 levels beyond their historic concentration of 280 ppm. The United States relies on fossil fuels to satisfy the majority of its energy needs. Of the 98 quadrillion Btu of energy consumed in the United States in 2010, 83% was generated by natural gas, coal, or petroleum. In contrast, only 8% of the energy consumed in the United States came from "alternative sources," which include hydropower, geothermal, wood, biofuels, solar, wind or other sources that do not involve the combustion of fossil fuels (U.S. Energy Information Administration, 2011). Unfortunately, no single existing alternative-energy technology is capable of meeting all of our national energy needs. As a result, a sustainable energy plan for the future will need to include a myriad of alternative energy sources to fill our various energy needs.

Aviation, shipping and ocean transportation, and agriculture will continue to require a source of fuel that is light, energy dense, safe, and easy to transport (Bang, 2011). One potential source of energy for these sources is liquid fuel grown from biological feedstocks such as plants. These are therefore considered renewable fuels because the carbon comes from the atmosphere where it is fixed by the feedstock plants and not from fossilized sources. These bio-based fuels, or biofuels, have an energy density comparable to fossil fuels, and release CO_2 that was recently sequestered from the atmosphere by the feedstock plant, resulting in lower net CO_2 emission compared to fossil fuels (Bessou et al., 2011). Biofuels such as ethanol and biodiesel can serve as an alternative to traditional fossil fuels. These fuels can be grown and processed in the United States, thereby reducing the United States' reliance on imported fuels.

FUELS AND BIOFUELS IN THE UNITED STATES

In 2010, the United States consumed 19,150,000 barrels of oil per day, which exceeded the next largest consumer, the European Union, by 5,470,000 barrels (CIA World Factbook, 2010). Beginning in the 1950s rising energy consumption in the United States began to outstrip production and the importation of petroleum fuel began. Today, petroleum imports comprise 45% of the total petroleum consumed in the United States (U.S. Energy Information Administration, 2011). Our imported petroleum comes from a variety of countries. The greatest petroleum exporter to the United States is Canada, which in 2011 provided 29% of the imported petroleum products, followed by Saudi Arabia (14%), Venezuela (11%), Nigeria (10%) and Mexico (8%) (U.S. Energy Information Administration, 2011). Some of these countries use fuel revenues to support governments that are either unfriendly to the interests of the United States or the rights of these people. Therefore, reducing our reliance on imported fossil fuels could reduce support for these governments.

In an effort to encourage the production and utilization of bio-based liquid fuel, the U.S. federal government enacted the Energy Policy act of 2005, which introduced the renewable fuel program (RFS1). This required a total of 7.5 billion gallons of renewable fuel to be blended into gasoline by 2012, primarily through the use of ethanol derived from corn (Renewable Fuel Standard, 2012). Following this program was the Energy Independence and Security Act (EISA) of 2007. As a result of the passage of this act, the Environmental Protection Agency established national minimum usage standards for biofuels. This program was known as the Renewable Fuel Standard 2 (RFS2). The RFS2 came into effect on July 1, 2010, with a mandate that the American energy economy would be using 36 billion gallons of renewable transportation fuel per year by 2022 (USDA Biofuels Strategic Production Report, 2010). Of these 36 billion gallons, 21 billion gallons must come from so called “advanced biofuel sources”, 16 billion from “cellulosic biofuels”, and 1 billion from bio-based diesel (Bessou et al., 2001; Carriquiry et al., 2001; Congressional Research Service, 2011)]. An advanced biofuel is defined as any renewable fuel, excluding ethanol derived from corn, which achieves a 50% GHG emissions reduction (U.S. Department of Energy, 2011). A cellulosic biofuel is defined as any fuel that is derived from cellulose, hemicellulose or lignin that achieves a 60% or greater greenhouse gas (GHG) reduction (U.S. Department of Energy, 2011). This can be achieved by utilizing second-generation feedstocks such as fast growing perennial grasses or woody biomass. Biomass-based diesel is defined as a renewable transportation fuel, transportation fuel additive, heating oil, or jet fuel that meets the definition of either biodiesel or non-ester renewable diesel, and achieves a 50% GHG emissions reduction (U.S. Department of Energy, 2011).

In 2010, the U.S. Department of Agriculture estimated each of the United States’ geographic region’s potential contribution to the overall national production of biofuels (USDA Biofuels Strategic Production Roadmap, 2010). The Central East and Southeast regions are projected to contribute 93.1% of the total volume. The American West, on the other hand, is only projected to contribute less than 0.3% of the total volume. Of this 0.3%, the only contributions are predicted to come from feedstocks derived from logging residues and sweet sorghum biomass. Despite the government predictions for the biofuel production Western States, interest is growing in this area for the on-farm production of biofuels. In addition, cooperative oilseed crushing facilities have begun to develop in rural communities in Colorado. These locally produced biofuels could help Agricultural producers in the Western United States reduce their dependence on fossil fuel.

The goal of this thesis is to do research related to increasing the percentage of biofuels contributed by the Western High Plains region. There has been controversy in the past, commonly referred to as the "food vs. fuel" argument, where it is supposed biofuel feedstocks drive up the prices of food crops due to competition for land use. The High Plains agricultural region has traditionally been a wheat-producing area. For Eastern Colorado this means that any successful biofuel feedstock must not interfere with the production of winter wheat. There are significant challenges to achieving this goal. The dry, variable nature of the High Plains climate is not conducive to growth of most biofuel-producing species that show promise in more humid eastern and central areas of the United States. Previous research has identified short-season oilseed species as strong potential biofuel feedstocks because they replace a fallow period and not wheat crop. Based on this research I have worked to carry out further characterization and development of these oilseed species to identify optimal climates and genotypes for the High Plains region.

COLORADO CROPPING SYSTEMS

Colorado is predominantly a wheat-producing state. According to the 2011 Colorado agricultural statistics database, Colorado is the eighth largest wheat-producing state nationwide (Clark, 2011). In 2010, out of 1,002,811 wheat hectares, 991,479 were planted with winter wheat. This resulted in a total value of $606,359,000 (Clark, 2011).

Wheat-Fallow Cropping System

In the wheat-fallow cropping system, winter wheat is alternated with a fallow period as part of a rotation. In the Southeastern area of Colorado where the climate is hotter and drier, wheat is planted in the fall and harvested the following summer, normally in late June to early July. The following year, the land is left fallow in order to allow for moisture recharge. The next year wheat is planted again and the cycle continues.

There is evidence that the traditional wheat-fallow cropping system is an inefficient use of soil moisture, in that much of the soil moisture that is supposedly recharged during the fallow period is lost to evaporation (Peterson and Westfall, 2004). This suggests that incorporation of a spring oilseed crop such as camelina would not deplete the soil of moisture any more than a fallow period that extended through the summer. Introducing camelina into this cropping system would intensify this system to a continuous rotation of wheat-camelina-wheat

Wheat-Spring Crop-Fallow System

In Northeastern Colorado, the predominant dryland cropping systems involve a three-year wheat-fallow-spring crop rotation. Common spring crops are corn, sunflower and proso millet. This system could increase the intensity by incorporating a spring- or winter-seeded oilseed during the fallow period. It is suggested that growing a summer crop might increase the water use efficiency by 37% relative to the continuous wheat-fallow system (Peterson and Westfall, 2004). More intensive cropping systems also have been shown to increase the soil organic carbon content by 39% (Peterson and Westfall, 2004). This is partly due to the increased plant residues left on the soil. A winter-seeded oilseed such as winter canola or camelina could be compatible with this rotation.

In the Northeastern part of the state, a typical rotation would start with wheat being planted in September and harvested the following July. The land would then be planted to a summer crop such as corn, millet, or sunflowers. These crops are harvested as late as November and the land remains fallow until the following September when it is planted with wheat again. This results in a cropping system that produces two crops in three years with a lengthy fallow period. The incorporation of a short-season oilseed could effectively produce another crop in the fallow period during the time between when the summer crop such as corn is harvested in November and the second wheat crop is planted in the following September as shown in Figure 1.1. Farahani et al. (1998) determined that the water storage efficiency is lowest from May to September. Therefore, having an oilseed crop planted during this period would reduce the inefficiency of the management system.

There are several oilseed crops that are potentially suitable for cultivation in Colorado. These include sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), canola (*Brassica napus*), Indian brown mustard (*Brassica juncea*), camelina (*Camelina sativa*), field pennycress (*Thlaspi arvense*) and soybean (*Glycine max*). Although each of these species can be grown in Colorado, not all of them can be effectively incorporated into a dryland winter wheat rotation. Soybean is a well-known crop that is already planted under irrigation on limited acreages in Colorado, however it is not sufficiently drought tolerant and therefore cannot be incorporated into dryland cropping systems (Johnson et al., 2008). Sunflower, and to a much lesser extent safflower, are grown in Colorado, but they are planted in May and harvested in October, rendering them incompatible with winter wheat (Johnson et al., 2008). This leaves *B. juncea*, canola and camelina as potential oilseed crops that may fit well into the dryland winter wheat crop rotation.

Each of these crops is classified as early maturing, meaning that they require a short growing season to reach full maturity (Johnson et al., 2009; Enjalbert, 2011). Of the three species, canola has been the subject of the most research and variety development (Johnson et al., 2009).

Camelina and Indian brown mustard remain experimental crops, but research interest is growing as studies illuminate their potential in the United States.

Of these three crops, camelina shows the most promise for Colorado (Enjalbert, 2011). Canola is cultivated in Colorado, but shows inferior cold tolerance and sensitivity to high temperatures, both of which can cause reductions in yield. In addition, canola and Indian brown mustard are susceptible to attacks by flea beetles, which cause extensive crop damage (Johnson et al., 2008).

CAMELINA AGRONOMY

Spring Camelina

Camelina sativa, or "gold of pleasure" belongs to the Brassicaceae family and has been cultivated in Europe as an oilseed since the Bronze Age, which began around 4000 BC. (Zubr, 1997). Numerous archeological studies have shown that camelina, flax and cereals constituted a significant portion of the human diet in Europe and Scandinavia during the Bronze Age (Zubr, 1997). For unknown reasons, cultivation of camelina waned until recent interest in low-input biofuels resulted in a reexamination of its value as an oilseed crop and as a potential source of omega-3 fatty acids for human and animal consumption (Zubr, 1997; Frohlich and Rice, 2005).

There have been limited breeding programs implemented in the past few years, however interest is increasing (Vollman et al., 2005). European varieties have been subjected to limited improvement and subsequently brought to the United States, Chile, Canada and other temperate areas where they have been tested and adapted to regional environments (Berti et al., 2011; Enjalbert and Johnson, 2011). Genetic screens using randomly amplified polymorphic DNA

(RAPD) markers have revealed that there is a low degree of diversity for seed characteristics (Vollman et al., 2005).

Camelina can be grown in a dryland winter wheat-based cropping system where it can be treated as a summer annual or a fall-seeded annual (Enjalbert and Johnson, 2011). Camelina is a small-seeded crop and can be broadcast or direct seeded using existing wheat or canola planting equipment at a shallow depth of no more than 12 mm, with 6.3 mm being optimal (Enjalbert and Johnson, 2011). The optimal seeding rate has been found to be 5.6 to 7.8 kg ha^{-1} depending on planting conditions such as seed bed quality, soil humidity, and weed pressure (Robinson, 1987). No-till conditions are appropriate for camelina planting, although there are some weed-control issues that arise from this method of planting due to the lack of herbicide resistant varieties (Lafferty et al., 2009).

Weed control is important. Camelina is generally a strong competitor, but can be overcome with weeds. Tillage prior to spring planting can reduce some of the weed pressure. The only herbicide for weed control in camelina is sethoxydim, or Poast (BASF, 2010), which is useful for control of grassy weeds (Hulbert et al., 2011; Lafferty et al., 2009). Poast can be applied at any point in the growth cycle of camelina, as it has no effect on broadleaved plants (Lafferty et al., 2009). This weed control measure therefore is not effective against thistles, bindweed and other common broadleaved weeds present in the High Plains. For this reason, planting in fields with a history of weed problems should be avoided. Camelina is sensitive to sulfonylurea herbicide residuals such as Ally, Amber and triazine, which are all labeled for use with wheat or corn (Enjalbert and Johnson, 2011).

Seeding of camelina can occur in spring or in the fall. Fall-seeded varieties have a growth cycle similar to wheat in that they establish a stand and overwinter in a dormant stage. Of course, this is dependent on the presence of fall rains. Spring-seeded camelina does best when planted early (Ehrensing and Guy, 2008; Enjalbert and Johnson, 2011). If camelina is planted in March before spring weed emergence, its earlier date of emergence will allow it to compete more vigorously against spring weeds (Lafferty et al., 2009; Enjalbert and Johnson, 2011). Camelina is a short-season crop, requiring roughly 80 days to reach maturity. Early spring planting or late winter planting will allow camelina to mature before summer temperatures cause heat stress and lower yields (French et al., 2009). The required cumulative growing degree days (GDD) for camelina are estimated to be 1,300 °C (Hunsaker et al., 2012). The temperature for germination is 3.3 °C and delay of planting from March until April results in yield reductions of up to 25% due to heat stress (Ehrensing and Guy, 2008). Dryland tests of camelina in Colorado have demonstrated superior yields compared to other oilseed crops (Johnson et al., 2008). Dryland field trials of spring varieties in 2007 and 2008 showed promising yields compared to canola (Johnson et al., 2009). The oil content of camelina ranges from 30% to 45% (Zubr, 1997; Johnson et al., 2009)

Camelina responds to fertilization, with the addition of supplemental nitrogen resulting in yield increases. A general rule of thumb is that camelina needs 2 to 2.7 kg of N to produce 45 kg of grain (Hulbert et al., 2011). This can be applied during the growing season, or if residual nitrogen is available from previous crops, this can be utilized by the plant as well (Hulbert et al., 2011).

During growth, camelina is not susceptible to insect pressure from flea beetles that have been shown to negatively affect yields of canola and *Brassica juncea* (Zubr, 1997). The resistance to flea beetles is thought to be the result of defense compounds present in the leaves of camelina. A class of compounds known as quercetin glycosides has been identified as contributing to its resistance to damage from the crucifer flea beetle (Onyilagha, 2012). The presence of additional leaf compounds means that camelina is naturally resistant to some fungal infections, which is important in irrigated situations (Browne et al., 1991). Camelina has also shown allelopathic relationships with flax, *Linum usitatissimum,* under controlled conditions (Lovett and Sagar, 1978; Lovett and Jackson, 1980).

Camelina can be direct harvested using existing wheat harvesting equipment with a screen of 3.6mm installed over the lower sieves (Enjalbert and Johnson, 2011; Lafferty et al., 2011). Harvesting efficiency can be improved if future varieties are selected to reduce shattering. If weeds are a problem camelina can be swathed when it is 65% yellow (Lafferty et al., 2009).

Camelina is well suited to growth in low-moisture environments. The minimum water requirement for camelina to reach its maximum yield potential has been calculated to be 333mm to 422mm in Arizona (French et al., 2009). The required minimum irrigation varies with climatic conditions and evapotranspiration rate. Below this minimum, yields are negatively affected, however irrigating above this level doesn't show any positive effect on seed yields. Irrigating above this level has been shown to raise evapotranspiration of the plant (Hunsaker et al., 2011). The root zone of camelina is relatively shallow compared to wheat, reaching a maximum depth of 1.4m (Sabu et al., 2000; Hunsaker et al., 2011).

<u>Fall-seeded Camelina</u>

There are two types of camelina varieties: Those vernalization response varieties that can be planted in the fall and allowed to overwinter in the rosette stage (fall-seeded) and those that do not have a vernalization requirement (spring-seeded) (Putnam et al., 1993). In the case of fall-seeded, the plant establishes itself in the fall and overwinters as a rosette. The following spring, when temperatures reach 3.3°C, growth is initiated and the plant emerges from the rosette and resumes growth (Ehrensing and Guy, 2008). Previous experiments have found that fall seeded camelina has enough winter hardiness to survive the harsh winters of Minnesota, where average winter air temperatures are far lower than those found in Colorado (Gesch and Cermak, 2011). As the plant is already established, it reaches maturity earlier than spring-seeded camelina. Earlier maturity means that the plants are not exposed to as much of the heat and drought stress that occurs during the warmest months of summer.

In addition to the potential for increasing yields, earlier harvest allows more time for moisture recharge in the field during the summer. This could result in higher yields for wheat that is planted after fall-seeded camelina than spring-seeded camelina. This may vary based on spring temperatures and moisture conditions. Another advantage of winter camelina is that fall planting is generally drier and the seeds are already planted when spring rainfall arrives. Winter seeding of camelina would be particularly advantageous in Southeastern Colorado, where the winters are warmer and the spring arrives earlier.

Overwintering ability is increased with snow cover (Aase and Siddoway, 1979; Sharratt et al. 1992). Aase and Siddoway (1979) determined that 7 cm of snow cover is sufficient to buffer wheat seedlings from temperatures as low as -40°C. With the increased stubble as a result of the implementation of no-till agricultural systems, there is a greater amount of snow capture on fields in Eastern Colorado.

FATTY ACIDS AND VEGETABLE OILS

Not all vegetable oils are created equal. The vast majority of commercially produced vegetable oils are destined for human or animal consumption. As a result, vegetable oils breeding programs have focused on improvement of the oils from a health perspective. This thesis will be focusing on the suitability of a vegetable oil for use as a diesel substitute. This creates an issue from the food vs. fuel perspective because oilseed varieties that are bred for the optimal production of biofuels are not necessarily optimal for human consumption.

The basic structure of vegetable oil is a triglyceride, which is formed when three fatty acid molecules are connected to a glycerol backbone (Ryan, 1984). The three fatty acid chains may be the same, or may vary in length and composition (Harrington, 1986). What we call vegetable oil is actually an amalgamation of various fatty acid chains of different lengths and degrees of saturation. Saturation is a measurement of the number of double bonds present in the carbon chain (Kahn, 1983; Harrington, 1986). The percentage of each type of carbon chain is known as the fatty acid profile. Different fatty acid profiles determine the chemical and physical properties of the vegetable oil. Depending on these properties, oil can be more or less suitable for one purpose or another. This creates a conundrum for those hoping to improve the oil of a species, because the improvement of food and fuel characteristics of oil involves fulfilling different goals.

The nomenclature that is traditionally utilized to represent the different fatty acids is as follows: Within the parenthesis, the letter C is followed by a number that represents the chain length, i.e., how many carbon molecules are contained within the chain. After the colon there is a second number representing the degree of saturation of the fatty acid chain. For example, linolenic acid is represented as (C18:3). This means that this fatty acid is composed of a carbon chain containing 18 carbon molecules with three double bonds somewhere in the chain. If there are no double bonds, and the second number is 0, the chain is fully saturated. As a rule of thumb, at room temperature, saturated fatty acids are solid because straight saturated chains are joined by the attraction of the carbon and the hydrogen atoms in close proximity (Lyons et al., 1964). Unsaturated fatty acids tend to be liquid at room temperature because the double bonds cause the formation of kinks in the chain. These kinks mean that the chains cannot lie flat on each other and are therefore not subject to the same attractive forces as saturated fatty acids (Lyons et al., 1964).

Velasco et al. (1998) use an estimation of the activity of various pathways that cause the elongation and desaturation of the fatty acids that represents these activities through a series of ratios. The elongation ratio (ER) estimates the activity of the pathway that converts oleic acid (C18:1) to eicosenoic (C20:1). The desaturation ratio (DR) estimates the activity of the pathway responsible for the conversion of linoleic fatty acid (C18:2) to linolenic (C18:3). This desaturation pathway is further subdivided into the oleic desaturation ratio (ODR) and the linoleic desaturation ratio (LDR). The ODR estimates the efficiency of the desaturation from oleic to linoleic fatty acid while the LDR estimates the efficiency of the linoleic to linolenic conversion pathway (Pleines and Friedt, 1988). Figure 1.2 shows how to calculate these ratios.

These ratios are useful when they are compared for many different species or varieties. This gives an idea of which varieties are more efficient producers of the desired fatty acids, which could be a valuable breeding tool for estimating genetic gain for oilseed improvement programs. Breeding for improvement of the fatty acid profile is very difficult. The metabolic pathways that produce the component fatty acids of the oil profile are composed of complex pathways that result in the production of different fatty acids in particular concentrations. These pathways are influenced by the environment (Tremolieres et al., 1982).

The fatty acid composition of vegetable oil determines its suitability as a food or fuel source. In the case with bio-based diesel fuels, there are two possibilities for on-farm production, conversion to biodiesel and utilization in an unrefined state as straight vegetable oil. Each has its benefits and drawbacks, but the main difference is that biodiesel can be blended with conventional diesel to help meet the RFS2 biodiesel blending mandate and SVO requires minimal processing and is therefore preferable for on-farm production.

Biodiesel

Biodiesel is the term for any triglyceride (derived from a vegetable oil or animal fat) that has been subjected to a chemical conversion process known as transesterification. Transesterification is the process of switching an organic group from an ester to an alcohol. This process makes the vegetable oil directly compatible with mineral diesel fuel and is outlined in Figure 3 (Van Gerpen, 2005).

During transesterification, raw vegetable or animal-derived triglycerides are combined with an alcohol such as methanol or ethanol and reacted with a catalyst. The primary products resulting from this process are glycerol, which formed the backbone of the triglyceride, and fatty acid methyl esters, or biodiesel. There is evidence that the oil profile of the triglyceride feedstock plays a large role in the efficiency of the process and the quality of the resulting biodiesel (Pinzi et al., 2009).

The oil profile of the feedstock vegetable oil influences the quality of the biodiesel product in a number of ways. The most common parameters that researchers use to judge the suitability of a biofuel feedstock based on its oil profile are iodine value, cetane number, cold weather performance, kinematic viscosity, and free fatty acid content. Iodine value is a measure of the degree of saturation of the fatty acid. High iodine value, meaning high unsaturation, has been shown to negatively effect oxidative stability, or shelf life, of the biodiesel (Azam et al., 2005; Pinzi et al., 2009). Oxidation, in turn, negatively affects engine performance over time (Van Gerpen, 2005). Cetane number is a quantification of the combustion quality of the fuel. High combustion quality reduces engine 'knocking'. Higher cetane number is correlated with higher ignition quality and leads to lower emission of harmful nitrogen oxide (NOx) pollutants (Pinzi et al., 2009). Cetane number increases with increased chain length and saturation (Knothe, 2008). Related to iodine value is cold weather performance. Polyunsaturated fatty acids show better cold weather performance than more saturated fatty acids (Pinzi et al., 2009). Kinematic viscosity is very important for several reasons. Increased viscosity decreases the efficiency of the fuel's flow through the engine. This decreases ignition efficiency leading to engine drag, greater fuel consumption and increased emission of NOx (Knothe, 2008). The presence of free fatty acids is important for the production of biodiesel. Low quality feedstocks contain more than 5% free fatty acids. The presence of these fatty acids causes loss of catalyst during the transesterification process, as when free fatty acids react with the alkali catalyst, insoluble soap is formed that must be removed before combustion. High percentages of free fatty acids are generally found in vegetable oils that have been used for cooking (Van Gerpen, 2005).

In comparison to petroleum-based diesel, production of biodiesel has a lower net emission of greenhouse gasses and it is less damaging to the environment due to its biodegradability and low toxicity (Azam et al., 2005). In terms of diesel oil substitutes, biodiesel is the only mineral diesel alternative that can be run in a traditional diesel engine with little or no engine modification (Johnson and Taconi, 2006). Certified biodiesel can also be sold to petroleum distributers where it is blended with petroleum in certain quantities so that the producers are able to meet federal blending requirements (Bang, 2011). When considering cost, the feedstock production costs are the most important, as they comprise nearly 80% of the production costs of making biodiesel (Demirbas, 2006)

As glycerol comprises approximately 10% of the weight of vegetable oil feedstocks, it is produced in substantial quantities as a secondary product (Thompson and He, 2006). As glycerol is denser than biodiesel, it naturally separates from the resulting mixture following the transesterification process (Van Gerpen, 2005). After it is produced, it is cleaned through the removal of the ethanol or methanol. The glycerol byproduct can be used in a number of ways. It can be sold to paper, food, chemical, cosmetic or other industries, or else it can be co-fired to create electricity or heat for the biodiesel production facility (Johnson and Taconi, 2007; Thompson and He, 2006).

Straight Vegetable Oil

Straight vegetable oil (SVO) can be used as an alternative to processed biodiesel. The principal difference between using SVO and biodiesel is the viscosity of the fuel. For this reason, a fuel-heating unit must be added to the vehicle's fuel line to decrease the viscosity of the vegetable oil (Nettles-Anderson, 2009).

In order to run SVO in a truck or piece of agricultural machinery, engine modifications are recommended along with the above mentioned fuel line modifications. A conversion kit designed by the German company Elsbett (Thalmässing, Germany) includes modified injector nozzles, duel fuel tank with a heater, a modified temperature control system, strong glow plugs, and dual fuel filters. With these minor modifications to the fuel line, it is reported that filtered highly saturated palm oil can meet international requirements for use as diesel fuel (Bari et al., 2002; Masjuki and Abdulmunin, 1996). The vegetable oil must first be degummed before it can be run through an engine. This inexpensive process involves heating the vegetable oil to 40°C briefly while a small amount of phosphoric acid is added. After one week, the gums are fully precipitated to the bottom where they can be used as a fertilizer (Haldar et al., 2009).

SVO can be made more directly compatible with conventional diesel engines through blending, where it is mixed with mineral diesel in different concentrations. Blending SVO from *Jatropha curcas* in India with diesel fuel in concentrations up to 30% produces a fuel that is nearly indistinguishable from mineral diesel with respect to fuel and emissions properties, meaning that engine modifications are not necessary (Agarwal et al., 2007). As the vegetable oil component of the blend increases, the viscosity and the energy value, or gross calorific value, is decreased (Wang et al., 2006). Blending is a good alternative for those who are thinking of using SVO on their farm, but are not ready to make the full conversion by purchasing a conversion kit. This would also be useful for those who are interested in buying SVO from a local producer.

One of the most significant advantages to using SVO instead of biodiesel is an energy savings from the reduced processing inputs and the environmentally advantageous lack of secondary products such as glycerol. A common method for comparing the energy savings of a type of biofuel is to represent its output in terms of an energy conversion ratio. This ratio is a measure of the energy consumed in the processing phases of these fuels over the energy gained from combustion of the fuel. Esteban et al. (2011) performed a lifecycle analysis comparing the energy allocation ratios of biodiesel and SVO. They found that the ratio was 2.34 for SVO and 1.77 for biodiesel. This is a substantial energy savings compared to biodiesel. Another important consideration for SVO is that it can be produced locally and doesn't need to be shipped to a processing facility and then entered into the petroleum distribution system. Small-scale crushing facilities already exist in several areas in Colorado. These are located in Stratton, Costilla County, and Rocky Ford, Colorado (Enjalbert and Johnson, 2011).

There are some issues with using SVO in a diesel engine. One of these is the lower energy value compared to mineral diesel, which means that a greater volume of fuel is consumed during engine operation (Agarwal et al., 2007). In addition, there is the expense of converting a vehicle or piece of machinery with a tank heater to increase the viscosity of the fuel. If the vegetable oil fuel is heated to more than 90°C there is the possibility of causing damage to engine components if the process is not properly monitored. There is also the possibility for an increase in harmful NOx emissions (Nettles-Anderson, 2009). There can be a delay while the oil is being degummed to reduce buildup on the injectors (Nettles-Anderson, 2009; Haldar et al., 2009).

The emissions from burning SVO vary depending on the oil profile of the feedstock vegetable oil. Generally, the emissions of NOx and carbon monoxide are lower than emissions from petroleum diesel (Wang et al., 2006). In the case of SVO, the most significant gaseous pollutants are NOx compounds. Generally, a higher percentage of polyunsaturated fatty acid in the oil and lower combustion temperature will result in higher NOx emissions (Bari et al., 2002). High viscosity also increases NOx emissions due to incomplete combustion however; this is avoided by preheating the vegetable oil (Wang et al., 2006). The most important factor for reducing NOx emissions is high engine temperature.

Camelina as a fuel oil

As a candidate feedstock for biodiesel production, camelina is suitable in all respects except its high iodine value due to the high degree of unsaturation of its oil (Frohlich and Rice, 2005). This high iodine value would result in higher than acceptable levels of oxidation and issues with pouring quality (Pinzi et al., 2005). The concentration of free fatty acids in camelina was found to be 3.1%, which is within the acceptable 5% range for biodiesel feedstocks (Budin et al., 1995). Frohlich and Rice (2005) performed engine tests for camelina fatty acid methyl ester and found that the high iodine value was within the acceptable specifications, meaning that it closely resembled the trial results for rapeseed oil, especially when the biodiesel was blended with petroleum diesel to improve the cold temperature and pour point qualities.

Camelina seeds are composed of 30%-40% oil (Zubr, 1997). Vegetable oil produced from camelina seeds is composed of over 50% polyunsaturated fatty acid (Figure 1.4), 30-45% of which are omega-3 alpha-linolenic acid. Due to this high concentration of omega-3 fatty acids, it is suggested that consumption of camelina oil is beneficial to human and animal health (Frohlich and Rice, 2005). This high concentration of polyunsaturated fatty acids and protein present in the press cake is a valuable addition to feed, but must be added in modest proportions. It is recommended that camelina meal comprise no more than 15% of the feed weight due to the concentration of toxic glucosinolates that can negatively affect the thyroid (Moriel et al., 2011). Camelina meal can also replace up to 5 percent of broiler chicken feed without negatively impacting the quality of the meat. The incorporation of this feed increases the intramuscular concentration of omega-3 fatty acid (Ryhanan et al., 2007). The protein content of the press cake left over from the hexane solvent extraction process is suitable for animal consumption. It is lower in fat due to the increased efficiency of the extraction technique but it has protein content similar to that of soybean meal. Any harmful compounds such as glucosinolates and erucic acid that are present in the seeds before pressing can be extracted by subsequent solvent treatment of the seed meal (Naczk et al., 1985). The consumer of vegetables within the Brassica family will recognize glucosinolates from the pungent odor that is released from the cooking of cabbagees and Brussels's sprouts. With these vegetables, and with leftover seed meal, glucosinolate content can be reduced by heating of the product prior to consumption due to leaching and some decomposition (Fenwick and Heaney, 1983).

The leftover seed meal from camelina pressing contains 40 to 45% crude protein and 10% fiber, which is lower than soybeans but comparable to rapeseed press cake (Ryhanen et al., 2007). Combined with the residual fatty acids and a lack of toxic erucic acid (C22:1), the molecular profile of the leftover seed meal indicates that it could be a potentially valuable coproduct as animal feed. Future improvements and plant breeding research will need to focus on optimizing the biofuel oil profile to raise the percentage of oleic acid (18:1) and decrease concentrations of linolenic acid (18:3) (Pinzi et al., 2009). This would eliminate some of the health benefits of consuming camelina seed meal, thereby affecting its value as an additive to animal feed, but it would make a better diesel alternative. Figure 1.4 compares the oil profiles of several oilseeds that are grown in different areas of the United States.

Oil extraction

There are two methods for expelling the oil from the feedstock oilseeds. The most common, easiest, and lowest cost method of oil extraction uses a mechanical oilseed crusher. This machine heats and crushes the seeds, which causes the separation of the oil from the seed meal (Kahn and Hanna, 1983). Figure 1.5 shows an example of a screw-press oil expeller. The resulting press cake contains approxinately 10% oil by weight and is considered to have an extraction efficiency of 75% (Boateng et al., 2010). Another common extraction method uses hexane as a solvent. This hexane extraction raises the efficiency to 95% (Esteban et al., 2011).

Another solvent-mediated extraction technology uses either 5% methanol and water or a two-phase system that consists of a 10% solution of ammonia in methanol (Naczk et al., 1985).

Oilseed press cake can be converted to liquid fuel using a thermochemical conversion process known as pyrolysis. This has been shown to have a carbon conversion efficacy of 60 to 80%. Coproduction of this fuel would provide additional fuel that could be used to co-fire an oilseed crushing facility (Boateng et al., 2010).

GENOTYPE BY ENVIRONMENT INTERACTION (GxE)

The characterization of plants with stable drought responses with respect to yield and oil quality characteristics is important for identifying individuals that are stable across the variable environmental conditions of the arid High Plains region. It is important to remember, when breeding for stability across environments, that a certain degree of phenotypic plasticity is not always negative (Bradshaw, 2006; Freeman et al., 1993). Plants that can respond and change their phenotype in response to their environment are useful in environments where they are likely to encounter a large degree of variability from year to year.

The term “environment” in this case generally refers to different geographic environments and years. The interaction between the genotypes and the environment occurs when the ranked performance of a cultivar changes among environments. This is known as crossover interaction (Fehr, 1987). Non crossover interaction can also occur when the performance of a genotype changes between environments, but this does not alter the ranking of a genotype compared to other entry genotypes (Fehr, 1987)

GxE is important to consider when making varietal recommendations. Farmers could suffer heavy losses if varietal recommendations are made based on yield data where overall yield stability is not taken into consideration. The stability of a variety depends on the heritability of its yield and yield components. The heritability component of yield is reduced if genotype x year x environments (G x Y x E) interactions are significant (Richards et al., 2010).

The High Plains region of the United States encompasses a large area stretching from Eastern Washington to Arizona that contains many different micro and macroclimates (Peterson et al., 2006). Dryland areas throughout this arid region vary greatly in terms of temperature, elevation, and precipitation, each bestowing its own stress on crop plants. The way that crop plants react to each of these stress factors can affect their ability to withstand environmental stresses (Bohnert et al., 1995; Nicotra and Davidson, 2010). The source of the plant's ability to adapt to stress conditions varies based on the degree of plasticity exhibited by the trait and the biochemical pathways controlling the responses (Bohnert et al., 1995; Nicotra et al., 2010). In earlier experiments, earlier-flowering varieties of camelina demonstrated higher yields presumably because of higher water availability earlier in the spring (Enjalbert et al., 2011). Another factor that could affect the suitability of different camelina cultivars in the High Plains is water use efficiency. A variety with high water use efficiency would use water efficiently under periods of drought but not under periods of water abundance (Nicotra and Davidson, 2010). This is an example of phenotypic plasticity that can contribute to the overall fitness of a variety across environments.

Oil profiles are affected by climatic conditions (Aslam et al., 2009). There has been a demonstrated instability across environments of the oil profile of soybean particularly with respect to concentrations of oleic fatty acid (C18:1) (Bachlava et al., 2008). This is important from the perspective of oilseed breeders working to optimize oil profiles for biofuels as there is evidence that oleic acid content has a negative correlation to the content of linolenic fatty acid

(C18:3) (Bachlava et al., 2008). Earlier flowering varieties of camelina are also shown to have higher linolenic fatty acid content in their seeds (Enjalbert et al., 2011). This could be because linolenic fatty acid contributes to cell membrane fluidity at lower temperatures, therefore contributing to increased cold-tolerance (Linder, 2000). Higher concentration of polyunsaturated fatty acids increases the cold or heat tolerance of oilseed species (Lyons et al., 1964; Seiler et al., 1983; Kodama et al., 1994; Murakami et al., 2000; Izquierdo et al., 2003; Baux et al., 2008; Sadras et al., 2009). This environmental variability in oil profile influences the suitability of oil for use as a fuel.

It is important to select for varieties that perform equally well in drought years as well as years with ample rainfall. In other crops such as wheat, efforts have been made to model the responses of these varieties to climatic conditions so that a check can be made for comparison to yields (Chapman, 2008). In experimental conditions, these modeling exercises have shown that much of the variation is due to rainfall timing rather than amount of rainfall (Hammer et al., 2005). To properly quantify the crop's response to environmental factors, it is important to have as many representative samples as possible.

Breeding for stability over environments will become more important in the future as climate change and global warming affect both absolute environmental conditions and variability of environmental conditions. With a changing climate, varieties will become outdated sooner while stability over years in the same environment will become a more pressing issue.

Wheat-Fallow-Oilseed Based Cropping System for Southeastern Colorado

Year	March	April	May	June	July	Aug	September	Oct	November	Dec to Feb
1	Oilseed Planted				Oilseed Harvested		Wheat planted			
2					Wheat Harvested					
3	Oilseed Planted				Oilseed Harvested		Wheat Planted			
4					Wheat Harvested					

Wheat-Spring Crop-Fallow-Oilseed Cropping System for Northeastern Colorado

Year	Mar	Apr	May	June	July	Aug	Sept	Oct	November	Dec to Feb
1							Wheat Planted			
2					Wheat Harvested					
3		Corn Planted							Corn Harvested	
4	Oilseed Planted				Oilseed Harvested		Wheat Planted			

Figure 1.1. Colorado cropping systems that could include an oilseed species

$$ER = \frac{\%C20{:}1 + \%C22{:}1}{\%C20{:}1 + \%C22{:}1 + \%18{:}1 + \%C18{:}2 + \%C18{:}3}$$

$$DR = \frac{\%C18{:}2 + \%C18{:}3}{\%C20{:}1 + \%C22{:}1 + \%C18{:}1 + \%C18{:}2 + \%C18{:}3}$$

$$ODR = \frac{\%C18{:}2 + \%C18{:}3}{\%C18{:}1 + \%C18{:}2 + \%C18{:}3}$$

$$LDR = \frac{\%C18{:}3}{\%C18{:}2 + \%C18{:}3}$$

Figure 1.2. Calculating the ratios to estimate fatty acid production efficiency (Pleines and Friedt, 1988; Velasco et al., 1998).

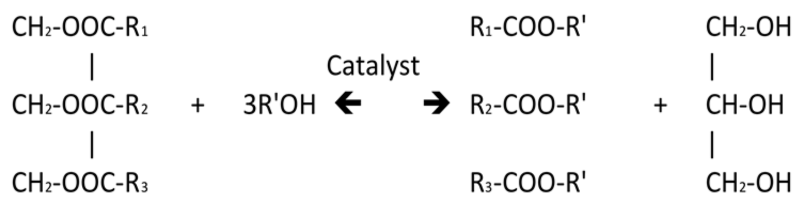


Figure 1.3. An outline of the transesterification process (Ma et al., 1999).

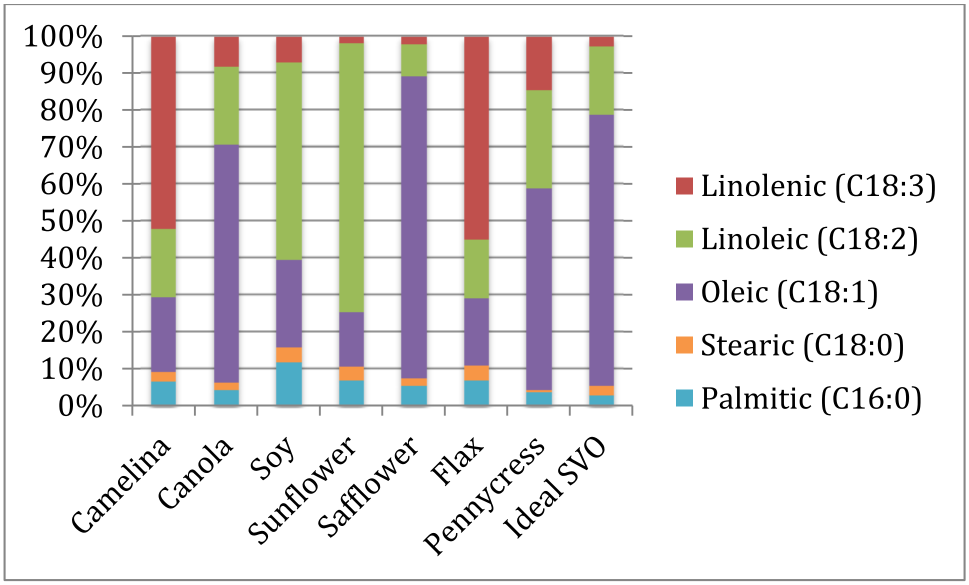


Figure 1.4- Oil profiles of different oilseed species (Zubr, 1997; Isbell, 2009; Pinzi et al., 2009).

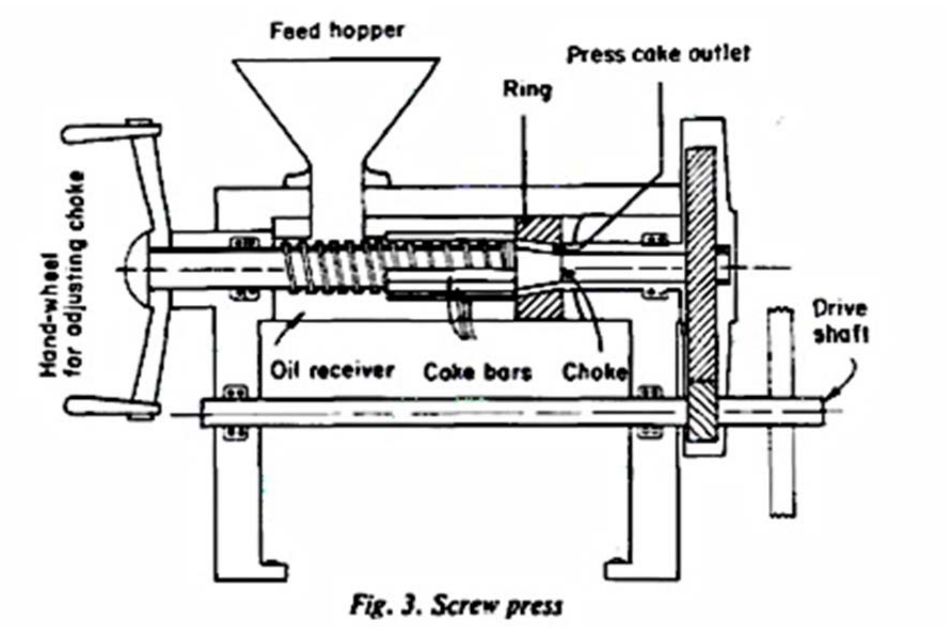


Figure 1.5. Diagram of a screw-press oil expeller (Kahn and Hanna, 1983)

REFERENCES

Aase, J.K., and F.H. Siddoway. 1979. Crown-depth soil temperatures and winter protection for winter wheat survival. Journal of the Soil Science Society of America 43:1229-1233.

Agarwal, D., and A.K. Agarwal. 2007. Performance and emissions characteristics of Jatropha oil (preheated and blends) in a direct injection compression engine. Applied Thermal Engineering 27:2314-2323.

Allison, N.L., Bindoff, R.A., Bindoff, R.A., Bindschadler, P.M., Cox, N., de Noblet, M.H., England, J.E., Francis, N., Gruber, A.M., Haywood, D.J., Karoly, G., Kaser, C., Le Quéré, T.M., Lenton, M.E., Mann, B.I., McNeil, A.J., Pitman, S., Rahmstorf, E., Rignot, H.J., Schellnhuber, S.H., Schneider, S.C., Sherwood, R.C.J., Somerville, K.Steffen, E.J., Steig, M., Visbeck, and A.J. Weaver. 2010. The Copenhagen diagnosis, 2009: Updating the world on the latest climate science. The University of New South Wales Climate Change Research Centre (CCRC), Sydney, Australia, 60pp.

Aslam M.N., Nelson M.N., Kailis S.G., Bayliss K.L., Speijers J., and W.A. Cowling. 2009. Canola oil increases in polyunsaturated fatty acids and decreases in oleic acid in drought-stressed Mediterranean-type environments. Plant Breeding 128:348-355.

Azam, M.M., Waris, A., and N.M. Nahar. 2005. Prospects and potential of fatty acid methyl esters of some non-traditional seed oils for use as biodiesel in India. Biomass and Bioenergy 29:293-302.

Bachlava, E., Burton, J.W., Brownie, C., Wang, S.B., Auclair, J., and A.J. Cardinal. 2008. Heritability of oleic acid content in soybean seed oil and its genetic correlation with fatty acid and agronomic traits. Crop Science 48:1764-1772.

Bang, G. 2010. Energy security and climate change concerns: triggers for energy policy change in the United States? Energy Policy 38(4):1645–1653.

Bari, S., Lim, T.H., and C.W. Yu. 2002. Effect of preheating crude palm oil (CPO) on injection system, performance and emission of a diesel engine. Renewable Energy 27:171-81.

BASF, 2010. Poast Herbicide Supplemental Label. For use in Gold of Pleasure. EPA Reg. No. 7969-58. BASF.

Baux A., Hebeisen, T., and D. Pellet. 2008. Effects of minimal temperatures on low-linolenic rapeseed oil fatty-acid composition. European Journal of Agronomy 29:102-107.

Bessou, C., Ferchaud, F., Gabrielle, B., and B. Mary. 2011. Biofuels, greenhouse gases and climate change. a review. Agronomy for Sustainable Development 31:1–79.

Berti, M., Wilckens, R., Fischer, S., Solis, A., and B. Johnson. Seeding date influence on camelina yield, yield components, and oil content in Chile. Industrial Crops and Products 34:1358-1365.

Boateng, A.A., Mullen, C.A., and N.M. Goldberg. 2010. Producing stable pyrolosis liquids from the oil-seed presscakes of mustard family plants: Pennycress (*Thlaspi arvense* L.) and camelina (*Camelina sativa*). Energy and Fuels 24:6624-6632.

Bohnert H.J., Nelson, D.E., and R.G. Jensen. 1995. Adaptations to environmental stresses. Plant Cell 7:1099-1111.

Bradshaw, A.D. 2006. Phenotypic plasticity: Why should we bother? New Phytologist 170(4):644-648.

Browne L.M., Conn, K.L., Ayer, W.A., and J.P. Tewari. 1991. The camelexins: new phytoalexins produced in the leaves of *Camelina sativa* (Cruciferae). Tetrahedron 47(24):3909-3914.

Campos, H., Cooper, A., Habben, J.E., Edmeades, G.O., and J.R. Schussler. 2004. Improving drought tolerance in maize: a view from industry. Field Crops Research 90:19–34
Carriquiry, M.A., Du, X., and G.R. Timilsina. 2011. Second generation biofuels: economics and policies. Energy Policy 39(7):4222–4234.
Chapman, S.C. 2008. Use of crop models to understand genotype by environment interactions for drought in real-world and simulated plant breeding trials. Euphytica 161:195-208.
CIA World Factbook. 2010. "Country comparison: Oil consumption." https://www.cia.gov/library/publications/the-world-factbook/rankorder/2174rank.html.
Clark, C. Colorado agricultural statistics. 2011. National Agricultural Statistics Service. http://www.nass.usda.gov/Statistics_by_State/Colorado/index.asp.
Congressional Research Service. 2011. Biofuel incentives: a summary of federal programs. Congressional Research Service, Washington, D.C.
Demirbas, A. 2006. Biodiesel production via non-catalytic SCF method and biodiesel fuel characteristics. Energy Conversion and Management 47:2271-2282.
Ehrensing, D.T., and S.O. Guy. 2008. Oilseed crops: Camelina. Oregon State University Extension Service EM 8953-E.
Enjalbert, J.N. 2011. An integrated approach to local based biofuel development. (Doctoral Dissertation). Colorado State University Libraries. 140p. http://hdl.handle.net/10217/46747.
Enjalbert, J.N., and J.J. Johnson. 2011. Guide for producing dryland camelina in Eastern Colorado. Colorado State University Extension factsheet No. 0.709. http://www.ext.colostate.edu/pubs/crops/00709.pdf.
Esteban, B., Baquero, G., Puig, R., Jordi-Roger, R., and A. Rius. 2011. Is it environmentally advantageous to use vegetable oil directly as biofuel instead of converting it to biodiesel? Biomass and Bioenergy 35:1317-1328.
Farahani, H.J., Peterson, G.A., Westfall, D.G., Sherrod, L.A., and L.R. Ahuja. 1998. Soil water storage in dryland cropping systems: the significance of cropping intensification. Soil Science of America Journal 62:984-991.
"Federal & State Incentives and Laws." 2011. Alternative Fuels Data Center: Federal and State Laws and Incentives, and U.S. Department of Energy. http://www.afdc.energy.gov/afdc/laws/law/US/390.
Fehr, W.R. 1987. Principles of crop development. Vol 1. Theory and Technique. Macmillan Publishing Co., New York, New York.
Fenwick, G.R., and R.K. Heaney. 1983. Glucosinolates and their breakdown products in cruciferous crops, food and feedingstuffs. Food Chemistry 11: 249-271,
Freeman, C.D., Graham, J.H., and J.M. Emlen. Developmental stability in plants: symmetries, stress and epigenesis. Genetica 89:97-119.
French, A.N. Hunsaker, D., Thorp, K., and T. Clarke. 2009. Evapotranspiration over camelina crop at Maricopa, Arizona. Industrial Crops and Products 29:289-300.
Frohlich, A., and B. Rice. 2005. Evaluation of *Camelina sativa* oil as a feedstock for biodiesel production. Industrial Crops and Products 21:25-31.
Gesch, R.W., and S.C. Cermak. 2011. Sowing date and tillage effects on fall-seeded camelina in the northern corn belt. Agronomy Journal 103(4): 980-987.
Haldar, S.K., Ghosh, B.B., and A. Nag. 2009. Studies on the comparison of performance and emission characteristics of a diesel engine using three degummed non-edible vegetable oils. Biomass and Bioenergy 33:1013-1018.

Hammer, G.L., Chapman, S., Van Oosterom, E., and D.W. Podlich. 2005. Trait physiology and crop modelling as a framework to link phenotypic complexity to underlying genetic systems. Australian Journal of Agricultural Research 56:947–960.

Harrington, K.J. 1986. Chemical and physical properties of vegetable oil esters and their effect on diesel fuel performance. Biomass 9:1-17.

Hulbert, S., Guy, S., Pan, B., Paulitz, T., Schillinger, B., Wysocki, D., and K. Sowers. 2011. Camelina production in the Pacific Northwest. Washington State University Extension Publication. http://css.wsu.edu/biofuels/publications/ Camelina_Production_PNW_Fact_Sheet.pdf.

Hunsaker, D.J., French, A.N., and K.R. Thorp. 2012. Camelina water use and seed yield response to irrigation scheduling in an arid environment. Irrigation Science. Published online: 31 July, 2012. DOI: 10.1007/s00271-012-0368-7

Hunsaker, D.J., French, A.N., Clarke, T.R., and D.M. El-Shikha. 2011. Water use, crop coefficients and irrigation management criteria for camelina production in arid regions. Irrigation Science 29:27-43.

IPCC Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (AR4). S. Solomon et al. eds (Cambridge University Press, Cambridge, UK & New York, NY, USA).

Izquierdo, N., Aguirrezabal, L., Andrade, F., and V. Pereyra. 2002. Night temperature affects fatty acid composition in sunflower oil depending on the hybrid and the phenological stage. Field Crops Research 77:115-126.

Johnson, D.T., and K.A. Taconi. 2007. Crude glycerol resulting from biodiesel production. Environmental Progress 26(4):338-348.

Johnson, J.J., Enjalbert, N., Schneekloth, J., Helm, A., Malhotra, R., and D. Coonrod. 2009. Development of oilseed crops for biodiesel production under Colorado limited irrigation conditions. Completion report no. 211. Colorado Water Institute.

Johnson, J.J., Enjalbert, N., Shay, R., Heng, S., and D. Coonrod. 2008. Investigating straight vegetable oil as a diesel fuel substitute: Final report to Colorado agricultural value-added development board. Fort Collins, Colorado.

Khan, L.M., and M.A. Hanna. 1983. Expression of oil from oilseeds-A review. Journal of Agricultural Engineering Research 28:495-503.

Knothe, G. 2008. "Designer" biodiesel: Optimizing fatty acid ester composition to improve fuel properties. Energy and Fuels 22:1358-1364.

Kodama, H., Hamada, T., Horiguchi, G., Nishimura, M., and K. Iba. 1994. Genetic Enhancement of cold tolerance by expression of a gene for chloroplast omega-3-fatty-acid desaturase in transgenic tobacco. Plant Physiology 105:601-605.

Lafferty, R.M., Rife, C., and G. Foster. 2009. Spring camelina production guide for the central high plains. Blue Sun Biodiesel special extension publication, ACRE. http://www.colorado.gov/cs/Satellite?blobcol=urldata&blobheader=application%2Fpdf&blobkey=id&blobtable=MungoBlobs&blobwhere=1251616501820&ssbinary=true

Linder, C. R., and J. Schmitt. 1995. Potential persistence of escaped transgenes: performance of transgenic, oil- modified Brassica seeds and seedlings. Ecological Applications 5:1056–1068.

Lovett, J.V, and A.M. Duffield. 1981. Allelochemicals of *Camelina sativa*. Journal of Applied Ecology 18(1):283-290.

Lüthi, D., LeFloch, M., Bereiter, B., Blunier, T., Barnola, J.M., Siegenthaler, U., Raynaud, D., Jouzel, J., Fischer, H., Kawamura, K., and T.F. Stocker. 2008. High-resolution carbon dioxide concentration record 650,000-800,000 years before present. Nature 453:379-382.

Lyons J.M., Pratt H.K., and T.A. Wheaton. 1964. Relationship between physical nature of mitochondrial membranes and chilling sensitivity in plants. Plant Physiology 39:262-268.

Masjuki, H., and M.Z. Abdulmunin. 1996. Investigations on preheated palm oil methyl esters in the diesel engine. Proceedings of the Institute of Mechanical Engineers 213:417-425.

Moriel, P., Nayigihugu, V., Cappellozza, B.I., Goncalves, E.P., Krall, J.M., Foulke, T., Cammak, K.M., and B.W. Hess. 2011. Camelina mean and crude glycerin as feed supplements for developing replacement beef heifers. Journal of Animal Science 89:4314-4324.

Murakami, Y., Tsuyama, M., Kobayashi, Y., Kodama, H., and K. Iba. 2000. Trienoic fatty acids and plant tolerance of high temperature. Science 287:476-479.

Naczk, M., Diosady, L.L., and L.J. Rubin. 1985. Functional properties of canola meals produced by a two-phase solvent extraction system. Journal of Food Science 50:1685-1688.

Nettles-Anderson, S.L., and D.B. Olsen. 2009. Survey of Straight Vegetable Oil Composition Impact on Combustion Properties. SAE International.

Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius, U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F., and M. van Kleunen. 2010. Plant phenotype plasticity in a changing climate. Trends in Plant Science 15(12):684-692.

Nicotra, A.B., and A. Davidson. 2010. Adaptive phenotypic plasticity and plant water use. Functional Plant Biology 37:117-127.

Onyilagha, J.C., Gruber, M.Y., Hallet, R.H. , Holowachuk, J., Buckner, A., and J.J. Soroka. 2012. Constitutive flavonoids deter flea beetle insect feeding in *Camelina sativa.* Biochemical Systematics and Ecology 42:128-133.

Peterson, G.A., Unger, P.W., Payne, B.A., Anderson, R.L., and R.L. Baumhardt. 2006. Dryland agriculture research issues. American Society of Agronomy Monograph Series 23:901-907.

Peterson, G.A., and D.G. Westfall. 2004. Managing precipitation use in sustainable dryland agroecosystems. Annals of Applied Biology 144:127-138.

Pinzi, S., Garcia, I.L., Lopez-Gimenez, F.J., Luque de Castro, M.D., Dorado, G., and M.P. Dorado. 2009. The ideal vegetable oil-based biodiesel composition: a review of social, economical and technical implications. Energy and Fuels 23:2325-2341.

Pleines, S. and W. Friedt. 1988. Breeding for improved C18-fatty acid composition in rapeseed (*Brassica napus* L.). Fat Science Technology 90: 167–171.

Putnam, D.H., J.T. Budin, L.A. Field, and W.M. Breene. 1993. Camelina: A promising low-input oilseed. p. 314–322. In J. Janick and J.E. Simon (eds). New crops. John Wiley & Sons, New York.

Raymo, M.E., Grant, B., Horowitz, M., and G.H. Rau. 1996. Mid-Pliocene warmth: stronger greenhouse and stronger conveyer. Marine Micropaleontology 27:313-326.

"Renewable Fuel Standard." 2012. United States Environmental Protection Agency: Fuels and fuel additives. http://www.epa.gov/otaq/fuels/renewablefuels/index.htm.

Richards, R.A., Rebetzke, G.J., Watt, M., Condon, A.G., Spielmeyer, W., and R. Dolferus. 2010. Breeding for improved water productivity in temperate cereals: phenotyping, quantitative trait loci, markers and the selection environment. Functional Plant Biology 37:85-97.

Robinson, R.G. 1987. "Camelina: A Useful Research Crop and a Potential Oilseed Crop." Minnesota Agricultural Research Station, Station Bulletin 579.

Ryan, T.W., Dodge, L.G., and T.J. Callahan. 1984. The effects of vegetable oil properties on injection and combustion of two different diesel engine. Journal of American Oil Chemists' Society 61(10):1610-1619.

Ryhanen, E.L., Perttla, S., Tupasela, T., Valaja, J., Eriksson, C., and K. Larkka. 2007. Effects of *Camelina sativa* expeller cake on performance and meat quality of broilers. Journal of the Science of Food and Agriculture 87(8):1489-1494.

Sabu, P. Panda, S.N., and D.N. Kumar. 2000. Optimal irrigation allocation: a multilevel approach. Journal of Irrigation and Drainage Engineering 126(3):149-156.

Sadras, V.O., Reynolds, M.P., de la Vega, A.J., Petrie, P.R., and R. Robinson. 2009. Phenotypic plasticity of yield and phenology in wheat, sunflower and grapevine. Field Crops Research 110:242-250.

Seiler, G.J. 1983. Effect of Genotype, Flowering Date, and Environment on Oil Content and Oil Quality of Wild Sunflower Seed. Crop Science 23:1063-1068.

Sharratt, B.S., D.G. Baker, D.B. Wall, R.H. Skaggs, and D.L. Ruschy. 1992. Snow depth required for near steady-state soil temperatures. Agricultural and Forest Meteorology 57:243–251.

Siddique, K.H.M., Tennant, D., Perry, M.W., and R.K. Belford. 1990. Water-Use and Water-use efficiency of old and modern wheat cultivars in a mediterranean-type environment. Australian Journal of Agricultural Research 41:431-447.

Thompson, J.C., and B.B. He. 2006. Characterization of crude glycerol from biodiesel production from multiple feedstocks. Applied Engineering in Agriculture 22(2):261-265.

Tremolieres, A., Dubacq, J.P., and D. Drapier. Unsaturated fatty acids in maturing seeds of sunflower and rape: regulation by temperature and light intensity. Phytochemistry 21(1):41-45.

Tripati, A.K., Roberts, C.D., and R.A. Eagle. 2009. Coupling of CO_2 and ice sheet stability over major climate transitions of the last 20 million years. Science 326:1394-1397.

Union of Concerned Scientists 2012. "Global Warming-Each Country's share of CO2 emissions." http://www.ucsusa.org/global_warming/science_and_impacts/science/each-countrys-share-of-co2.html.

USDA Biofuels Strategic Production Report. 2010. http://www.usda.gov/documents/USDA_Biofuels_Report_6232010.pdf.

U.S. Energy Information Administration. 2011. "Annual energy review." Retrieved Aug 27, 2012. http://www.eia.gov/totalenergy/data/annual/perspectives.cfm.

Van Gerpen, J. 2005. Biodiesel processing and production. Fuel Processing Technology 86:1097-1107.

Velasco, L. Fernanez-Martinez, J.M., and A. De Haro. 1999. Intraspecific breeding for reduced glucosinolate content in Ethopian Brown mustard (*Brassica carinata* A. Braun). Euphytica 106:125-130.

Velasco, L., Goffman, F.D., and C. Becker. 1998. Variability for the fatty acid composition of the seed oil in a germplasm collection of the genus *Brassica*. Genetic Resources and Crop Evolution 45:371-382.

Vollman, J., Grausgruber, H., Stift, G., Dryzhyruk, V., and T. Lelly. 2005. Genetic diversity in camelina germplasm as revealed by seed quality characteristics and RAPD polymorphism. Plant Breeding 124:446-453.

Wang, Y.D., Al-Shemmeri, T., Eames, P., McMullan, J., Hewitt, N., Huang, Y., and S. Rezvani. 2006. An experimental investigation of the performance and gaseous exhaust emissions

of a diesel engine using blends of a vegetable oil. Applied Thermal Engineering 26:1684-1691.
Zubr, J. 1997. Oil-seed crop: Camelina sativa. Industrial Crops and Products 6:113-119.

CHAPTER TWO:

ENVIRONMENTAL, GENOTYPE, AND GENOTYPE BY ENVIRONMENT (GXE)EFFECTS ON *CAMELINA SATIVA* YIELD AND OIL CHARACTERISTICS IN THE AMERICAN WEST

CHAPTER SUMMARY

Volatility in fuel prices and rising levels of atmospheric carbon dioxide have fueled interest in the on-farm production of biofuels. Increasing the on-farm production of biofuels contributes to national food security and stability of production costs, as they are less dependent on the price of petroleum fuel. *Camelina sativa* is an oilseed species that has been identified as a potential feedstock for the on-farm production of biofuels in the Colorado agricultural area. This project included five varieties (listed with prefix SSD) derived from a mapping population and identified as containing favorable alleles at QTL for yield and drought tolerance. Our project conducted a two-year trial of 15 camelina varieties over a diversity of environments in two main geographic areas. The first area included high-latitude environments in the Pacific Northwest and Montana. The second included high-altitude environments in the Intermountain West region of Colorado and High Plains environments in Colorado and Wyoming. The overall mean yield was 813 kg ha^{-1} and significant GxE interaction was present when all environments were combined. We found that environment played a larger role in determining yield and oil profile than genotypes. Of the environmental factors, latitude (model-R square % 0.76) and temperature (model R-square % 0.59) during the growing season had the greatest impact on yield and oil profile characteristics while precipitation had the least (model R-square % 0.01). The average maximum temperature had a significant negative effect on yield (p <0.001) and a significant

negative correlation (*p* <0.001) with the percent of oil composed of polyunsaturated fatty acid. The significant GxE interaction for polyunsaturated fatty acid percent suggests that heat impacts the development of these oils. With the exception of Yellow Jacket, Colorado, in all of our low-yielding environments (yields of under 500 kg ha^{-1}), warm days comprised more than 22% of the growing season. When environments were grouped so that GxE interaction was not significant, we found that six varieties in the trial (Ligena, SSD10, SSD177, SSD87, SSD138, and Celine) ranked in the highest significance group across a variety of environments that included irrigated and dryland conditions. Although many of the SSD varieties ranked in the highest-yielding group of varieties, they were not significantly higher-yielding than their parental varieties (p= 0.9954). Further work should focus on the development of camelina germplasm that is more resistant to heat stress of lower-latitude environments.

INTRODUCTION

American agriculture uses more than 5% of the total distillate fuel oil consumed annually in the United States, mostly in the form of diesel fuel (DOE Energy Information Administration, 2009). It is possible to increase the energy independence of American agriculture by reducing the overall consumption of fossil fuels. Price fluctuations of petroleum products and dependence on imported fossil fuels reduce the overall sustainability of the food production system. Promoting energy independence of American agriculture can lead to improved security of the food and feed production sectors. The production of fuels derived from contemporaneous plant sources, or biofuels, can provide a domestic source of petroleum that can lower the agricultural sector's overall dependence on fossil fuels. In addition to reducing the relationship between food and fuel prices, this will reduce the overall carbon footprint of the agricultural sector. The combustion of conventional fossil fuels releases carbon in the form of carbon dioxide (CO_2) that was sequestered by plants and fossilized beneath the earth's surface millions of years ago. In the case of biofuels, the combustible carbon comes from the modern atmosphere instead of from non-renewable subterranean carbon sources, and, depending on the source, can reduce the net contribution of atmospheric CO_2 compared to petroleum fuel sources (Bessou et al., 2011).

Oilseed species in the mustard family Brassicacae have generated much interest as potential sources for the production of biofuels in the American Midwest. One species, *Camelina sativa,* has potential for yielding in dryland conditions (Angelini et al., 1997). Camelina is able to produce yields quickly and with minimal inputs. It is also resistant to many of the problems that have plagued other, better-established Brassicaceae species such as canola (*Brassica napus*). The oil extracted from camelina can be burned directly as a diesel substitute or blended with petroleum diesel fuels to offset the annual consumption of petroleum diesel. The potential of this crop as a feedstock for the production of biofuel in Eastern Colorado and the High Plains region must be evaluated through multi-environment yield and oil quality testing of existing varieties.

The climate of the arid Western American High Plains region of Eastern Colorado and Wyoming is characterized by frequent droughts, low humidity and seasonal high temperatures, which mean that soil evaporation throughout the growing season commonly exceeds precipitation (Peterson and Westfall, 2004). In the High Plains region annual precipitation ranges from 250 to 450 mm per year and on average 75% of the total annual precipitation falls between April and September (Peterson and Westfall, 2004). In the inland Pacific Northwest dryland cropping region, annual precipitation ranges from less than 250 mm to over 600 mm per year and on average only 35% of total annual precipitation occurs between April and September (S.O. Guy, personal communication, 2013).

The agricultural landscape of Eastern Colorado is dominated by two cropping systems. The first is a dryland winter wheat-fallow rotation and the second is a dryland winter wheat rotation that incorporates one or more spring crops such as corn, proso-millet, sorghum or sunflowers. In these rotations, there is a fallow period that extends from the time that the wheat or spring crop is harvested until the following September when wheat is planted again. In the lower intermediate and intermediate precipitation zones of the Pacific Northwest (PNW), 250-450 mm annual precipitation, camelina would fit into a winter wheat-fallow crop sequence. In this cropping system, a fallow period would extend from the time wheat is harvested until the fall when wheat is planted again. Camelina would serve as an addition to this rotation and would be planted in the spring of the fallow period and would be harvested in mid summer before wheat is planted again in late summer (S.O. Guy, personal communication, 2013). In the high precipitation zones, 450-600 mm, camelina could fit in as part of a winter wheat-spring cereal-camelina rotation (S.O. Guy, personal communication, 2013). In Colorado and lower precipitation areas of the PNW, a biofuel-producing oilseed could be incorporated during a fallow period without the displacement of any food-producing crops (Johnson et al., 2009). In the higher precipitation areas of the PNW, camelina could serve as an alternative crop in a three-crop rotation.

Winter wheat is an avoidance crop, meaning that it matures ahead of the hottest, driest part of summer, in order to avoid heat and drought stress (Blum and Ebercon, 1981). As a result, the summer months are left fallow. Summer precipitation is not effectively captured in fallow fields due to high evaporation during that time, so replacing summer fallow with an additional crop can increase overall productivity by 75-100% percent (Peterson and Westfall, 2004). *C. sativa*, is a short season crop that requires between 90 and 120 days to reach maturity. It can be incorporated into a dryland rotation where it is planted in the early spring and harvested in the early summer (Zubr, 1997; Johnson et al., 2008).

Archeological evidence has shown that *C. sativa*, or false flax, has been cultivated by humans for nearly 4000 years (Zubr, 1997). Although camelina has shown highly variable yields under dryland conditions, it has a suitable oil profile for use as a diesel substitute, is relatively tolerant to cold temperatures and is resistant to flea beetles, a common pest of other oilseed crops (Browne et al., 1991; Zubr, 1997; Frohlich and Rice, 2005; French et al., 2009; Enjalbert, 2011; Enjalbert and Johnson, 2011). Yield stability depends on the genotype and how it is affected by environmental conditions compared with other genotypes.

Camelina produces seeds that contain 30%-40% oil, although this percentage varies due to environment and genotype (Zubr, 1997; Enjalbert, 2011). These oils are particularly rich in unsaturated fatty acids (90%) such as oleic (C18:1), linolenic (C18:2) and linolenic fatty acid (C18:3) with linolenic comprising the largest percentage at 25 to 42% (Vollmann et al., 2007). Camelina oil can be burned directly in a modified diesel engine or else it can be converted to biodiesel and used in a traditional diesel engine. The fatty acid profile is considered adequate for the production of biofuel (Frohlich and Rice, 2005). Increasing the portion of the vegetable oil that is unsaturated decreases the combustion quality of the oil (Pinzi et al., 2009). On the other hand, increasing the unsaturated portion contributes to cold-temperature performance of the oil as well as the cold and drought tolerance of the plant (Linder, 2000; Pinzi et al., 2009; Vollmann et al., 2007; Enjalbert, 2011).

Prior to this study, two European camelina varieties, “Lindo” and “Licalla”, were crossed and 187 recombinant inbred lines (RILs) were evaluated for their performance with respect to yield, oil content, and drought adaptation (Gehringer et al., 2006; Enjalbert, 2011). Of the mapping population derived from these parents, 24% evidenced transgressive segregation by outperforming the parents (Gehringer et al., 2006). Previous studies carried out at Colorado State University have identified several significant alleles at quantitative trait loci (QTL) for yield and drought tolerance in individuals from this mapping population (Enjalbert, 2011). Our study aims to test the performance of five of these lines identified as highest-yielding compared to 10 other existing camelina varieties in multiple environments across the High Plains and Pacific Northwest. Through large scale, multi-environment testing we can determine if these alleles contribute to higher, more stable yields.

Analyses of genotype and environment interaction for camelina will allow us to assess varietal stability and analyze what subsets of the environments constitute a single variety recommendation domain. Oil content and profile information will help assess how environmental effects and genotypes affect these traits.

MATERIALS AND METHODS

Environments

In 2011, field environments included five High Plains environments in Colorado: Fort Collins, Iliff, Rocky Ford, Yellow Jacket, and Craig; and two Pacific Northwest environments in Lind, Washington, and Bozeman, Montana. In 2012, the environments were in Greeley and Rocky Ford, Colorado, Creston, Montana, and Lind, Washington. The Fort Collins, Greeley and Rocky Ford environments were under a limited irrigation regime for stand establishment and to prevent excessive drought stress. In all cases, the precipitation and irrigation were combined and reported as precipitation.

Plot sizes measured 1.8 m by 6.1 m, for a total plot area of 10.8 m^2. For each environment, there were four replications except at Rocky Ford and Yellow Jacket, where there were three replications. The planting was a randomized complete block design. The seeding rate was 2,802,000 seeds $acre^{-1}$. Fertilizer application varied among environments depending on plant needs. The herbicide Sonalan (Ethalfluralln, DowAgrosciences, Indianapolis IN) was applied prior to planting at a rate of 1.17 L ha^{-1}.

Plant Material

The names and origins of the varieties included in this study are listed in Table 2.1. The varieties with the prefix SSD were all developed as part of a mapping population. These were identified as containing alleles for yield and drought tolerance (Enjalbert, 2011). They were originally part of a mapping population derived from a cross between the parental varieties "Lindo" and "Licalla" at the University of Giessen in Germany (Gehringer et al., 2006). BSX G22, BSX G24 and Cheyenne came from the Blue Sun Biodiesel company in Colorado. Blaine Creek and Suneson came from Montana State University. Celine and Ligena are two European varieties that are commonly available. Yellowstone is a variety from Great Plains Oil and Exploration in Ohio.

Data Collection

Yields were recorded at each environment after combining with plot combine of varying makes and models. Seeds were allowed to air dry to 8% moisture level. The seed oil profile analysis for 2011 environments was carried out at a USDA research station in Peoria, Illinois, using gas chromatography mass spectrometry (GC-MS). Oil profile analysis for the 2012 locations are not yet completed. The oil profile traits included percent composition of total saturated fatty acid (C16:0, C18:0, C20:0, and C22:0), C18:1, C18:3, C22:1, percent monounsaturated fatty acid (C18:1, C20:1, C22:1, and C24:1), percent polyunsaturated fatty acid (C18:2, C18:3, and C20:2), and total percent oil (% Oil).

Climatic Variables

Weather data were obtained from the Western Regional Climate Center, the Colorado Agricultural Meteorological network (COAGMET) or the NOAA National Climatic Data Center. Climate data collected for the growing season were latitude, precipitation, average maximum temperature, average minimum temperature, number of warm days (days where maximum temperatures exceeded 32°C), number of cold days (days where minimum temperatures fell below 5°C), growing degree-days (GDD), and elevation. These data are found in Table 2.2.

Baux et al. (2008) outlined a method for determining whether extreme temperature affects the oil profile of rapeseed. This was adapted by Enjalbert (2011) to camelina development and used as a threshold for influence on oil production and vegetative growth. Heat stress days were those days during the growing season where the maximum temperature rose above 32°C. Cold days were recorded as those where the minimum temperature fell below 5°C.

Analysis

The data were analyzed using the SAS statistical program, version 9.3 (SAS Institute Cary, NC). The general linear model (GLM) was used to analyze yields among genotypes and environments. The mixed procedure was used to evaluate genotype by environment interactions. The regression and correlation procedures were used to analyze the relationships among traits and environmental variables. Climate variables were considered independent variables. The dependent variable was yield. Pearson's correlation was used to measure the correlation between the oil characteristics and three environmental variables, precipitation, latitude, and average maximum temperature as well as yield.

RESULTS

Table 2.2 shows the weather variables at each environment. The higher-latitude environment group includes the Pacific Northwest and Montana environments into one group above 43°N while lower-latitude environments were those below 42.06°N. Lower-latitude environments include those in the High Plains region of Eastern Colorado and Torrington, WY, as well as two Intermountain environments in Craig and Yellow Jacket, CO. Growing season precipitation included irrigation in Greeley, Rocky Ford 2011 & 2012, Fort Collins, and Torrington, WY. The precipitation throughout the growing season varied from 117 mm in Lind, WA, (2012) to 364 mm in Fort Collins, CO. The average maximum temperature for the Colorado and Wyoming environments was 25.9°C while the average temperature in the Pacific Northwest environments was 22.7°C and in Montana was 23.5°C. Table 2.2 shows the number of warm days, which are days where the daily temperature exceeded 32°C, as a percentage of the growing season. This provides a variable to evaluate the potential effect of high temperatures on camelina yield. This was calculated by dividing the total number of warm days by the total days in the growing season. Craig, CO, had the highest percentage of warm days (35%), although this was a higher-altitude environment. Conversely, Yellow Jacket had a low percentage of warm days (6%) at a similarly high altitude. These environments were both environments in the Intermountain region of Colorado. The length of the growing season between the dates of planting and harvest was the most important factor in determining the number of GDD at each environment. Regardless of the length of the growing season, the GDD were sufficient to produce high yields, as all were above 2100 GDD for the season. There was no apparent geographical pattern to the number of GDD between environments. Lind, WA, in 2011 had 2387 GDD for the growing season, but had 3050 GDD for the 2012 season.

Table 2.3 shows the seed yield for each variety at each environment and the overall mean yields by variety and environments. There was only a difference of 91 kg ha^{-1} between the overall highest and lowest performing varieties. The overall mean yield was 813 kg ha^{-1} but significant GxE interaction was present when all environments were combined. Nevertheless, Ligena had the highest mean yield of all the varieties at 842 kg ha^{-1}. Similarly, the SSD lines all yielded higher than the variety trial average and higher than their parental lines, Lindo and Licalla. Lines that yielded below the trial average included varieties from Blue Sun Biodiesel, Great Plains Oil, Montana State University, and some European varieties. According to Table 2.3, the highest yielding environments were in Kalispell and Bozeman, MT, Lind, WA and Fort Collins, CO. The range between the highest- and lowest-yielding environments was 1914 kg ha^{-1} (the highest was Montana 2012 at 2037 kg ha^{-1} and the lowest was Greeley 2012 at 123 kg ha^{-1}). Higher-yielding environments (above 500 kg ha^{-1}) tended to be either higher altitude or higher latitude, and tended to have a lower average maximum temperature. The lowest-yielding environment was Greeley, CO. Greeley experienced high temperatures between June 20, 2012, and July 5, 2012, during which floral abortion and early ripening were observed. Similarly, heat stress might have contributed to the low yields in Rocky Ford, CO for both years. The Torrington, Wyoming, environment had heavy weed infestation, which contributed to low yields. Yields in Yellow Jacket were affected by low precipitation and heavy, late season rainfall resulting in stand regrowth that caused shattering and reduced yields. Iliff experienced heavy rainfall (Table 2.2) and a high percentage of warm days that resulted in soil cracking and lower yields. Significant differences were observed between the varieties in Montana 2011, Montana 2012 and Yellow Jacket. The least significant difference (LSD) values for Montana were 92.3 kg ha^{-1} for 2012 and 132.56 kg ha^{-1} for 2011. Yields were so low in Yellow Jacket that significant differences among varieties were not informative.

Table 2.4 looks at groups of environments to attempt to evaluate genotype by environment (GxE) interactions. In the first column, all environments were included and there was significant GxE interaction. When the higher-yielding environments (yields greater than 500 kg ha^{-1}, with the exception of Montana 2012) were combined, there was no significant GxE interaction and there were significant differences among varieties. Above 500 kg ha^{-1} stands appeared healthy, below this, stands appeared to be thin and stressed. At the lower-yielding environments, there was no significant GxE interaction but there were no significant differences among varieties. The model R-square value was greater for the higher-yielding environments (0.92) than for the lower-yielding environments. Performance among the environments differed significantly (p <0.001). The highest-yielding environment in Colorado was Fort Collins in 2011, and the lowest-yielding was Greeley in 2012.

Table 2.5 compares the performance of different varieties at groupings of environments. These groupings include all environments, the maximum number of environments where GxE interaction was not significant and higher-yielding environments (environments where yields exceeded 500 kg ha^{-1}). In each case, the rankings of the varieties change; however, varieties with above-average yield tended to be the same in all groups. In the case of the maximum number of environments, the range between the highest-yielding variety, SSD 10, and the lowest-yielding variety, Yellowstone, was 102 kg ha^{-1}. The LSD value (67 kg ha^{-1}) means that the performance of the top six varieties are not significantly different from one another. In the higher-yielding environments, the LSD value was 101 kg ha^{-1}, meaning that the yield of the top six varieties did not differ significantly. The difference between the highest- and lowest-yielding varieties among higher-yielding environments was 199 kg ha^{-1}. The varieties that were included in the top significance group in all combinations of environments were, Ligena, SSD10, SSD177, SSD87, SSD138, and Celine. In the case with maximum environments and high-yielding environments, three of the experimental lines, SSD10, SSD87, and SSD177, ranked significantly higher than one parental variety, Lindo, but not the other, Licalla. The yields of the SSD lines and their parent lines, Lindo and Licalla, were compared in an analysis of variance using Tukey's honestly significant difference (HSD) test to determine significant yield differences. No significant differences were found in analyses comparing all SSD lines to the parental varieties across all environments ($p = 0.9954$), the top two yielding SSD lines, SSD 10 and SSD 177, to the parents at all environments ($p = 0.9933$), and all SSD lines to the parents at only the high-yielding environments ($p = 0.8066$) (Table 2.4).

Regression of climatic and weather variables on yield shows that all of the weather variables have a significant effect on yield (Table 2.6). Latitude had the strongest effect on yield, and precipitation (including irrigation) had the smallest effect on yield compared to all other climatic variables. Three climatic variables related to high temperatures (average maximum temperature, number of warm days, GDD) all had negative slopes, which indicates a negative effect on yield.

Table 2.7 shows the results of analysis of yield and oil characteristics of varieties at 2011 environments. The % Oil at Rocky Ford was very low compared to other environments and was the only environment where the % Oil was lower than 30%. The % Oil varied significantly among varieties at all environments except in Yellow Jacket, CO and Lind, WA in 2011. The mean % Oil varied significantly between environments (p <0.001). The mean for the trial was 35%, but the range was from a low of 11% in Rocky Ford to a high of 42% in Washington. Percentage monounsaturated fatty acid (MUFA) varied less; with a mean of 36%, it varied from 36 to 37% and there was still significant variation among varieties and environments (p <0.001). Percent polyunsaturated fatty acid (PUFA) also varied significantly among environments (p <0.001). The trial mean was 56% and was higher than MUFA. The least significant differences (LSD) were 0.80 for the % Oil, 0.65 for MUFA, and 0.82 for PUFA. The percentage of MUFA varied significantly among varieties in all environments. The percent of the oil composed of PUFA varied significantly among varieties at all environments except in Rocky Ford in 2011.

The effects of genotype, environment and genotype x environment (GxE) interaction on the different oil profile characteristics of importance to camelina researchers and biofuel producers are shown in Table 2.8. In addition to % Oil, percentage saturated fatty acid (SAT), MUFA, and PUFA, we chose to include C18:1, C18:2, C18:3, and C20:1 because they comprise the majority of the fatty acid profile of camelina and their concentrations have been shown to vary among environments (Vollmann et al., 2007; Johnson et al., 2009; Enjalbert, 2011). C22:1 was included because of the deleterious health effects of this fatty acid, although it normally exists in lower concentrations in camelina than in other Brassica oilseed crops (Velasco et al., 1998). There were highly significant differences among varieties for all of the oil profile characteristics and differences among these values at different environments. There was significant GxE interaction for percent C18:3, C20:1, C22:1, and % Oil. The model R-square was high for all oil characteristics, varying from 0.79 for SAT to 0.98 for % Oil. This means that the models captured a large percentage of the total variation for all of the oil characteristics.

Table 2.9 illustrates the relationship between the environmental conditions and yield with the oil characteristics. With increase in latitude, there was a decrease in the percent concentration of C18:1, C18:2, the percent of SAT, and the percentage MUFA. Conversely, for C18:3, C20:1, % Oil and PUFA, an increase in latitude correlated to an increase in concentration. C22:1 was not significantly correlated with latitude. Precipitation had a significant negative correlation to the C20:1, % Oil, and percent PUFA. The other fatty acids were positively correlated to precipitation, except for C22:1 and MUFA. Average maximum temperature was negatively correlated to the production of C18:3, C22:1, % Oil, and PUFA. There was a positive correlation between average maximum temperature and C18:1, C18:2, SAT, and percent MUFA. An increase in the average maximum temperature was negatively correlated to lower concentrations of C18:3, C22:1, % Oil, and percent PUFA. C18:3, SAT and MUFA were positively correlated with average maximum temperature. The concentration of C20:1 was not correlated with average maximum temperature. Increased yield had a highly significant positive correlation with the concentration of PUFA, % Oil, concentrations of C18:3, and concentrations of C20:1. This means that as yield increased, the concentrations of PUFAs and the percent oil in the seeds increased. Conversely, the concentrations of MUFA, SAT, C18:1 and C18:2 decreased with increasing yields.

DISCUSSION

The trial average of 813 kg ha^{-1} falls within the range reported in trials in Western Nebraska in 2005 and 2006, which fell between 556 and 1456 kg ha^{-1} depending on the date of planting (Pavlista et al., 2011). Yields in Minnesota were also reported to fall within the range reported in this study (Gesch and Cermak, 2011). Camelina in Arizona under irrigation yielded over 1500 kg ha^{-1} in 2009 and 2010 (Hunsaker et al., 2012). Camelina yields in Chile have also been reported to vary between 420 kg ha^{-1} and 2314 kg ha^{-1} for 2008 and 2009 (Berti et al., 2011). Mean yields across several environments in Germany ranged from 1460 kg ha^{-1} to 1715 kg ha^{-1}, which exceeded our yields (Gehringer et al., 2006).

Yield trends were observed in our study. Higher-latitude environments with cooler climates experienced higher yields. Lower than expected yields were mostly a result of environmental stresses. These environments experienced heat during flowering, intense rainfall, weed infestations and regrowth due to uneven rainfall patterns. Hunsaker et al. (2012) found the maximum seasonal water use of camelina to be 470-490 mm, which is more than was received by any of our environments, including those under irrigation. These factors together may have contributed to overall lower average yields in this trial. The low yields at Greeley, Rocky Ford, and Yellow Jacket in Colorado and Torrington in Wyoming emphasize the importance of site selection and management to produce adequate yields.

As with any crop, favorable environmental conditions produce higher yields. Environments in the Pacific Northwest and Montana produced higher yields (Table 2.3). These higher-latitude environments provided comparisons to the lower-latitude environments to help determine the effect of environmental conditions on yield due to their lower number of GDD, lower average maximum temperatures, and % warm days. We made combinations of the different environments to analyze GxE interaction (Table 2.4). When all environments were included, there was significant GxE interaction. Enjalbert (2011) also reported significant GxE interaction for yield in a variety trial grown throughout the High Plains region. When three of the environments were removed, one of which had harvest problems stemming from stand regrowth and another had low yields, the GxE interaction was no longer significant. The same was true when only the highest-yielding environments were included in the analysis. When all low-yielding environments (environments with mean yields below 500 kg $ha^{-1)}$ were grouped together for analysis, there was significant GxE and no significant differences among the genotypes. This was likely due to the fact that the varieties did not reach their yield potential due to stress. Six varieties were significantly higher yielding than the others in all of the environment groupings (Table 2.5). These were Ligena, SSD10, SSD177, SSD87, SSD138, and Celine. From these analyses, we can conclude that across a wide range of environments some varieties produced significantly higher yields. Enjalbert (2011) also found Celine and Ligena to be well adapted to a wide range of environments throughout the High Plains region. With the exception of SSD186, the yields of the SSD varieties, which contained alleles identified at quantitative trait loci (QTL) for yield and drought tolerance, performed well, placing in the higher categories in all of the groups of environments. Gehringer et al. (2006) performed a similar multi-environment trial in Germany using the same mapping population. They found that Lindo was the best-performing parent and that in certain environments, SSD10 and SSD177 yielded significantly higher than the parents, although the mean yield across all environments did not exceed that of the parental varieties.

Across all environments, there were significant differences for oil characteristics among varieties and environments. Table 2.7 shows that with the exception of the Rocky Ford environment, the mean % Oil for our environments, 32%, in this study exceeded those reported by Pavlista et al. (2011) in Nebraska. The Rocky Ford 2011 environment had a very low oil percentage (11%) and was the only environment below 32%. Rocky Ford exceeded the average maximum temperature of 25.1°C and the average % warm days for the growing season (Table 2.2). It had the most GDD, despite a relatively early date of harvest, suggesting that the oil content was affected by heat. Rocky Ford also had the lowest percent of PUFA and there was no significant difference among the genotypes. This may have to do with warm temperatures later in the growing season, as Berti et al. (2011) found that in Chile, the concentration of C18:3 was negatively affected by later date of planting. Table 2.8 shows that there were significant differences among varieties and environments for all of the oil characteristics. Significant GxE was only present for C18:3, C20:1, and C22:1. This differs from the lack of GxE interaction for these characteristics observed by Vollmann et al. (2007), perhaps because our trials were subjected to environmental conditions that were not optimal for the production of a stable fatty acid profiles. C18:3, C20:1, and C22:1 are also the most unsaturated and longest-chain fatty acids that comprise a significant portion of the oil profile of camelina. The desaturation pathway, produces increasingly unsaturated fatty acids and the elongation pathway produces longer-chain fatty acids (Velasco et al., 1998). These pathways were affected by conditions present in these environments. Enjalbert (2011) reported significant GxE interaction for % Oil and C18:3. This suggests that the production of MUFA and SAT are less affected by the environment than the production of PUFA.

Latitude and temperatures during the growing season had the greatest influences on the yields and oil profile characteristics of the varieties in this study (Table 2.6). These two variables are closely related, as higher latitudes have lower average maximum and minimum temperatures. A decrease in average maximum temperature or an increase in latitude results in increased yields (Table 2.6). It appears that camelina would yield higher in cooler, higher-latitude environments with fewer GDD, and lower average temperatures during the growing season. There were heat-related yield reductions, which is consistent with reports that camelina shows yield reductions beginning at 25°C (Berti et al., 2011). Our mean for the average maximum temperature was 25.1°C .With the exception of Craig, CO, number of warm days comprise no more than 17% of the total days throughout the growing season of the high yielding environments (Table 2.2). The opposite is true for the low-yielding environments, with the exception of Yellow Jacket; all of the low-yielding environments had warm days comprising more than 22% of the growing season. The effect of high temperatures was also evidenced by the small stature of plants in high heat environments (data not shown), which suggested that heat was affecting the physiological maturity of the plants. Heat had a negative effect on yield but altitude was a confounding factor. Craig and Yellow Jacket, both part of the Intermountain region of Colorado, were the two highest-altitude environments at 1855 m and 2070 m, respectively, which was above the average of 1234 m for all environments. These were also the only two environments that were the exception to the effect of number of warm days on yield.

Lower temperatures and higher latitude were also positively correlated with concentrations of C18:3, C20:1, and % Oil. It has been observed that % Oil is a characteristic related to temperature and precipitation (Berti et al., 2011). This means that at those cooler, higher-latitude environments, the elongation and desaturation pathways were more active. In other oilseed species, it has been reported that lower daily temperatures result in a higher concentration of C18:3 (Deng and Scarth, 1998). This is caused by the downregulation of fatty acid desaturase 3 (FAD3), the enzyme responsible for the production of C18:3 (Baux et al., 2008). Higher concentration of PUFA increases the cold tolerance of oilseed species (Lyons et al., 1964; Seiler, 1983; Kodama et al., 1994; Murakami et al., 2000; Izquierdo et al., 2002; Baux et al., 2008; Sadras et al., 2009). In our study, increased yield is correlated with an increase in

C18:3 and C20:1, suggesting that where conditions are conducive to high yields, they are also conducive to the activity of the desaturation pathway. Precipitation was the weather variable with the lowest R-square value, meaning that it accounted for the least amount of variability with respect to yield. Precipitation had no clear association with the activity of any pathway, but it was negatively correlated with PUFA. Enjalbert (2011) found that drought stress reduced C18:3 content by 17% compared to a well-watered trial.

From this study, it would seem that with the exception of Fort Collins in 2011, and Craig in 2011, the low-latitude environments in this study are not conducive to the production of high-yielding camelina stands. Higher-latitude environments with lower average temperatures and lower precipitation seem to be better producers of camelina with more unsaturated oil. Environment plays a larger role than genotype in determining the yields of camelina. Oil profile characteristics, with the exception of the production of linolenic and linoleic fatty acids, are more genetically fixed. Environment and related stress factors such as drought and heat can lead to variation in oil profile and decreased seed oil content, which can impact seed viability and suitability as a biofuel feedstock.

There was no significant difference between the SSD varieties and the other entries in the variety trial. This does not mean that these varieties are not good performers. These topped the trial and should be recommended for this area. The lack of significant differences in varietal yields points out a need for new germplasm to augment a lack of germplasm diversity in the possible entries for camelina

CONCLUSION

The camelina variety trial compared the performance of 15 varieties in two distinct geographical regions in the Western United States. Six of the varieties, Ligena, SSD10, SSD177, SSD87, SSD138, and Celine, were in the highest-yielding group of varieties in all of our combinations of environments, including irrigated environments. Gehringer et al. (2006) found that two of the varieties, SSD10 and SSD177, performed well in Germany, suggesting that these two lines are very widely adapted. The SSD varieties yielded well in our study but did not significantly outperform their parental varieties across all environments.

Lower-latitude environments in Colorado were not as high-yielding as higher-latitude environments. Camelina did not perform well at low latitudes even under irrigated conditions, during the two years of our study, so the low yields can be attributed to above-average, high temperatures. Heat affected the physiological maturity of camelina, which is related to oil production pathways, yield, and seed fill. Varietal improvement efforts need to focus on development of heat-resistant camelina varieties for lower-latitude environments. From an agronomic perspective, the focus might be on reducing the number of warm days so that they comprise no more than 17% of the growing season. One way this can be achieved is through earlier planting. As shown by the results of the higher-latitude environments, a decrease in average maximum temperature during the growing season resulted in increased yield and was positively correlated with an increase in % Oil and PUFA and a decrease in SAT. As a potential biofuel feedstock, camelina is better adapted to higher-latitude environments and further development of lines is needed for adaptability of camelina in lower-latitude environments.

Table 2.1. Names, sources, and maturity dates of the camelina varieties included in this trial**.**

Variety	Maturity	Source
SSD 177[¶]	M[#]	University of Giessen
Ligena	L	Europe
SSD 10[¶]	L	University of Giessen
Celine	M	Europe
BSX G22	E	Blue Sun Biodiesel
SSD 138[¶]	L	University of Giessen
Licalla	M	University of Giessen
BSX G24	M	Blue Sun Biodiesel
SSD 186[¶]	M	University of Giessen
Suneson	M	Montana State University
Yellowstone	E	Great Plains Oil
SSD 87[¶]	M	University of Giessen
Lindo	M	University of Giessen
Blaine Creek	E	Montana State University
Cheyenne	M	Blue Sun Biodiesel

¶Indicates entry identified as containing significant quantitative trait loci for yield and drought tolerance
[#] Maturity classification of the varieties. L= Late Maturing, M= Medium Maturing, E= Early Maturing

Table 2.2. Date of planting, date of harvest, and environmental variables for the environments included in this trial.

Year	**Environments**			**Environmental Variable**					
		Date of planting	Date of harvest	Latitude	Growing season precipitation[1]	Avg max temp[2]	% Warm days[3]	GDD[4]	Elevation
				°N	mm	°C	°C		m
2011	Lind, WA	4/19/11	8/8/11	46.79	181	20.8	4	2387	417
2011	Iliff, CO	4/20/11	8/1/11	40.75	300	26.6	35	2491	1147
2011	Fort Collins, CO	3/25/11	7/26/11	40.58	364	21.9	24	2369	1533
2011	Rocky Ford, CO	3/18/11	7/18/11	38.05	226	26.5	26	3086	1254
2011	Craig, CO	5/12/11	9/1/11	40.51	182	28.3	35	2561	1856
2011	Bozeman, MT	5/12/11	8/23/11	45.67	201	25.7	17	2173	1341
2011	Yellow Jacket, CO	4/20/11	8/16/11	37.53	121	24.3	6	2833	2070
2012	Rocky Ford, CO	3/19/12	6/28/12	38.05	361	27.0	26	2683	1254
2012	Torrington, WY	3/16/12	7/20/12	42.06	229	27.8	34	2354	1231
2012	Greeley, CO	4/9/12	7/11/12	40.42	220	27.1	22	2193	1405
2012	Lind, WA	4/3/12	8/9/12	46.79	117	23.6	15	3050	417
2012	Creston, MT	3/20/12	8/18/12	48.18	302	21.4	10	2255	887

[1] Growing season precipitation includes irrigation from date of planting to date of harvest

[2] Average maximum temperature from date of planting to date of harvest

[3] Percentage of the growing season composed of warm days (days above 32°C) from date of planting to date of harvest

[4] GDD=Growing degree days $(T_{max}+T_{min})/2-T_{base}$. T_{base}=4.4°C (McMaster and Wilhelm, 1997)

Table 2.3. Seed yields for all environments and varieties within the variety trial. Varieties are ranked in order of decreasing mean yields across all environments. Environments are organized in order of decreasing mean yield. Environments are abbreviated: MT 2011 Bozeman, Montana; MT 2012 Crestone, Montana; WA Lind, Washington; FC Fort Collins, Colorado; RF Rocky Ford, Colorado; WY Torrington, Wyoming; YJ Yellow Jacket, Colorado; GR Greeley, Colorado.

Variety	**Environments**												
	MT 2012	WA 2011	WA 2012	MT 2011	FC 2011	Craig 2011	Iliff 2011	RF 2011	WY 2012	RF 2012	YJ 2011	GR 2012	Mean
	Yield kg ha^{-1}												
Ligena	2113	1820	1555	1101	1072	699	348	396	334	311	210	150	842
SSD10	2060	1976	1413	1171	995	714	383	391	318	297	204	92	835
SSD177	2083	2071	1198	1160	1017	609	552	420	339	206	202	140	833
SSD87	2063	1901	1578	1034	934	682	331	372	300	260	144	113	809
SSD138	2130	1936	1256	1105	855	690	549	405	286	281	109	105	809
Celine	1835	1957	1487	1018	1037	682	312	365	309	272	189	141	800
SSD186	2211	1744	1367	1022	900	549	530	376	236	246	193	126	792
Suneson	2139	1778	1492	930	894	675	359	365	314	228	206	98	790
Licalla	1825	1883	1352	1055	992	650	376	292	296	353	120	205	783
BSX G22	2013	1782	1146	1062	965	629	573	265	225	236	225	111	769
Yellowstone	2133	1824	1317	935	736	570	486	297	362	212	233	112	768
BSX G24	2026	1787	1312	825	774	731	453	400	270	342	150	121	766
Lindo	2039	1862	1168	1124	820	655	372	360	245	261	182	95	765
Blaine Creek	1989	1794	1342	932	745	608	426	306	329	282	190	76	752
Cheyenne	1892	1817	1338	1001	798	630	315	339	304	244	182	155	751
Mean	2037	1862	1355	1032	902	651	424	357	298	269	183	123	813
p- varieties	***	NS‡	NS	**	NS	NS	NS	NS	NS	NS	*	NS	-[1]

* Significant at the 0.05 probability level
**Significant at the 0.01 probability level
***Significant at the 0.001 probability level
‡ NS indicates lack of significance
[1] Genotype x environment interaction was significant

Table 2.4. Results of multiple genotype by environment interaction analysis. 'All environments' includes all the environments of the variety trial, 'maximum' includes the most environments without significant GxE interaction (p <0.05), 'high yielding' environments are those where average yields exceed 500 kg ha^{-1}, and 'low yielding' environments are those with average yields below 500 kg ha^{-1}.

Year	Environments	Groups of environments				
		All environments	**Maximum**	**High yielding**	**Low yielding**	**Mean**
						kg ha^{-1}
2012	Crestone, MT	††				2037
2011	Lind, WA	††	††	††		1862
2012	Lind, WA	††	††	††		1355
2011	Bozeman, MT	††	††	††		1037
2011	Fort Collins, CO	††	††	††		902
2011	Craig, CO	††	††	††		652
2011	Iliff, CO	††	††		††	424
2011	Rocky Ford, CO	††	††		††	357
2012	Torrington, WY	††	††		††	298
2012	Rocky Ford, CO	††	††		††	269
2011	Yellow Jacket, CO	††			††	183
2012	Greeley, CO	††			††	123
Mean kg ha^{-1}		813	807	1160	276	
LSD kg ha^{-1}		52	67	101	56	
			ANOVA			
Model R-square		0.97	0.95	0.92	0.71	
CV, %		16	18	14	34	
GxE		***	NS‡	NS	NS	
Genotype		**	**	***	NS	
Environment		***	***	***	***	

†† Indicates grouping
* Significant at the 0.05 probability level
**Significant at the 0.01 probability level
***Significant at the 0.001 probability level
‡ NS indicates lack of significance

Table 2.5. Comparative yields and analysis of variance (ANOVA) results for each combination of environments listed in Table 2.4. The varieties are ranked in order of decreasing average yield. Max environments includes the maximum number of environments that can be combined without significant GxE interaction. High-yielding environments are those environments where yields exceeded 500 kg ha^{-1}.

Groups of Environments					
All Environments		**Max Environments**		**High Yielding**	
Variety	Yield	Variety	Yield	Variety	Yield
	kg ha^{-1}		kg ha^{-1}		kg ha^{-1}
Ligena	842	SSD10	851	SSD10	1254
SSD10	835	Ligena	848	Ligena	1249
SSD177	833	SSD177	841	Celine	1236
SSD87	809	Celine	827	SSD87	1226
SSD138	809	SSD87	821	SSD177	1210
Celine	800	SSD138	818	Licalla	1186
SSD186	792	Licalla	805	SSD138	1168
Suneson	790	Suneson	782	Suneson	1153
Licalla	783	SSD186	774	Lindo	1126
BSX G22	769	BSX G24	766	BSX G22	1117
Yellowstone	768	BSX G22	765	Cheyenne	1117
BSX G24	766	Lindo	763	SSD186	1086
Lindo	765	Cheyenne	754	BSX G24	1084
Blaine Creek	752	Blaine Creek	752	Blaine Creek	1084
Cheyenne	751	Yellowstone	749	Yellowstone	1076
ANOVA					
R-square	0.97	R-square	0.95	R-square	0.92
CV, %	16	CV, %	18	CV, %	14
GxE	***	GxE	NS‡	GxE	NS
Genotype	**	Genotype	**	Genotype	***
Environment	***	Environment	***	Environment	***
Mean	816	Mean	807	Mean	1160
p-value varieties	**	*p*-value varieties	**	*p*-value varieties	***
LSD (0.05)	52	LSD (0.05)	67	LSD (0.05)	101

**Significant at the 0.01 probability level
***Significant at the 0.001 probability level
‡ NS indicates lack of significance

Table 2.6. Climatic variables regressed on yield across all environments in order of decreasing model R-square value.

Climate Variable	**Significance**	**Intercept**	**Slope**	**Model R-square**
Latitude (°N)	***	-5598	152.5	0.76
Average maximum temperature (°C)	***	9156	-107.7	0.59
Elevation (m)	***	1914	-0.3	0.42
Number of warm days (days above 32°C)	***	1558	-29.6	0.32
Growing degree days (GDD)	***	2706	-0.7	0.10
Precipitation (mm)	**	1084	-0.9	0.01

**Significant at the 0.01 probability level
***Significant at the 0.001 probability level

Table 2.7. Yield, percent oil (% Oil), percent monounsaturated fatty acid (MUFA) and percent polyunsaturated fatty acid (PUFA) by variety at each environment for 2011. The *p*-values indicate significance among the varieties at each environment. The varieties are ranked in order of mean yield across all environments for 2011. Varieties are abbreviated, FC: Fort Collins, Colorado; RF: Rocky Ford, Colorado; YJ: Yellow Jacket, Colorado; WA: Lind, Washington; MT: Bozeman, Montana.

Variety	Oil	Environments							
		Iliff 2011	FC 2011	Craig 2011	RF 2011	YJ 2011	WA 2011	MT 2011	Mean
					% Oil				
SSD177	% Oil	33.9	38.2	40.8	11.0	38.2	41.9	39.5	34.8
					MUFA				
	MUFA	39.7	41.3	40.6	41.6	40.5	39.7	40.9	40.6
					PUFA				
	PUFA	48.9	47.3	48.8	45.3	48.6	49.9	49.2	48.3
SSD10	% Oil	27.9	33.9	40.5	11.0	37.3	40.8	36.9	32.6
	MUFA	40.2	40.4	39.5	41.2	40.4	39.6	39.9	40.2
	PUFA	47.3	47.1	49.8	44.6	48.0	50.4	48.9	48.0
SSD138	% Oil	34.1	39.4	40.4	11.3	37.3	42.5	38.9	34.8
	MUFA	37.7	35.4	35.9	37.1	35.4	35.3	34.6	35.9
	PUFA	50.9	53.4	53.6	48.0	53.9	55.0	55.0	52.8
Ligena	% Oil	26.1	39.2	40.9	11.8	36.3	43.3	39.6	33.9
	MUFA	34.5	33.5	34.0	35.9	33.2	33.5	33.0	33.9
	PUFA	52.8	54.9	55.3	51.3	55.7	56.8	56.8	54.8
Celine	% Oil	32.1	38.8	40.2	10.2	38.1	42.2	38.3	34.3
	MUFA	37.1	35.1	35.6	36.8	35.5	35.3	34.7	35.7
	PUFA	50.9	53.7	54.0	46.9	53.8	55.0	55.5	52.8
BSXG22	% Oil	33.8	39.2	40.3	11.3	38.2	41.9	40.1	35.0
	MUFA	35.6	35.1	35.9	36.3	35.2	34.8	34.8	35.4
	PUFA	52.9	53.5	53.6	48.0	54.2	55.2	54.6	53.2
SSD87	% Oil	30.8	37.7	38.9	9.7	35.2	39.2	37.9	32.8
	MUFA	36.2	36.3	36.3	36.1	36.0	35.6	35.6	36.0
	PUFA	52.7	52.6	53.3	49.1	53.5	54.4	54.4	52.9
Lindo	% Oil	31.6	36.9	41.5	11.8	37.3	43.0	39.9	34.6
	MUFA	37.4	35.2	35.7	37.1	35.9	35.4	35.2	36.0
	PUFA	51.0	52.9	53.5	48.3	52.5	54.6	54.2	52.4
Licalla	% Oil	32.4	36.0	41.0	11.1	36.7	41.7	39.3	34.0
	MUFA	37.3	37.5	37.9	37.7	36.9	36.6	37.5	37.3
	PUFA	51.2	51.3	52.0	45.8	52.4	53.9	52.6	51.3
SSD186	% Oil	32.2	37.2	40.7	9.5	37.5	42.5	39.1	34.1
	MUFA	35.1	33.7	34.7	35.6	33.6	34.1	34.0	34.4
	PUFA	52.8	54.0	54.5	47.4	54.7	55.7	55.1	53.5
Suneson	% Oil	30.9	38.0	39.4	10.9	35.2	41.6	39.1	33.6
	MUFA	36.5	36.0	36.9	37.4	36.4	35.3	35.8	36.3

	PUFA	52.1	53.0	52.7	49.1	52.8	54.7	53.9	52.6
BSX G24	% Oil	33.8	37.7	40.3	9.2	37.9	41.5	39.6	34.3
	MUFA	36.5	36.0	36.4	37.2	36.1	35.4	35.5	36.1
	PUFA	51.9	52.3	53.2	44.3	53.5	54.9	54.2	52.1
Cheyenne	% Oil	30.3	36.9	41.3	11.4	36.6	41.9	39.3	34.0
	MUFA	35.3	36.9	35.8	37.9	36.5	35.1	35.9	36.2
	PUFA	53.1	52.0	53.7	49.1	52.8	55.2	54.0	52.9
Yellow Stone	% Oil	32.8	37.8	40.4	11.1	37.4	41.1	39.8	34.3
	MUFA	35.6	33.3	34.5	35.6	34.7	33.5	33.4	34.4
	PUFA	53.2	55.7	55.4	51.9	54.6	57.1	56.7	54.9
Blaine Creek	% Oil	32.1	36.6	41.5	11.3	38.7	42.4	39.3	34.5
	MUFA	36.5	35.2	36.4	36.8	36.4	35.4	34.8	35.9
	PUFA	52.2	52.7	53.2	48.9	52.7	55.0	54.5	52.7
ANOVA									
p-value varieties	% Oil	32**	38*	41*	11	37	42***	39***	35***
	MUFA	37*	36***	36***	37***	36***	36***	36***	36***
	PUFA	52*	52***	53***	48	53***	55***	54***	52***

* Significant at the 0.05 probability level
**Significant at the 0.01 probability level
***Significant at the 0.001 probability level
‡ NS indicates lack of significance

Table 2.8. Mean values plus genotype, environment and GxE effects on important oil characteristics for all 2011 locations. The oils in this table include oleic (C18:1), linoleic (C 18:2), linolenic (C18:3), eicosenic acid (C 20:1) erucic (C22:1), percent oil (% Oil), percent saturated fatty acid (SAT), percent monounsaturated fatty acid (MUFA), percent polyunsaturated fatty acid (PUFA).

	Oleic C18:1	**Linoleic C18:2**	**Linolenic C18:3**	**Eicosenic C20:1**	**Erucic C22:1**	**% Oil**	**SAT**	**MUFA**	**PUFA**
					% Composition				
Mean	19.0	20.7	30.0	12.8	3.5	34.9	10.8	36.3	52.5
					ANOVA				
Genotype	***	***	***	***	***	***	***	***	***
Environment	***	***	***	***	***	***	***	***	***
GxE	NS‡	NS	*	***	**	***	NS	NS	NS
CV, %	6	4	4	4	5	4	9	3	3
Model R-Square	0.81	0.86	0.94	0.76	0.85	0.98	0.79	0.80	0.84

* Significant at the 0.05 probability level
**Significant at the 0.01 probability level
***Significant at the 0.001 probability level
‡ NS indicates lack of significance

Table 2.9. Results of Pearson's correlation analysis displaying the correlation coefficients and significance between oil characteristics and environmental variables for the 2011 environments. The oils in this table include oleic (C18:1), linoleic (C 18:2), linolenic (C18:3), eicosenic acid (C 20:1), erucic (C22:1), percent oil (% Oil), percent saturated fatty acid (SAT), percent monounsaturated fatty acid (MUFA), percent polyunsaturated fatty acid (PUFA).

Environment Variable	**C18:1**	**C18:2**	**C18:3**	**C20:1**	**C22:1**	**% Oil**	**SAT**	**MUFA**	**PUFA**
Latitude	-0.22 ***	-0.45 ***	0.59 ***	0.12 *	0.01 NS	0.53 ***	-0.47 ***	-0.18 ***	0.43 ***
Precipitation	0.15 **	0.33 ***	0.34 ***	-0.26 ***	-0.09 NS	-0.15 **	0.23 ***	0.04 NS	-0.17 ***
Average Max temp	0.18 ***	0.42 ***	-0.44 ***	-0.08 NS	-0.19 ***	-0.31 ***	0.23 ***	0.14 **	-0.23 ***
Yield	-0.20 ***	-0.53 ***	0.57 ***	0.13 *	0.10 NS	0.49 ***	-0.41 ***	-0.14 **	0.34 ***

* Significant at the 0.05 probability level

**Significant at the 0.01 probability level

***Significant at the 0.001 probability level

‡ NS indicates correlation coefficient was not significant

REFERENCES

Angelini, L.G., Moscheni, E., Colonna, G., Belloni, P., and E. Bonari. 1997. Variation in agronomic characteristics and seed oil composition of new oilseed crops in central Italy. Industrial Crops and Products 6:313-323.

Baux, A., Hebeisen, T., and D. Pellet. 2008. Effects of minimal temperatures on low-linolenic rapeseed oil fatty-acid composition. European Journal of Agronomy 29:102-107.

Bessou, C., Ferchaud, F., Gabrielle, B., and B. Mary. 2011. Biofuels, greenhouse gases and climate change. A review. Agronomy for Sustainable Development 31:1-79.

Berti, M., Wilckens, R., Fischer, S., Solis, A., and B. Johnson. 2011. Seeding date influence on camelina yield, yield components, and oil content in Chile. Industrial Crops and Products 34:1358-1365.

Blum, A., A. Ebercon. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Science 21(1):43-47.

Browne, L.M., Conn, K.L., Ayer, W.A., and J.P. Tewari. 1991. The camelexins: new phytoalexins produced in the leaves of *Camelina sativa* (Cruciferae). Tetrahedron 47(24):3909-3914.

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

DOE Energy Information Administration. 2009. Fuel Oil and Kerosene Sales. EIA-0535(09).

Enjalbert, J.N. 2011. An integrated approach to local based biofuel development. Ph.D. diss. Colorado State University Libraries. 140p. http://hdl.handle.net/10217/46747.

Enjalbert, J.N., and J.J. Johnson. 2011. Guide for producing dryland camelina in Eastern Colorado. Colorado State University Extension factsheet No. 0.709. http://www.ext.colostate.edu/pubs/crops/00709.pdf.

French, A.N., Hunsaker, D., Thorp, K., and T. Clarke. 2009. Evapotranspiration over camelina crop at Maricopa, Arizona. Industrial Crops and Products 29:289-300.

Frohlich, A., and B. Rice. 2005. Evaluation of *Camelina sativa* oil as a feedstock for biodiesel production. Industrial Crops and Products 21:25-31.

Gehringer, A., Freidt, W., Lühs, W., and R.J. Snowdon. 2006. Genetic mapping of agronomic traits in false flax (*Camelina sativa* subsp. *sativa*). Genome 49:1555-1563.

Gesch, R.W., and S.C. Cermak. 2011. Sowing date and tillage effects on fall- seeded camelina in the northern corn belt. Agronomy Journal 103(4):980-987

Hunsaker, D.J., French, A.N., and K.R. Thorp. 2012. Camelina water use and seed yield response to irrigation scheduling in an arid environment. Irrigation Science. Published online: 31 July, 2012. DOI: 10.1007/s00271-012-0368-7.

Izquierdo, N., Aguirrezabal, L., Andrade, F., and V. Pereyra. 2002. Night temperature affects fatty acid composition in sunflower oil depending on the hybrid and the phenological stage. Field Crops Research 77:115-126.

Johnson, B.L., Berti, M.T., and P.J. Petersen. 2008. Seeding date influence on winter camelina performance in North Dakota. Abstract. In: 7–11 September 2008, 20th Annual Meeting New Crops and Bioproducts Development. Texas A&M University, College Station, TX.

Johnson, J.J., Enjalbert, N., Schneekloth, J., Helm, A., Malhotra, R., and D. Coonrod. 2009. Development of oilseed crops for biodiesel production under Colorado limited irrigation conditions. Completion report no. 211. Colorado Water Institute. Fort Collins, Colorado.

Kodama, H., Hamada, T., Horiguchi, G., Nishimura, M., and K. Iba. 1994. Genetic enhancement of cold tolerance by expression of a gene for chloroplast omega-3-fatty-acid desaturase in transgenic tobacco. Plant Physiology 105:601-605.

Linder, C.R. 2000. Adaptive evolution of seed oils in plants: Accounting for the biogeographic distribution of saturated and unsaturated fatty acids in seed oils. American Naturalist 156:442-458.

Lyons, J.M., Pratt, H.K., and T.A.Wheaton. 1964. Relationship between physical nature of mitochondrial membranes and chilling sensitivity in plants. Plant Physiology 39:262-268.

McMaster, G.S. and W.W. Wilhelm. 1997. Growing degree days: one equation, two intepretations. Agricultural and Forest Meterology 87:291-300.

Murakami, Y., Tsuyama, M., Kobayashi, Y., Kodama, H., and K. Iba. 2000. Trienoic fatty acids and plant tolerance of high temperature. Science 287:476-479.

Pavlista, A.D., Isbell, T.A., Baltensperger, D.D., and G.W. Hergert. 2011 Planting date and development of spring-seeded irrigated canola, brown mustard and camelina. Industrial Crops and Products 33:451-456.

Peterson, G.A., and D.G. Westfall. 2004. Managing precipitation use in sustainable dryland agroecosystems. Annals of Applied Biology 144:127-138.

Pinzi, S., Garcia, I.L., Lopez-Gimenez, F.J., Luque de Castro, M.D., Dorado, G., and M.P. Dorado. 2009. The ideal vegetable oil-based biodiesel composition: a review of social, economical and technical implications. Energy and Fuels 23:2325-2341.

Sadras, V.O., Reynolds, M.P., de la Vega, A.J., Petrie, P.R., and R. Robinson. 2009. Phenotypic plasticity of yield and phenology in wheat, sunflower and grapevine. Field Crops Research 110:242-250.

Seiler, G.J. 1983. Effect of genotype, flowering date, and environment on oil content and oil quality of wild sunflower seed. Crop Science 23:1063-1068.

Velasco, L., Goffman, F.D., and C. Becker. 1998. Variability for the fatty acid composition of the seed oil in a germplasm collection of the genus *Brassica*. Genetic Resources and Crop Evolution 45:371-382.

Vollmann, J., Moritz, T., Kargl, C., Baumgartner, S., and H. Wagentristl. 2007. Agronomic evaluation of camelina genotypes selected for seed quality characteristics. Industrial Crops and Products 26:270-277.

Zubr, J. 1997. Oil-seed crop: *Camelina sativa* Industrial Crops and Products 6:113-119.

CHAPTER THREE:

FACTORS AFFECTING CAMELINA YIELD COMPONENTS IN COLORADO AND WYOMING

CHAPTER SUMMARY

Yield components are a useful tool for crop scientists to evaluate physiological characteristics that contribute most to yield and how these traits are affected by environmental conditions. Camelina is an experimental oilseed species that has been identified as a candidate oilseed for the on farm production of biofuel in Colorado and the High Plains of the Western United States. In this experiment, we calculated the yield components seeds per pod, pods per plant, plants per hectare and thousand seed weight of several environments within a larger camelina variety trial. This study included three environments in the Colorado High Plains region, one in the Wyoming High Plains region and two in the Intermountain region of Colorado. Path analysis showed that plants per hectare had the strongest direct and indirect effects on yield and the other yield components. In addition, it had a consistent positive relationship with yield at all environments ($p<0.001$). There was also significant GxE interaction for plants per hectare in our study. This suggests that farmers looking for a quick way to increase yields should increase their planting density which increases the number of plants per hectare.

Plants per hectare had no significant correlation to the production of polyunsaturated fatty acids. Thousand seed weight and pods per plant had significant positive correlations to the production of linolenic (C18:3) fatty acid and a negative relationship with linoleic (C18:2). Average maximum temperature during the growing season is negatively correlated with high thousand seed weight, number of pods per plant, and the production of unsaturated fatty acids. Increasing precipitation had a significant positive relationship only with the number of seeds per

pod. This suggests that the other yield components are more affected by temperature than precipitation.

INTRODUCTION

Camelina sativa is an oilseed species with potential for use as a feedstock for the production of triglycerides for use as feedstocks for the production of biodiesel, surfactants, lubricants and many other industrial products (Zubr, 1997). Production of bio-based petroleum substitutes, or biofuels, in dryland farms could help offset the annual consumption of diesel fuels in Colorado farms. Enthusiasm for camelina in Eastern Colorado stems from its drought tolerance, suitable oil profile for use as a diesel substitute, compatibility with a dryland wheat-based cropping system, and resistance to flea beetles, a common pest of canola (*Brassica napus*) (Browne et al., 1991; Frohlich and Rice, 2005; French et al., 2009; Zubr, 1997). Camelina has demonstrated variable yields under dryland conditions and needs further assessment to judge its potential for growth in Colorado agricultural areas (Johnson et al., 2009).

Camelina seeds are composed of 30%-40% oil (Zubr, 1997). Vegetable oil produced from camelina is composed of over 50% polyunsaturated fatty acid, 30% to 45% of which are omega-3 alpha linolenic acid. Due to the high concentration of omega-3 fatty acids and low concentrations of erucic acid (C22:1) it is suggested that consumption of camelina oil is beneficial to human and animal health (Frohlich and Rice, 2005). High concentrations of polyunsaturated fatty acid can result in elevated emissions of nitrogen oxide (NO_x) pollutants (Pinzi et al., 2009). Optimizing the biofuel oil profile of camelina will need to focus on raising the percentage of oleic acid (C18:1) and decreasing concentrations of linolenic fatty acid (C18:3) (Pinzi et al., 2009).

Colorado is a predominantly wheat-producing state. According to the 2011 Colorado agricultural statistics database, Colorado is the eighth largest wheat-producing state nationwide. In 2010, out of 1,002,811 wheat hectares in Colorado, 991,479 were planted with winter wheat. This resulted in a total value of $606,359,000 (National Agricultural Statistics Service, 2011). Therefore, any candidate oilseeds for use in the production of bio-based petroleum substitutes in Eastern Colorado will need to be compatible with a dryland winter wheat rotation.

The Western Great Plains region of the United States is a challenging environment for any crop species due to its seasonal precipitation, low humidity and temperature fluctuations (Peterson and Westfall, 2004). Nevertheless, a bio-based petroleum substitute must be high-yielding in order for Colorado farmers to adopt this method of offsetting their annual diesel consumption.

It is important to identify varieties with stable yield and oil quality responses the diverse climatic conditions of the Colorado agricultural region. It is important to remember, when breeding for stability across environments, that a certain degree of phenotypic plasticity is not always negative (Bradshaw, 2006; Freeman et al., 1993). Plants that can respond and change their phenotype in response to their environment are useful in environments where they are likely to encounter a large degree of variability from year to year.

Yield stability is assessed by comparing the genotype and how it is affected by environmental conditions compared with other genotypes. The interaction between the genotypes and the environment, commonly known as genotype by environment (GxE) interaction, can occur in two ways, crossover and non-crossover interaction (Fehr, 1987). GxE interaction is important to consider when making varietal recommendations. Farmers could suffer heavy losses if varietal recommendations are made based on yield data where yield stability is not taken into consideration. The stability of a variety depends on the heritability of its yield and yield components. The heritability component of yield is reduced if genotype x year x environment (G x Y x E) interactions are significant (Richards et al., 2010).

Yield is a difficult selection criterion for plant breeders. There are numerous qualities that contribute to the overall yield of a crop species. A useful way to divide yield into more specific units is to measure yield in terms of yield components. There are many potential yield components and their relevance to yield improvement varies with the type of crop. For Brassica oilseed crops species, these include stand density, number of flowers, number of seed-bearing pods, seed weight, and number of branches. Measuring yield as a component of these characteristics gives breeders and crop scientists a way to measure yield as a function of different contributing factors. The advantage of breeding for components of yield is that heritability of the components of yield can be higher than heritability of total yield. For example, Vollman et al. (1996) calculated that the heritability of seed weight in camelina was 97.6 while the heritability for yield was lower, at 86.5. Yield components facilitate the identification of large-effect quantitative trait loci related to yield as yield is a complex trait that is governed by many genes and environmental effects while yield components are more specific traits that are controlled by fewer genes (Ribaut et al., 1997). Additionally, information about yield components better characterizes the specific roles stresses, such as drought, play in reducing overall yield (Ribaut et al., 1997).

Understanding how yield components are affected by climatic conditions and stresses can improve our understanding of how to breed for stability with respect to these characteristics. Oil profile characteristics can be affected by temperature and other environmental conditions during the growing season (Vollman et al., 2007). The level of linolenic fatty acid (C18:3) content in the seed-oil and plant biomass of camelina has a strong positive correlation with latitude, as the percent of this oil increases in cooler, wetter conditions (Linder, 2000; Enjalbert, 2011).

In addition, yield components have been shown to affect other yield components. Previous studies on yield components in camelina have found that large-seeded varieties have lower oil content than smaller-seeded varieties (Vollman et al., 2007). Increased planting density for camelina results in a decreased number of pods per plant (Agegnehu and Hornermeier, 1997).

A useful addition to the analysis of yield components is path analysis, a technique first described by Wright (1921). This technique has been used to analyze the relationships between yield components in wheat, crested wheatgrass and winter rapeseed (Dewey and Lu, 1959; Bhatt, 1973; Basalma, 2008; Cooper et al., 2012). Performing a correlation measures the association between two traits without identifying any of the specific causes or effects. By weighting the suspected causes and expressing their relative contributions to overall yield, path analysis uses a partial regression coefficient to give a more detailed analysis of the relationship between yield and yield components (Wright, 1921; Dewey and Lu, 1959; Bhatt, 1973). A visual representation is illustrated below in Figure 3.1.

Velasco et al. (1998) use a series of ratios to estimate the activity of various pathways that cause the elongation and desaturation of the fatty acids. This is a helpful tool for comparing the activity of metabolic pathways that form these fatty acids between species or varieties. The desaturation ratio (DR) estimates the activity of the pathway responsible for the conversion of linoleic fatty acid (C18:2) to linolenic (C18:3). The linolenic desaturation ratio (LDR) estimates the efficiency of the linoleic to linolenic conversion pathway. Figure 3.2 shows how to calculate these ratios. These pathways are a useful way to represent oil content because the production pathways of these oils are related.

This study calculated the yield components for camelina within a regional variety trial in order to quantify the variation in yield components in a diversity of environments within Eastern Colorado, Wyoming and the Intermountain West environments. The effect of the climatic variations on yield components will give us a better idea of the variation of these components in Colorado and Wyoming farming environments. In addition, the relationship of these components to each other will allow breeders to better understand the consequences of selecting for certain traits.

The yield components measured in this study were plants per hectare, pods per plant, seeds per pod and thousand seed weight. These yield components were analyzed for possible correlation with overall yield, oil content, oil profile, and climatic conditions. The climatic variables in each site were site elevation, total irrigation and precipitation, average daily maximum temperature, number of warm (above 32°C) and cold (below 5°C) days, as adapted from Baux et al. (2008) and used by Enjalbert (2011) as a stress threshold for oil development in oilseed species. From these measurements, we determined the stability of these yield component traits in a diversity of Colorado and farming conditions. By quantifying how these traits vary over a number of environments, we will be able to provide a useful tool for breeders hoping to produce high, stable yields across a number of drought environments.

MATERIALS AND METHODS

Environments

The environments in 2011 were Craig, Fort Collins, Yellow Jacket and Iliff, Colorado. In 2012, the environments were Greeley, Colorado, and Torrington, Wyoming. The sites in this study were selected from within a larger multi-environment variety trial. The environments were selected to represent a range of elevations and climatic conditions within the High Plains region. The two higher-altitude environments in this study were Yellow Jacket, Colorado, located at 2,103 m above sea level, and Craig, Colorado, which is located at 1,885 m. The lower elevation environments, more typical of Eastern Colorado agricultural areas, were Fort Collins, located at 1,557 m, Greeley at 1,427 m, Torrington, Wyoming, at 1,251 m, and Iliff at 1,165 m.

These sites were planted at different times depending on the planting conditions. In each environment except for Yellow Jacket, irrigation was applied as needed if drought conditions were excessive. The earliest planting date was at Torrington, Wyoming, which was planted on March 16, 2012. The latest date was at Craig, Colorado, which was planted on May 12, 2012. The harvesting dates varied from the earliest, July 11, 2012, at Greeley, to the latest, September 1, 2011, at Craig.

Planting and Harvesting

The entries were planted at a constant rate of 300 seeds m^{-2}. The seeds were planted in rows using a wheat planter. The plots measured 11 m^2 or 6 m long by 1.9 m wide, and were organized in a randomized complete block design with four replications. The herbicide Sonalan (Ethalfluralln, DowAgrosciences, Indianapolis IN) was applied prior to planting at a rate of 1.17 L ha^{-1} at all environments for all years. Fertilizer application varied among environments depending on how much was needed so that N, P and K were not limiting yield.

Genotypes Evaluated

A total of 15 varieties were included in this trial. The goal was to test a number of varieties from a diversity of breeding programs. Several of these were identified at Colorado State University as containing alleles speculated to contribute to increased yield performance in drought conditions (Enjalbert, 2011). These varieties, named with the prefix SSD, for single seed descent, were derived from a mapping population developed from the German varieties Lindo and Licalla (Enjalbert, 2011). The names and origins of the varieties included in this study are listed in Table 3.1.

Data Collection

For each environment, three yield components were measured within seven days before harvest. Three plants were selected randomly from within the plot and the number of pods per plant (PPP) was recorded. Open space between rows increased the amount of branching and caused an increase in the number of pods on plants at the perimeter of a plot, so plants were selected from within the interior of the plot. The number of seeds per pod was calculated using data from 10 pods from plants throughout the plot. The weight of 30 seeds was measured and this was used to calculate the average weight of a single seed for each variety and multiplied by a thousand for the thousand seed weight (TSW). The seeds from 10 pods from each plot were pooled and divided by the weight of a single seed for that plot and then by 10 to get the average number of seeds per pod (SPP).

Plants per hectare (PPH)= (((Yield (kg) per hectare/(TSW/1000))/SPP)/PPP)

Following collection of yield component data, the plots were harvested following drying to full harvest maturity and the yield was calculated after the seeds were allowed to dry to 8%

moisture. The harvesting was carried out with a Wintersteiger plot combine in Fort Collins and Iliff. In Craig, and Yellow Jacket, harvesting was done with a Hege plot combine. In Torrington, the harvesting was done with a 35' Massey-Ferguson.

Seed oil profiles for the 2011 environments were analyzed at a USDA research station in Peoria, Illinois, using gas chromatography mass spectrometry (GC-MS). Environments in 2012 have not been analyzed yet for oil profile and content. The weather data was gathered from the CoAgMet or USDA meteorological station nearest to the trial. The growing degree days were calculated using a base of 4.4°C and an upper threshold of 29.4°C. The equation for calculating growing degree days was described by McMaster et al. (1997) in instances where an upper threshold is used. This is:

$GDD= [(T_{MAX}+T_{MIN})/2]-T_{BASE}$ (McMaster and Wilhelm, 1997).

In our calculations, if the T_{MAX} exceeds the upper threshold, then the upper threshold temperature is used. The inverse is the case with the T_{MIN} and the lower threshold.

Analysis

SAS 9.3 (SAS Institute, Cary, NC) was used to perform the statistical analysis for this study. The General linear model (GLM) procedure was used to analyze the yield data for each environment individually and across all environments. The mixed procedure was used to calculate the analysis of variance (ANOVA) for the yield and yield components across varieties and environments. Pearson's correlation was used for correlation analysis. Path coefficients, both direct and indirect, were computed using a SAS macro developed by Cramer et al. (1999), using techniques originally pioneered by Wright (1921). Regression was carried out in SAS PROC REG, where the dependent variables were the yield components; seeds per pod, thousand seed weight, pods per plant, and plants per acre and the independent variables were the environmental variables latitude, precipitation, average maximum and minimum temperatures, number of cold and warm days, growing degree days, and elevation.

RESULTS

Table 3.2 shows the mean yields at each environment for each of the 15 varieties included in this study. All 15 varieties were included at each environment. The *p*-value denotes whether there are significant differences among the varieties at each environment. The varieties are listed according to mean performance across all environments. SSD177 was the overall highest yielding variety in this set of environments.

Table 3.1 shows the correlations of the yield components with the overall seed yield for each variety included in this trial across all environments and years. For eight of the fifteen entries, pods per hectare was positively correlated with seed yield. For six of the fifteen entries, thousand seed weight was positively correlated with seed yield. For two of the entries, seeds per pods was negatively correlated with seed yield. Pods per plant was not significantly correlated with seed yield. For five of the entries, none of the components of yield were correlated with seed yield.

The average values for the seed yields and yield components for each of the environments are shown in Table 3.3. They are arranged by order decreasing total seed yield. Fort Collins was the highest yielding trial. Low seeds per pod was observed at the Craig environment. There were four environmental conditions that may have influenced yield this includes dense stand establishment at Craig, high temperatures between June 20, 2012 and July 5, 2012 at Greeley (during which floral abortion and early ripening were observed), and high temperatures between June 20, 2012 and June 30, 2012 at Iliff. At Yellow Jacket, a dry period in early to mid-July followed by heavy rains on July 25, 2011 resulted in pod shattering and subsequent secondary flowering of the plants. Excessive weeds at Torrington were a problem for that trial.

There are several significant correlations reported in Table 3.4. Thousand seed weight is negatively correlated with the number of seeds per pod in all environments and across the combined environments. Thousand seed weight showed a significant negative correlation with the number of pods per plant but the correlation was significant only at Iliff. The number of plants per hectare had the strongest relationship with the other yield components. Seeds per pod and plants per hectare were negatively correlated in all the individual environments and across the combined environments. Pods per plant demonstrated significant negative correlations with number of plants per hectare in each individual environment and across all environments. At all environments, yield had a consistent positive relationship with the number of plants per hectare.

The underlined numbers in Table 3.5 express the direct relationship between the yield components and seed yield. The indirect effect expresses the negative or positive contribution of that yield component to the overall correlation with yield. The sum of the direct and the indirect effects across the rows is equal to the correlation coefficient between the yield component and the seed yield.

The direct positive effect of seeds per pod on yield is almost always offset by a medium or large indirect negative effect of plants per hectare and thousand seed weight. Likewise, the direct effect of thousand seed weight on yield was nearly always positive, but was usually counteracted by a negative indirect effect of seeds per pod. Pods per plant did not show a significant correlation with yield across all environments or at any individual environment. Plants per hectare always showed a large positive direct effect on yield. The indirect effects of plants per hectare on yield were usually small and ngative, except at Iliff, Fort Collins, and Craig, where plants per hectare had medium indirect negative effects on pods per plant and seeds per pod.

Table 3.6 shows the effect of varieties, environment and genotype by environment (GxE) for the various yield components in this study. Pods per plant and plants per hectare both show significant genotype by environment interaction. In all cases except for plants per hectare, the genotypes showed significant differences with respect to the yield components. The high coefficient of variation (%CV) suggest that these data might not be normally distributed. It is especially high for PPH, SPP and PPP.

Table 3.7 shows the weather and descriptive date for each environment. Three of the environments, Torrington, WY, Fort Collins, CO, and Greeley, CO were irrigated. Table 3.8 shows the relationship among climatic variables and the yield component data for all environments combined. The number of seeds per pod has a significant negative relationship with the average maximum temperature, the number of days above 32°C (warm days), the number of days below 5°C (cold days), and elevation. On the other hand, it has a significant positive relationship with the amount of available water and the average minimum temperature.

Thousand seed weight has a significant negative relationship with latitude, the average maximum and minimum temperatures, and the number of warm days. It has significant positive relationships with the number of cold days, number of growing degree days and elevation.

Increasing the average maximum temperature negatively affected the number of pods per plant and increasing the average minimum temperature, number of cold days, and number of growing degree days led to increased number of pods per plant.

The number of plants per hectare is negatively influenced by the average minimum temperature. Pods per hectare if negatively influenced by the average maximum temperature, number of warm days, number of cold days, number of growing degree days and elevation.

Overall, yield decreases with an increase in average maximum and minimum temperatures. The latitude, the amount of available water, the number of cold days, and number of growing degree days all contribute to higher yields.

Table 3.9 shows the results of a correlation between yield components and the oil profile characteristics that were analyzed for 2011 environments. The fatty acids in this table were used because they contribute to the calculation of the linoleic desaturation ratio (LDR) and the desaturation ratio (DR). Yield had a strong positive correlation with percent oil and a negative correlation to percent erucic acid. Seeds per pod showed a strong negative correlation with percent oil, percent eicosenoic acid, percent linolenic acid and LDR. There was a positive correlation between seeds per pod and percent linoleic and erucic acid.

Thousand seed weight was positively correlated with percent oil, percent linolenic acid and LDR. Thousand seed weight was negatively correlated with linoleic acid. Pods per plant was positively correlated with percent oil, linolenic acid, LDR and DR and negatively correlated with oleic, linoleic and eicosenoic acid. Plants per hectare was positively correlated with percent oil and eicosenoic acid, and negatively correlated with percent erucic acid. Percent oil was positively correlated with linolenic, eicosenoic, LDR and DR and negatively correlated with oleic, linoleic, and erucic acid.

DISCUSSION

The heat and drought stresses experienced at all environments except Fort Collins environment resulted in the high yield variability. In Fort Collins in 2011, precipitation was high and the average maximum temperatures were lower than average. In other drier or hotter environments, heat resulted in lower yields. Heat stress during flowering stage between June 20, 2012, and July 5, 2012, may have caused the floral abortion and lack of seed fill observed in Greeley, Colorado. In Iliff, Colorado, a lower yielding environment (mean yield 379 kg ha^{-1}), high temperatures were recorded between June 20, 2011, and June 30, 2011, which was around the flowering period at this environment. In Yellow Jacket, heavy rains on July 25, 2011, followed a low precipitation period. This resulted in pod shattering and subsequent secondary flowering of the plants which reduced the yields severely (mean yield=162.3 kg ha^{-1}). The yields at Torrington (266.7 kg ha^{-1}) were reduced because of heavy weed competition. In the Craig environment, seeds per pod were lower than other environments, but moisture conditions were optimal at planting so the stand densities were higher as a result.

The optimal seeding rate for camelina varies depending upon different environments and climatic conditions. Some (such as our program) prefer to row plant in high population densities, which for us was 710 seeds m^{-2}. Depending on the thousand seed weight, the planting rate in kg ha^{-1}varied but was approximately 7.84 kg ha^{-1} for our plots. This planting technique is compatible with wheat planting equipment if the planting depth is set to 6.3mm. Koncius and Karcauskiene (2010) found that the optimal yields and thousand seed weight of camelina were reached with a planting rate of 8 kg ha^{-1}. Other programs plant camelina by broadcasting the seeds throughout the field at lower densities closer to 3 kg ha^{-1} and pressing them with a press-wheel (Francis and Campbell, 2003). There is some disagreement as to whether one should plant at lower densities to increase the number of pods per plant or plant at higher densities with fewer pods per plant for optimal yields. Agegnehu and Hornermeier (1997) found that optimal stand density for camelina was 400 seeds m^{-2} while Crowley and Frolich (1998) found a lower seeding rate of 300 seeds m^{-2} to be adequate. Ehrensing and Guy (2008) reported that seeding at a rate as low as 134 seeds m^{-2} would produce adequate stands. This is far lower than our planting rate of 300 seeds m^{-2}.

As shown in Table 3.4, the number of pods per plant did not have a significant correlation with yield at any individual environment or across environments in this experiment. A higher number of plants per acre has a stronger relationship to seed yield than the number of pods per plant. The number of plants per hectare showed significant positive correlations with seed yield across many of the varieties as shown in Table 3.4. In previous experiments, it has been observed that optimal seeding rates result from planting rates that ranged from 300 to 600 seeds m^{-2} (Agregnehu and Honermeier, 1997; Crowley and Frolich, 1998; Uabaniak et al., 2008). At heavy plant densities, reductions in pods per plant were also observed in our study as well as others (Agregnehu and Honermeier, 1997; Uabaniak et al., 2008). In canola, plants have been observed to compensate for low stand establishment as a result of phenotypic plasticity in yield components (Angadi et al., 2003). Above a certain plant density, which for canola has been observed to be 8 plants m^{-2}, the plants yield better due to interplant competition (Angadi et al., 2003). Below that threshold, yield increases resulting from interplant competition are no longer present and yields drop precipitously (McGregor, 1987). It has been shown in camelina that stand reductions up to 50% during rosette and bolting stages had no significant effect on seed yields over a two-year period in Montana (McVay and Kahn, 2011).

Thousand seed weight and the number of seeds per pod were negatively correlated to each other at all environments. This suggests that there is a finite metabolic allotment to each pod, and therefore, the total weight of the seeds within a pod is more or less constant regardless of how many seeds are produced. This means that seeds per pod would not be a good trait to select for due to its negative relationship with thousand seed weight. According to Table 3.4, the direction and strength of correlation between yield and thousand seed weight varies in individual environments but the correlation is significant and positive when all of the environments are combined. Among individual varieties, shown in Table 3.2, there are many cases where thousand seed weight is significantly positively correlated with yield. This would suggest that thousand seed weight could be a yield component to select for in an effort to increase yields. Vollman et al. (1996) found that this trait has a high heritability, making it a good trait for selection by breeders. Due to the negative relationship between oil content and larger-seeded camelina seen in other studies, this increase in yields could come at the expense of the amount of oil per acre (Vollman et al., 2007).

The path analysis in Table 3.5 reveals some of the more nuanced relationships between the yield components and yield. The strongest direct effect on yield was the number of plants per hectare. It is interesting that the direct effects of this yield component always have a negative indirect effect from seeds per pod and pods per plant. It has been previously observed that branching and pods per plant are positively correlated (Urbaniak et al., 2008) This would suggest that increasing the number of plants per hectare has a negative effect on the pods per plant and number of seeds per pod, but an overall positive effect on seed yield. The number of seeds per pod also has a strong direct effect on seed yield but this is always countered by a strong opposite indirect effect of the number plants per hectare by increasing the overall number of pods per acre.

This effect is similar for the number of pods per plant, where it has a large direct effect on yield that is countered by large indirect effects from the number of plants per hectare. Follow up experiments could further elaborate on this idea by comparing the harvest index, or percent of the total biomass that is dedicated to seed. This would verify whether stand density has a significant positive effect on seed production.

According to our analysis, plants per acre would be a useful yield component for breeders interested in increasing yield. As shown in Table 3.6, plants per hectare and pods per plant showed significant GxE interaction across the environments. In addition, there were no significant differences for the varieties for plants per hectare. This suggests that this yield component is dependent on the environment and is not a characteristic that should be selected for as part of a breeding program.

The influence of environmental variables on the yield components, shown in Table 3.8, is strong. Latitude, average maximum temperature, and days above 32°C had the strongest effects on thousand seed weight (model R-square=0.324, 0.197, and 0.246, respectively). The negative slopes of latitude (-0.1314) and warmer average temperature (-0.3383) show that environments at higher latitude or warmer temperatures have lower thousand seed weights. This result is confounding as higher latitudes would be assumed to have a lower average maximum temperature, which had a negative relationship with yield. An explanation for this might be the effect of elevation on temperature for the environments in this study. The highest elevation environments in this study, Craig, and Yellow Jacket, Colorado, were also some of the lowest latitudes.

Irrigation and precipitation had a strong influence on yield. It has been estimated that camelina planted in Arizona consumed between 333 and 423 mm of applied water (French et al., 2009). Hergert et al. (2011) found that camelina exhibited a curvilinear response to irrigation that plateaued between 200 to 250 mm which when combined with stored soil moisture resulted in a maximum seasonal water use of 500-700 mm in the growing season. Our recorded combined precipitation and irrigation in this study varied from 360 mm in Fort Collins, Colorado, to 121 mm in Yellow Jacket, Colorado. Water stress during flowering in canola has been shown to cause a yield reduction of 29.5% (Ahmadi and Bahrani, 2009).

The average maximum temperature had a negative effect on yield and thousand seed weight. This reflects the susceptibility of camelina to heat stress as warmer environments yielded poorly. Also, the average minimum temperature had a strong negative effect on thousand seed weight and number of plants per hectare and yield. Conversely, it had a strong positive effect on the number of seeds per pod and pods per plant. This suggests that in colder environments, there is superior stand establishment and higher yields but fewer seeds in each pod and fewer pods per plant than in warmer climates. This possibly explains the low number of seeds per pod in the Craig environment as it had some of the lowest average minimum temperature and highest elevation. The result of the positive relationship between number of cold days with both yield and plants per hectare reinforced this idea, higher yields were associated with colder temperatures and number of plants per hectare.

The accumulated growing degree days throughout the growing season had a positive effect on yield and on most yield components. This was likely a result of the plant’s reaction to increased number of days of appropriate temperature, which resulted in more growth and therefore more plants, more seeds, and heavier seeds. In the Greeley environment, flowering was terminated early because of high temperatures early in the season. Experiments with canola have found earlier sowing dates, and more time before high summer temperatures, cause stress have resulted in higher yields (Ozer, 2003).

High temperatures seemed to result in deformed, light seeds as were seen in the negative correlation between thousand seed weight and average maximum temperature. We found that thousand seed weight and plants per hectare had positive correlations with oil content, suggesting good establishment conditions promote the formation of oil. This was observed by McVay and Khan (2011) who found decreasing stand density reduced seed oil content. Enjalbert (2011) found that cooler environments had an increased level of linolenic fatty acid. This is because of the down-regulation of fatty acid desaturase 3 at higher temperatures (Baux et al., 2008). The thousand seed weight and its relationship to the ratio of linoleic (C18:2) acid and linolenic (C18:3) acid is of interest here. Thousand seed weight has a negative relationship with C18:2, and a positive relationship with C18:3 suggesting that C18:2 might be formed early and then increasingly desaturated as the seed develops until there is a greater percentage of C18:3. High temperatures decrease oil content which is shown through the positive correlation between thousand seed weight and the LDR. This could mean that the linoleic to linolenic conversion pathway are responsive to the same stresses that cause a reduced thousand seed weight. The negative relationship between C18:2 and % Oil could suggest that as the seed develops, the percentage that is C18:2 is fixed and the plants continue to produce more of the other oils that form the components of the oil profile. Increasing yields and denser stands had a negative effect on concentrations of erucic acid, which is detrimental to the health of livestock and humans (Hulan et al., 1976). This suggests that dense camelina stands have less erucic acid and higher value as an animal feed.

CONCLUSION

The number of plants per hectare had the largest effect on yield. According to the path analysis, there were indirect effects from the other yield components but they were not sufficient to outweigh the direct effect of the plants per hectare. The result of our study suggest that within the locations of this study, areas with environmental conditions favoring high yields were also those that favored denser stands. All of our locations were seeded at a constant rate but the stand density varied among locations. The significant GxE interaction and variation among environments for plants per hectare suggests that this component is influenced by environmental conditions and not related to genotype. In a dryland agricultural system, this increased density may have a negative tradeoff in the form of increased water usage of the crop suggesting that a density threshold would be reached where yield increases would decrease because of competition for moisture, however Hunsaker et al. (2011) observed when low and limited irrigation conditions exist, high plant population contributed to yield increases. On the other hand, Agegnehu and Honermeier (1997) found that in higher-precipitation areas, pods per plant were the most important contributor to yields. Further investigation into the water use of a densely planted stand verses a low density, highly branched stand in low moisture, low humidity environment would be a useful follow-up experiment.

According to Table 3.1, the highest yielding variety in this trial was SSD 177, however, the seed yield of this variety did not show any significant correlation with yield components across all environments. If breeders are interested in choosing a variety for seed yield improvement, it would be beneficial to choose one that shows significant correlation with yield components that are highly heritable and related to genotype. It has been reported that camelina TSW shows a heritability of 97.6 (Vollman et al., 1996). TSW would be the best yield component for breeders interested in increasing yield because it doesn't show any significant GxE and varies significantly among varieties.

Based on the effect environment has on the yield components, it would appear that camelina does better in cooler climates where the chances of heat stress are low. In warmer climates, such as those in Colorado, where the chances of encountering excessively warm days, early planting would be a useful way to decrease the chances of flowering during a warm period.

According to our trials, the number of pods per plant has little relationship with the overall yields for camelina. The number of seeds per pod was observed to vary significantly among environments. This was most apparent in Craig, Colorado, where the number of seeds per pod was very low. This could have been related to the dense stand establishment suggesting a response to altitude.

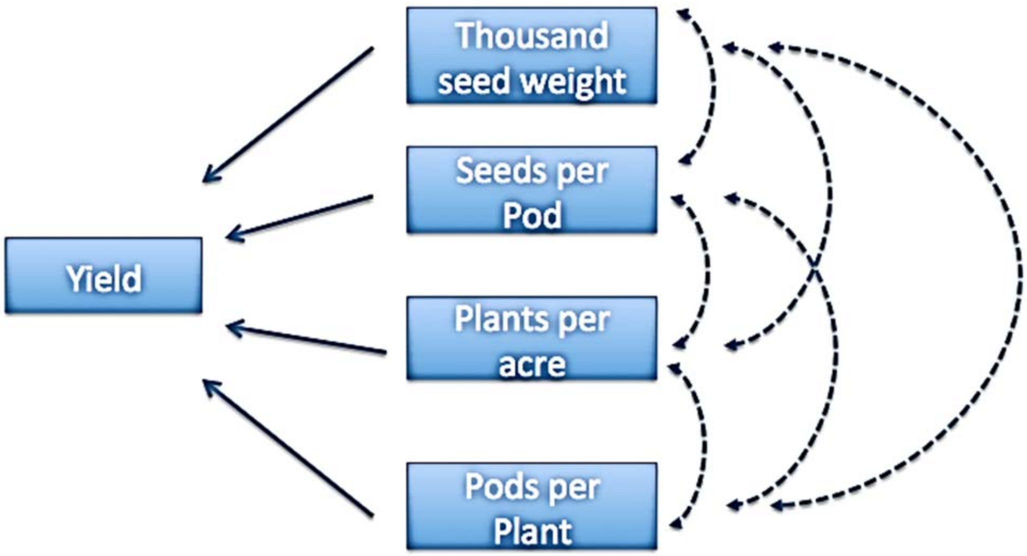


Figure 3.1. The direct and indirect effects of the yield components. The solid lines represent the direct effects on yield while the dashed lines represent the indirect effects. The indirect effect of plants per acre via pods per plant would be calculated by the correlation coefficient between the two variables multiplied by the direct path coefficient for pods per plant (Cooper et al., 2012).

$$DR = \frac{\%C18{:}2 + \%C18{:}3}{\%C20{:}1 + \%C22{:}1 + \%C18{:}1 + \%C18{:}2 + \%C18{:}3}$$

$$LDR = \frac{\%C18{:}3}{\%C18{:}2 + \%C18{:}3}$$

Figure 3.2. Calculating the ratios to estimate fatty acid production efficiency (Velasco et al., 1998).

Table 3.1. Pearson's correlations of the yield components with the yield of the varieties across all environments. Entries are ranked by order of yield performance across the trial. Includes sources of the varieties included in this trial with their respective maturity dates.

Variety	Maturity	Source	Avg. Yield	SPP†	TSW	PPP	PPH
			kg ha^{-1}				
SSD 177§	M¶	Univ. Giessen	484	NS‡	NS	NS	NS
Ligena	L	Univ. Giessen	478	NS	NS	NS	NS
SSD 10§	L	Univ. Giessen	451	NS	0.44	NS	NS
Celine	M	Europe	445	NS	0.50	NS	NS
BSX G22	E	Blue Sun Biodiesel	443	NS	NS	NS	0.53
SSD 138§	L	Univ. Giessen	437	-0.52	0.50	NS	0.55
Licalla	M	Europe	437	NS	NS	NS	NS
BSX G24	M	Blue Sun Biodiesel	428	NS	0.46	NS	0.59
SSD 186§	M	Univ. Giessen	427	NS	NS	NS	0.44
Suneson	M	MT State Univ.	424	-0.44	0.48	NS	0.43
Yellowstone	E	Great Plains Oil	423	NS	NS	NS	0.55
SSD 87§	M	Univ. Giessen	412	NS	NS	NS	NS
Lindo	M	Univ. Giessen	401	NS	0.50	NS	0.46
Blaine Creek	E	MT State Univ.	398	NS	NS	NS	0.44
Cheyenne	M	Blue Sun Biodiesel	395	NS	NS	NS	NS

§Indicates entry identified as containing significant favorable quantitative trait alleles for yield and drought tolerance

¶ Maturity classification of the varieties. L= Late Maturing, M= Medium Maturing, E= Early Maturing

†Yield components: SPP= Seeds per pod, TSW= Thousand seed weight (g), PPP=Pods per plant, PPH=number of pods per hectare

‡ NS indicates correlation coefficient was not significant (α=0.05)

Table 3.2. LSMEAN yields for each variety at each environment. *P*-value signifies whether there is a significant difference among the varieties at each environment.

Variety	Fort Collins	Craig	Iliff	Torrington	Yellow Jacket	Greeley	Mean
				kg ha^{-1}			
SSD177	1017	609	551	339	202	140	476
Ligena	1072	699	348	334	210	150	469
BSX G22	965	629	572	225	225	111	455
SSD10	995	714	383	318	204	92	451
Celine	1037	682	312	309	188	141	445
Licalla	992	650	376	296	120	205	440
SSD138	855	690	549	286	109	105	432
Suneson	894	675	359	314	206	98	424
SSD186	900	549	530	235	193	126	422
SSD87	934	682	331	300	144	113	417
BSX G24	774	731	453	270	150	121	417
Yellowstone	736	570	486	362	233	112	416
Cheyenne	798	630	315	304	182	155	397
Blaine Creek	745	608	426	329	190	76	396
Lindo	820	655	372	245	182	95	395
Mean	902	651	424	298	182	123	433
p val	0.092	0.758	0.198	0.444	0.029	0.299	-

Table 3.3. Average yields and yield component values at each environment across all 15 varieties included in this study.

Year	Environment	Yield	Yield Component† SPP	TSW	PPP	PPH
		kg ha^{-1}		g		
2011	Fort Collins	804	10.0	1.11	121	134,884
2011	Craig	592	3.3	1.12	56	709,502
2011	Iliff	379	10.1	0.77	62	189,872
2012	Torrington	267	9.7	0.57	180	78,116
2011	Yellow Jacket	162	9.7	1.20	143	31,228
2012	Greeley	109	9.5	0.80	72	45,657

† Yield components: SPP= Seeds per pod, TSW= Thousand seed weight (g), PPP=Pods per plant, PPH=number of pods per hectare

Table 3.4. Correlation coefficients and significances among yield components.

Environment	Yield Component				
	SPP†	TSW	PPP	PPH	YIELD
All environments					
SPP	1	-0.37***	0.13*	-0.56***	-0.16**
TSW	-	1	-0.16**	0.20***	0.37***
PPP	-	-	1	-0.41***	-0.08
PPH	-	-	-	1	0.43***
Fort Collins					
SPP	1	-0.51***	0.03	-0.44**	-0.23
TSW	-	1	-0.02	0.04	0.32
PPP	-	-	1	-0.61***	0.03
PPH	-	-	-	1	0.50***
Iliff					
SPP	1	-0.49**	0.15	-0.47**	0.08
TSW	-	1	-0.42***	0.28*	0.28*
PPP	-	-	1	-0.49***	0.10
PPH	-	-	-	1	0.35**
Craig					
SPP	1	-0.37*	0.04	-0.32	0.13
TSW	-	1	-0.09	-0.10	-0.12
PPP	-	-	1	-0.75**	-0.17
PPH	-	-	-	1	0.40**
Yellow Jacket					
SPP	1	-0.57***	-0.21	-0.09	-0.19
TSW	-	1	-0.10	-0.18	0.19
PPP	-	-	1	-0.56***	-0.20
PPH	-	-	-	1	0.45**
Greeley					
SPP	1	-0.16	0.01	-0.34**	-0.09
TSW	-	1	-0.16	0.23	-0.54***
PPP	-	-	1	-0.32*	0.21
PPH	-	-		1	0.62***
Wyoming					
SPP	1	-0.04	-0.13	-0.38**	-0.09
TSW	-	1	-0.03	0.45**	0.05
PPP	-	-	1	-0.40**	-0.17
PPH	-	-	-	1	0.350**

* Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
† Yield components: SPP= Seeds per pod, TSW= Thousand seed weight (g), PPP=Pods per plant, PPH=number of pods per hectare

Table 3.5. Path analysis showing direct and indirect effects and total correlation coefficients of yield and yield components. Effect values are considered large if they exceed 0.50, medium if they are near 0.30, and small if they are less than 0.10 (Suhr, 2008).

Environment	**Yield Component**				
	SPP†	TSW	PPP	PPH	YIELD
All environments					
SPP	0.29‡	-0.14	0.02	-0.33	-0.16**
TSW	-0.11	0.38	-0.03	0.12	0.37***
PPP	0.04	-0.06	0.19	-0.24	-0.08
PPH	-0.16	0.08	-0.08	0.59	0.43***
Fort Collins					
SPP	0.52	-0.28	0.02	-0.50	-0.24
TSW	-0.27	0.55	-0.01	0.04	0.32
PPP	0.02	-0.01	0.72	-0.69	0.03
PPH	-0.23	0.02	-0.44	1.14	0.50***
Iliff					
SPP	0.71	-0.34	0.10	-0.39	0.08
TSW	-0.35	-0.69	-0.29	0.23	0.28*
PPP	0.10	-0.29	0.69	-0.41	0.10
PPH	-0.34	0.19	-0.34	0.83	0.35**
Craig					
SPP	0.56	-0.10	0.03	-0.37	0.13
TSW	-0.21	0.26	-0.06	0.11	-0.12
PPP	0.02	-0.02	0.67	-0.83	-0.17
PPH	-0.19	-0.03	-0.50	1.11	0.40**
Yellow Jacket					
SPP	0.20	-0.26	-0.06	-0.06	-0.19
TSW	-0.11	0.46	-0.03	-0.12	0.19
PPP	-0.04	-0.04	0.29	-0.40	-0.20
PPH	-0.02	-0.08	-0.16	0.70	0.45**
Greeley					
SPP	0.24	-0.08	0.00	-0.26	-0.09
TSW	-0.04	0.49	-0.09	0.17	-0.54***
PPP	0.00	-0.08	0.54	-0.25	0.21
PPH	-0.08	0.11	-0.17	0.76	0.62***
Torrington					
SPP	0.16	-0.01	-0.01	-0.23	-0.09
TSW	-0.01	0.33	0.00	-0.27	0.05
PPP	-0.02	-0.01	0.09	-0.25	-0.17
PPH	-0.06	-0.15	-0.04	0.60	0.350**

* Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.
† Yield components: SPP= Seeds per pod, TSW= Thousand seed weight (g), PPP=Pods per plant, PPH=number of pods per hectare
‡ Direct effects denoted with underline

Table 3.6. ANOVA for the effects of genotype, environment and genotype by environment (GxE) on the four yield components.

	df	SPP†	TSW	PPP	PPH
		P	*P*	*P*	*P*
Source of variation					
Genotype	14	**	***	**	NS
Environment	5	***	***	***	***
GxE	70	NS‡	NS	*	*
R-Square		0.63	0.80	0.72	0.85
CV%		29.90	18.23	39.10	64.94
Mean		8.92	0.91	106.29	182282.01

* Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

† SPP= Average number of seeds per pod, TSW=Thousand seed weight, PPP= Average number of pods per plant, PPH=Average number of plants per hectare.

‡ NS indicates correlation coefficient was not significant

Table 3.7. The weather data collected for each environment in this study. Precip=Growing season precipitation (combination of precipitation and irrigation). Avg Max Temp= Average maximum temperature for the growing season. Avg Min Temp= Average minimum growing season temperature.

Year	**Environment**	**Latitude**	**Date of Planting**	**Date of Harvest**	**Precip**	**Avg max temp**	**Avg min temp**	**Growing degree days**	**Elevation**
					mm	°C	°C		m
2011	Fort Collins, CO	40.58	3/25/11	7/26/11	364	21.9	6.6	2369	1533
2011	Craig, CO	40.51	5/12/11	9/1/11	182	28.3	6.1	2561	1856
2011	Iliff, CO	40.75	4/20/11	8/1/11	300	26.6	8.4	2491	1147
2011	Yellow Jacket, CO	37.53	4/20/11	8/16/11	121	24.3	8.4	2833	2070
2012	Torrington, WY	42.06	3/16/12	7/20/12	229	27.8	8.4	2354	1231
2012	Greeley, CO	40.42	4/9/12	7/11/12	220	27.1	8.6	1250	1405

Table 3.8. Regression of yield and yield components on climatic variables over all environments.

Yield Component	Intercept	Slope	Model R-square	Intercept	Slope	Model R-square
	Latitude			**Precipitation+ Irrigation**		
SPP†	NS‡	NS	NS	6.22	0.28	0.06***
TSW	6.22	-0.13	0.30***	NS	NS	NS
PPP	NS	NS	NS	NS	NS	NS
PPH	NS	NS	NS	NS	NS	NS
Yield	-1398.74	45.31	0.03**	-150.89	60.89	0.37***
	Avg. Max temp			**Avg. min temp**		
SPP	28.46	-0.25	0.07***	-27.87	0.80	0.16***
TSW	3.57	-0.03	0.20***	4.40	-0.08	0.21***
PPP	295.61	-2.40	0.02**	-166.14	5.90	0.03**
PPH	-3297289	47562	0.10***	10253142	-212316	0.40***
Yield	3240.26	-35.63	0.23***	6393.86	-129.10	0.63***
	Days above 32°C			**Days below 5°C**		
SPP	10.51	-0.06	0.04***	11.49	-0.07	0.03***
TSW	1.22	-0.01	0.25***	0.68	0.01	0.04***
PPP	NS	NS	NS	66.56	1.02	0.03**
PPH	-42113	18243	0.15***	-447807	23059	0.16***
Yield	NS	NS	NS	-474.62	23.30	0.68***
	GDD			**Elevation**		
SPP	NS	NS	NS	13.91	-0.00	0.08***
TSW	0.52	0.00	0.08***	-0.03	0.00	0.41***
PPP	45.73	0.03	0.04***	NS	NS	NS
PPH	-263265	313.80	0.07***	-317242	153.35	0.07***
Yield	-42.01	0.21	0.12***	NS	NS	NS

*Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level
† SPP= Average number of seeds per pod, TSW=Thousand seed weight, PPP= Average number of pods per plant, PPH=Average number of plants per hectare.
‡ NS indicates lack of significance

Table 3.9. Correlation of yield and yield components with oil profile characteristics for environments in Fort Collins, Yellow Jacket, Craig and Iliff, CO, in 2011.

Oil Component	**Yield Component**					**% Oil**
	Yield	**SPP†**	**TSW**	**PPP**	**PPH**	
% Oil	0.332***	-0.353***	0.499***	0.135*	0.352**	NS
C18:1 Oleic	NS‡	NS	NS	-0.172*	NS	-0.150*
C18:2 Linoleic	NS	0.142*	-0.442**	-0.189**	NS	-0.385***
C18:3 Linolenic	NS	-0.169*	0.431***	0.378***	NS	0.643***
C20:1 Eicosenoic	NS	-0.185**	NS	-0.175*	0.214**	0.162*
C22:1 Erucic	-0.145*	0.172*	NS	NS	-0.141*	-0.158*
LDR$^{\alpha}$	NS	-0.177*	0.511***	0.311***	NS	0.583***
DR	NS	NS	NS	0.213**	NS	0.197**

* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level
† SPP= Average number of seeds per pod, TSW=Thousand seed weight, PPP= Average number of pods per plant, PPH=Average number of plants per hectare.
‡ NS indicates correlation coefficient was not significant
α LDR= Linolenic desaturation ratio, DR= Desaturation Ratio

REFERENCES

Agegnehu, M., and B. Honermeier. 1997. Effects of seeding rates and nitrogen fertilization on seed yield, seed quality and yield components of false flax (*Camelina sativa* Crtz.) Die Bodenkultur 48(1):15-21.

Ahmadi, M., and M.J. Bahrani. 2009. Yield and yield components of rapeseed as influenced by water stress at different growth stages and nitrogen levels. American-Eurasian Journal of Agriculture and Environmental Science 5(6):775-761.

Basalma, D. 2008. The correlation and path analysis of yield and yield components of different winter rapeseed (*Brassica napus* ssp. *olifera* L.) cultivars. Research Journal of Agriculture and Biological Sciences 4(2):120-125.

Bhatt, G.M. 1972. Significance of path coefficient analysis in determining the nature of character association. Euphytica 22:338-343.

National Agricultural Statistics Service. 2011. Colorado agricultural statistics. http://www.nass.usda.gov/Statistics_by_State/Colorado/index.asp.

Cooper, J.K., Ibrahim, A.H.M., Subas Malla, J.R., Hays, D.B., and J. Baker. 2012. Increasing hard red winter wheat yield potential via synthetic wheat: I. Path-coefficient analysis of yield and its components. Crop Science 52:2014-2022.

Cramer, C., Wehner, T., and S. Donaghy. 1999. PATHSAS: A SAS computer program for path coefficient analysis of quantitative data. Journal of Heredity 90: 260-262.

Crowley, J. G., and A. Frohlich. 1998. Factors affecting the composition and use of camelina. Teagasc. Crops Research Centre, Oak Park, Carlow, Ireland.

Dewey, D.R., and K.H. Lu. 1959. A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agronomy Journal 51:515-518.

Ehrensing, D.T., and S.O. Guy. 2008. Oilseed crops: Camelina. Oregon State University Extension Service EM 8953-E.

Enjalbert, J.N. 2011. An integrated approach to local based biofuel development. (Doctoral Dissertation). Colorado State University Libraries. Fort Collins, CO 140p. http://hdl.handle.net/10217/46747.

Enjalbert, J.N., and J.J. Johnson. 2011. Guide for producing dryland camelina in Eastern Colorado. Colorado State University Extension factsheet No. 0.709. http://www.ext.colostate.edu/pubs/crops/00709.pdf.

Fehr, W.R. 1987. Principles of crop development. Vol 1. Theory and Technique. Macmillan Publishing Co., New York, New York.

Francis, C. M. and M.C. Campbell. 2003. New high quality oil seed crops for temperate and tropical Australia. A Report for the Rural Industries Research and Development Corporation. Crawley, Australia.

French, A.N., Hunsaker, D., Thorp, K., and T. Clarke. 2009. Evapotransipration over a camelina crop in Maricopa, Arizona. Industrial Crops and Products 29:289-300.

Harrington, K.J. 1986. Chemical and physical properties of vegetable oil esters and their effect on diesel fuel performance. Biomass 9:1-17.

Hergert, G.W., Marghiem, J., Pavlista, A., Burgener, P., Lyon, D., Hazen, A., Martin, D., Supalla, R., and C. Thompson. 2011. Yields and ET of deficit to fully irrigated canola and camelina. Proceedings of the 23rd Annual Conference, Burlington, Colorado.

Hulan, H.W., Kramer, J.K.G., Mahadevan, S., Sauer, F.D., and A.H. Corner. 1976. Relationship between erucic acid and myocardial changes in male rats. Lipids 11(1):9-15.
Hunsaker, D.J., French, A.N., Clarke, T.R., and D.M. El-Shikha. 2011. Water use, crop coefficients and irrigation management criteria for camelina production in arid regions. Irrigation Science 29:27-43.
Koncius, D., and D. Karcauskiene. 2010. The effect of nitrogen fertilizers, sowing time and seed rate on the productivity of *Camelina sativa*. Zemdirbyste-Agriculture 97(4):37-46
Khan, L.M., and M.A. Hanna. 1983. Expression of oil from oilseeds-A review. Journal of Agricultural Engineering Research 28:495-503.
Linder, C.R. 2000. Adaptive evolution of seed oils in plants: Accounting for the biogeographic distribution of saturated and unsaturated fatty acids in seed oils. American Naturalist 156:442-458.
McGregor, D.I. 1987. Effect of plant density on development and yield of rapeseed and its significance to recovery from hail injury. Canadian Journal of Plant Science 67:43–51.
McMaster, G.S. and W.W. Wilhelm. 1997. Growing degree days: one equation, two intepretations. Agricultural and Forest Meterology 87:291-300.
McVay, K.A., and Q.A. Kahn. 2011. Camelina yield response to different plant populations under dryland conditions. Agronomy Journal 103(4):1256-1269.
Ozer, H. 2003. Sowing date and nitrogen rate effects on growth, yield and yield components of two summer rapeseed cultivars. European Journal of Agronomy 19(3):453-463.
Ribaut, J.M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G.O., and D.A. Hoisington. 1997. Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. Theoretical and Applied Genetics 94:887-896.
Ryan, T.W., Dodge, L.G., and T.J. Callahan. 1984. The effects of vegetable oil properties on injection and combustion of two different diesel engines. Journal of American Oil Chemists' Society 61(10):1610-1619.
Suhr, D. 2008. Step your way through path analysis. Western Users of SAS Software Conference Proceedings. Retrieved Oct 4, 2012. http://www.wuss.org/.
Urbaniak, S.D., Caldwell, C.D., Zheljazkov, V.D., Lada, R., and L. Luan. The effect of seeding rate, seeding date and seeder type on performance of *Camelina sativa* L. in the Maritime Provinces of Canada. Canadian Journal of Plant Science 88(3):501-508.
Velasco, L., Goffman, F.D., and C. Becker. 1998. Variability for the fatty acid composition of the seed oil in a germplasm collection of the genus *Brassica*. Genetic Resources and Crop Evolution 45:371-382.
Vollmann, J., Damboeck, A., Eckl, A., Schrems, H., and P. Ruckenbauer. 1996. Improvement of *Camelina sativa*, an underexploited oilseed. p. 357-362. In: J. Janick (ed.), Progress in new crops. ASHS Press, Alexandria, VA.
Vollman, J., Moritz, T., Kargl, C., Baumgartner, S., and H. Wagentristl. 2007. Agronomic evaluation of camelina genotypes selected for seed quality characteristics. Industrial Crops and Products 26:270-277.
Wright, S. 1921. Correlation and causation. Journal of Agricultural Research 20:557-585.
Zubr, J. 1997. Oil-seed crop: *Camelina sativa*. Industrial Crops and Products 6:113-119.

CHAPTER 4:

FALL SEEDING OF CAMELINA IN COLORADO

INTRODUCTION

Camelina sativa, or false flax, is an oilseed species that has been identified as a promising feedstock for the on-farm production of biodiesel due to its favorable oil profile, resistance to flea beetles, and cold and drought tolerance (Angelini et al., 1997; Zubr, 1997; French et al., 2009; Onyilagha, 2012). Camelina can be planted in agricultural areas utilizing both tillage or no-till techniques (Gesch and Cermak, 2011).

There is evidence that incorporating different crops into an otherwise continuous winter wheat-fallow rotation can result in increased wheat yields (Bushong et al., 2012). Bushong et al. (2012) found in Western Oklahoma that wheat yield of a winter wheat-canola rotation showed an increase that varied from 10 to 22%. They also found that based on the historical wheat and canola prices, the net economic returns from the canola-wheat rotation were greater than those for continuous wheat. This analysis included the economic impacts of purchasing herbicide treatments and losses due weed pressure. The yield effect of incorporating camelina into the rotation may be comparable to canola, as their growing season lengths are comparable. Figure 4.2 presents a possible planting schedule comparing how spring and winter camelina are compatible with a winter wheat rotation as opposed to other common oilseeds in Colorado such as sunflowers.

There are two types of camelina that differ from an agronomic perspective. Winter varieties are planted in the fall and overwinter in a rosette stage. These resume growth in the spring when temperatures rise above 3.3°C (Ehrensing and Guy, 2008). Spring seeded varieties, on the other hand, are planted in early spring and do not undergo a dormancy stage (Putnam et al., 1993). Both planting regimes have their benefits and drawbacks but are equal with the winter wheat-fallow rotation in the American High Plains.

Advantages of spring planting include better weed control if spring tillage is utilized, lack of winter-kill of dormant seedlings, and the ability to plant into moist spring conditions. Drawbacks of this regime include later spring emergence, and dependency on spring moisture for emergence.

The majority of the research on camelina has focused on spring varieties. For dry areas in Colorado, fall planting of camelina might result in a yield advantage similar to that of winter wheat over spring wheat. Advantages of fall-seeded winter varieties include earlier initiation of growth, reduced risk of unfavorable planting conditions, and less time to reach maturity than spring-seeded camelina. Earlier maturity means that the plants are not exposed to as much heat and drought stress that occurs in the warmest months of summer. Drawbacks of this planting regime include increased weeds and insect pests.

Seeding camelina in the fall requires modification of the weed and pest management regimes that are utilized for spring camelina. Fall planting means that tillage or herbicide applications occur in the fall prior to planting, instead of the spring. This means that weed pressure could be increased because spring tillage is not available as a control measure. Good fall stand establishment and vigorous spring growth can alleviate some of the weed pressure through shading and vigorous competition (Crowley, 1999).

A concern for the viability of overwintering camelina seedlings is their tolerance to cold winter temperatures. Previous experiments have found that camelina is hardy enough to survive the harsh winters of Minnesota, where average winter air temperatures are far lower than those found in Colorado (Gesch and Cermak, 2011). Overwintering ability is increased with snow cover (Aase and Siddoway, 1979; Sharratt et al., 1992). Aase and Siddoway (1979) determined that 7 cm of snow cover is sufficient to buffer wheat seedlings from temperatures as low as -40°C. With the increased stubble as a result of the implementation of no-till agricultural systems, there is a greater amount of snow capture on fields in Eastern Colorado than is present with traditional tillage (Nielsen, 1998).

There are insect pests of winter camelina that are not present in spring-seeded camelina. Previous winter camelina trials in Akron, Colorado, have encountered failures related to the presence of *Ceutorhynchus cyanipennis* and *Ceutorhynchus americanus* (Boris Kondratieff, personal communication, Apr 8, 2011; Gary L. Hein, personal communication, Feb 18, 2006). These insect pests appear frequently on plant species in the Brassicaceae family, which includes camelina (Cripps et al., 2006). These insects most strongly affect winter camelina that is planted in earlier in the fall, especially in August, as this is the time adult insects lay eggs (Cripps et al., 2006). Later fall planting dates have been shown to reduce the impact of these pests. Studies of winter camelina in Ireland have shown that earlier planting dates are prone to high rates of lodging (Crowley, 1999). This may be due to the damage from these insects, as the larvae feed on stems at or below the soil line (Gary L. Hein, personal communication, Feb 18, 2006).

Fall seeding of camelina would be particularly advantageous in Southeastern Colorado, where early spring temperatures are warmer and spring regrowth can be initiated earlier. In this area, high summertime temperatures result in a high evapotranspiration rate, meaning earlier establishment can allow camelina to avoid the hot, dry conditions of early summer.

There were three specific objectives to this study within a larger goal of evaluating the potential of fall-seeded camelina in Colorado. The first was to determine the optimal date of planting for fall seeded camelina. The second was to determine the optimal variety for fall planting among the varieties included in this study. The third goal was to evaluate the overwintering survival of spring varieties compared to winter varieties

MATERIALS AND METHODS

The initial evaluation of fall-seeded camelina was carried out in 2010-2011 in two environments, Fort Collins and Rocky Ford, Colorado. In both environments, there were fourteen total entries (Table 4.2). These included eleven winter-seeded varieties from the Great Plains Oil breeding program in Torrington, Wyoming. In addition, three spring types were included for comparison; these were Yellowstone, Celine and Ligena. In Fort Collins and Rocky Ford, these varieties were seeded in plots measuring 1.3 m^2. The Fort Collins environment also included two treatments: irrigated and dryland. The date of planting was randomized within each treatment. In Rocky Ford, there was only an irrigated treatment and three dates of planting. In both environments there were two replications. The varieties planted in Fort Collins were planted at 5 different dates throughout the fall of 2010 and one date in the spring of 2011. These dates of planting are listed in Table 4.1. The spring planting date reflected when spring camelina is typically planted in the High Plains. These entries were hand planted in multiple rows at a rate equivalent to 7.8 kg ha^{-1}.

For the irrigated treatment, sprinkler irrigation was applied as needed to prevent excessive drought stress of the plots. Irrigation water was shut off on October 13, 2010.

Throughout the fall and spring, emergence and flowering data for the fall and spring-seeded entries were recorded on a biweekly basis. At harvest, the plots were hand-harvested and hand threshed at different times depending on the date of maturity.

The yields were recorded and the data were processed using a general linear model (GLM) as provided in the SAS statistical software (SAS institute Cary, North Carolina). The independent variables were date of planting, variety, environment, irrigation treatment, and replication. There were no significant differences among the environments in this study (p=0.6683) so the environments were combined for analysis. For both environments, the least squared means (LSMEAN) were calculated from yields across both environments. The dependent variable was yield.

RESULTS

Spring varieties that were seeded in the fall all emerged in the fall but failed to overwinter. Weeds were also a problem and resulted in many plots being abandoned. Plots that were abandoned due to weeds were recorded as missing data in the analysis. This ensured that plot failure due to outside factors was not counted against the true performance of the varieties. Due to the unbalanced design because of the missing entries, least squared (LS) means were used.

The fall of 2010 was dry. The first significant precipitation event occurred on October 12, 2010, so fall germination was not observed prior to that in the non-irrigated trials. Following that rain, the next precipitation event occurred on Oct 22, 2010 and again on Nov 9, 2010. The total precipitation that fell from Sept 15, 2010 to Nov 23, 2010, when the soil temperature fell below 0°C was 31mm.

Fall emergence was only observed in the irrigated treatments for those entries planted in the first, second and third fall planting dates. At the time of the fourth and fifth planting, temperatures were below the minimum required for camelina germination. The winter camelina varieties established a small, purple rosette that remained dormant throughout the winter. In the following spring, the rosette resumed growth. All entries planted on the third date of planting failed to emerge in the spring. The winter camelina varieties planted on the fourth and fifth dates germinated in the spring when conditions were optimal. Those winter camelina entries that emerged in the fall were ready for harvest on July 19, 2010 while those that did not germinate until the spring were not ready until July 24, 2010. The spring varieties planted in any fall date failed to emerge in the spring.

Spring precipitation in 2011 was sufficient to result in a yield of the dryland camelina entries. From March 11, 2011, when soil temperatures rose above 0°C until July 15, 2011 on the first harvest date, there was 219 mm of total precipitation. This is close to the 333mm total water requirement at which camelina has been observed to reach its maximum yield potential in Arizona (French et al., 2009).

There were several issues that led to low yields of the entries in this trial. The entries that germinated in the earliest dates of fall planting may have been affected by the presence of an insect pest, *Ceutorhynchus americanus*, which was positively identified in the field as living in the rosette of overwintering individuals. This may have contributed to the die-off of many of the smaller individuals that emerged in the fall but failed to reemerge in the spring. In addition to insect pressures, weeds were also a problem. Lodging of the camelina due to bindweed resulted in the abandonment of many plots. Another issue affecting those entries planted on the first and second dates of planting was uneven establishment. Of the winter camelina varieties, some of the seeds planted in the fall remained dormant until the spring. These seeds emerged in the spring later than the individuals that established a rosette in the fall. As a result, there was a discontinuity in the maturity within some of the plots.

Despite the poor performance of some of the varieties included in this study, yield data were recorded for many of the entries. The results of the analysis of variance carried out for this trial showed that there were significant differences among three of the treatments in this experiment. Those were variety (p =0.0485), date of planting (p <0.001) and irrigation treatments (p =0.0358). The mean yield for the irrigated entries in this trial was 468 kg ha^{-1}, while the mean yield for the dryland entries was 400 kg ha^{-1}. There were no significant differences among the environments in this study (p =0.6683) so the environments were combined for analysis. The yield mean for the winter camelina in this trial was 434 kg ha^{-1}, while the mean yield for the spring varieties that were planted in the spring as a check was 479 kg ha^{-1}. Table 4.2 shows the averages for the different varieties included in the trial. Figure 3 shows a comparison of the mean yields for the winter varieties at each date of planting, irrigation treatment and environment.

DISCUSSION

Camelina entries showed much variability for yield. The highest performer was a winter variety HPX-WG2 at 567 kg ha^{-1}. The lowest yielding varieties were HPX-WG1-29 and HPX-WG4, which yielded 342 kg ha^{-1} and 324 kg ha^{1}, respectively. Overall the spring varieties seeded on the final date of planting outperformed the winter varieties in this trial.

Our yields were comparable to the winter camelina yields observed by Gesch and Cermak (2011) in Minnesota. Their average yield for the variety Joelle, which was an entry in this trial, was 552 kg ha^{-1}, which is higher than the 470 kg ha^{-1} recorded here. Overall the entries in this study yielded lower than the average for a nearby spring camelina variety trial, which had a trial average of 1033 kg ha^{-1}.

Figure 4.3 shows the performance for each of the dates of planting. The fourth, fifth and sixth dates of planting were the best performers. Varieties planted on these dates germinated and emerged in the spring. Planting dates 4 and 5 were allowed to overwinter as seed and germinate when spring conditions were optimal. This might have some implications for those interested in planting camelina. Planting a winter variety in the late fall when average temperatures are below the germination threshold for camelina means that seeds stay dormant throughout the winter. This means that the seeds emerge when spring moisture arrives. This does not, however, work for spring camelina varieties, as low winter temperatures result in die-off.

CONCLUSION

From this study, we affirmed that is it possible to grow winter camelina in Eastern Colorado. We also discovered that spring varieties seeded in the fall not will emerge in the spring. It is possible that planting in the late fall and allowing camelina to overwinter as a seed could be an effective part of an agricultural rotation that effectively utilizes subsoil moisture. This planting strategy ensures the seeds are able to germinate at the earliest moment that spring conditions are optimal. Treating camelina as a winter annual and allowing it to germinate and emerge in the fall is not a recommended strategy because of the insect and drought pressure in this area.

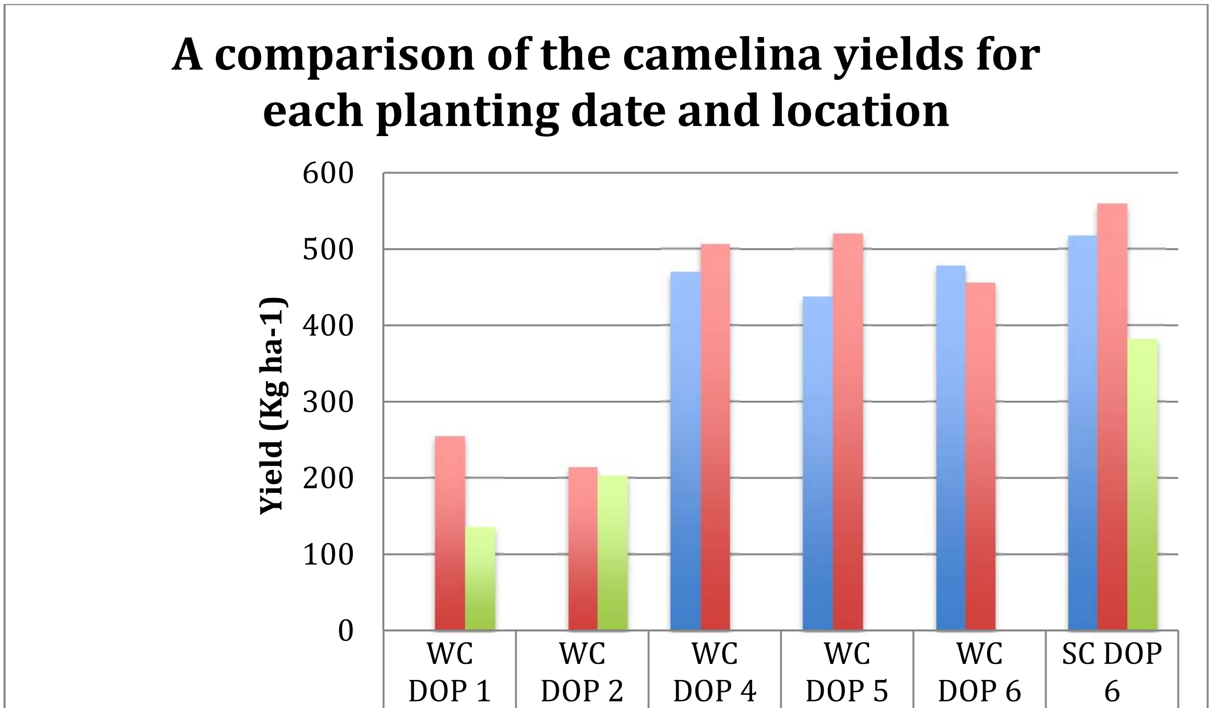
Wheat-Spring Crop-Fallow Cropping System for Northeastern Colorado

Year	Mar	Apr	May	June	July	Aug	Sept	Oct	November	Dec to Feb
1							Wheat Planted			
2					Wheat Harvested					
3		Corn Planted							Corn Harvested	
4	Oilseed Planted				Oilseed Harvested		Wheat Planted			

Figure 4.1. The chronology of a rotation for Eastern Colorado that produces three crops in three growing seasons by displacing fallow period with a short season oilseed crop (Johnson et al., 2009).

Crop	Aug	Sep	Oct-Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Winter Wheat	Planted							Harvest			
Winter Camelina		Planted						Harvest	Planted with wheat		
Spring Camelina				Planted				Harvest	Planted with wheat		
Sunflower							Planted				Harvest

Figure 4.2. Potential cropping sequences for winter oilseeds in Eastern Colorado (Johnson et al., 2009).



	WC DOP 1	WC DOP 2	WC DOP 4	WC DOP 5	WC DOP 6	SC DOP 6
Fort Collins irrigated			470	438	479	519
Fort Collins dryland	256	215	507	521	457	560
Rocky Ford irrigated	137	204				383

Figure 4.3. Comparison of the average winter camelina (WC) yields for each date of planting (DOP) with the spring camelina (SC) yields included in the trial. The spring camelina emerged on DOP 6 only and failed to overwinter.

Table 4.1. Planting dates for camelina in Fort Collins and Rocky Ford in 2011.

Planting Date	Date of planting	Date of harvest
1	9/15/10*	7/19/11
2	9/29/10*	7/19/11
3	10/13/10	No spring emergence
4	10/27/10	7/19/11
5	11/8/10	7/19/11
6	3/9/11*	7/24/11

*Indicates a date of planting at Rocky Ford, Colorado. No asterisk indicates that planting was done only in Fort Collins.

Table 4.2. A list of the LSMEAN yields for each winter variety over all dates of plating across both environments.

Variety	Yield LSMEAN (kg ha^{-1})
HPX-WG2	524.4
HPX-WG1-33	500.0
Spring variety mean	479.0
HPX-WG1-6	475.9
Joelle	470.7
HPX-WG1-24	405.7
HPX-WG1-35	446.7
HPX-WG1	446.0
HPX-WG4-1	423.9
HPX-WG3	420.9
HPX-WG1-29	342.3
HPX-WG4	324.0

REFERENCES

Aase, J.K., and F.H. Siddoway. 1979. Crown-depth soil temperatures and winter protection for winter wheat survival. Journal of the Soil Science Society of America 43:1229-1233.

Angelini, L.G., Moscheni, E., Colonna, G., Belloni, P., and E. Bonari. 1997. Variation in agronomic characteristics and seed oil composition of new oilseed crops in central Italy. Industrial Crops Production 6:313–323.

Bushong, J.A., Griffith, A.P., Peeper, T.F., and F.M. Epplin. 2012. Continuous winter wheat versus a winter canola- winter wheat rotation. Agronomy Journal 104(2):324-330.

Cripps, M.G., Schwarzlander, M., McKenny, J.L., Hinz, H.L., and W.J. Price. 2006. Biogeographical comparison of the arthropod herbivore communities associated with *Lepidium draba* in its native, expanded and introduced ranges. Journal of Biogeography 33:2107-2119.

Crowley, J.G. 1998. Evaluation of Camelina sativa as an alternative oilseed crop. End of Project Report No. 7. ISBN 1 84170 049 5 Crops Research Centre, Oak Park, Carlow, Ireland.

Ehrensing, D.T., and S.O. Guy. 2008. Oilseed crops: Camelina. Oregon State University Extension Service EM 8953-E.

French, A.N. Hunsaker, D., Thorp, K., and T. Clarke. 2009. Evapotranspiration over camelina crop at Maricopa, Arizona. Industrial Crops and Products 29:289-300.

Gesch, R.W., and S.C. Cermak. 2011. Sowing date and tillage effects on fall-seeded camelina in the northern corn belt. Agronomy Journal 103(4):980-987.

Johnson, J.J., Enjalbert, N., Schneekloth, J., Helm, A., Malhotra, R., and D. Coonrod. 2009. Development of oilseed crops for biodiesel production under Colorado limited irrigation conditions. Completion report no. 211. Colorado water Institute. Fort Collins, Colorado

Nielsen, D.C. 1998. Snow catch and soil water recharge in standing sunflower residue. Journal of Production Agriculture 11:476–480.

Putnam, D.H., Budin, J.T., Field, L.A., and W.M. Breene. 1993. Camelina: A promising low-input oilseed. p. 314–322. In J. Janick and J.E. Simon (eds.) New crops. John Wiley & Sons, New York.

Sharratt, B.S., Baker D.G., Wall, D.B., Skaggs, R.H., and D.L. Ruschy. 1992. Snow depth required for near steady-state soil temperatures. Agricultural and Forest Meterology 57:243–251.

Onyilagha, J.C., Gruber, M.Y., Hallet, R.H. , Holowachuk, J., Buckner, A., and J.J. Soroka. 2012. Constitutive flavonoids deter flea beetle insect feeding in *Camelina sativa.* Biochemical Systematics and Ecology 42:128-133.

Zubr, J. 1997. Oil-seed crop: Camelina sativa. Industrial Crops and Products 6:113-119.

CHAPTER 5:

YIELD EVALUATION OF PENNYCRESS AND OTHER OILSEEDS IN DRYLAND CONDITIONS OF EASTERN COLORADO

INTRODUCTION

Another promising summer oilseed for the Colorado winter wheat-fallow cropping system is another member of the Brassicaceae family known as field pennycress (*Thlaspi arvensi*). This weedy species is native to Europe but has become widely established throughout midwestern agricultural areas of North America. Colorado farmers will recognize this plant, as it is commonly found growing near irrigation ditches throughout Eastern Colorado. Like *Camelina sativa*, another promising oilseed species in the Brassicaceae family, there are two varieties of pennycress that can be grown depending on the cropping system most prevalent in the area. Pennycress can be seeded in the fall where it is allowed to overwinter as a rosette or else it can be planted in the spring (Isbell, 2009). This plant is less cold tolerant than camelina and requires a higher soil temperature and more soil moisture for germination than camelina (Hazebroek and Metzger, 1990). The optimal daily temperature for germination and growth of this species is 20°C (Hume et al., 1995).

Much of the research that has been carried out on pennycress has focused on defining its characteristics as a weed. Some promising experimental lines have been identified that are high yielding and have potential as a field crop. These advanced lines exclude some of the weedy characteristics that would cause problems for potential producers. The characteristics that are excluded include cold-induced secondary dormancy, uneven annual germination and seed vernalization requirements (Hazebroek and Metzger, 1990). Pennycress seeds collected from wild germplasm have been reported to have a germination rate of less than 50% due to various dormancy effects (Hume, 1994). In addition to germination traits, pennycress improvement programs have also focused on identifying varieties that are early-flowering, meaning that they flower 30 to 60 days after germination (Hume, 1994; Hume et al., 1995). In comparison, later-flowering strains need 100 to 130 days to reach full flower (Hume, 1990). Pennycress does not compete well as a weed in dryland agricultural systems and is easily controlled with existing herbicides meaning that it will not take over when incorporated into a dryland agricultural rotation.

Some pennycress varieties have a winter vernalization requirement and some do not. Annual varieties of pennycress, meaning those that do not have a vernalization requirement, flower in April or May depending on whether they were planted in the spring or fall. Preliminary studies have shown yields of 1,420 kg ha^{-1} in test plots (Isbell, 2009).

The seed oil profile determines the potential uses of this feedstock and its suitability as a feedstock for the on-farm production of biodiesel and vegetable oil. The seed oil of pennycress is composed of a higher percentage of monounsaturated fatty acids. The main components of pennycress oil are erucic (C22:1): 32.8%, linoleic (C18:2): 22.4%, linolenic (C18:3): 11.8%, and oleic (C18:1): 11.8% (Moser et al., 2009). The oil content of this species is lower than for other oilseeds at 29% (Isbell, 2009; Moser et al., 2009). Figure 5.1 compares the oil profile of pennycress to that of other potential oilseed species in the Brassicaceae family.

Pennycress biodiesel meets or exceeds all of the biofuel feedstock requirements set out in the American Society for Testing Materials (ASTM) D6751 (Moser et al., 2009; American Society for Testing Materials, 2008). High concentration of polyunsaturated fatty acids in pennycress oil means that it has low overall oxidative stability however high concentrations of antioxidative compounds such as tocopherols increase the stability (Moser et al., 2009). The cetane number, which determines combustion quality of the oil, exceeds the minimum ASTM D6751 standard for biodiesel production.

The process involved with the on-farm production of biofuels produces a large amount of secondary products in the form of press cake. Finding economically viable uses for this press-cake is an important part of ensuring the economic sustainability of pennycress-based biofuel. Pennycress oil and seed meal is high in concentrations of erucic (C22:1) acid (Moser et al., 2009). This fatty acid has been shown to be toxic to animals and humans and causes cardiac lesions in test situations but has industrial uses (Hulan et al., 1976). Other studies have shown that phytotoxic compounds such as glucosinolates present in pennycress press cake can inhibit the germination of certain crop species and in other cases greatly reduce the ability of these plants to grow and thrive meaning pennycress meal could be utilized as a weed control agent (Vaughn et al., 2005).

Pyrolysis, or the heating of the seed meal under pressure to produce liquid fuel, would be a potential option for value-added use of the leftover seed meal from pennycress because of the deleterious effects of pennycress seed meal when used for feed or fertilizer (Boateng et al., 2010).

The objective of this study was to test the initial performance of pennycress in a Colorado agricultural setting. Based on the initial observations of the growth and yields of pennycress in a smaller variety trial, a second larger trial was conducted to test the dryland yields of fall and spring-seeded pennycress compared to winter camelina and an entry of lesquerella (*Lesquerella fendleri*).

MATERIALS AND METHODS

Within a variety trial and date of planting study of winter and spring camelina outlined in Chapter 4, one entry of pennycress was included. This was planted at two dates throughout the fall in $3.9m^2$ mini-plots. The dates of planting were Oct 14, 2010 and Oct 28, 2010. These entries were hand planted in multiple rows at a rate of 4.48 kg ha^{-1}. Sonalan (Dow Agrosciences, Indianapolis, IN) was applied as a pre-planting weed control measure at a rate of 1.4 L ha^{-1} and the field was tilled to incorporate the herbicide. The fields were fertilized to ameliorate deficiencies identified through soil testing. The plots were hand weeded in the spring but heavy weed pressure was a problem throughout the trial.

Throughout the fall and spring, emergence and flowering data for the fall-seeded varieties were recorded on a biweekly basis. The plots were hand-harvested July 17, 2011 and hand threshed at different times depending on the date of maturity.

The pennycress was hand cut and threshed in the same manner as the winter camelina. The spring of 2011 was wetter than normal, with the result that between the date of March emergence and harvest, over 219mm of precipitation and irrigation water was applied to the irrigated varieties.

As a follow up to the initial pennycress seeding study, we tested the dryland potential of pennycress. Three winter pennycress varieties, W12, Patton and Beecher, were planted in a randomized complete block with four replications at the USDA research station in Akron, Colorado. Parallel to this block for comparison was another randomized block that included the top winter camelina performer from the 2011 date of planting study: HPX-WG2. An entry of lesquerella (*Lesquerella fendleri*) and a spring variety of pennycress were also included in this block. With the exception of the spring pennycress, all of these varieties were planted on October 10, 2011.

RESULTS

Pennycress seeded in the winter of 2010 did not germinate until May 5, 2011 when soil temperatures exceeded 4.4°C and was ready for harvest on July 12, 2010.

Figure 5.2 shows the performance of thlaspi in a 2010-2011 variety trial compared to entries of spring and winter camelina. Its performance far exceeded that of any of the individual varieties of winter or spring camelina in this trial. The highest yielding camelina variety was the winter line HPX-WG2 that yielded 524 Kg Ha^{-1}. This is far less than the 1,392 kg ha^{-1} yield of the pennycress.

From October 10, 2011, the date of planting, until April 2, 2012, there was a total of 38mm of precipitation in Akron, Colorado where the follow up dryland thlaspi trial was held. This was insufficient to raise a dryland crop of pennycress and as a result, there was no fall or spring emergence. Insufficient spring moisture (47mm in April) led to the failure of the pennycress and lesquerella. The spring camelina emerged and reached maturity under dryland conditions, but yield data was not gathered because the field was used as a demonstration during the wheat field day presentation.

DISCUSSION

The biggest surprise in this trial was the yield potential for pennycress under limited irrigation as shown in the 2010-2011 variety trial. This entry topped the trial with a yield of 1392 kg ha^{-1}. As a result of this study we discovered the superiority of pennycress as a potential oilseed crop under irrigated conditions. These results led to a follow-up study in 2011 to assess the dryland potential of pennycress in a larger experiment. From this, we can conclude that thlaspi and Lesquerella require more than 38mm of precipitation throughout the growing season in order to yield a crop.

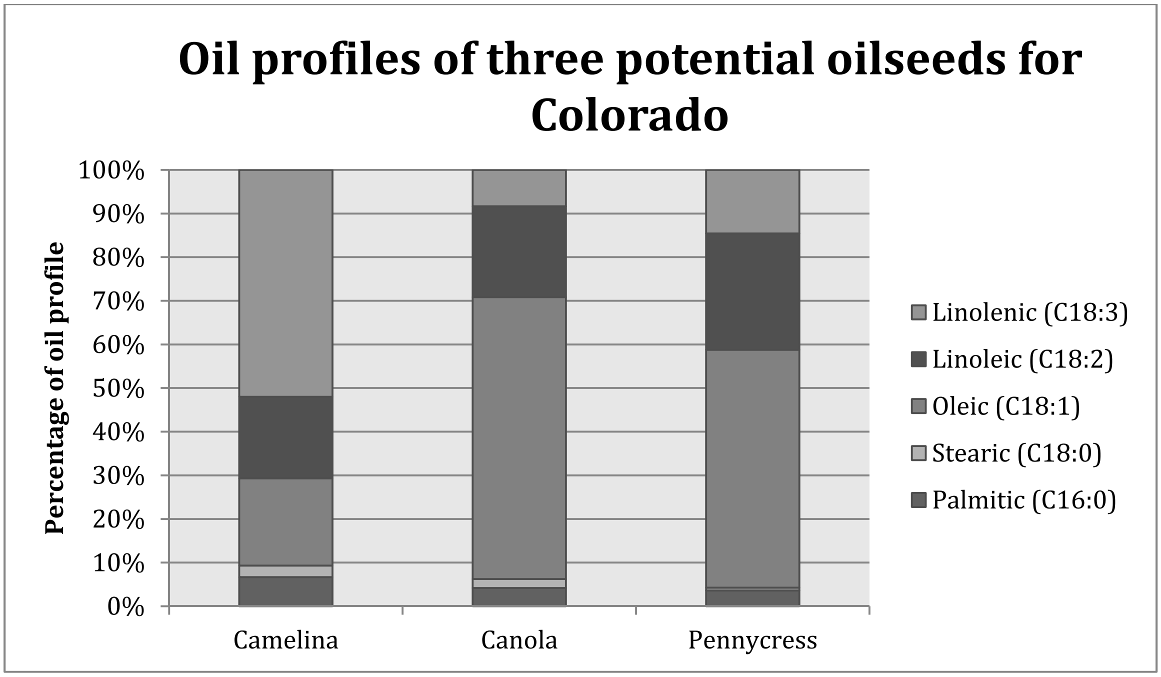


Figure 5.1. Oil profiles of different oilseeds with potential for use as a biofuel feedstock in Colorado (Zubr, 1997; Isbell, 2009; Pinzi et al., 2009).

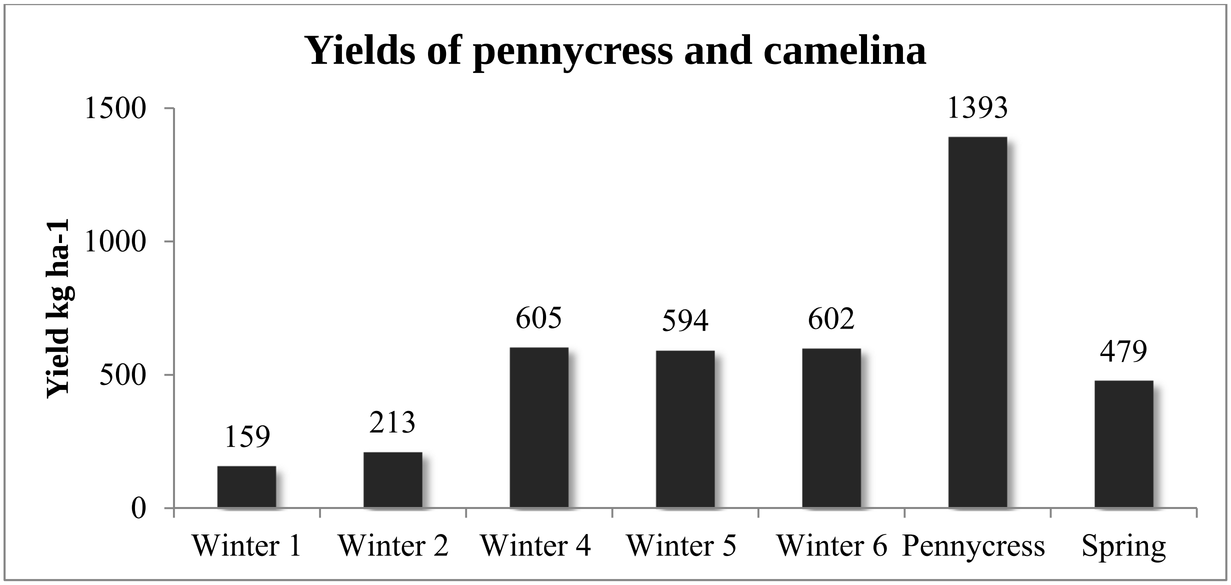


Figure 5.2. Comparing the 2010-2011 variety trial yields of winter camelina, pennycress, and spring camelina entries (Spring). Winter 1,2,4,5, and 6 refers to winter camelina varietal performance at different dates of planting.

REFERENCES

American society for Testing and Materials. Standard Specification for biodiesel fuel blend stock (B100) for middle distillate fuels, ASTM D6751-08. In *ASTM Annual Book of Standards*; American Society for Testing and Materials: West Conchohocken, PA, 2008.

Boateng, A.A., Mullen, C.A., and N.M. Goldberg. 2010. Producing stable pyrolosis liquids from the oil-seed presscakes of mustard family plants: Pennycress (*Thlaspi arvense* L.) and camelina (*Camelina sativa*). Energy and Fuels 24:6624-6632.

Hazebroek, J.P., J.D. Metzger. 1990. Environmental control of seed germination in *Thlaspi arvense* (Cruciferae). American Journal of Botany 77(7):945-953.

Hulan, H.W., Kramer, J.K.G., Mahadevan, S., Sauer, F.D., and A.H. Corner. 1976. Relationship between erucic acid and myocardial changes in male rats. Lipids 11(1):9-15.

Hume, L. 1990. Influence of emergence date and strain on phenology, seed production, and germination of *Thlaspi arvense* L. Botanical Gazette 151(4):510-515.

Hume, L. 1994. Maternal environmental effects on plant growth and germination of two strains of *Thlaspi arvense* L. International Journal of Plant Science 155(2):180-186.

Hume, L., Devine, M.D., and S. Shirriff. 1995. The influence of temperature upon physiological processes in early-flowering and late-flowering strains of *Thlaspi arvense* L. International Journal of Plant Science 156(4):445-449.

Isbell, T.A. 2009. US effort in the development of new crops (lesquerella, pennycress coriander and cuphea). Oleagineux Corps Gras Lipides 16:205-210.

Moser, B.R., Knothe, G., Vaugn, S.F., and T.A. Isbell. 2009. Production and evaluation of biodiesel from field pennycress (*Thlaspi arvense* L.) oil. Energy and Fuels 23(8):4149-4155.

Pinzi, S., Garcia, I.L., Lopez-Gimenez, F.J., Luque de Castro, M.D., Dorado, G., and M.P. Dorado. 2009. The ideal vegetable oil-based biodiesel composition: a review of social, economical and technical implications. Energy and Fuels 23:2325-2341.

Vaughn, S.F., Isbell, T.A., Weisleder, D., and M.A. Berhow. 2005. Biofumigant compounds released by field pennycress (*Thlaspi arvensi*) seedmeal. Journal of Chemical Ecology 31(1):167-177.

Zubr, J. 1997. Oil-seed crop: Camelina sativa. Industrial Crops and Products 6:113-119.

CHAPTER 6:

HYBRID COMBINING ABILITY OF CAMELINA

INTRODUCTION

Crop scientists have long recognized the phenomenon known as hybrid vigor, whereby a particular hybrid cross outperforms the parental varieties with respect to growth and reproductive vigor. This phenomenon was first observed in corn and has since been recognized in many crop species (Shull, 1908). When making a cross, it is important to consider that some varieties make superior pollen donors or recipients and some crosses produce stronger, more fertile offspring.

The value of a parent is measured in terms of 'combining ability.' This is divided into two types of combining ability, general and specific. General combining ability refers to a single parental variety's average performance in combination with all other parents in the study. Specific combining ability, on the other hand, is a measurement of the specific crosses that exceed the average within a set of crosses (Sprague and Tatum, 1942). Specific combining ability is an important part of most breeding programs, especially those for maize and other outcrossing species. It is estimated that maize hybrids yield 15% more than open-pollinated varieties (Lippman and Zamir, 2006). This is a large reason why nearly 100% of corn planted in the US today is the derived from hybrid crosses (Duvick, 1999).

Camelina sativa is an oilseed species within the Brassicaceae family that has been identified as a potential feedstock species for the production of biofuel in dryland agricultural systems. This species is primarily self-pollinating and its degree of outcrossing has been estimated to be between 0.09% and 0.28% (Walsh et al. 2012).

Being able to identify good specific crosses can contribute significant yield increases to developing breeding programs. Some oilseed breeding programs have adopted hybrids as a way to increase oil content and overall yields (Duvick, 1999).

MATERIALS AND METHODS

This study was carried out using common varieties that were identified by a collaborator as optimal maternal or paternal lines. These pollen recipients were Glacier, Orovada, Blaine Creek, and Jasper. Glacier, Orovada, Blaine Creek and Jasper were developed in Big Fork, Montana. The varieties were grown in the Colorado State University greenhouse facility and a proprietary chemical, provided by one of our collaborators, was applied at the bolting stage to create male-sterile plants to receive pollen from the other entries. The entries in this case were 5 varieties that were identified as part of a mapping population and bred at CSU using single seed descent (SSD) after identification of quantitative trait loci for drought tolerance in a previous experiment. In addition to these and the 4 varieties used as female parents, several check varieties were added in order to create a diverse and well-rounded selection of individuals from a diversity of backgrounds.

The crossing plan was to create a half-diallel analysis where crosses are only made in one direction. This involved sowing 10 seeds in a 1in Jiffy pot, and repeating so that each individual has six replications. Next, Glacier and Blaine Creek were grown in the same type of pot and a proprietary sterility-inducing chemical was applied. This crossing experiment failed to produce male-sterile plants due to unknown problems with the greenhouse or sterility-inducing chemical.

Do to the failure to produce male-sterile individuals, the parental varieties Glacier, Orovada, Blaine Creek and Jasper were sent from Montana along with individual hybrid crosses between each possible parental combination. These were planted in Greeley, Colorado with three replications. Figure 6.1 outlines the planting design and the environment of the hybrids relative to the parents. The hybrid entries were Blaine Creek x Orovada (BCxO), Glacier x Orovada (GxO), Glacier x Jasper (GxJ), and Blaine Creek x Jasper (BCxJ). The plots 11.6 m^2 blocks where the parents were planted adjacent to the hybrid to facilitate visual comparison between them.

Hand weeding was carried out throughout the trial. Irrigation was applied as needed to prevent excessive drought stress. The yields were recorded and the hybrid and parental yields were processed using a one-way analysis of variance (ANOVA) model as provided in the SAS statistical software (SAS institute, 2011). Precipitation data was gathered from the Colorado Agricultural Meteorological network (COAGMET) website.

RESULTS AND DISCUSSION

The greenhouse portion of this experiment was not successful. The varieties were grown separately and the proprietary male-sterilant was applied, but the resulting flowers emerged fertile and produced seed. This was the case with both attempts to create male-sterile camelina lines for crosses.

The analysis of variance analysis carried out through this study showed that the hybrid varieties in this study did not differ significantly from the non-hybrid varieties (P=0.43). In addition, there was not significant variation among the varieties in this experiment (P=0.85). Overall, the mean of the hybrid varieties was 131.3 kg ha^{-1}while the mean for the non-hybrid varieties was 117.1 kg ha^{-1}. This lack of significant difference among the varieties was likely due to the overall poor yields for the camelina plots in Greeley, Colorado. High temperatures and lack of precipitation during pod fill likely contributed to flower abortion and early termination of pod creation. The seeds in these varieties were small and withered, as was the case with the adjacent camelina variety trial, where the yields were similarly low. Without a significant difference between the hybrid and non-hybrid varieties as a result of environmental stresses, it is impossible to calculate general and specific combining ability (Sprague and Tatum, 1942). Perhaps in another environment, hybrid vigor could have been recorded.

When the flanking parental varieties were compared directly with the hybrids, the relationships were all found to be nonsignificant, as illustrated in Figure 6.2. There was one significant difference where the variety jasper was found to be significantly different from the Jasper x Glacier cross (P=0.0376). Unfortunately, the parent was found to be significantly higher yielding than the hybrid.

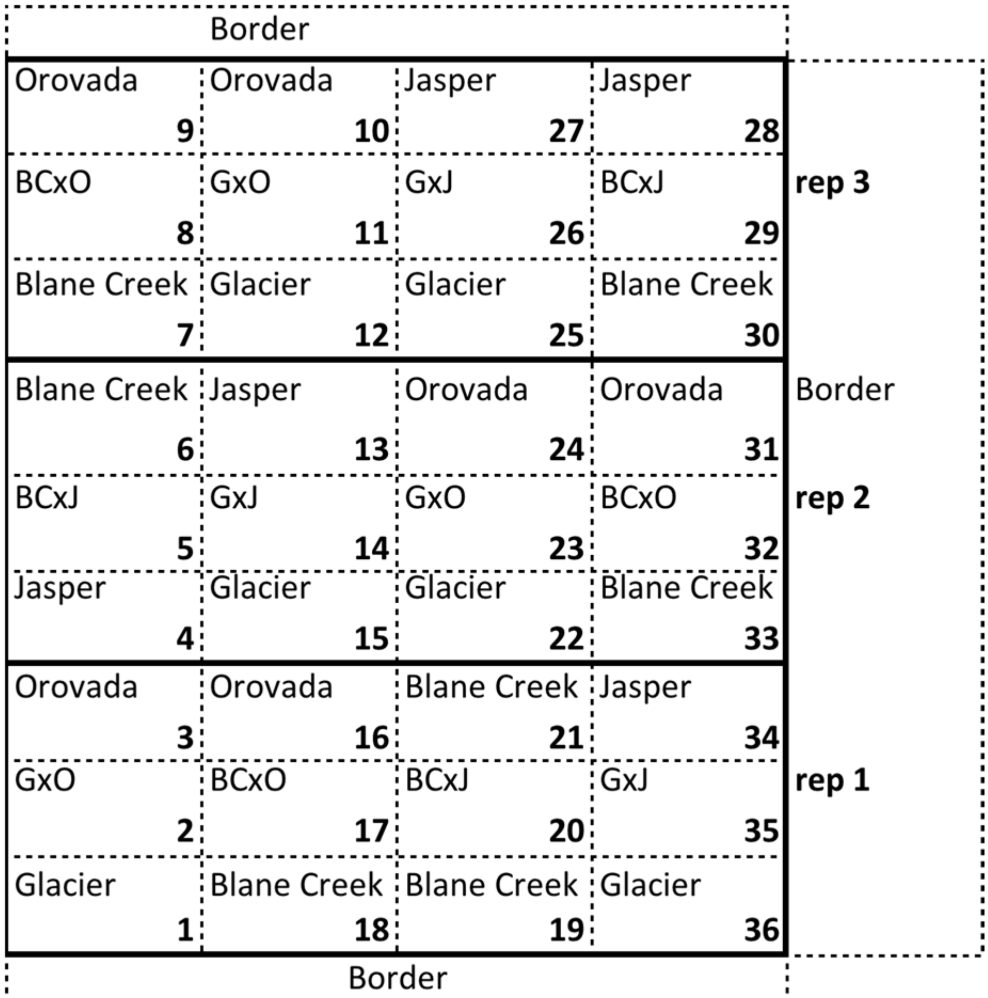


Figure 6.1. Diagram of the planting layout of the combining ability field design.

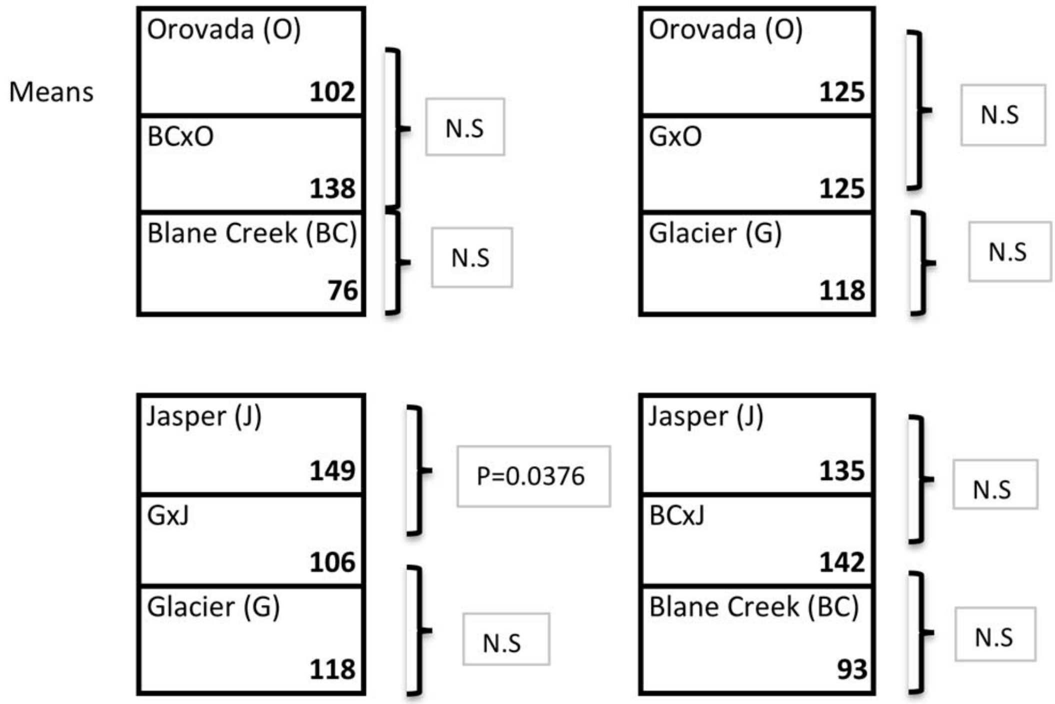


Figure 6.2. The relationship between the mean yields of the parental varieties and those of the hybrids. The parental varieties are Orovada, Blaine Creek, Orovada, Glacier and Jasper. The hybrids are named with the letter symbols of the parental varieties and a lowercase x between them to indicate the lineage.

REFERENCES

Duvick, D.N. 1999. Heterosis: feeding people and protecting natural resources. In The Genetics and Exploitation of Heterosis in Crops (Coors, J.G. and Pandey, S., eds), pp. 19–29, American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

Lippman, Z.B., and D. Zamir. 2006. Heterosis: revisiting the magic. Trends in Genetics 23(2):60-66.

Shull, G.H. 1908. The composition of a field of maize. American Breeders Association 4:296–301.

Sprague, G.F., and L.A. Tatum. 1942. General vs. specific combining ability in single crosses of corn. Journal of the American Society of Agronomy 34:923–932.

Walsh, K.D., Puttick, D.M., Hills, M.J., Yang, R., Topinka, K.C., and L.M. Hall. 2012. First report of outcrossing rates in camelina (*Camelina sativa* (L.) Crantz), a potential platform for bioindustrial oils. Canadian Journal of Plant Science 92:681-685.