USE OF LATE-SEASON HERBICIDES ON WINTER CANOLA (*Brassica napus*) AND IT'S IMPACTS ON YIELD AND SEED QUALITY

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Abstract: Canola (*Brassica napus* L.) was adopted in the Southern Great Plains as a means to increase the productivity and decrease weed pressure in traditional monoculture wheat. While canola has been effective at achieving this task, the difficulty to apply herbicides in the spring following dormancy can result in high weed pressure within the canola system and potentially the successive wheat crop. Herbicide applications during or around flowering are often prohibited as they have been linked to decreased viability of pollen. However, this information has not been strictly noted in Southern Great Plains. To evaluate this phenomenon in the Southern Great Plains region a trial was initiated at the Cimarron Research Station in Perkins, OK from 2014-2016. Experimental design was a three factorial randomized complete block design with four replications. Factor one was herbicide: glyphosate, quizalofop, and clethodim, factor two being the high or low rate, based on labeled rates, and factor three was canola growth stage: bolting, early flower, and mid flower. Overall, the application of herbicides during reproductive growth negatively affected crop yield. Also, it was especially true for glyphosate, which decreased yields by an average of 389, 483, and 182 kg ha^{-1} during bolting, early flower, and mid flower, respectively. Higher herbicide application rates usually resulted in decreased yield, but was only statistically significant with the glyphosate applications. Seed quality was significantly decreased; in a similar way to the yield. These results suggest that late-season herbicide applications, especially glyphosate, should be avoided when possible as decreased yield and seed quality can be expected.
Nomenclature: Canola, *Brassica napus* L., clethodim, quizalofop, glyphosate.
Key words: Oklahoma, mid flower, early flower, bolting

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION ... 1

II. REVIEW OF LITERATURE ... 1

Canola Basics ... 2
Canola Growth and Development ... 4
Integrating Winter Canola in Winter Wheat Systems ... 5
Canola Herbicides ... 8
Herbicide Timings in Winter Canola Production ... 10
Hypothesis ... 12

III. MATERIAL AND METHODS ... 21

Locations, soils and weather ... 13
Experimental design ... 15
Spray procedure ... 18
Evaluations ... 18
Statistical analysis ... 19

IV. RESULTS AND DISCUSSION ... 20

Grain Yield ... 20
Oil Content ... 35

V. CONCLUSION ... 39

REFERENCES ... 42

LIST OF TABLES

1. Agronomic management practices utilized during 2014, 2015 and 2016 at Cimarron Research Station in Perkins, OK 15

2.1 Herbicides and application rates for 2014, 2015 and 2016 trials during bolting, early flower and mid flower at Cimarron Research Station in Perkins, OK 17

2.2.Treatments in 2014, 2015 and 2016 trials at Cimarron Research Station in Perkins, OK....................17

3. Yield difference (kg ha $^{-1}$) and significance levels of treatments during 2014 in Perkins, OK....................20

4. Glyphosate treatments grain yield with high and low rates in 2014 in Perkins, OK...23

5. Yield difference (kg ha $^{-1}$) and significance levels of treatments during 2015 in Perkins, OK....................24

6. Glyphosate treatments grain yield with high and low rates in 2015 in Perkins, OK...26

7. Yield difference (kg ha $^{-1}$) and significance levels of treatments during 2016 in Perkins, OK....................28

8. Glyphosate treatments grain yield with high and low rates in 2016 in Perkins, OK...30

LIST OF FIGURES

1. Weather conditions during study at Cimarron Research Station at Perkins, OK in 2014, 2015 and 2016 seasons..15

2. Canola grain yield following applications of glyphosate, quizalofop, and clethodim during bolting, early flower, and mid flower with lower and higher rates based on herbicide labels in 2014 at Perkins, OK. Check grain yield was 1543 kg ha $^{-1}$. Critical values are indicated by the lines in each bar, meaning that if the line passed the number zero the treatment is nonsignificant...20

3. Grain yield interaction of glyphosate by timing x rate in 2014 at Perkins, OK. Means within a bar followed by the same letter are not significantly different in comparison to glyphosate rates and application timing according to Tukey Protected LSD Test at $p<0.05$. 23

4. Canola grain yield following applications of glyphosate, quizalofop and clethodim during bolting, early flower and mid flower with lower and higher rates based on herbicide labels in 2015 at Perkins, OK. Check grain yield was 2400 kg ha $^{-1}$. Critical values are indicated by the lines in each bar, meaning that if the line passed the number zero the treatments is nonsignificant. 25

5. Grain yield interaction of glyphosate by timing x rate in 2015 at Perkins, OK. Means within a bar followed by the same letter are not significantly different in comparison to glyphosate rates and application timing according to Tukey Protected LSD Test..26

6. Canola grain yield following applications of glyphosate, quizalofop and clethodim during bolting, early flower and mid flower with lower and higher rates based on herbicide label in 2016 at Perkins, OK. Check grain yield was 2158 kg ha $^{-1}$. Critical values are indicated by the lines in each bar, meaning that if the line passed the number zero the treatment is nonsignificant...29

7. Grain yield interaction of glyphosate by timing x rate in 2016 at Perkins, OK. Means within a bar followed by the same letter are not significantly different in comparison to glyphosate rates and application timing according to Tukey Protected LSD Test..30

8. Glyphosate injury showing unformed pods, pale flowers and aborted pods after applications during bolting, early flower and mid flower...33

9. Number of aborted pods with the application of glyphosate, quizalofop and clethodim with low and high rates during bolting, early flower and mid flower in 2016 in Perkins, OK....34

10. Percentage of seed oil content with application of glyphosate, quizalofop and clethodim during bolting, early flower and mid flower with lower and higher rates based on herbicide labels in 2016 at Perkins, OK. Check oil content was 39.5%. Critical values are indicated by the lines in each bar, meaning that if the line passed the number zero the treatment is nonsignificant....36

CHAPTER I

INTRODUCTION

There has been an increasing interest in winter canola (*Brassica napus* L.) in production systems in the Southern Great Plains. There are many beneficial aspects that canola can bring into Oklahoma production systems, and one of the greatest is the benefits to a successive winter wheat crop, with the greatest of these benefits being an increase in winter and perennial weed control options. This introduction of winter canola in Oklahoma makes herbicide management in crop a critical component to achieve all the benefits of winter canola. However, improper herbicide management can result in decreased canola yields and ineffective weed management (Harker, 1995).

CHAPTER II

REVIEW OF LITERATURE

Canola Basics:

Canola is a broadleaf plant in the *Brassica* or mustard family, and was originally developed by breeding several types of wild mustard and domesticated rapeseed (Stamm et al., 2005). Rapeseed has been utilized for centuries and was grown extensively in Europe and Asia during the 13th century. The primary use for rapeseed was as an industrial lubricant and lamp oil (Stamm et al., 2005). Rapeseed is toxic to human if consumed in high levels (30-60%) of erucic acid and high levels of glucosinolates (Canola Council of Canada, 2001). Canola is actually an acronym that stands for CANadian Oil Low in Acid and requires the crop to have a lower percentage of erucic acid and less than 30 micromoles of glucosinolates per gram of oil-free meal. In 1985, the U.S. Food and Drug Administration deemed canola a safe consumable with less than 2 percent erucic acid (Stamm et al., 2005), making it a viable source of consumable oil.

The primary uses of canola in the United States are for both oil and meal. Canola oil is used as both a cooking oil and a biofuel. In 2006, the U.S. Food and Drug Administration stated that canola oil was a heart healthy oil and had the benefits of reduced the risk of coronary heart disease due to the lower percentage of saturated fats and lack of trans fats (Stamm et al., 2005). Since the concern about the use of petroleum and its hazard related to global climate change, an area of oil use as a fuel has been in the production of biofuels, specifically biodiesel. (Fore et al., 2011). According to Fore et al. (2011) the use of canola as a biofuel is economically efficient and also is one of the alternatives to reduce the use of petroleum based fuels. In addition to oil, canola can be used as a valuable feedstock. The most notable use of canola as a feedstock is in the dairy industry because of the higher fat content, which can improve milk production (Ash, 2017). Canola also can be a valuable feedstock in the beef, poultry, aquaculture, and equine industries. However, the use by these industries is limited due to Canola's higher fiber content, limited crushing locations, and lower palatability at feeding rates needed Ash, (2017).

With many uses for canola, the demand for canola for both oil and feedstock is increasing. In order to fill this demand, the United States imports an equivalent of 2 million production acres every year. As a result, domestic production for the crop has been increasing across the United States with increasing acres in the Southeast, Pacific Northwest, and the Southern Great Plains. The domestic production of canola in United States in 2016 was around 1,400,000 metric tons with an average yield of 2006 kg ha^{-1} (NASS, 2016). In Oklahoma, the production in 2016 was near 51 thousand metric tons with an average yield of 1672 kg ha^{-1} (NASS, 2016).

Canola can be grown as both a winter and a spring crop. The highest acres of canola grown in the United States is the spring variety. Spring canola is primarily grown in the northern Great Plains, however, it also is produced in the Pacific Northwest and is grown in rotations with cereals and legumes (Lofton et al., 2015). Contrary to spring canola, winter varieties need a period of vernalization, or a period of chilling weather, in order to break the reproductive dormancy (Edwards & Hertel, 2011). The winter canola cropping system matches that of other winter crops, especially wheat, and thus provides a valuable rotation with this crop.

Canola Growth and Development:

Winter canola is typically planted in late-September through early-October (Lofton et al., 2015). After emergence, the canola plant starts the vegetative portion of its growth cycle. During the fall, the plant will develop and enter in the rosette growth stage, which the plant will overwinter. During this stage, canola plant will develop leaves and the rooting system. In the rosette, older leaves will be at the base and the base of the canopy. Smaller and newer leaves will continue to develop, emerge, and grow from the center of the leaf structure. Following the first major frost event, the growth of the plant will slow and many visible changes will occur. In more severe winters and in colder production regions, a majority of the developed leaves will dieback.

Growth will resume in late winter or early spring as temperatures increase and day length becomes longer. New leaves will begin to emerge once temperatures consistently reach greater than 4°C. The amount of leaf growth that will occur in the spring depends on several factors, including cultivar, environment, length of winter, and moisture. Once temperatures rise and night lengths continue to decrease, the flowering structure will emerge from the rosette. Once the flower structure has emerged, which is called bolting, the plant has shifted from vegetative to reproductive growth. At this stage, leaves will continue to grow at the base and the flower cluster and main stem continues to elongate (Oplinger et al., 1989). The main stem will reach 30-60% of its total height prior to flowering. Once flowering begins, maximum canopy development occurs (Oplinger et al., 1989). At this time, majority of photosynthesis is occurring at the upper leaves and the green tissue on the stem. Flowering begins from the base of the main stem. The flowering structure is called a raceme. Flowering will continue and average five or more flowers a day. Flowering of the main stem will continue for 2 to 4 weeks following initial flower development (Oplinger et al., 1989). Unlike wheat, winter canola exhibit indeterminate growth. This growth pattern means the plant can continue flowering beyond the typical two to four weeks, especially in the axial stems, to compensate for late-season freezes (Lofton et al., 2015).

Maturation and ripening occurs once the last flowers fade from the main raceme. It means that the plant can be at the ripening stages on the main raceme and flowering or bolting on the secondary branches. Additionally, many pods on the lower portion of the raceme can begin to mature while still flowering at the top of the raceme. During early maturation, most of the photosynthetic activity comes from the stem and the pods. Young seed is translucent as the embryo develops rapidly. Seed weight increases and it is complete approximately 35 to 45 days after flowering. As the seed turns green, the seed weight has been set and the oil content begins to develop. As the plant continues maturity, seed will shift from green to brown to black. When seeds reach brown to black, the seeds begin to dry down. Harvest typically occurs when the seed reaches 6 to 10% moisture (Oplinger et al., 1989).

Integrating Winter Canola in Winter Wheat Systems:

Winter wheat is the primary production crop in the Southern Great Plains. In 2017, Oklahoma harvested over 2.8 million acres of winter wheat with an additional 1.5

million acres being grazed for beef cattle production but not harvested. While the acreage of wheat a valuable crop for the state of Oklahoma and the Southern Great Plains in general, stagnation in yields and lower quality provide several challenges for the crop in the region (Patrignani et al., 2014). The main cause of yield stagnation has been theorized as a monocrop wheat production system (Patrignani et al., 2014). Crookston et al. (1991) reported the benefits of crop rotation. They noted a 10-15% increase in corn yields and an 8-17% increase in soybean yields when under a corn-soybean rotation compared to monoculture systems alone. Kirkegaard et al. (2008) noted similar yield response in yield increase for winter wheat. They indicated that wheat had 20% increased yields when rotated with other crops compared to wheat rotated with wheat or another cereal grain. While wheat can benefit by a rotation with several crops, the yield benefits of rotating wheat with canola have been consistently seen throughout the literature. Bourgeois & Entz (1996) showed that wheat grown after canola yielded 8% higher than wheat grown after wheat. Gardner et al. (1998) showed a greater benefit of rotating wheat with canola, indicating a 72% increase in grain yield. They indicated that this greater increase in yield from wheat following canola compared to wheat following wheat was due to increased biotic pressure in the monoculture production, which can lead to stagnation of grain yield. These benefits also have been observed in a winter wheat-winter canola rotation in the Southern Great Plains. Bushong et al. (2011) reported that wheat yields following canola increased by 10-22% compared to wheat following wheat in Oklahoma production systems.

One of the primary reasons canola was brought into Oklahoma was to aid in weed management in traditional monoculture wheat production systems. The primary concern in wheat production systems are problematic grasses, such as Italian ryegrass (*Lolium multiflorum*), various Brome species (*Bromus* spp.) and wild oat (*Avena fatua*) (Lofton et al., 2015). With an addition canola into the production system, producers are able to integrate several new herbicide modes of action unavailable in a wheat monoculture. These grassy weeds not only have the ability to decrease wheat yields due to increased in-field competition but also can decrease wheat seed quality resulting in high dockage and low test weights at the point of sale (Lofton et al., 2015). Siddiqui et al. (2010) reported that weeds in a wheat based system resulted in a 60-76% decrease in yields when grown in a 1:1 wheat-weed relationship. Additionally, they found that certain weed species, *Rumex dentatus* (toothed dock), *Coronopus didymus* (Lesser swinecress), and *Phalaris minor* (littleseed canarygrass), resulted in a significant decrease in 100 grain weights. Chhokar et al. (2007) noted that. They found that wheat yields decreased by 100% in weedy checks compared to when weeds were controlled. Additionally, they noted that using either a graminicide alone or graminicide paired with a broadleaf herbicide increased yields compared to using a broadleaf herbicide alone. These studies demonstrate the yield limitation with grassy weeds present in a wheat system. Fast et al. (2009) reported that cheat, feral rye, and Italian ryegrass resulted in the high dockage, weed seed and plant material, while feral rye was the main culprit for increased foreign material. Ferreira et al. (1990) found that a wheat fields with at least 116 cheat plants per m^2 resulted in dockage values of 5.1-24%. Barnes et al. (2001) reported similar results, showing that 100-150 cheat plants per m^{-2} resulted in 11.4-19.3% dockage. This decline in wheat seed quality can be controlled. Ratliff & Peeper (1987) noted that high percentage of cheat resulted in increased dockage but post-emergence of ethylthio analog of metribuzin herbicide controlled 91-100% of the weeds and thus decreased dockage.

The challenge with grassy weeds in a monoculture wheat production system is that there are very limited herbicides available that can control them in-season. However, if canola is incorporated into a production system, growers can spray graminicides that are not traditionally available in a wheat system. In order to take advantage of incorporating canola into a wheat production system, growers have to be proactive to manage weeds within the canola crop. To help control grasses, growers can utilize graminicides that specifically target these problematic grassy weeds but have no control of broadleaves. Two of the most common graminicides producers use is clethodim and quizalofop. While these two herbicides can control grasses effectively, they have no impact on broadleaf weeds due to their mode of action, which calls Acetyl CoA Carboxylase (ACCase) inhibitors and they can only inhibit grasses grow. However, broadleaf weeds also is an issue for canola systems, therefore, in order to control both broadleaf and grassy weeds, many growers plant glyphosate tolerant canola, so that they can apply glyphosate, a nonselective herbicide over the top of the crop to help with weed management (Lofton et al., 2015).

Canola Herbicides:

Clethodim is a product that inhibits the acetyl CoA carboxylase (ACCase) enzyme, and it is the first enzyme in the fatty acid synthesis pathway. Fatty acids are important for membrane structure. Therefore, this inhibition will consequently leads to membrane disruption and plant death. Broadleaves are not affected by the lack of susceptible ACCase enzyme. A study done by Vidrine et al. (1995) focused on grass control in soybean with graminicides applied alone and in mixtures stated that the use of clethodim alone controlled almost 99% of Barnyardgrass (*Echinochloa crus-gali*). Concerning the use of this herbicide in canola, a study done by Bushong et al. (2006) stated that the application of clethodim during the fall presented a high control of Italian ryegrass in the state of Oklahoma. In other two studies, Bushong et al. (2012; 2011) observed that an application of clethodim resulted in higher yield and grassy weed control in comparison to the non-herbicide treatment.

Another graminicide used for weed control in canola is quizalofop. This product acts in the same way as clethodim, inhibiting the ACCase enzyme. A report written by Alberta Agriculture (1996) shows the benefits of quizalofop for full season control of quackgrass (*Elymus repens*) in different broadleaf crops. According to one study done by Vidrine (1989), the use of quizalofop in soybean resulted in effective control of Johnsongrass (*Sorghum halepense*) from 86 to 100%. Another study done with chickpea state that the use of quizalofop controlled grassy weeds effectively and also increased the crop yield (Goud et al., 2013). In winter canola, the use of quizalofop did not cause any injury on the crop and it was effective for weed control (Cogdill et al., 2013). Bushong (2011) also stated that the use of quizalofop in canola resulted in higher yields and weed control in comparison to the non-treated check.

The herbicides that were described above mainly control grassy weeds, thus in order to increase the spectrum of weed control in canola, glyphosate is commonly used. Glyphosate is a herbicide which translocates in the plant and inhibits the synthesis of aromatic amino acids. Many authors have observed the efficacy of glyphosate applications on Roundup Ready (RR) crops. Johnson et al. (2000) evaluated the efficacy of the use of glyphosate on RR, and observed that of the treatments that were used, glyphosate provided more than 90% of weed control. Another study regarding soybean stated that the use of glyphosate in RR soybean controlled at least 90% of weeds such as common lambsquarters (*Chenopodium album*), fall panicum (*Panicum dichotomiflorum*) and smooth pigweed (*Amaranthus hybridus*) (Stanley Culpepper et al., 2000).

In canola, the use of RR or glyphosate-resistant varieties, which allows the use of this herbicide over the top of the crop, provides effective weed management, increasing. According to Harker et al. (2000), glyphosate was the herbicide that provided the higher percentage of wild oat (*Avena fatua*) and stickywilly (*Galium aparine*) control in canola. Bushong et al. (2012) showed that the use of glyphosate resulted in higher yield and weed control in winter canola in comparison to treatments with no applications of glyphosate and applications of other herbicides.

These herbicides can be applied in winter canola during the fall and/or spring for weed control. The fall season is the best period to control troublesome species in canola; however, when canola starts its regrowth after the period of vernalization, some weeds could grow as well, which could affect crop production. In order to control this issue, farmers usually apply herbicides during the spring to maintain a field free of difficult-to-control weed species and decrease weed/crop competition.

Herbicide Timings in Winter Canola Production:

During the fall, the incidence of winter annual and perennial weeds in Oklahoma such as Italian ryegrass (*Lolium multiflorum*), cheatgrass (*Bromus tectorum*), henbit (*Lamium amplexicaule*) and field bindweed (*Convolvulus arvensis*) can affect canola growth and development and consequently decrease grain yield (Bushong et al., 2011).

Therefore, producers apply herbicides during this period in order to control these invasive species. For fall applications, the best period of control is between 4 and 6 leaf, which is the stage that canola has a higher weed/crop competition susceptibility due to a small amount of vegetative tissue available to compete with weeds (Lofton et al., 2015). The best period of applicationalso was confirmed by Harker et al. (2003) by doing a multiyear field experiment in order to evaluate the best application timing in canola, and they stated that the best period of weed control which caused the highest yields was at the four-leaf stage. If the application is delayed, yield and plant height could be reduced and the number of aborted pods could increase (Tozzi et al., 2016). Furthermore, all of the herbicides labeled for use in canola allow application during the fall.

After fall, canola has minimal growth until the spring, when the temperatures start to increase and the crop starts to regrow to complete its life cycle. On the other hand, during this period, weeds also start to regrow from the seedbank that is in the soil. Furthermore, during this time, canola starts its reproductive growth, which is when the crop starts to lose biomass in order to produce flowers and pods. Due to this fact, there are some weeds that can compete with canola, and can decrease yield. Therefore, a spring application is recommended in order to maintain crop yield potential; however, the window for spring application during some seasons is narrow in Oklahoma due to adverse environmental conditions, rapid crop growth, and high amount of land to cover with a herbicide. All of these factors lead farmers to apply herbicides sometimes later than recommended, such as during bolting, early flower or mid flower. These applications can cause crop injury, decrease yield and lower percentage of oil in the seed (Tozzi et al., 2015).

However, there is limited information on the impact of late-season herbicide applications in canola and how they impact crop growth and development. Therefore, it is important to determine the impact of these herbicides on winter canola yield and productivity. If little negative impact is found, this would potentially allow for winter canola producers in the Southern Great Plains to push for later applications of herbicides to help control critical weeds present during the early spring.

HYPOTHESIS

Null - The use of late-season herbicides will negatively influence winter canola yield and development.

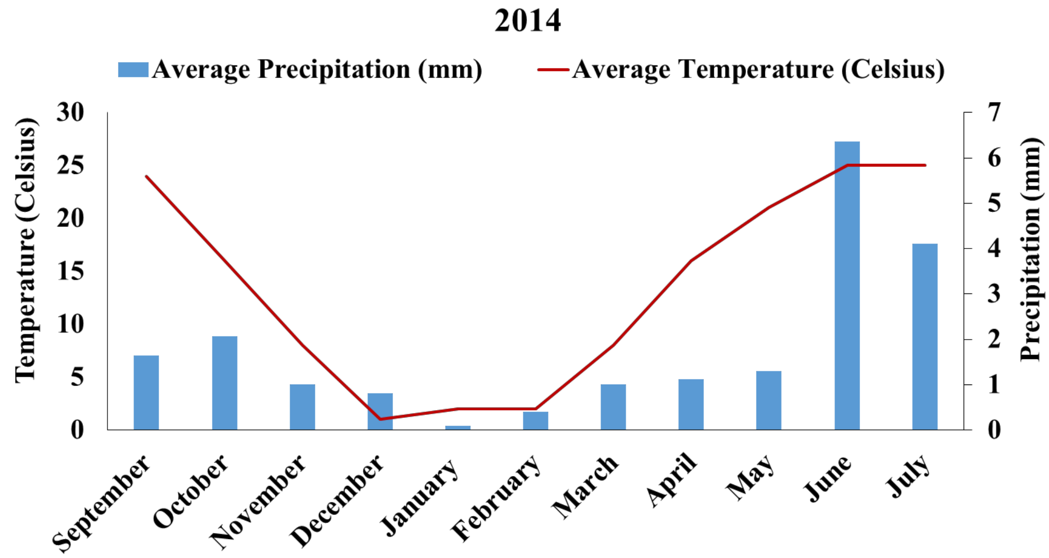
Alternative – The use of late-season herbicides will not cause any differences in grain yield and seed quality.

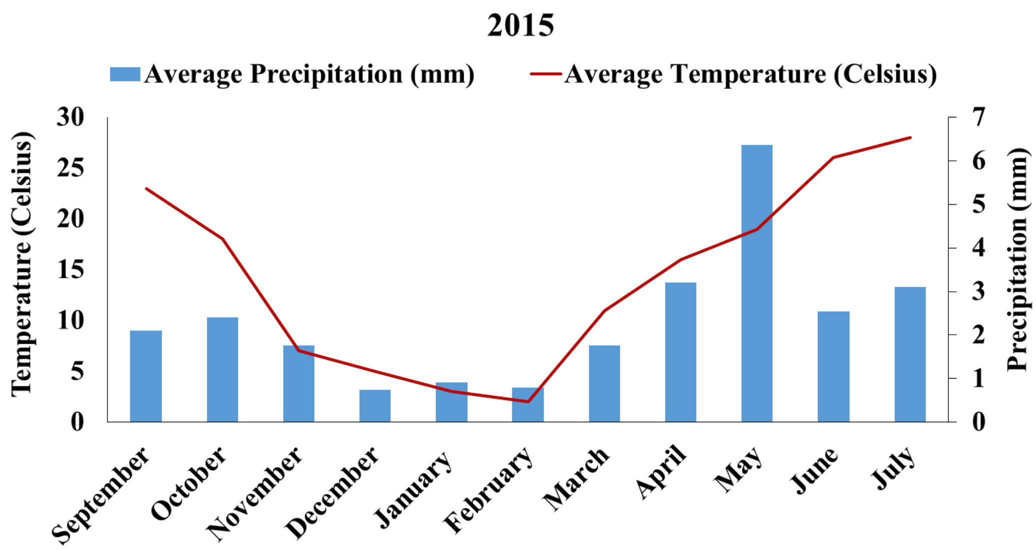
CHAPTER III

MATERIAL AND METHODS

Locations, soils and weather:

A trial was established in 2014, 2015 and 2016 at the Cimarron Research Station in Perkins, OK (35°59'60"N 97°03'03"W). Dekalb 44-10 (Monsanto Co, St Louis, MO) was utilized in 2014 and 2015. The 44-10 cultivar was not available during the 2016 season, therefore Dekalb 46-15 (Monsanto Co, St Louis, MO) was utilized instead. These two cultivars are both glyphosate tolerant and very similar in growth and productivity. All agronomic management practices utilized for all three years are highlighted in Table 1. Trials for all three years were planted using a custom-built planter on a Fabro base with a 0.19 meters row spacing. Winter canola was planted at varying dates for all three years based on environmental conditions; however, all plots were planted within the recommended planting guidelines from September 10^{th} through October 12^{th} (Table 1). Canola plots were planted at a targeted population of 926,250 seed ha^{-1}. The soil type are Teller loams and sandy loams with a pH range between 5.1 and 6.5 and the annual average precipitation is 941.32 millimeters with an average temperature of 15 degrees Celsius. All years were conducted under conventionally tilled dryland cropping systems. Figure 1 shows the weather conditions during 2014, 2015 and 2016 seasons, respectively.


2014
Average Precipitation (mm)
Average Temperature (Celsius)
Temperature (Celsius)
Precipitation (mm)
30
25
20
15
10
5
0
7
6
5
4
3
2
1
0
September
October
November
December
January
February
March
April
May
June
July


2015
Average Precipitation (mm)
Average Temperature (Celsius)
Temperature (Celsius)
Precipitation (mm)
30
25
20
15
10
5
0
7
6
5
4
3
2
1
0
September
October
November
December
January
February
March
April
May
June
July

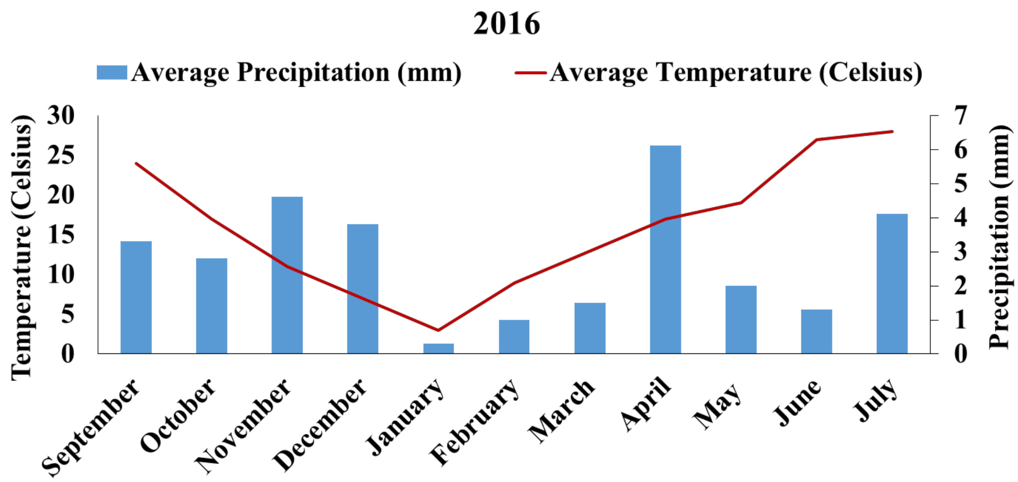


Figure 1. Weather conditions during study at Cimarron Research Station at Perkins, OK in 2014, 2015 and 2016 seasons.

Table 1. Agronomic management practices utilized during 2014, 2015 and 2016 at Cimarron Research Station at Perkins, OK.

Year	Planting	Herbicide application	Herbicide application date	Nitrogen fertilization	Nitrogen fertilization date
2014	September 27th	Glyphosate; 650 mL	February 5th	99 kg ha^{-1}	January 18th
2015	October 5th	Glyphosate; 650 mL	February 16th	99 kg ha^{-1}	January 27th
2016	October 12th	Glyphosate; 650 mL	March 1st	67 kg ha^{-1}	February 14th

Experimental design:

The studies were established as a factorial with 3 factors arranged as a randomized complete block design with 19 treatments and four replications with each individual plot measuring 1.67 meters wide and 3.33 meters long. Three different herbicides at two different application rates were evaluated at three different crop growth stage timings. The three evaluated herbicides were glyphosate (RoundUp PowerMAX®;Monsanto Company, St. Louis, Missouri), quizalofop (Assure II®;(DuPont USA, Wilmington, Delaware), and clethodim (Select; Valent, Walnut Creek, California).

The designated herbicides were evaluated at the full-labeled rate as well as partial application rate. Exact rates for the individual herbicides were 473 and 650 mL ha^{-1}, 207 and 354 mL ha^{-1}, and 118 and 177 mL ha^{-1} at the high and low rates for glyphosate, quizalofop, and clethodim, respectively (Table 2.1). Glyphosate treatments were added ammonium sulfate at 2.2 kg ha^{-1} as an adjuvant following the herbicide label in order to improve the efficacy and translocation of herbicide. Clethodim and quizalofop also received a nonionic surfactant as an adjuvant at 1% v/v following the herbicide labels. The treatments also were assessed in three different application timings: bolting, early flower, and mid-flower (Table 2.2). Bolting applications were applied after the bud cluster had emerged but prior to the first flower, individual application timing varied slightly between years due to environmental conditions. Early flower was distinguished as half of the field with less than 20% flowering and mid-flower was defined as at least half the field having over 30% flowering. Weed control in all the plots were maintained throughout the fall with glyphosate applications at critical thresholds. Additionally, an early season herbicide application was made in the spring in order to minimize the effect of weed pressure between treatments, as the focus of the treatments was phototoxicity and not weed control.

Table 2.1 Herbicides and application rates for 2014, 2015 and 2016 trials during bolting, early flower and mid flower at Cimarron Research Station in Perkins, OK

Herbicides common names	Brand names or designations	Application rates (mL/ha^{-1})	Manufacturer
Glyphosate	RoundUp PowerMAX	473	Monsanto Company, St. Louis, MO,
Glyphosate	RoundUp PowerMAX	650	Monsanto Company, St. Louis, MO
Quizalofop	Assure II	207	DuPont USA, Wilmington, DE
Quizalofop	Assure II	354	DuPont USA, Wilmington, DE
Clethodim	Select	118	Valent, Walnut Creek, CA
Clethodim	Select	177	Valent, Walnut Creek, CA

Table 2.2. Treatments in 2014, 2015 and 2016 trials at the Cimarron Research Station in Perkins, OK

Treatment	Rate	Timing
Check	N/A[a]	N/A
Glyphosate	High[a]	Bolting
Glyphosate	Low[a]	Bolting
Quizalofop	High	Bolting
Quizalofop	Low	Bolting
Clethodim	High	Bolting
Clethodim	Low	Bolting
Glyphosate	High	Early Flower
Glyphosate	Low	Early Flower
Quizalofop	High	Early Flower
Quizalofop	Low	Early Flower
Clethodim	High	Early Flower
Clethodim	Low	Early Flower
Glyphosate	High	Mid Flower

Glyphosate	Low	Mid Flower
Quizalofop	High	Mid Flower
Quizalofop	Low	Mid Flower
Clethodim	High	Mid Flower
Clethodim	Low	Mid Flower

[a] N/A – not applicable, glyphosate high: 650 mL ha^{-1}, glyphosate low: 473 mL ha^{-1}, quizalofop high: 354 mL ha^{-1}, quizalofop low: 207 mL ha^{-1}, clethodim high: 177 mL ha^{-1}, clethodim low: 118 mL ha^{-1}.

Spray procedure:

All the treatments were sprayed using 3-liter bottles with a CO_2-pressurized backpack sprayer with water as the carrier. The spray tips used were a Teejet XR11002 nozzles (TeeJet® Technologies, Glendale Heights, IL) with 140L ha^{-1} of spray volume at a speed of 4.8 km/h and pressure of 206 kPa. Each application was applied with a maximum of 16 km/h wind speed in order to prevent spray drift among the treatments. At the end of each application, the sprayer tank and boom was purged and washed using deionized water as a means to minimize contamination between treatments.

Evaluations:

In order to assess canola grain yield, the crop was swathed at 50% color change and then harvested less than 14 days after swathing. Plots were harvested with a Delta Wintersteiger (Wintersteiger USA, Salt Lake City, UT) self-propelled small plot combine. Plot weights were used to estimate grain yields on a per hectare basis. All the grain yields were collected for each plot with were the moisture adjusted for 6% moisture. In 2016 season was evaluated number of aborted pods by collecting 10 canola plants of each treatment and counting the numbers of total pods and aborted pods in each plant collected, and after that an average was taken. Also, in the 2016 season, subsamples were collected for each plot in order to analyze winter canola seed quality. Subsamples were utilized to determine oil and protein content, using a DA 7000 near-infrared spectroscopy analyzer (Perten Instruments, Hagersten, Sweden). This instrument performs an assessment of seed oil content by absorbing near infrared (NIR) energy, and consequently the vibration and rotation of chemical bonds within molecules leads to the generation of a great amount of energy levels and consequently the oil content reading (Velasco et al.,1999).

Statistical analysis:

Grain yield and seed quality was analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC). For grain yields, analysis was conducted to determine the response of canola crop yield to treatments between years. Results of that analysis suggested that crop yield should be analyzed separately between years. Therefore, an analysis of variance (ANOVA) using Procedure MIXED with a Tukey adjustment at a 95% confidence level was used to determine all pairwise differences between treatment means for each year individually. A SLICE function within LSMeans was used in order to evaluate significant differences of interactions of herbicide type, rate, and application. All figures were constructed in Microsoft EXCEL with confidence limits adjusted to the critical LSD-value.

CHAPTER IV

RESULTS AND DISCUSSION

Grain Yield:

In the 2014 season, the check treatment yielded an average of 1543 kg ha^{-1}. While the herbicide applications did result in lower yields, none of the application treatments resulted in any significant differences in comparison to the nontreated-consistent check (Table 3 and Figure 2). The glyphosate treatments presented a numerical decrease in yield in comparison to the nontreated check (Figure 2). Higher application rates of herbicides did result in lower yields compared to the same application applied at a lower rate; however, no significant declines in yields compared to those found in under the check treatments were detected at a 95% of confidence level.

Table 3. Yield difference (kg ha-1) and significance levels of treatments during 2014 in Perkins, OK.

Herbicide	Timing	Yield difference[b]	Significance[a]
Glyphosate HR[a]	Bolting	-16.2	NS
Glyphosate LR	Bolting	-30.5	NS
Glyphosate HR	Early Flower	-73.7	NS

Glyphosate LR	Early Flower	-33.0	NS
Glyphosate HR	Mid Flower	-60.8	NS
Glyphosate LR	Mid Flower	-11.0	NS
Clethodim HR	Bolting	-16.0	NS
Clethodim LR	Bolting	-27.5	NS
Clethodim HR	Early Flower	7.4	NS
Clethodim LR	Early Flower	-2.8	NS
Clethodim HR	Mid Flower	-3.3	NS
Clethodim LR	Mid Flower	-27.8	NS
Quizalofop HR	Bolting	0.6	NS
Quizalofop LR	Bolting	-48.7	NS
Quizalofop HR	Early Flower	7.2	NS
Quizalofop LR	Early Flower	0.8	NS
Quizalofop HR	Mid Flower	-25.0	NS
Quizalofop LR	Mid Flower	42.1	NS

[a]Abreviation – HR: high rate, LR: low rate, NS: non-significant according to Tukey Protected LSD test at $p<0.05$.

[b]Yield check: 1543 kg ha^{-1}

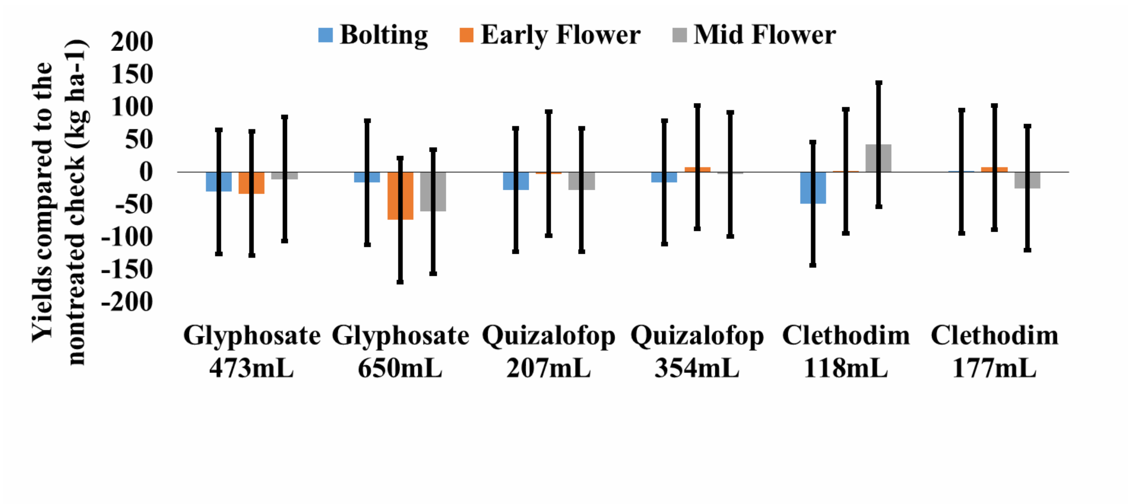


Figure 2. Canola grain yield following applications of glyphosate, quizalofop, and clethodim during bolting, early flower, and mid flower with lower and higher rates based on herbicide labels in 2014 at Perkins, OK. Check grain yield was 1543 kg ha $^{-1}$. Critical values are indicated by the lines in each bar, meaning that if the line passed the number zero the treatment is nonsignificant.

Due to the higher decrease in canola grain yield among glyphosate treatments, the study assessed an interaction of timing x rate on these treatments in order to evaluate the difference of grain yield (Table 4 and Figure 3). In the results presented on Figure 3 and Table 3, there were no significant differences observed among this interaction on glyphosate treatments.

Table 4. Glyphosate treatments grain yield with high and low rates in 2014 in Perkins, OK.

Rate[a]	Timing	Yield[b] (Kg ha^{-1})
High	Bolting	1526.3 a
High	Early Flower	1468.8 a
High	Mid Flower	1481.7 a
Low	Bolting	1512.0 a
Low	Early Flower	1509.5 a
Low	Mid Flower	1531.5 a

[a] Glyphosate rate – High: 650 mL/ha^{-1}, Low: 473 mL/ha^{-1}
[b] Means within a column followed by the same letter are not significantly different according to Tukey Protected LSD test at $p<0.05$.

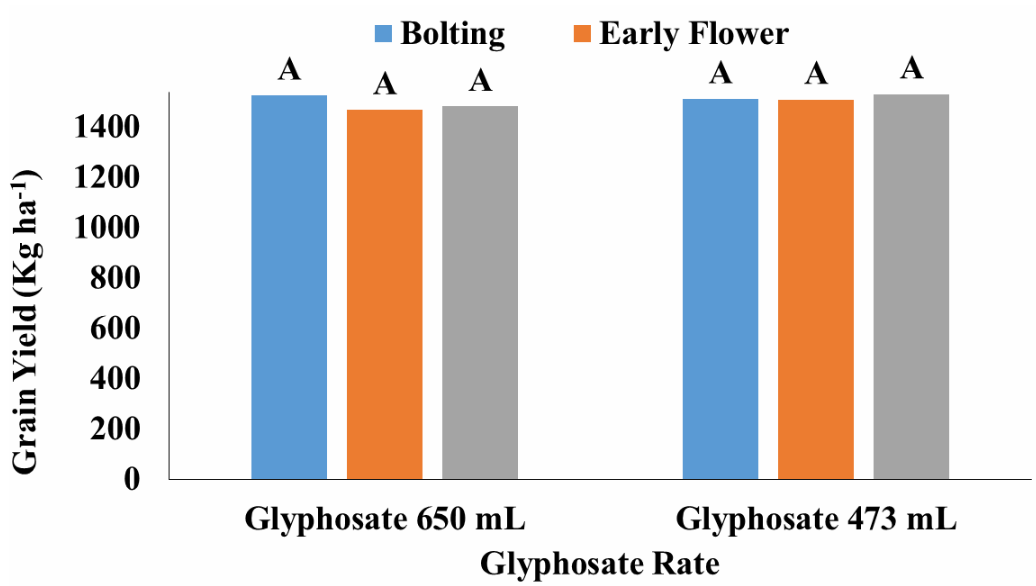


Figure 3. Grain yield interaction of glyphosate by timing x rate in 2014 at Perkins, OK. Means within a bar followed by the same letter are not significantly different in comparison to glyphosate rates and application timing according to Tukey Protected LSD Test at $p<0.05$.

In 2015, was observed an average yield for the nontreated check of 2400 kg ha^{-1}, with several application treatments resulting in significant declines in crop yields, especially for glyphosate applications. For glyphosate treatments, all applications resulted in significant differences. (Table 5 and Figure 4). Similar to the previous season, no significant effect was found with clethodim or quizalofop applications.

Table 5. Yield difference (kg ha^{-1}) and significance levels of treatments during 2015 in Perkins, OK.

Herbicide	Timing	Yield difference[b]	Significance[a]
Glyphosate HR[a]	Bolting	-696.1	$p<0.001$[c]
Glyphosate LR	Bolting	-465.9	$p<0.001$
Glyphosate HR	Early Flower	-942.5	$p<0.001$
Glyphosate LR	Early Flower	-677.1	$p<0.001$
Glyphosate HR	Mid Flower	-234.9	$p<0.01$[c]
Glyphosate LR	Mid Flower	-190.6	$p<0.05$ [c]
Clethodim HR	Bolting	115.8	NS [a]
Clethodim LR	Bolting	-30.8	NS
Clethodim HR	Early Flower	-0.6	NS
Clethodim LR	Early Flower	105.6	NS
Clethodim HR	Mid Flower	7.2	NS
Clethodim LR	Mid Flower	48.7	NS
Quizalofop HR	Bolting	109.2	NS
Quizalofop LR	Bolting	-8.6	NS
Quizalofop HR	Early Flower	172.2	NS

Quizalofop LR	Early Flower	68.0	NS
Quizalofop HR	Mid Flower	127.4	NS
Quizalofop LR	Mid Flower	140.6	NS

[a]Abreviation – HR: high rate, LR: low rate, NS: non-significant according to Tukey Protected LSD test at $p<0.05$.

[b]Yield check: 2400 kg ha^{-1}

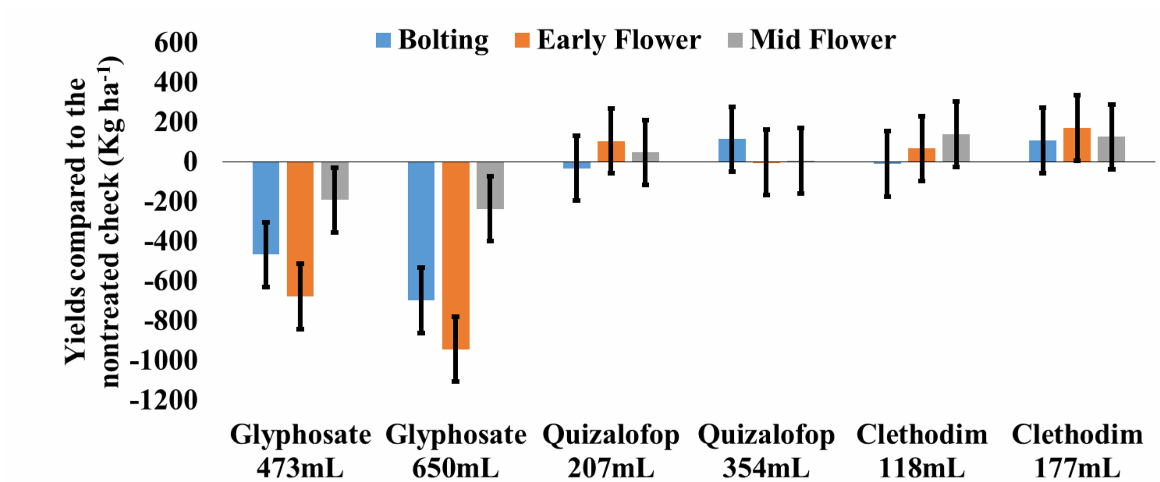


Figure 4. Canola grain yield following applications of glyphosate, quizalofop, and clethodim during bolting, early flower, and mid flower with lower and higher rates based on herbicide labels in 2015 at Perkins, OK. Check grain yield was 2400 kg ha $^{-1}$. Critical values are indicated by the lines in each bar, meaning that if the line passed the number zero the treatment is nonsignificant.

Results presented in Figure 5 and Table 6, regarding the interaction of timing x rate among glyphosate treatments, showed that the application of glyphosate at a higher rate during early flower presented the lowest grain yield followed by glyphosate at a higher rate during bolting and lower rate during early flower. Glyphosate applications during mid flower at higher rates and at the bolting stage at lower rates resulted in a significant decrease in grain yield compared to the nontreated check. Applications of glyphosate at the low rate during mid flower resulted in the highest yield in comparison to all other glyphosate treatments.

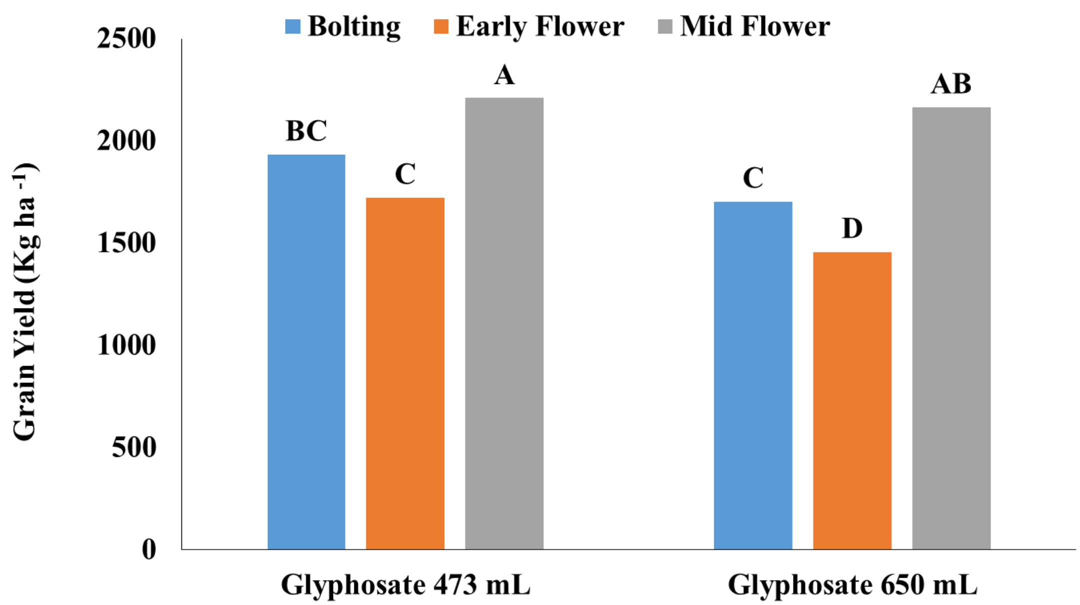


Figure 5. Grain yield interaction of glyphosate by timing x rate in 2015 at Perkins, OK. Means within a bar followed by the same letter are not significantly different in comparison to glyphosate rates and application timing according to Tukey Protected LSD Test.

Table 6. Glyphosate treatments grain yield with high and low rates in 2015 in Perkins, OK.

Rate[a]	Timing	Yield[b] (kg ha^{-1})
High	Bolting	1703.6 c
High	Early Flower	1457.2 d

High	Mid Flower	2164.8 ab
Low	Bolting	1933.8 bc
Low	Early Flower	1722.6 c
Low	Mid Flower	2209.1 a

[a] Glyphosate rate – High: 650 mL/ha^{-1}, Low: 473 mL/ha^{-1}
[b] Means within a column followed by the same letter are not significantly different according to Tukey Protected LSD test at $p<0.05$.

During the 2016 season, an average yield of 2158 kg ha^{-1} was found for the nontreated check. Similar to that seen during the 2015 harvest season, glyphosate treatments significantly decreased canola yields and resulted in the highest yield decrease among the three herbicides evaluated in. (Table 7 and Figure 6). Quizalofop applications resulted in a significant decrease in crop yield compared to the untreated check ($p<0.05$); however, none resulted in as high as a yield decrease than glyphosate. Alternatively, where the previous two seasons showed no detrimental impacts from clethodim, significant yield declines were seen when it was applied during bolting and early flower with low and high rates respectively ($p<0.05$). It must be noted that while there was a significant decline compared to the check plot, yield decline associated with application of clethodim did not result in as high of yield a decline as those associated with glyphosate at either application rate or growth stage in 2016.

Table 7. Canola grain yield with application of low and high rates of glyphosate, quizalofop and clethodim during bolting, early flower and mid flower in 2016 harvest season at Perkins, OK.

Chemical	Timing	Yield difference[b]	Significance[a]
Glyphosate HR[a]	Bolt	-752.4	$p<0.001$ [c]
Glyphosate LR[a]	Bolt	-373.5	$p<0.001$
Glyphosate HR	Early Flower	-720.7	$p<0.001$
Glyphosate LR	Early Flower	-453.1	$p<0.001$
Glyphosate HR	Mid Flower	-307.3	$p<0.001$
Glyphosate LR	Mid Flower	-288.7	$p<0.01$ [c]
Clethodim HR	Bolt	-120.4	NS[a]
Clethodim LR	Bolt	-159.6	$p<0.05$ [c]
Clethodim HR	Early Flower	-129.8	$p<0.05$
Clethodim LR	Early Flower	-140.7	NS
Clethodim HR	Mid Flower	-96.0	NS
Clethodim LR	Mid Flower	-96.4	NS
Quizalofop HR	Bolt	-66.8	$p<0.05$
Quizalofop LR	Bolt	-225.5	$p<0.05$
Quizalofop HR	Early Flower	-185.4	$p<0.05$
Quizalofop LR	Early Flower	-66.4	$p<0.05$
Quizalofop HR	Mid Flower	26.7	$p<0.05$
Quizalofop LR	Mid Flower	10.6	$p<0.05$

[a]Abreviation – HR: high rate, LR: low rate, NS: non-significant according to Tukey Protected LSD test at $p<0.05$.

[b]Yield check: 2158 kg ha^{-1}

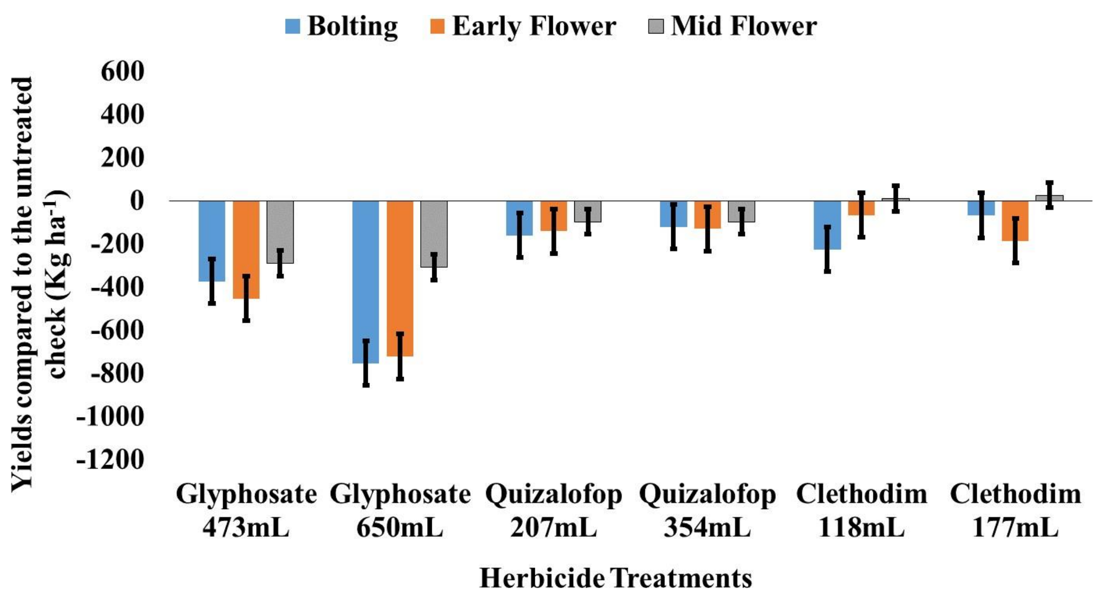


Figure 6. Canola grain yield following applications of glyphosate, quizalofop, and clethodim during bolting, early flower, and mid flower with lower and higher rates based on herbicide labels in 2016 at Perkins, OK. Check grain yield was 2158 kg ha $^{-1}$. Critical values are indicated by the lines in each bar, meaning that if the line passed the number zero the treatment is nonsignificant.

Results presented in the Table 8 and Figure 7 glyphosate time x rate interaction in 2016 season shows that applications of glyphosate at high rates presented lower yields in comparison to applications of glyphosate at low rates. Glyphosate applications at high rate during bolting and early flower showed the lowest yields, followed by glyphosate application at low rate during early flower, and glyphosate at low rate during bolting. Glyphosate at low rate during mid flower and high rate during mid flower was the treatments that presented the highest yield among glyphosate treatments.

Table 8. Glyphosate treatments grain yield with high and low rates in 2016 in Perkins, OK.

Rate[a]	Timing	Yield[b] (kg ha^{-1})
High	Bolting	1406 c
High	Early Flower	1437 c
High	Mid Flower	1851 a
Low	Bolting	1785 ab
Low	Early Flower	1705 b
Low	Mid Flower	1869 a

[a] Glyphosate rate – High: 650 mL/ha^{-1}, Low: 473 mL/ha^{-1}
[b] Means within a column followed by the same letter are not significantly different according to Tukey Protected LSD test at $p<0.05$.

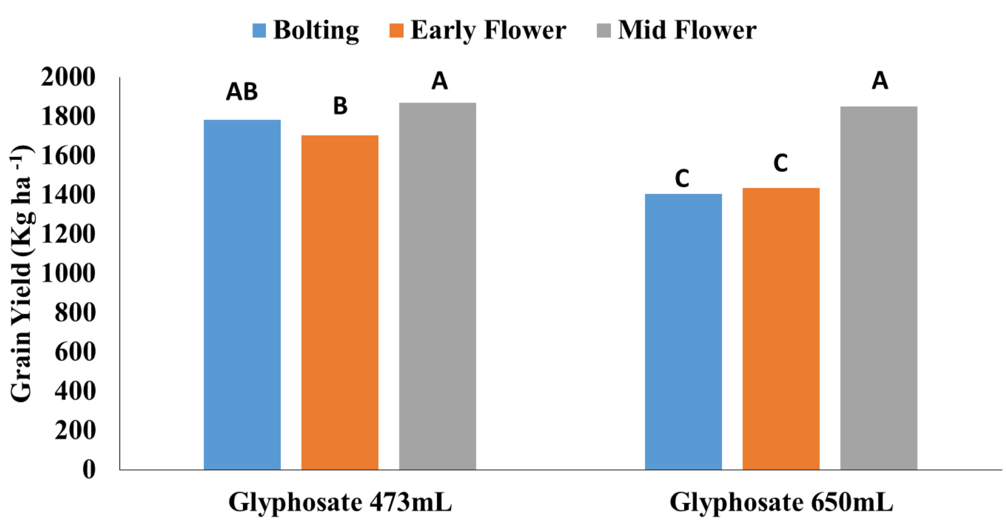


Figure 7. Grain yield interaction of glyphosate by timing x rate in 2016 at Perkins, OK. Means within a bar followed by the same letter are not significantly different in comparison to glyphosate rates and application timing according to Tukey Protected LSD Test.

Based on the results presented, the application of herbicides, especially glyphosate, can result in a significant decrease in overall productivity and yield. In 2014, these results were not observed and could be explained; however, environmental conditions during the reproductive growth, which was highlighted by a cooler winter and sporadic periods of dry conditions, could have played as a role. The bad environmental conditions can be seen by the overall lower yields associated with the 2014 season, which yielded nearly 500 kg ha^{-1} lower than the state average of Oklahoma. Nuttall et al. (1992) also observed a yield decrease in canola when the temperature changed, and Stoker & Carter (1984) assessed that the temperature at the later stages is one of the most important factors to affect grain yield in canola. Furthermore, the adverse conditions could lead to decrease of herbicide efficacy and consequently the influence of the results presented in this study (Coetzer et al., 2001). In 2015 and 2016, environmental conditions were more favorable for canola growth for much of the early spring through reproductive growth. This can be highlighted again when comparing the nontreated check to state yield averages, where both seasons resulted in 300-500 kg ha^{-1} higher yields than the state average. This could explain the response in 2015 and 2016 but not in the 2014 season.

In the 2015 and 2016 seasons, the application of glyphosate presented a significant yield decreases in comparison to the nontreated check. The yield increase is not an uncommon result in the literature (Norsworthy, 2004; Thomas et al., 2004). Norsworthy (2004) found that application of glyphosate during reproductive stage (R2) on glyphosate-tolerant soybean varieties resulted in nearly a 20% yield decline. In canola, Clayton et al. (2002) and Tozzi et al. (2015) observed that the application of glyphosate during the reproductive stage can increase the chance of yield reduction. Clayton et al.

(2002) showed that glyphosate-tolerant canola resulted in significantly higher yields when glyphosate was not applied and when glyphosate applications were made later in the season, significant yield reductions occurred. Tozzi et al. (2015) reported similar findings. They indicated that no negative effects where observed when glyphosate was applied prior to 6-leaf canola; however, when glyphosate was applied later, plants had decreased seed yield.

Several factors could be the cause of this decreased crop yield. One reason could be that glyphosate applications during late-season can cause the abortion and injury of pods, leading a decrease in seed formation and consequently, a yield decrease (Figure 8). Tozzi et al. (2015) indicated that canola plants that had lower yields, associated with late-season glyphosate applications, had less seeds per pod and an increased number of aborted pods. This was also reported by the Canola Council (2016), which stated that the application of a late season herbicide is one of the causes of missing pods, pale flowers, pod sterility and blanks, consequently decreasing the grain yield. Similarly, Schilling et al. (2006) also observed canola injury after late-season glyphosate applications under greenhouse conditions, such as epidermal damage and chlorosis. Tozzi et al. (2015) observed a decrease in number of pods per plant and number of seeds per pod on glyphosate-resistant canola that received late glyphosate application and Pline et al. (2002) observed aborted pollen and reduced pollen viability in cotton. Similar results were found for this study. Figure 8 and Figure 9 highlight the effect that herbicide treatment had on pod malformation in 2016. The application of glyphosate, at any growth stage, resulted in an increased percentage of malformed pods. It must be noted that no other herbicide application resulted in an increase of malformed pods as glyphosate compared to the nontreated check.

Figure 8. Glyphosate injury showing unformed pods, pale flowers and aborted pods after applications during bolting, early flower and mid flower.

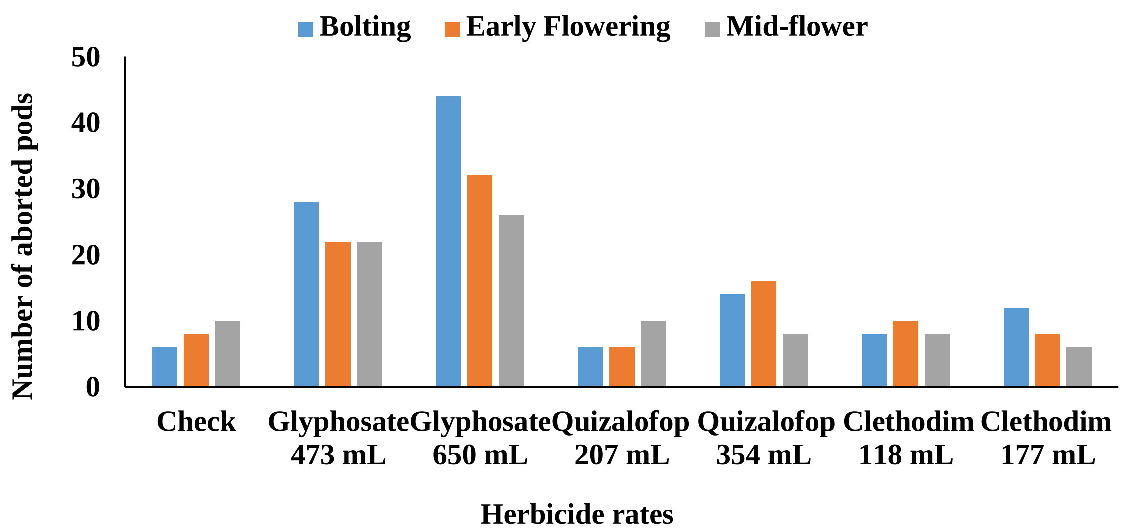


Figure 9. Number of aborted pods with the application of glyphosate, quizalofop and clethodim with low and high rates during bolting, early flower and mid flower in 2016 in Perkins, OK.

The negative impact of glyphosate on winter canola yields with higher application rates was expected. (Jason K Norsworthy & Grey, 2004). Grey & Prostko (2010) noted that when glyphosate was applied in peanut during reproductive growth and at higher applications, but still within the recommended guidelines, seed weights decreased by as much as 88%. This has even been shown in canola. Thomas et al., (2003) showed that not only did canola yield decreased significantly with late-season glyphosate but also when increases in application rates. They indicated that the decreased yield when higher applications were used, also decreased number of pods, decreased seeds per pod, and lower seed weight.

For both quizalofop and clethodim, the lack of any consistent yield response was expected. Lemerle & Hinkley (1991) also observed similar results, they did not notice significant injuries in canola with the application of clethodim and quizalofop. However, there are studies in the literature reporting decrease in grain yield with the application of graminicides such as clethodim and quizalofop. Zerner & Wheeler (2013) observed significant yield loss with clethodim application, especially when the herbicide was applied during later growth stages. These results vary from the results of this study; however, varying differences between the trials, environments and canola cultivars could result in these differences. Additionally, Zerner and Whealer did not compare clethodim applications to any other chemistries and therefore could be a result of various other factors.

Oil Content:

The results for percentage of seed oil content presented values that analyzed during 2016 harvest season only due to lack of availability of seed for the previous two seasons. The percentage of oil content on the untreated check was 39.5% (Figure 10). According to Figure 10, the treatment with glyphosate in both rates and all application timings caused a significant decrease of oil percentage compared to the check. The application of glyphosate at the lower rate caused a reduction of oil content in the seed of 4%, 2.1% and 1.3% during bolting, early flower and mid flower respectively. For higher rates of glyphosate, the reduction observed was higher, reaching 6.7%, 3.9% and 1.1% during bolting, early flower and mid flower respectively.

For the results of quizalofop applications, the percentage of oil content in response to herbicide applications with a significant difference was observed just with the application of quizalofop at a low rate during bolting (1.2%) and at a high rate during early flower (0.9%). A significant increase of canola oil content also was observed at lower rates of quizalofop during mid flower applications; however, the cause of this result could not be determined. According to the results showed in Figure 6 for clethodim applications, the only significant result observed was at a low rate during mid flower (0.6%).

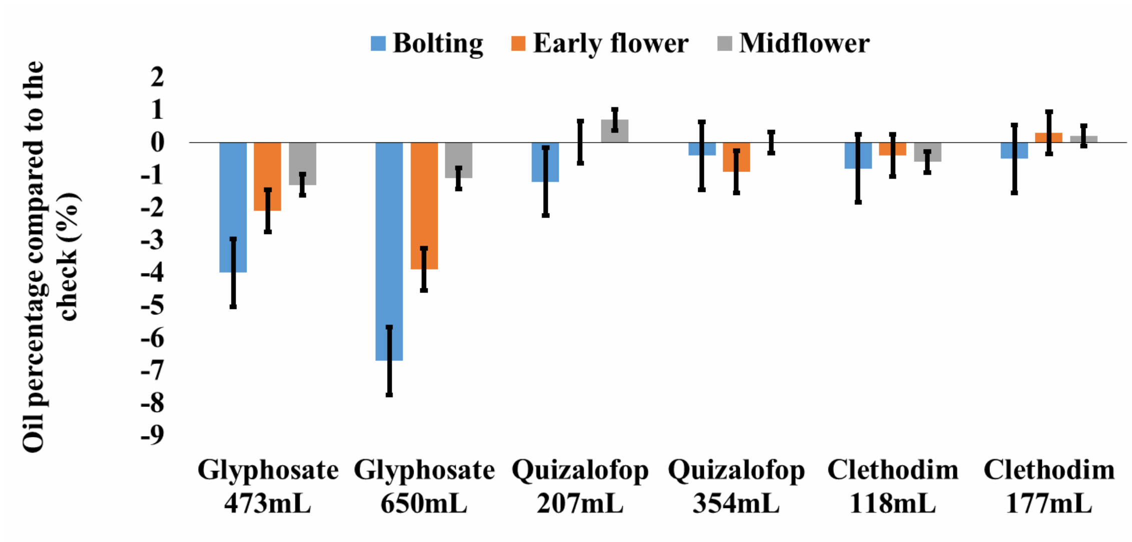


Figure 10. Percentage of seed oil content with application of glyphosate, quizalofop and clethodim during bolting, early flower and midflower with lower and higher rates based on herbicide labels in 2016 at Perkins, OK. Check oil content was 39.5%. Critical values are indicated by the lines in each bar, meaning that if the line passed the number zero the treatment is nonsignificant.

According to these results, the decrease of oil content by glyphosate applications could be predicted due to the herbicide mode of action of the glyphosate, which inhibits the shikimate pathway (Saltveit & Mencarelli, 1988). This pathway is important for the synthesis of some compounds such as vitamins, lignin, auxins and proteins, and due to the inhibition of this pathway by glyphosate, canola will not synthesize important compounds to produce oil, therefore causing a decrease of canola oil content (Herrmann & Weaver, 1999). Previous studies also observed a negative impact on oil content from seed following late-season glyphosate applications. According to Albrecht et al. (2011), they observed a decrease of soybean oil and protein content after higher rates of glyphosate applications during the reproductive stage. In addition, Zobiole et al. (2010) observed a decrease in lignin and amino acid content in glyphosate applications on glyphosate-resistant soybean. As previously discussed, these applications could result in significant damage to the pod during early development, which consequently leads to improper seeds and thus less percentage of oil content (Pline et al., 2002; Tozzi et al., 2015). Part of the issue could be a result of poor or improper pollination of the crop that has been show to result from these later applications (Thomas et al., 2004).

According to the results observed for quizalofop and clethodim, any seed quality aspects that were influence by either herbicide were not an impactful as glyphosate. Adomas (2003) observed some herbicides that did not present a decrease in oil percentage after applications. This could explain the inconsistent results regarding clethodim and quizalofop applications.

Overall, the decrease in seed oil content percentage after herbicide applications during late-season in canola could be predicted by the herbicide mode of action as was discussed before and when the crop is under stress. Therefore, what could be discussed concerning the percentage of oil content is because of late-season applications of clethodim, glyphosate and quizalofop in canola could cause a development of stress condition. During this period the crop needs a good environmental and management conditions in order to complete its cycle (Ávila & Albrecht, 2010). Hence, when the environmental and management conditions is not suitable, it will lead to decreases in the percentage of oil in canola. Moreover, as well as in grain yield, off-label applications also could affect canola oil content and the farmer should be aware of herbicide applications during this period (Tozzi et al., 2015).

CHAPTER V

CONCLUSION

One of the primary benefits of winter canola within a winter wheat rotation is the control of grassy weeds, and the best period to control these weeds is during the fall. However, when canola starts to regrow after a period of vernalization, weeds will also start to grow, which could cause some issues on canola development. This leads to herbicide application during the spring; however, best results are typically found when those applications are made prior to reproductive growth of the canola crop. However, due to environmental conditions and rapid plant growth, there are some seasons when the window for spring applications is too narrow. Therefore, growers are forced to apply herbicides during the reproductive stage. Based on the results shown in this study, the application of glyphosate, quizalofop and clethodim during bolting, early flower and mid flower did not cause impact on canola grain yield in 2014. It was theorized that this occurred mainly because of environmental conditions that led to decreased grain yield in all treatments. Furthermore, lower herbicide efficacy could have resulted due to applications in cooler conditions and when water was limited. In 2015, the application of glyphosate caused a higher yield decrease at both rates during bolting and early flower, while applications of clethodim and quizalofop did not cause significant impacts on canola grain yield. In 2016, glyphosate continued to show a higher yield decrease, while quizalofop showed significant differences in all application timings and rates and clethodim showed a grain yield decrease only at the low rate during bolting and high rate during early flower, while not as highe as the glyphosate yield decrease. In percentage of seed oil content analysis, evaluated only in 2016, glyphosate also presented a higher decrease in oil percentage, as was observed with grain yield. Quizalofop applications in low rate and high rate during bolting and early flower, respectively also presented a significant decrease but not as high as glyphosate applications. Overall, the use of glyphosate during bolting, early flower and mid flower in winter canola in Oklahoma can create a negative impact on canola grain yield and percentage of oil content on the harvested seed, making these applications a high risk for farmers across the state. In addition, glyphosate applications from bolting are off label and growers have to be aware of the consequences for the use of this herbicide during this period. For clethodim, even if the results show significant differences only during bolting and early flower, this herbicide is also off label for applications from bolting. Therefore, canola producers also has to be aware of the application during this period. Quizalofop is the only herbicide that could be applied at bolting stage for winter canola among those herbicides observed in this study. According to the Assure II label, which was the herbicide brand name for quizalofop applications, this herbicide could be applied before 60 days of harvest in winter canola during spring. as the results showed significant differences just on 2016 season. In conclusion, clethodim and glyphosate should not be applied during bolting, early flower and mid flower in winter canola in Oklahoma due to a yield decrease and percentage of oil in the seed that especially glyphosate caused in canola, and also due the off label applications according to Select (clethodim) and Roundup (glyphosate) labels.

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