

STRATEGIES TO IMPROVE *BRASSICA NAPUS L.*
(CANOLA) ESTABLISHMENT AND WINTER
SURVIVAL IN CONSERVATION SYSTEMS

By

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Bachelor of Science in Plant and Soil Sciences

Oklahoma State University

Stillwater, Oklahoma

2015

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2015

STRATEGIES TO IMPROVE CANOLA
ESTABLISHMENT AND WINTER SURVIVAL IN
CONSERVATION SYSTEMS

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ACKNOWLEDGEMENTS

First and foremost, I would like to thank my wife, Shawntel, and the rest of my family for the never-ending support and love throughout this process. I would also like to thank Dr. Jason Warren for the patience and willingness to provide support and mentorship as my faculty advisor.

Additionally, I would like to express appreciation to my committee for their support and guidance during this journey at Oklahoma State University: Dr. Angela Post and Dr. Hailin Zhang.

Lastly, I would like to thank Wendal Vaughan for the support and guidance in the field. I would also like to thank the current and past graduate and undergraduate students that have assisted in my research. Without your help and participation, this project would not have been possible.

Name: JOHN WILLIAM JONES

Date of Degree: DECEMBER, 2015

Title of Study: STRATEGIES TO IMPROVE CANOLA ESTABLISHMENT AND
WINTER SURVIVAL IN CONSERVATION SYSTEMS

Major Field: PLANT AND SOIL SCIENCES

Abstract: The introduction of winter canola to the U.S. has allowed producers to diversify their winter wheat systems by offering an additional rotational crop. Canola acreage has been on a steady incline in the southern Great Plains as it has proven beneficial to removing grassy weeds from continuous wheat systems. Winter canola production has also proven to be a challenge with winter kill particularly in no-till systems within the southern Plains. There is little data available to evaluate the impact of wheat residue and freeze intensity on the winter survival of canola in the southern Plains. Therefore a study was conducted in Fairview and Chickasha, OK to evaluate the effects of shallow tillage and burn on the winter survival of canola during and following the winter. There are few studies investigating the screening of varieties in a controlled environment for winter hardiness. The majority of winter survival assessment is conducted in the field, mainly in the National Canola Variety Trials. Therefore a study was conducted in Stillwater, OK to develop a variety screening program for cultivars commonly used in the southern Plains. Residue management methods impacted canola plant density at both locations but only resulted in yield differences in Chickasha. Canola subjected to a three hour freeze at -4.4C° and -8.8C° did not result in any conclusive winter kill however a six hour freeze with the same temperature resulted in complete plant loss. This data will provide insight into the potential need of a residue management method in canola to increase productivity and provide a better understanding of how freeze intensity and duration impacts canola survival.

TABLE OF CONTENTS

Chapter	Page
I. REVIEW OF LITERATURE	1
Canola in the Southern Great Plains	1
Impact of Burning and Single Pass Tillage on Soil Characteristics	3
Stand Establishment and Winter Hardiness: No-till vs Conventional	5
Physiology of Winter Hardiness in Canola	6
Literature Cited	9
II. STRATEGIES TO IMPROVE CANOLA ESTABLISHMENT AND WINTER SURVIVAL IN CONSERVATION SYSTEMS	12
Abstract	12
Introduction	13
Hypothesis	15
Materials and Methods	16
Results and Discussion	20
Crop Assessment	20
Canola Stand Count	20
Canola Yield	21
Thermal Imaging	23
Canopy Cover and NDVI	24
Grain Sorghum Yield	24
Soil Assessment	25
Glomalin	25
Soil pH	25
Summary	26
Literature Cited	27

Chapter	Page
III. CONTROLLED ENVIRONMENT <i>BRASSICA NAPUS</i> WINTER SURVIVAL SCREENING PROTOCOL AND ASSESSMENT.....	35
Abstract.....	35
Introduction.....	36
Hypothesis.....	38
Materials and Methods.....	39
Results and Discussion	41
Plant and Crown Height.....	41
Canopy Cover	42
Summary.....	43
Literature Cited	44

LIST OF TABLES

CHAPTER II

Table	Page
1. ANOVA table for stand count at Fairview and Chickasha.....	29
2. Mean number of plants per linear meter by treatment for Fairview canola.....	29
3. Mean number of plants per linear meter by treatment for Chickasha canola	29
4. Mean winter kill for Fairview and Chickasha.....	30
5. Fairview rainfall between June and May of the following year	30
6. Chickasha rainfall between June and May of the following year	31
7. Mean grain yield for Chickasha canola by treatment	31
8. Mean grain yield for Fairview canola by treatment.....	32
9. Fairview thermal image temperatures by treatment measured during freeze on 1 December 2014	32
10. Fairview sorghum NDVI and canopy cover readings 65 days after planting.....	33
11. Mean Fairview grain sorghum yield.....	33
12. Mean glomalin content from a 10cm composite soil sample	34
13. Mean soil pH values following canola harvest.....	34

CHAPTER III

Table	Page
1. Mean plant height and crown height prior to a three hour freeze.....	46
2. Mean plant area measured by Canopeo ten days following a three hour freeze.....	46
3. Mean plant height and crown height prior to a six hour freeze	47
4. Mean plant area measured by Canopeo ten days following a six hour freeze	47

CHAPTER I

REVIEW OF LITERATURE

CANOLA IN THE SOUTHERN GREAT PLAINS

Canola (*Brassica napus* L.) is a broadleaf oilseed crop grown mainly for the consumable oils and quality of the meal (Bell, 1993). Canola was developed using traditional plant breeding techniques from rapeseed (Cowling, 2007). The lower erucic acid in the oil make canola marketable for human consumption for cooking oil and the low glucosinates in the meal make it suitable as a feed supplement for livestock (Raymer, 2002).

Prior to the development of winter type varieties and hybrids, canola production in the southern United States was limited. Spring planted canola, which is the dominate type widely grown across Canada and the northern United States, is not feasible in the southern Great Plains. Spring planted canola in the southern Great Plains does not have enough time to set and fill pods prior to the onset of summer and this reduction in grain fill time greatly lowers the yield potential of the Spring planted canola (Angus et al., 1991; Angadi et al., 2003; Boyles et al., 2006). In addition, the lack of processing plants has kept production at a minimum due to the increased costs associated with shipping and storage.

Continuous wheat production systems in the region are common and overrun with weed and disease pressures which limit increased productivity, despite advancements in wheat varieties and crop protection chemicals. In fact, the five-year average wheat yield in Oklahoma between 1983 and 1987 was 2112.7 kg ha⁻¹ compared to the five-year average between 2003 and 2007 of

2126.1 kg ha⁻¹. There was only a gain of 14 kg ha⁻¹ over this thirty year period (USDA-National Agricultural Statistics Service, 2014).

Canola benefits wheat productivity in the region. Bushong et al. (2012) showed a 14% increase in wheat yields following canola. Wheat yields were increased from 2530 kg ha⁻¹ in continuous wheat production system to 2800 kg ha⁻¹ when the wheat followed a canola crop (Boyles and Sanders, 2009). An economic analysis conducted by DeVuyst et al. (2011) showed that the increased wheat yields along with competitive gross revenue from the canola make a rotation of wheat with canola more profitable than continuous wheat with a wheat price around \$4.75 and canola around \$6.00.

Benefits from canola in rotation with winter wheat combined with the development of local markets have resulted in a rapid increase in the production of canola. In fact, production has increased in Oklahoma from 14,000 ha to 110,000 planted ha from 2009 to 2014 (USDA-National Agricultural Statistics Service, 2014). Despite the benefits of canola production in the region, there are many challenges in planting canola, such as stand establishment and winter survivability. In fact, as production area has increased the harvested area has declined, from 90% in 2009 with an average yield of 1400 kg ha⁻¹ to 70 % in 2013 with an average yield of 1570 kg ha⁻¹ (USDA-National Agricultural Statistics Service, 2014).

Fertilizer management and soil fertility influence yield, quality, and winter survival of fall planted canola. Management practices for canola are very similar to wheat, but more intensive. Fertility requirement is also similar to wheat, but careful consideration should be used when applying nitrogen fertilizers in the fall. Too much nitrogen applied prior to dormancy may result in excessive fall growth (Grant and Bailey, 1993).

The excessive application of nitrogen may lead to crown elongation and the susceptibility to winter freezes is greatly increased (Conley et al., 2004). A fall nitrogen application of 33 kg ha⁻¹

to 55 kg ha⁻¹ is recommended prior to planting instead of applying all of the required nitrogen before planting which is common in wheat production systems (Boyles et al., 2006). Canola also requires more nitrogen and sulfur than wheat for the same yield goal. Applications of phosphorus, potassium, sulfur and any other soil amendments should be applied prior to the final tillage pass (Boyles et al., 2006; Jackson, 2000). This ensures complete soil mixing in conventional systems.

IMPACT OF BURNING AND SINGLE PASS TILLAGE ON SOIL CHARACTERISTICS

The burning of crop residue is a common method of residue management and is used in an effort to improve canola establishment under no-till conditions. Weed and disease pressure can be reduced with prescribed burning, along with a variety of other benefits, such as a warmer seedbed due to the removal of residue (The USCA Canola Grower Manual 2008; Bailey and Lazarovits, 2003). However, removing crop residue exposes soil to erosion and carbon and nitrogen are lost through combustion. The lack of surface cover can influence both soil physical characteristics and chemical properties.

Root penetration and distribution may be limited due to an increase in soil strength following a burn event (Govaerts et al., 2006). In contrast, recent research by Virto et al. (2007) indicates burning has no impact on total soil organic carbon in no-till; however, the effects of tillage were observed in the 0–5 cm depth. In this study, differences were discovered in the concentration of particulate organic matter, in the 0–5 cm and 5–10 cm depths. At both depths, no-till soils contained more particulate organic matter than burned treatments. Virto et al. (2007) attributed this to particulate organic matter being comprised of primarily partially decomposed plant litter and residue.

Removal of surface residue allows erosion to occur resulting in offsite soil deposition and alteration of soil physical characteristics. Soil crusting at the surface is more likely to occur following a scheduled burn event, even more so if followed by rain (Mills and Fey, 2004). Soil

crusting reduces infiltration rates and soil becomes dry at the surface (Certini, 2005). The low hydraulic conductivity associated with dry soil is due to macro-pore size voids being filled with clay and silt size soil particles. Structural change occurs in the near-surface, resulting in influences on root growth and elongation, particularly in water limited environments (Grossnickle, 2005).

Another residue management option that is currently used in an effort to improve establishment of canola is single pass vertical tillage prior to planting. Research data is currently not available to evaluate the impact of this practice on canola establishment or winter survival. Furthermore, data is not available to evaluate the impact of this practice on beneficial soil characteristics that develop in otherwise continuous no-till systems. However, research is available to suggest that periodic tillage in no till systems has some positive influences mainly on soil fertility (Pierce et al., 1994). Incorporating and redistributing soil nutrients within the plow layer stimulates the mineralization of nitrogen and will eliminate the stratification of phosphorus and potassium found near the soil surface (Garcia et al., 2007).

Mechanical disruption of soil physical properties is short lived. Pierce et al. (1994) suggests that physical properties of no till soil are similar to conventional systems following a tillage event. However, the soil will aggregate to an intermediate state the year following tillage and effects of tillage can be evident for two years. After four or five years the impacts of tillage are no longer evident although the redistribution of phosphorus and potassium were still present (Pierce et al., 1994). Benefits of no-till are seen more so in summer crops as compared to winter crops due to the increased water demand present during the summer and the increased soil water storage potential to overcome that demand (Nielsen et al., 2005). Summer crop yield can be improved in the southern Great Plains in long term no-till. Kochenower (2010) indicated that winter wheat planted in the panhandle did not respond to no-till. However, grain sorghum yields have been significantly higher in no-till than conventional after the first three years of no-till adoption.

Soil aggregation begins with the assembly of soil particles into micro-aggregates, which are less than 0.25 mm. Miller and Jastrow (1990), showed that arbuscular mycorrhizal fungi were involved in the stable aggregation of loamy soils. Arbuscular mycorrhizal fungi produce an immunoreactive glycoprotein which is very stable and somewhat difficult to extract (Wright and Upadhyaya, 1998). This protein, glomalin, is produced by the hyphae of arbuscular mycorrhizal fungi and it accumulates in the surface of stable soil systems since it is not water soluble and not susceptible to leaching. Findings from Wright and Upadhyaya (1998) indicated that disturbed soils, tillage to a depth of 15 cm, had significantly less extracted amounts of glomalin and percent aggregate stability compared to an undisturbed system. A fine sandy loam from Texas was shown to have 0.3 mg g⁻¹ of extractable glomalin and 9% aggregate stability in the disturbed system compared to 0.8 mg g⁻¹ of glomalin and 22% aggregate stability in the undisturbed system. This indicates that glomalin content is correlated to aggregate stability and it was stated by Jastrow (1987) that soil disturbance can have lasting effects on aggregate stability.

Single moldboard tillage as stated by Garcia et al. (2007) reduces arbuscular mycorrhizal fungi and did not recover over the duration of the three year study. It was suggested that an increase in phosphorus concentration in plant roots following the tillage event contributed to the reduction of arbuscular mycorrhizal fungi. If phosphorus runoff is a concern, a single tillage pass can reduce the risk of soluble phosphorus loss to surface water through incorporation; however, the risk of particulate phosphorus loss through erosion is possible following soil disturbance (Fraser et al., 1999).

STAND ESTABLISHMENT AND WINTER HARDINESS

The amount of fall growth greatly impacts the survival rate of canola during winter (Conely et al., 2004). Plants require an extensive root system for carbohydrate storage that is used during the period of dormancy when temperatures are low. When the root growth is impeded, success of

winter survival is reduced (Koenig et al., 2011). A large crown growing close to the soil surface improves winter survival (Holman et al., 2011). During periods of insufficient moisture, drought stress will reduce stands of larger plants due to their increased soil moisture requirement.

The problems of stand establishment and winter survival are compounded with no-till production systems. Godsey et al. (2008) showed that planting canola in no till residue results in a 40% stand loss, but up to 80% stand loss is not uncommon. This increased winter kill observed in no-till systems offset the benefits of residue resulting from improved soil moisture retention and soil temperature regulation (Godsey et al., 2008; Teasdale and Mohler, 1993).

Canola that is planted directly into standing or laying residue in no-till which results in delayed emergence and an elevated crown, as compared to conventional planted canola (Wuest et al., 2000). As stated earlier, the elevated crown is vulnerable to winter kill (Godsey et al., 2008). Row cleaners and aggressive coulters are additional tools on the planting implement which aid in the removal or sweeping of the residue away from the furrow, allowing for a lower crown set.

There are several factors in addition to crown height at onset of freeze that influence winter survivability. Chemical properties of the soil also have a great effect on seedling vigor. Soil pH and nutrient status can impede seedling growth, ultimately resulting in stunted stands (Grant and Bailey 1993; Islam et al., 1980). It is suggested that canola have four to six leaves and have a main root that is at a minimum of 1 cm in diameter prior to winter dormancy (Boyles et al., 2006).

PHYSIOLOGY OF WINTER HARDINESS IN CANOLA

During cold periods, various physical changes occur in canola and plant growth slows, but does not cease. As day length shortens and temperatures decrease, canola goes through a process similar to vernalization (Zanewich and Rood, 1995). The plant requires prolonged periods of near freezing temperatures to begin the developing processes which result in winter hardiness (Boyles

et al., 2006). Periods of near freezing or freezing temperatures can result in tissue damage due to ice formation.

Ice usually develops in the intercellular areas of plant cells and tissues due to the relatively low concentration of ice-nucleating or anti-freeze substances compared to the intracellular space concentrations. This accrual of ice may result in the physical disturbance of tissues and cells due to the adhesion of ice to the cell walls (Thomashow, 1998). The chemical potential of ice is less than liquid water at freezing temperatures. As the ice forms in the intercellular spaces, water potential decreases outside of the cells. Therefore, water moves from inside the cells to the intercellular areas. The initial solute concentration of the fluid within the intercellular space influences the amount of water required to move across the gradient to equilibrate the system; chemical potential of ice is directly influenced by the temperature during the freezing period (Thomashow, 1998). Thomashow (1998) stated that 90% or more of the osmotically active water moves into the intercellular areas from the cells at subzero temperatures. This loss of cellular water results in dehydration of the cell, which can lead to cellular damage by precipitating various molecules and degradation of proteins (Thomashow, 1998). Dehydration causes injury at the membrane level (Webb and Steponkus, 1993) and membrane lesions in various forms result from freeze-induced dehydration (Thomashow, 1998). At higher freezing temperatures, most cellular damage occurs from expansion and contraction resulting in lysis, or breakdown of cells. During a hard freeze or temperatures below -10°C , lesions occur due to the severe state of dehydration and extreme low water potential (Thomashow, 1998).

Canola is a desired component of conservation systems in the southern Plains due to the rotation benefits in continuous wheat systems. Stand establishment and winter kill are perceived as significant challenges to canola, particularly in no-till. However, the impact of periodic tillage or other residue reduction practices on beneficial soil characteristics needs to be evaluated to understand the consequences of the efforts to improve canola productivity. Furthermore, winter

kill is still a significant challenge for winter canola in the southern Plains and an improved understanding of the conditions that result in winter kill is needed. This combined with the development of a controlled environment screening protocol will allow for identification of cultivars that are more resistant to winter kill in this region.

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CHAPTER II

STRATEGIES TO IMPROVE CANOLA ESTABLISHMENT AND WINTER SURVIVAL IN CONSERVATION SYSTEMS

ABSTRACT

Inclusion of canola in conservation wheat production systems in the southern Great Plains is desirable due to increased wheat performance and improved weed control. This study included two field experiments to evaluate the effects of no-till, vertical tillage (VT) [Great Plains unit with a gang angle of 0°, 3°, and 6° and a Landoll unit], burn, harrow, and a cover crop on establishment, winter survival, and yield of canola (*Brassica napus* L.). Composite soil samples were also collected to a depth of 10 cm to measure the effects of treatments on glomalin content. Vertical tillage resulted in increased stand counts when averaged across locations and sample dates by 2.5 plants m⁻¹ when compared to no-till plantings. Alternatively, the influence of the harrow and burn treatments on canola stand was inconsistent between locations. Yields were not improved by VT, burn, harrow or cover crop treatments when compared to no-till planting. In fact, at the Chickasha location the no-till treatment produced the highest yield of 2146.4 kg ha⁻¹, which was significantly higher than the burned, harrowed and Great Plains 0° treatments.

INTRODUCTION

Implementing winter canola into continuous no-till production systems in the southern Great Plains is of interest to producers looking to diversify their cropping systems and to take advantage of the yield increase in winter wheat following winter canola (Bushong et al., 2012). Canola production in Oklahoma has increased from 14,000 hectares in 2009 to 110,000 hectares in 2014 (USDA-National Agricultural Statistics Service, 2014). Bushong et al. (2012) demonstrated that rotating winter canola into a continuous wheat system will result in a 14% wheat grain yield increase. There are many other benefits from diversifying the rotation to include canola. Since winter annual grasses such as Italian ryegrass, are difficult to control in Oklahoma wheat systems, rotating canola will allow for the use of other herbicides to help combat and control annual grasses (Bushong et al., 2012).

Since weeds can decrease the quality and ultimately, the value of the crop, rotations have become a popular way to decrease the risk of economic loss due to weeds. Stripe rust and leaf rust are common diseases found in our winter wheat systems. By rotating canola, the disease cycle is broken, allowing for a decreased presence in the wheat following the canola (Peters et al., 2003).

Production of winter canola is preferred over spring types in the southern Great Plains. In this region, spring canola will flower during the hottest part of the summer which reduces yield potential (Holman et al., 2011). The development of winter canola cultivars for the southern Plains focuses on the rosette or crown height growth characteristic (Stamm et al., 2015). Cultivars in which the crown height was shorter were developed to be more resistant to winter kill. By selecting cultivars that do not grow excessively long or tall crowns, the potential for winterkill is lessened for fall planted canola (Boyles et al., 2006).

Stand establishment and winter survival in no-till conditions has proven to be problematic as compared to conventional tillage. The loss of stand can be attributed to winter injury from elevated growing points, or crowns, of the canola (Boyles et al., 2006; Stamm et al., 2015). Holman et al. (2011) stated that the elevated crowns may be a result of planting canola into the residue instead of the soil, deep residue on the surface forcing the plant crown to elevate above the residue rather than the surface of the soil, and lastly excessive fall growth resulting in large plants going into the winter. Since these field conditions are only present in no-till, conventionally tilled canola will result in an increased winter survival rate compared to no-till (Assefa et al., 2014).

As a result of these challenges with production of no-till canola, producers in the southern Great Plains, have an interest in using a form of residue management to increase the success of canola. Since canola seed is small in size, planting in residue poses a challenge for seed germination and seedling vigor due to the lack of seed to soil contact. Vertical tillage, harrow, and burn are the common choices when it comes to residue management. However, the removal of surface residue may allow soil erosion to occur resulting in the alteration of the physical characteristics. Soil crusting may occur after a burn event which is followed by rainfall (Mills and Fey, 2004). The structural changes of the soil may result in root growth and elongation problems, more so in water limited environments (Grossnickle, 2005).

Ogle et al. (2012) stated that adoption of no-till management of winter wheat and corn will increase yields in the southern United States. In contrast, the meta-analysis indicated that no-till management of these crops would result in decreased yields in the Corn Belt and northern areas of the United States. The increased soil moisture and cooler soil temperatures resulting from no-till were cited as the factors controlling the differential response between the southern and northern United States. Grain sorghum, soybean, and cotton are also predicted to have increased yields in the southern United States. Furthermore, previous research has found that the increase in

grain sorghum yields following adoption of no-till is due to enhanced root growth from improved soil structure and a reduction in soil moisture losses (Jones and Popham, 1997; Ogle et al., 2012).

Oklahoma is in a transition area between the moist, cool northern United States and the dry, warm southern United States. Ogle et al. (2012) indicated that Oklahoma is expected to see no yield response to even a slightly negative response to no-till winter wheat. However, grain sorghum is expected to result in a positive response to no-till. Kochenower (2010) indicated that winter wheat yield in the panhandle did not respond in a no-till system, however, grain sorghum yields have been significantly higher in no-till than conventional systems after the first four years of no-till adoption. This data suggests a need to evaluate the impact of periodic tillage, used to promote successful canola production, on the productivity of summer crops in rotation with winter wheat and canola.

There is limited data available to evaluate the impact of these practices on winter survival and yield of canola. Furthermore there is no data available to evaluate the impact of these practices on soil health characteristics and yield of the following crop. Therefore, the objectives of this study are to compare the establishment, winter survival and grain yield of canola planted into wheat stubble to those of canola planted after wheat stubble management practices such as burning, harrowing and vertical tillage. In addition, the impact of these stubble management alternatives on glomalin (Wright and Upadhyaya, 1998) concentrations, which has been suggested as an indicator of improvement in soil health resulting from long term no till management will be evaluated. Lastly, the yield of double crop grain sorghum planted after canola harvest will be evaluated.

HYPOTHESIS

The null hypothesis for this study is wheat stubble management will not influence the establishment, winter survival, or grain yield of canola following winter wheat and soil

characteristics will also not be affected. Alternatively, the most aggressive vertical tillage will improve establishment, winter survival and grain yield of canola following winter wheat, while decreasing glomalin.

MATERIALS AND METHODS

Site Description

Field experiments were established in the fall of 2014 on a producer's field one mile southwest of Fairview, Oklahoma on a McLain (fine, mixed, superactive, thermic Pachic Argiustoll) soil and at the South Central Research Station in Chickasha, Oklahoma on Dale (fine-silty, mixed, superactive, thermic Pachic Haplustoll) and McLain (fine, mixed, superactive, thermic Pachic Argiustoll) soils following wheat harvest. The experimental design at both locations was a randomized complete block design consisting of six treatments which were replicated four times. The geographical coordinates for the site in Fairview are 36.255° North Latitude, 98.501° West Longitude and the geographical coordinates at the South Central Research Station location are 35.035° North Latitude, 97.912° West Longitude. Weeds and insects at both locations were controlled as needed.

Plot dimensions at Fairview were 9.2 meters wide by 30.5 meters long and plot dimensions at the South Central Research Station were 13.72 meters wide by 27.43 meters long with a 21.34 meter long alley. The treatment structure at Fairview and the South Central Research Station included standard treatments of no till, burn, harrow, and vertical tillage (3 vertical tillage treatments were applied in Fairview and 2 vertical tillage treatments were applied at the South Central Research Station). In addition to the standard treatments, the South Central Research Station had a cover crop treatment that was swathed and baled prior to canola planting.

At Fairview, vertical tillage treatments were applied using a Great Plains Turbo-Max 3000TM (Great Plains Manufacturing, Inc., Salina, Kansas) on 14 August 2014 using three different gang angles of 0°, 3°, and 6° set at 7.5 cm deep, which represent the 3 vertical tillage treatments at this site. The vertical Turbo-Max was pulled at 15.3 kph. The harrow was also applied on the same date and it was pulled twice in opposite directions ensuring proper residue removal. On 2 September 2014, a roto-tiller was applied to the borders of the burn treatment to facilitate containment and fire was prescribed eight days later. Sitro canola (Rubisco Seeds LLC, Philpot, Kentucky) was planted on 17 September 2014 at 4 kg ha⁻¹ on 38 cm rows. Twelve kg ha⁻¹ of nitrogen in the form of urea was surface broadcasted prior to planting. At planting, 11 kg ha⁻¹ of diammonium phosphate was applied in furrow and on 21 November 2014, an application of 140 L of urea ammonium nitrate ha⁻¹ and 28 L of 12-0-0-26S ha⁻¹ was applied via surface broadcast.

At the South Central Research Station, a mixture containing 50% by weight Iron and Clay cowpeas (*Vigna unguiculata*) and 50% by weight AS6201 BMR Sorghum-Sudangrass (*Sorghum bicolor*) (Johnston Enterprises, Inc., Billings, Oklahoma) was planted at a seeding rate of 22.2 kg ha⁻¹ on 26 June 2014 using a John Deere 1590 no till drill pulled by a John Deere 6150R (Deere & Company, Moline, Illinois) tractor. The cover crop was swathed on 11 August 2014 using a John Deere 450R (Deere & Company, Moline, Illinois) swather with a cut height of 10 cm and baled eight days later using a John Deere 468 (Deere & Company, Moline, Illinois) baler. On 19 August 2014, a Landoll 3710VT (Landoll Corporation, Marysville, Kansas) was used at a fixed gang angle of 10° set at 10 cm deep. It was pulled at 20.1 kph. A Great Plains Turbo-Max 1800TM (Great Plains Manufacturing, Inc., Salina, Kansas) was used on 21 August 2014 with a gang angle of 0° set at 7.5 cm deep. It was pulled at 15.3 kph. The harrow treatment was also applied on 19 August 2014 and pulled across twice to ensure sufficient residue removal. A roto-tiller was applied to the borders of the burn treatment to help contain fire on 2 September 2014 and burning was prescribed on 16 September 2014. Dekalb 46-15 (Monsanto Company, St.

Louis, Missouri) canola was planted on 8 October 2014 at a rate of 5.5 kg ha⁻¹ on 38 cm rows and 33.3 kg ha⁻¹ of diammonium phosphate was applied in furrow.

Canola was harvested on 6 June 2015 at Fairview and on 24 June 2015 at Chickasha. Fairview canola was swathed 5 days prior to harvest and picked up by a Case 2388 combine. Each plot was then transferred to a weigh wagon where the amount of canola could be recorded. In Chickasha, the canola was direct harvested using a small plot combine.

After canola harvest, grain sorghum was planted to provide a sound assessment of the treatments in the no-till system over multiple seasons. Grain sorghum was planted at Fairview on 38 cm rows. There was 100 kg ha⁻¹ of urea applied between the rows. There was a surface broadcast of 123 kg ha⁻¹ of urea and 55 kg ha⁻¹ of DAP applied. At Chickasha, grain sorghum was planted on 76 cm rows.

Variables measured

Composite soil samples across the site were collected with a 22.5 mm diameter hand probe to a depth of 15 cm at establishment on 4 August 2014. Composite soil samples were also collected in the same manner at Chickasha on 11 August 2014. Pre-plant samples were analyzed by the Soil, Water and Forage Analytical Laboratory at Oklahoma State University to determine pH using a glass electrode in a 1:1 soil:water suspension (Sims, 1996), nitrate extracted with a 0.008M calcium phosphate and quantified by the cadmium-reduction method (LACHAT, 1994), and plant available phosphorus and potassium content using Mehlich-3 solution (Tucker, 1992). On 11 August 2014, biomass samples were collected from the cover crop treatments at the South Central Research Station to determine forage production. Sampling method consisted of clipping three, one meter strips and drying at 65° C.

Canola stand counts were conducted on 3 October and 19 November 2014 and 13 February 2015 at Fairview. Stand counts were conducted on 19 November 2014 and 18 February 2015 in

Chickasha. Five one-meter long areas were randomly chosen and marked to allow for assessment of same plants throughout the season.

Digital and infrared images were taken across all treatments with a FLIR E8 (FLIR Systems, Inc., Wilsonville, Oregon) at a height of 1 m at Fairview prior to an overnight freeze event and again the following morning. Images were collected on 11, 14, and 19 November 2014 and 1 December 2014.

Images were exported into Microsoft Excel as a csv file from the FLIR software platform. Each csv file represented the number of pixels contained in each image. Each box, or pixel, had the temperature of that pixel recorded. From this, the mean, minimum and maximum temperatures for each image were calculated.

Composite soil samples were collected on 16 July 2015 at Fairview and 20 July 2015 at Chickasha to a depth of 10 cm to determine glomalin content. The method used to evaluate glomalin was an easily extractable glomalin extraction (Wright and Upadhyaya, 1998).

Grain sorghum stand thickness was determined by analyzing digital images in Canopeo and NDVI was measured using the hand-held GreenSeeker. These readings were collected 65 days after planting in Fairview and 105 days after planting in Chickasha.

Statistical Analysis

Statistical analyses on the crop and soil variables were performed using the PROC MIX procedure in SAS v. 9.4 (SAS Institute, 2008) to determine significant treatment effects. Treatments were not identical at each location because of differences in the availability of equipment. Because treatments were not the same each location was analyzed independently.

RESULTS AND DISCUSSION

Crop Assessment

Canola Stand Counts

There was no treatment by date interaction for the stand count data at either location (Table 1), therefore stand count was averaged across dates to reveal significant differences ($\alpha=0.05$) between treatments at both locations. At Fairview, the Great Plains 0°, 3°, and 6° had significantly higher average stand counts than the remaining treatments, with 11.6, 11.7, and 11.6 plants m⁻¹, respectively (Table 2). The no-till, burn and harrow treatments contained average populations of 8.7, 9.7, and 9.5 plants m⁻¹, respectively.

At Chickasha, the Landoll treatment contained an average of 15.6 plants m⁻¹ during the season, which was significantly greater than the no-till and cover crop treatments as shown in Table 3. However, the Landoll treatment was not significantly different from the remaining treatments. The cover crop treatment contained the lowest average stand count with 11 plants m⁻¹, which was not significantly lower than the stand counts for the no-till and harrowed treatments.

Table 4 shows the stand loss as the percent difference between the initial and final stand counts at each location. It is noteworthy that the harrow treatment lost 50.7 % of the initial stand while the burn option resulted in the loss of only 34.7 % of the initial stand at Chickasha. However, these differences were not significant. In contrast, at the Fairview location the harrow treatment lost 33.1 % of the initial stand and the burn treatment lost 61.2% of the initial stand. Yet again these differences are not significant. This data shows that at these two locations, although treatment did influence the average stand observed during the growing season, the variation in winter kill was too great to isolate a significant difference influenced by residue management.

Fairview experienced the first freeze event on 31 October 2014 when the nighttime low reached 0 C° and the following night the low was -1.1 C°. Between the sampling date of 3 October and 19

November, there was a stand loss of 24.3% and between 19 November 2014 and 13 February 2015 there was a loss of 35.8% when averaged across treatments. Chickasha experienced the first freeze event on 31 October 2014, only 23 days after planting where the nighttime low dipped to -1.1 C°. The following night, the air temperature was -3.3 C°. Between 19 November 2014 and 18 February 2015, there was a loss of 45.2% of stand when averaged across treatments.

We hypothesized that the lack of rainfall in the fall and winter impacted the response to the residue management methods. Fairview only received 9.0 cm of rainfall between November 2014 and March 2015 as shown by the Table 5, whereas Chickasha received 23.7 cm of rainfall as shown in Table 6. Although the rainfall experienced at Chickasha was 16.4 cm more than that experienced at Fairview, the average stand loss were similar with 45.2% at Chickasha and 48.3% at Fairview. It is important to note that at Chickasha, November had over 12.5 cm of rainfall, but it fell in just two events. These high intensity rainfall events likely resulted in significant runoff which decreased the effective rainfall infiltrating the soil. This combined with the higher density of canola stand and later planting date may be responsible for Chickasha experiencing a stand loss similar to Fairview despite the greater amount of rainfall. Specifically, the higher density stand likely resulted in more inter-species competition and the later planting date reduced plant growth prior to the onset of the first freeze.

Canola Yield

Canola grain yields from Chickasha for the 2014-2015 growing season are presented in Table 7. Analysis of variance detected significant differences ($\alpha=0.05$) between treatments and found mean grain yield from the no-till to be the highest with 2146.4 kg ha⁻¹. The harrow treatment resulted in the lowest yield of 1188.1 kg ha⁻¹, which was not significantly different from the burned or Great Plains treatment which had yields of 1412.3 and 1558.0 kg ha⁻¹, respectively. Furthermore, the Great Plains treatment was not significantly different from the Landoll and

cover crop treatments which had yields of 2023.1 and 2034.3 kg ha⁻¹. Contrary to previous research (Assefa et al., 2014), the no-till treatment was the highest yielding treatment with 2146.4 kg ha⁻¹ although it was not significantly greater than the Landoll and cover crop treatments. This data suggests that the suppressed stand observed in the no-till and cover crop treatments (Table 3) did not play an important role in impacting final grain yield.

The Landoll was the most aggressive of the three tillage treatments and provided soil disturbance and residue burial to 10 cm of depth whereas the Great Plains and harrow treatments simply disturbed surface residue. This indicates that the aggressive vertical tillage provided no benefit over the undisturbed no-till system for this individual growing season. Previous research by Meeks (2014) has shown that summer cover crops can reduce soil profile moisture prior to planting a fall crop. Summer cover crops planted in rotation with continuous winter wheat reduced soil moisture on average at planting by 2.2 cm (Meeks 2014). Since the rainfall was above average during the 2014-2015 growing season, it is hypothesized that contrary to Meeks (2014) the cover crop treatment did not result in a moisture depleted soil profile but was able to maintain productive soil moisture levels well into the canola growing season.

We hypothesize that the difference between the highest yielding treatment in Chickasha, which was no-till, and the lowest yielding harrow treatment was due to the formation of soil crusting after initiation of treatment. The harrow completely pulverized and powdered the surface soil and left it exposed to erosion and crust formation. This resulted in the soil having a state of hydrophobicity and loss of all structure, which decreased infiltration and increased the risk of runoff and erosion (Pagliai et al., 2004). The reduced infiltration and presence of hydrophobic characteristics may have caused lower water availability in this treatment throughout the growing season resulting in the lower yields.

The initial stand count assessment on 19 November 2014 and the 18 February 2015 stand count for the harrow treatment were the same as the stand count in the cover crop and no-till treatments (Table 3). This confirms that the reduction in emergence is not the only factor influencing yield differences. These effects may have been exaggerated due to the heavy rainfall events occurring on 4 November and 22 November. The residue remaining in the cover and no-till treatments allowed more effective infiltration and reduced evaporative water loss thereby allowing for improved yields compared to the harrow treatment (Doran et al., 1984). The cover crop was swathed at a height of 10 cm and although 6 metric tons of cover crop was removed as hay prior to planting, there was an apparent benefit in having the cover as compared to removing the wheat residue with fire or a harrow. We speculate that the cover crop allowed for a greater infiltration of water during the high intensity rainfall events, resulting in an increase in soil moisture.

Canola grain yields from Fairview for the 2014-2015 growing season are presented in Table 8. Analysis of variance detected there were no significant differences ($\alpha=0.05$) among treatments. However, the harrow method, which had a grain yield of 1464.1 kg ha⁻¹, resulted in 325.4 kg ha⁻¹ less than burn, which was the highest yielding treatment of 1789.5 kg ha⁻¹, although there were no significant differences detected.

Thermal Imaging

There were no differences detected between treatments for the mean and minimum temperature (Table 9). However, there were differences detected for the maximum temperatures. No-till had the warmest maximum temperature of -1.7 C° while burn and harrow had the coldest maximum of -3.1 C° and -3.0 C°, respectively. Inspection of the thermal images revealed that the maximum temperature in no-till treatments was measured from small (less than 3 cm²) areas of exposed soil surrounded by wheat residue. This observation was likely the result of the soil temperature being higher in these areas due to the insulating effects of the residue (Godsey et al., 2008). Despite this

difference in the average maximum temperatures, the thermal images did not prove useful in identifying surface conditions responsible for winter kill. One limitation to the use of this technology in evaluating the impact of freeze events is the presence of frost on plant and residue. The frost alters the emissivity of the surface thereby confounding the results of the thermal imagery.

Canopy Cover and NDVI

There were significant differences ($\alpha=0.05$) detected between treatments in NDVI and canopy cover at Fairview as shown in Table 10. The Great Plains 3° had the highest NDVI reading of 0.71 while no-till, burn and Great Plains 0° had the lowest readings of 0.66. There was no correlation between NDVI and canopy cover at Fairview. Harrow had the highest canopy cover of 90% while the Great Plains 3° had the lowest of 86%. Chickasha was infested with head worms which caused severe damage to the canopy at 65 days after planting. Therefore, no differences ($\alpha=0.05$) were detected in NDVI readings and Canopeo images were not analyzed due to the lack of green vegetation.

Grain Sorghum Yield

In Fairview, no-till and Great Plains 3° treatments had a significantly higher ($\alpha=0.05$) yield of 5705.6 kg ha⁻¹ and 5592.6 kg ha⁻¹, respectively, while the Great Plains 0° treatment had the lowest yield of 4243.1 kg ha⁻¹ (table 11). Burn, Great Plains 6°, and harrow were found to be no different than the highest or lowest yielding treatment. The elevated yields in the no-till treatment as compared to the burn treatment are consistent with research reported by Biederbeck et al. (1980) who found that burning residue resulted in little difference between burn and no-till yields. However, the lack of significant difference between no-till and the remaining residue reduction treatments suggest that their influence on the productivity of an otherwise no-till system is negligible and/or inconsistent.

Soil Assessment

Glomalin

There were no significant differences ($\alpha=0.05$) among treatments at Fairview and Chickasha for the glomalin contents (Table 12). The Fairview location had been maintained in continuous no-till for 5 years prior to the initiation of this study. This data suggest that although the no-till soil contained numerically greater glomalin concentrations the residue management practices did not significantly reduce it.

Rillig et al. (2001) suggested that glomalin will accumulate in undisturbed soils. No data is available to provide insight into the rate of glomalin accumulation after conversion to no-till. However, radio carbon dating showed that glomalin may persist in soil up to 42 years as a function of the environment. The canola planted at Chickasha was the first no-till cash crop planted in this field. Therefore it is not surprising that no differences were found.

Despite the fact that the Fairview location had been in no-till for 5 years, the Chickasha location contained as much as 2.0 mg g^{-1} as compared to the highest concentration of 1.4 mg g^{-1} at Fairview. Wright and Upadhyaya (1998) reported glomalin concentrations of 0.2 to 4.5 mg g^{-1} and found they varied as a function of soil disturbance, geographic location, and soil texture.

Soil pH

There were no significant differences ($\alpha=0.05$) between treatments at Fairview and Chickasha (Table 13). The pH at these locations was below the optimum pH of 5.84 which could have influenced and reduced crop establishment and vigor (Arnall, 2013). The mean pH at Chickasha was higher than the mean pH at Fairview.

SUMMARY

Canola yield at Fairview was not influenced by any residue management method, but Chickasha experienced a significant difference in canola yield. No-till resulted in the highest yielding treatment while harrow and burn were the lowest yielding.

At Fairview, canola yield was not improved despite the increased stand counts resulting from the vertical tillage treatments. We hypothesize that the lack of an increased yield may be attributed to the ability of canola to compensate for a reduced stand. Research conducted by Angadi et al. (2003) showed that as stand count or plant population decreased, the number of pods produced on main and secondary branches increased. This compensation removes any differences in yield by redistributing the bulk of seed production to other areas of the plant as the number of pods per plant was the most important factor in yield compensation. This illustrates that although yield was significantly different at Chickasha, canola stand count cannot be used to indicate final yield.

Mean glomalin results from Fairview and Chickasha indicated that there were no differences at both locations. However, continuous no-till produced the highest sorghum yields which suggests that it is prudent to maintain continuous no-till systems for the benefit of the following crops but the yield was only significantly higher than one of the five other residue reduction treatments suggesting that this effect is inconsistent.

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Table 1. ANOVA table for stand count at Fairview and Chickasha.

	Location	
	Fairview	Chickasha
	—— Stand Count ——	
Treatment	*	***
Date	***	*
Treatment*Date	NS	NS

*Indicates significance at $\alpha=0.05$.***Indicates significance at $\alpha<0.0001$.

NS Indicates no significance.

Table 2. Mean number of plants per linear meter by treatment for Fairview canola.

Treatment	Oct. 3	Nov. 19	Feb. 13	Mean
	—— Plants m ⁻¹ ——			
No Till	10.9bc†	10.3a†	5.0a†	8.7b†
Burn	15.0ab	8.6a	5.5a	9.7b
Great Plains 0°	15.2a	11.4a	8.3a	11.6a
Great Plains 3°	16.8a	11.2a	7.0a	11.7a
Great Plains 6°	15.1ab	11.4a	8.2a	11.6a
Harrow	10.7c	10.9a	6.9a	9.5b
Average	14.0	10.6	6.8	10.5

† Means with the same letter are not significantly different. Alpha = 0.05.

Table 3. Mean number of plants per linear meter by treatment for Chickasha canola.

Treatment	Nov. 19	Feb. 18	Mean
	—— plants m ⁻¹ ——		
No Till	16.2a†	9.1a†	12.7bc†
Burn	18.5a	12.0a	15.3ab
Great Plains 0°	18.2a	10.2a	14.2ab
Landoll	20.5a	10.6a	15.6a
Harrow	17.8a	8.7a	13.3abc
Cover Crop	14.7a	7.3a	11.0c
Average	17.7	9.7	13.7

† Means with the same letter are not significantly different. Alpha = 0.05.

Table 4. Mean winter kill for Fairview and Chickasha.

Treatment	Location	
	Fairview†	Chickasha‡
	——Percent loss——	
No-till	51.9 (52.4)a§	42.2 (38.2)a§
Burn	61.2 (63.2)a	34.7 (30.1)a
Great Plains 0°	44.3 42.7)a	45.5 (42.1)a
Great Plains 3°	58.5 (59.1)a	—
Great Plains 6°	40.6 (40.3)a	—
Landoll	—	48.2 (44.9)a
Harrow	33.1 (28.9)a	50.7 (48.5)a
Cover Crop	—	49.7 (47.0)a
Average Loss	48.3	45.2

† Determined by difference between stand counts on Oct. 3 and Feb. 13.

‡ Determined by difference between stand counts on Nov. 19 and Feb. 18.

Values in parenthesis represent the coefficient of variation (CV).

§ Means with the same letter are not significantly different. Alpha = 0.05.

Table 5. Fairview rainfall between June and May of following year.

Month	Year										
	2015	2014	2013	2012	2011	2010	2009	2008	2007	2006	2005
	—— cm ——										
June	4.7	15.2	12.7	12.1	6.1	5.2	2.8	20.3	23.4	6.1	11.6
July	22.4	8.7	20.8	1.7	0.1	9.6	4.2	7.3	8.1	8.7	7.0
August	5.0	4.1	9.3	2.9	4.6	1.6	11.9	2.4	2.9	8.5	7.4
September		2.9	5.5	6.6	3.5	5.7	7.5	30.4	14.5	1.8	3.3
October		6.5	6.1	1.2	6.3	1.9	15.3	11.3	7.3	1.4	7.4
November		2.9	2.4	0.8	8.2	7.5	0.4	1.1	0.2	0.7	0.0
December		1.9	0.7	0.8	8.6	0.6	0.3	1.4	5.8	6.7	0.5
January		1.5	0.2	2.0	1.9	0.4	0.9	0.2	0.1	2.3	0.6
February		0.5	1.2	11.7	8.3	1.3	3.8	0.9	5.7	0.8	0.0
March		2.2	1.2	1.0	8.6	3.0	4.7	4.0	6.4	14.4	7.3
April		19.2	0.6	7.7	14.7	2.9	10.1	15.4	5.4	6.3	3.8
May		25.9	8.5	10.0	1.8	5.3	12.8	5.8	8.2	18.2	4.2
Total		91.6	69.3	58.5	72.6	45.1	74.8	100.5	88.2	75.7	53.0
Mean		72.9									

Table 6. Chickasha rainfall between June and May of following year.

Month	Year										
	2015	2014	2013	2012	2011	2010	2009	2008	2007	2006	2005
	—— cm ——										
June	12.5	15.0	11.3	7.1	5.6	7.9	5.3	14.2	40.0	4.2	13.1
July	10.1	6.4	14.5	4.8	0.5	14.1	8.5	2.4	12.7	3.3	4.2
August	4.9	3.7	2.4	4.3	7.4	1.2	11.3	10.8	14.8	14.9	16.0
September		3.1	4.9	11.7	3.2	15.5	8.3	3.4	5.3	7.3	4.3
October		5.7	6.7	1.4	10.4	6.2	18.2	3.9	6.8	5.8	3.2
November		12.6	3.7	2.2	9.4	1.6	0.9	3.3	1.3	2.4	0.0
December		1.9	0.7	2.2	4.0	0.4	3.4	1.1	2.0	6.9	0.8
January		3.6	0.1	3.8	5.0	0.1	3.8	1.8	0.4	4.0	0.7
February		0.3	0.9	7.3	1.6	1.2	7.0	1.8	5.8	2.3	0.8
March		5.3	3.6	2.7	11.3	0.1	2.2	3.8	6.1	17.1	6.8
April		7.3	6.4	26.9	7.9	0.0	8.0	14.0	10.8	5.0	10.3
May		43.0	4.0	7.6	15.0	10.5	5.1	16.2	11.0	20.9	5.6
Total		108.0	59.2	82.0	81.3	58.9	81.8	76.7	116.8	94.3	65.6
Mean		82.5									

Table 7. Mean grain yields for Chickasha canola by treatment.

Treatment	Yield
	—— kg ha ⁻¹ ——
No-till	2146.4a†
Burn	1412.3c
Great Plains	1558.0bc
Landoll	2023.1ab
Harrow	1188.1c
Cover Crop	2034.3ab

†Means with the same letter are not significantly different. Alpha = 0.05

Table 8. Mean grain yields for Fairview canola by treatment.

Treatment	Yield
	—— kg ha ⁻¹ ——
No-till	1667.5a†
Burn	1789.5a
Great Plains 0°	1708.1a
Great Plains 3°	1708.1a
Great Plains 6°	1748.8a
Harrow	1464.1a

†Means with the same letter are not significantly different. Alpha = 0.05

Table 9. Fairview thermal image temperatures by treatment measured during freeze on 1 December 2014.

Treatment	Mean	Min	Max	Standard Deviation	Coefficient of Variation
	—— C° ——				
No-till	-9.1a	-12.8a†	-1.7a†	1.8a†	-19.7a†
Burn	-8.7a	-12.2a	-3.1c	1.5a	-17.3a
Great Plains 0°	-9.4a	-13.1a	-2.4b	1.7a	-18.6a
Great Plains 3°	-9.6a	-13.7a	-2.4b	1.7a	-17.9a
Great Plains 6°	-8.9a	-12.7a	-2.3b	1.7a	-18.9a
Harrow	-9.6a	-13.6a	-3.0c	1.8a	-18.9a

† Means with the same letter are not significantly different. Alpha = 0.05.

Table 10. Fairview sorghum NDVI and canopy cover readings 65 days after planting.

Treatment	NDVI	Canopy Cover
No-till	0.66b†	88.3ab†
Burn	0.66b	87.7ab
Great Plains 0°	0.66b	86.3ab
Great Plains 3°	0.71a	85.5b
Great Plains 6°	0.69ab	87.1ab
Harrow	0.69ab	90.9a

†Means with the same letter are not significantly different. Alpha = 0.05.

Table 11. Mean Fairview grain sorghum yield

Treatment	Yield
	—— kg ha ⁻¹ ——
No-Till	5705.6†a
Burn	4927.3ab
Great Plains 0°	4243.1b
Great Plains 3°	5592.6a
Great Plains 6°	5379.2ab
Harrow	4720.1ab

†Means with the same letter are not significantly different. Alpha = 0.05.

Table 12. Mean glomalin content from a 10cm composite soil sample

Treatment	Glomalin	
	Fairview	Chickasha
	—— mg kg ⁻¹ ——	
No-till	1.4†a	1.5a
Burn	1.3a	1.6a
Great Plains 0°	1.4a	2.0a
Great Plains 3°	1.3a	—
Great Plains 6°	1.3a	—
Landoll	—	1.9a
Cover Crop	—	1.5a
Harrow	1.3a	1.9a

† Means with the same letter are not significantly different. Alpha = 0.05.

Table 13. Mean soil pH values following canola harvest.

Treatment	Fairview	Chickasha
	2015	2015
	—— 1:1, H ₂ O ——	
No-Till	5.3a†	5.7a†
Burn	5.2a	5.8a
Great Plains 0°	5.2a	5.6a
Great Plains 3°	5.3a	—
Great Plains 6°	5.3a	—
Harrow	5.3a	5.6a
Landoll	—	5.8a
Cover Crop	—	5.8a

† Means with the same letter are not significantly different. Alpha = 0.05.

CHAPTER III

CONTROLLED ENVIRONMENT *BRASSICA NAPUS L.* WINTER SURVIVAL SCREENING PROTOCOL

ABSTRACT

Winter hardiness screening is critical to assess the potential winter survivability of fall planted canola in the southern Great Plains. Currently, most of the effort involves field measurements of winter survivability which provides an indication to the cold tolerance of that particular variety or hybrid. Controlled environment screening is necessary to allow for the complete control of environmental variables. This study evaluated the effects of temperature and duration of freeze on the winter survival of canola. Canola was planted in a greenhouse and grown to four to six leaf stage prior to the freeze. Freezing was carried out in modified chest freezers to maintain temperatures of -4 C° and -9 C° for two durations of three and six hours. Viable vegetation measured by Canopeo resulted in no difference among varieties and hybrids, but there was a significant difference in crown height between varieties. At canola growth stage 4-6 leaf, DKW44-10 had the lowest measured crown height of 5.7 cm while Inspiration had the highest crown height of 10.3 cm. These results will provide preliminary information needed to develop a protocol to assess winter hardiness under controlled environmental conditions which will provide a better tool for the selection and adoption of cultivars in the southern Great Plains.

INTRODUCTION

Expansion of winter canola production in the southern Great Plains has been halted in recent years in part due to crop loss resulting from winter kill. When introducing new crops to an area, best management practices must be developed, as well as trait selection, which allows for the crop species to perform to its potential. Cultivars that express high levels of freeze tolerance and winter survivability are critical for the continued success and expansion of harvested canola acreage in the southern Great Plains.

Fall planted canola is exposed to frost and winter kill during the vegetative stage of crop growth. The temperature stresses endured during the fall and winter vegetative stages have an impact on the reproductive cycle during the spring (Angadi et al., 2000). These temperature-driven stress events are expected to intensify due to the increase of greenhouse gasses and the resulting changes in the climate. The surface temperature of the Earth is projected to increase by 1 to 11 C° by 2100 (Stainforth et al., 2005). According to Meehl and Tebaldi (2004), extreme climate events are predicted to occur more frequently in the future, but with less duration compared to the overall climate change. Reddy et al. (1997) indicated that these short episodes of extreme climatic events drastically lower crop yield due to the possibility of unexpected early fall frosts or late spring frosts. These unexpected freeze events may occur prior to the development of winter hardiness in crops such as wheat and canola.

Winter crops, such as wheat and canola, require a period of near freezing temperatures prior to a hard freeze to allow the plants to undergo physiological changes critical to winter survival. Teutonico et al. (1993) stated that freeze tolerance is the capability of a plant to persist in freezing temperatures and is the main factor in winter survival. The ability of a plant to increase its freeze tolerance is also known as acclimation ability. The survival of the plant after a period of cold temperatures will result in increased freeze acclimation or the capability of the plant to survive below-freezing conditions (Teutonico et al., 1993). Thomashow (1999) stated that numerous

mechanisms are involved in the initiation of the acclimation response. Lipid composition and sugar accumulation that likely contribute to freeze tolerance are not solely dependent upon gene expression, but may be a reaction or response by adjustments in the activities of enzymes responsible for their synthesis.

Thomashow (1998) stated that acclimated plants have undergone a process to stabilize membranes against freeze damage. The stabilization is a result of changes in the membrane lipid composition (Steponkus et al., 1993). Additionally, it has been well established that membrane damage resulting from freezing is a result of the severe dehydration associated with the freeze (Thomashow, 1999). When temperatures drop below freezing, ice begins to form in the intercellular spaces as a result of the extracellular fluid having a higher freezing point than the intercellular fluid (Xin and Browse, 2000). Due to the decreased chemical potential of ice as compared to water, there is a drop in water potential outside of the cell and at this point, liquid water from inside the cell begins to move down the potential gradient and into the intercellular spaces. Once temperatures reach -10°C , more than 90% of the osmotically active water has moved outside of the cells (Thomashow, 1999).

The insulating effect of snow against soil temperature changes is well known as stated by Aase and Siddoway (1979). Changnon et al., (2006), indicated that the southern Great Plains only receives one to five snowstorms per ten year period based on historical records from 1901 to 2001. Alternatively, the northern Plains receive between 15 and 20 snow events per ten year period. It was also stated that the snowstorms that do occur, fall between November and March for the southern Plains and between October and May for the northern Plains. The lack of snowfall experienced in the southern Plains may very well be a major contributing factor to the intensity of cold damage experienced by winter crops.

Although an in-field evaluation of winter hardiness is beneficial for the winter survival assessment of canola, the use of these in-field evaluations is limited by the erratic occurrence of severe winters (Changon et al., 2006) with differential winter kill severities (Levitt, 1980). Winter survival of canola is a compound system of stresses and environmental factors that alter the ability of the plant to endure sub-freezing temperatures. Much of the winter survival ratings are conducted in the field under varying weather conditions and exposures. The main limitation of in-field trials is the uncertain results following a complete or lack of winter kill (Limin and Fowler, 1991).

Similar to winter wheat, canola grown in the southern Great Plains is susceptible to environmental impacts such as heaving soil, cyclic freeze and thawing periods, insect damage, and water stress (Gusta et al., 1997). Additionally, variations in localized soil temperature (Dabney et al., 2001) and moisture (Medeiros and Pockman, 2011) may also result in irregular freeze acclimation and winter kill.

There is little data available on the controlled environment screening of canola varieties for cold tolerance and winter hardiness. Therefore, a study was conducted using popular cultivars in the southern Great Plains to develop a protocol for controlled environmental screening of canola. This will help solidify a complete and comprehensive winter survival indicator tool which may help in the adoption of certain cultivars in the area. The objective of this study is to evaluate the use of a controlled environment screening method to identify freeze tolerant canola cultivars.

HYPOTHESIS

Due to the inconsistent winter survival characteristic of canola grown in the southern Great Plains, winter survival must be assessed in a controlled environment to isolate potential experimental and environmental variables. The null hypothesis is that freezing temperature, duration, and cultivar will not influence winterkill. Alternatively, canola subject to the freezing

temperature of -4 C° will result in winter kill of susceptible cultivars during a 6 hour freeze event. However, the canola exposed to a -9 C° freeze will result in complete winterkill after 6 hours of exposure.

MATERIALS AND METHODS

Establishment

A growth chamber experiment was established at Oklahoma State University's Controlled Environment Research Lab using a greenhouse and freezing chambers constructed out of chest freezers.

The freezing chambers were constructed by first removing the lids from the chest freezers. A PVC table was constructed to fit inside the freezer. Expanded metal was used as the table top and a small fan was placed under the table to homogenize the air temperature within the freezer. An Intermatic timer was wired to a thermostat which had a thermocouple inside the chamber. The freezer was then plugged into the thermostat to allow the timer to turn the thermostat on and off which resulted in the freezer maintaining a constant temperature set on the thermostat. A thermometer was placed inside each freezer to monitor air temperature during the freeze and leaf temperature was measured at the end of the freezing period with a Fluke 62 MAX IR (Fluke Corporation, Everett, WA) thermometer.

The greenhouse was set to a daytime temperature of 26.6°C and a nighttime temperature of 21.1°C. The daytime and nighttime relative humidity was 45%. The day length was not altered and the canola was planted on 23 August, 9 September, 6 October, and 8 October 2014 representing freeze events one through four, respectively.

Canola plants were planted in 3.8 liter plastic pots filled with soil-less media and placed in the greenhouse in a completely randomized design. The growing media was well watered ensuring

sufficient moisture for germination and plant growth. After germination and complete emergence, canola was thinned to one plant per pot. Approximately 12 days following the initial planting, a second set of plants were planted as a replicate of the first round of freezing. A third and fourth set of plants was planted at the same interval and the temperature was maintained as the previous freeze, but the duration of the freezing was adjusted to 4 hours. The third and fourth sets of plants were not watered for 12 days prior to the freeze. The lack of water simulated droughty conditions which are common during the late fall and early winter months in the southern Great Plains.

Once the plants reached 4-6 leaves, they were transported from the greenhouse to the pre-cooled freeze chambers. At that time, plant and crown height was measured and a picture was taken on a picture board to obtain an average percentage of leaf area prior to the freeze. The picture board was 0.4 square meters in area providing a known background area to calculate percent green cover of the canola.

The pots were arranged in a completely randomized design consisting of 9 varieties and 8 replications across 2 freezing temperatures for each planting of canola. The hybrids included DKW46-15 (Monsanto Company, St. Louis, Missouri), DKW47-15, DKW 41-10, DKW 44-10, HyCLASS 125W (Croplan Genetics, Mentor, MN), Sitro (Rubisco Seeds, LLC, Philpot, KY), Edimax CL (Rubisco), Inspiration (Rubisco), and Mercedes (Rubisco). The first two freeze events consisted of temperature regimes of -4 C° and -9 C° when the plants reached 4-6 leaves and were watered to capacity following the freeze. The temperature was held for 3 hours during the first freeze event and 6 hours for the second freeze event. A third freeze event consisted of a -4 C° and -9 C° temperature regime held for 4 hours and the group of plants were not watered for twelve days prior to the freeze event and received no water following the freeze. The fourth freeze event also had a duration of 4 hours and the plants were not watered twelve days prior to the freeze, but the plants were watered to capacity immediately following the freeze.

Variables Measured

Leaf necrosis was measured using Canopeo (Oklahoma State University, Stillwater, OK), which measures the percent area occupied by green, viable vegetation. After the freeze was induced, the plants returned to the greenhouse for 14 days. At the end of this time, freeze damage was measured which provided a percentage of green material.

Statistical analysis on the canopy cover, crown height, and plant height was performed using the PROC MIX procedure in SAS v. 9.4 (SAS Institute, 2008) to determine significant treatment effects.

RESULTS AND DISCUSSION

Plant and Crown Height

There were no significant differences ($\alpha=0.05$) detected in plant height between cultivars tested prior to the first freeze; however, there were differences in crown height between cultivars as shown in Table 1. DKW 44-10 had the lowest crown height of 5.7 cm while DKW 41-10 was not different with a crown height of 6.3 cm. Inspiration had the highest of 10.3 cm with Edimax CL and DKW 47-15 being no different with crown heights of 9.0 cm for both. The differences in crown heights can be attributed to the genetic variability between cultivars and hybrids. A field study conducted by Assefa et al. (2014) included eight varieties in both conventional and no-till systems and found significant differences in crown height between varieties. DKW 46-15 had the highest crown height of 4.7 cm and was taller than Griffin and Kadore with crown heights of 3.5 cm and 3.8 cm, respectively. Assefa et al. (2014) indicated that, although it is hypothesized that crown height may be related to winter survival and yield, their analysis showed no correlation between crown height and either yield or winter survival. Furthermore, the researchers noted that no-till results in a higher crown height than conventional till but that planting date has more of an effect on winter survival.

Canopy Cover

There were no significant differences ($\alpha=0.05$) in leaf area following the first freeze (Table 2). The check presented in table 2 is the canopy cover prior to the freeze event. It is important to note that, while insignificant, the cooler temperature regime resulted in a lower percentage of cover as compared to the warmer regime at 14 days after the freeze event. The second freeze event resulted in no significant differences due to complete loss of vegetation as measured 14 days after this 6 hour freeze event. This suggests that the freeze duration at which differences in freeze survival may be expected is between 3-6 hours. A similar study conducted by Waalen et al. (2011), investigated the duration of freeze on the tolerance of winter canola. The short-term freeze consisted of a cooling rate of 3°C h^{-1} and a long-term freeze was -8°C for up to 24 days. Plant survival from the short-term and long-term events was poorly correlated. However, the long-term test did identify differences in cultivars as compared to the short-term test. Waalen et al. (2011) stated that tolerance to freezing over a longer duration is critical for the survival of canola and the long-term test may allow for the screening of subtle, important differences in freeze tolerance than a short-term test.

Furthermore, the results of our study detected a high level of variation in the canopy cover as measured by Canopeo (Patrignani and Ochsner, 2015) following the three hour freeze, indicated by the coefficient of variations in Table 2 and following the six hour freeze as shown in Table 3. Due to the variation in plant size prior to the freeze event, this indicated that canopy cover may not be an appropriate measurement to assess differences between varieties and freeze temperatures. Consequently, a visual rating may be beneficial in monitoring the vegetative damage during the days following the freeze event. A rating of ten would indicate a healthy plant with no visible damage while a rating of one would indicate a plant with extensive leaf and crown damage resulting in plant necrosis.

SUMMARY

A three hour freeze with temperatures of -4 C° and -9 C° did not result in significant vegetation loss. However, differences in crown height were detected prior to the freeze event. DKW44-10 had the lowest crown height while Inspiration had the highest. Following a six hour freeze of the same temperature, complete plant necrosis occurred.

Further investigation should include the addition of a simulated drought at various intensities. This is similar to field conditions at time of freezing and the effects of cellular drought stress may influence survival during the freeze (Thomashow, 1998; Burke et al., 1976). Simulated ground cover or no-till is also another avenue to pursue. The presence of a soil cover may also influence the growth habit of the plant, but also the available moisture. Lastly, fertility levels should be evaluated to gain a better understanding of winter hardiness of sufficiently fertilized plants compared to plants under reduced fertility program.

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Table 1. Mean plant height and crown height prior to a three hour freeze

Variety	Plant Height	Crown Height
	—— cm ——	
DKW 46-15	24.7†a	7.3†cd
DKW 47-15	29.3a	9.0ab
DKW 41-10	26.3a	6.3de
DKW 44-10	24.3a	5.7e
HyClass 125	22.3a	8.0bc
Edimax CL	29.7a	9.0ab
Inspiration	27.7a	10.3a
Sitro	25.3a	8.7bc
Mercedes	25.3a	8.7bc

†Means with the same letter are not significantly different. Alpha = 0.05.

Table 2. Mean plant area by Canopeo ten days following a three hour freeze.

Variety	Canopy Cover		
	Check	-4.4°C	-8.8°C
		---- % ----	
DKW 46-15	3.69(15.2)†a	6.00(31.1)†a	6.21(15.7)†a
DKW 47-15	5.09(58.4)a	5.32(49.8)a	4.32(74.8)a
DKW 41-10	4.93(26.3)a	7.99(40.9)a	6.70(58.5)a
DKW 44-10	3.52(36.0)a	8.35(48.3)a	3.44(50.2)a
HyClass 125	7.79(50.0)a	2.76(71.3)a	7.03(101.6)a
Edimax CL	7.69(49.3)a	7.72(34.9)a	6.18(14.4)a
Inspiration	4.72(29.4)a	4.91(49.1)a	2.36(98.5)a
Sitro	11.21(45.7)a	5.35(69.1)a	5.65(63.8)a
Mercedes	4.33(54.3)a	5.12(59.7)a	4.19(111.1)a

†Means with the same letter are not significantly different. Alpha = 0.05.

Values in parenthesis represent the coefficient of variation (CV)

Table 3. Mean plant height and crown height prior to a six hour freeze

Variety	Plant Height	Crown Height
	—— cm ——	
DKW 46-15	29.3†a	6.0†cd
DKW 47-15	34.7a	7.0bc
DKW 41-10	30.7a	4.7d
DKW 44-10	27.0a	5.7cd
HyClass 125	29.7a	7.0bc
Edimax CL	28.0a	8.7a
Inspiration	29.7a	8.3ab
Sitro	30.0a	8.3ab
Mercedes	29.0a	8.7a

†Means with the same letter are not significantly different. Alpha = 0.05.

Table 4. Mean plant area by Canopeo ten days following a six hour freeze.

Variety	Canopy Cover		
	Check	-4.4°C	-8.8°C
		----- % -----	
DKW 46-15	4.30†a	0.42(48.0)†a	0.66(28.4)†a
DKW 47-15	2.65a	0.34(45.8)a	0.45(32.7)a
DKW 41-10	4.53a	0.38(90.1)a	0.33(45.8)a
DKW 44-10	5.57a	0.69(17.3)a	0.49(65.5)a
HyClass 125	3.31a	0.56(44.4)a	0.26(45.8)a
Edimax CL	2.56a	0.44(41.7)a	0.63(10.8)a
Inspiration	2.70a	0.63(36.7)a	0.33(33.3)a
Sitro	2.08a	0.60(50.0)a	0.56(44.6)a
Mercedes	2.66a	0.55(20.4)a	0.40(48.0)a

†Means with the same letter are not significantly different. Alpha = 0.05.

Values in parenthesis represent the coefficient of variation (CV)

VITA

John William Jones

Candidate for the Degree of

Master of Science

Thesis: STRATEGIES TO IMPROVE CANOLA ESTABLISHMENT AND WINTER
SURVIVAL IN CONSERVATION SYSTEMS

Major Field: Plant and Soil Sciences

Biographical:

Education:

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