EVALUATING THE IMPACT OF VARIABLE PLANTING DATES AND THE USE OF PLANT GROWTH REGULATORS ON FALL STAND ESTABLISHMENT, WINTER HARDINESS, AND YIELD OF WINTER CANOLA IN OKLAHOMA

By

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Abstract: This thesis includes two research papers about winter canola.

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Chapter II

INTRODUCTION

Chapter I of this thesis is a manuscript to be submitted for publication in Agronomy Journal, a journal of the American Society of Agronomy. Chapter II of this thesis is a manuscript to be submitted for publication in Crop Science, the official publication of the Crop Science Society of America.

CHAPTER I

PLANTING DATES AND THE USE OF PLANT GROWTH REGULATORS ON FALL STAND ESTABLISHMENT, WINTER HARDINESS, AND YIELD OF WINTER CANOLA

Running Title: Planting Dates and PGR Effects on Winter Canola

ABSTRACT

Winter canola (*Brassica napus* L.) growth in the southern Great Plains is limited by fall stand establishment and winter survival. This experiment was conducted to determine the effects of planting dates and fall-applied plant growth regulators on winter canola growth and production. The effect of planting date and fall-applied plant growth regulators on winter canola was investigated at four sites across Oklahoma. At 4 wk after treatment, winter canola biomass decreased with each week that planting was delayed. Population density increased (68%) when winter canola was planted after 8 Sept. Winter canola height when treated with 51.3 g a.i. ha^{-1} metconazole resulted in an 11% reduction in plant height compared to the non-treated plants at 4 wk after treatment. Winter canola planted on 8 Sept. resulted in the shortest canola plants (18 cm) at 4 wk after treatment. Winter canola yield increased by 15% when planted after 1 Sept. Planting winter canola after 8. Sept. resulted in a 1% increase of protein content and planting winter canola on 25 Aug. resulted in a 1% increase in oil content. Later fall planting increased population density, reduce plant height, and increase winter canola yield and may improve the overwintering of winter canola when treated with 51.3 g a.i. ha^{-1}metconazole.

Introduction

Canola (*Brassica napus* L.) is one of the most important oilseed crops produced in the world. Canola meal is the second-largest protein source for animal rations, and its oil is the third-most produced vegetable oil in the world (USDA Economic Research Service, 2012). There were 72.7 million tonnes of oilseed produced worldwide in the 2012-2013 season and the United States ranked 11th worldwide in canola, producing 1.1 million tonnes (FAO, 2014).

The United States produces both spring and winter canola. Spring canola is generally grown in the northern areas of the United States, whereas, winter canola is grown in the southern

regions. Growers in the southern Great Plains (Oklahoma, Texas, and Kansas) planted 162,000 hectares of canola in 2014 (USDA NASS, 2014), and Oklahoma (739,000 ha) ranks second in canola production in the United States behind North Dakota (3,288,000 ha), which produces spring canola (USDA NASS, 2014).

Winter canola was introduced to the southern Great Plains as a rotational crop with winter wheat (*Triticum aestivum)* as a tool to control problematic weedy grasses in wheat fields (Bushong et al., 2012; Peeper et al., 2000; Trusler et al., 2007; Kumar et al., 2007). Cheat (*Bromus secalinus* L.), feral rye (*Secale cereal* L.), Italian ryegrass (*Lolium perenne* L. ssp. *multiflorium),* jointed goatgrass (*Aegilops cylidirica* L.), and wild oat (*Avena* fatua L.) are reported as the most problematic weeds in winter wheat production due to their similar life cycles with the crop and ability to reduce yields of up to 49% (Fast et al., 2009; Webster 2004). Oklahoma winter wheat producers who had traditionally produced continuous wheat were seeking options for controlling weed infestations and improving economic returns from the infested fields (Bushong et al., 2012; Trusler et al., 2007, Duke et al., 2009). Herbicide control of weed populations was limited by cost, efficacy, and resistant biotypes (Trusler et al., 2007). Crop rotations have long been recognized as an effective strategy for weed control and because canola is a broadleaf crop, herbicides with different modes of action than those used in cereal crops could be used to control weedy grasses (Assefa et al., 2014; Anderson, 1997; Bushong et al., 2012 Francis et al., 1989; Vasilakoglou et al., 2010).).

The canola-wheat rotation has made improvements in Oklahoma's cropping systems, but more improvements are needed, especially in the overwintering and fall stand establishment of winter canola (Holman et al., 2015). It has been estimated that 4.1 million hectares of canola production will be needed to fulfill domestic consumption and replace the canola seed imported into the United States (Holman et al., 2011). If the challenges of stand establishment and winter

survival were overcome in the southern Great Plains, the potential for increased production is possible.

The growth and development of canola as winter approaches is heavily dependent on environmental conditions, which in Oklahoma, are characteristically drier during the recommended planting window of 10 Sept. to 10 Oct. (Mesonet, 2015). According to Holman et al. (2015), establishing a canola crop in the semi-arid region of the southern Great Plains is difficult without irrigation due to the dry soil conditions and shallow seeding requirement of canola.

There is speculation about what factor is most directly responsible for the poor winter survivability of canola. Winter survival of canola can be influenced greatly by winter hardiness (Rapacz et al., 1998), planting date (Assefa et al., 2014; Alford, 2003; Boyles et al., 2004; Holman et al., 2015), developmental stage (Balodis et al., 2010), cultivar, and weather conditions (Holman et al., 2011; Balodis et al., 2010). The potential for vigorous fall growth of canola increases in the southern United States and could result in greater susceptibility to winter kill or premature bolting in the fall season (Assefa et al., 2014; Boyles et al., 2004). The exposed growing point of canola seedlings makes the crop more susceptible to freeze damage; however, Boyles et al. (2004) reported that canola plants with three to four leaves are more tolerant to freezing conditions than larger plants. Climatic changes may increase plant damage due to fast, frost-induced tissue desiccation (Webb et al., 1994). Winter canola may break dormancy during warm periods in late winter or early spring and then be damaged or killed by returning cold temperatures and under the varying climate in the southern Great Plains, wide temperature fluctuations are likely and greatly impact winter survival (Holman et al., 2014; Laaniste et al. 2007 and Rapacz et al., 1998).

The height of the crown is speculated to be an important factor in winter survival (Holman et al., 2011, 2015; Assefa et al., 2014). Producers in the southern Great Plains reported greater canola winter injury when the plant crowns were elevated above the soil surface (Holman et al., 2011, 2015). Crown height decreased with later planting dates and canola planted after 15 Sept. in the southern Great Plains did not have an elevated crown because the overall size of the plant was smaller (Holman et al. 2011). Elevated crown heights can be caused by genetics, shallow planting into residue, tillage type, or vigorous fall growth (Assefa et al., 2014).

Laaniste et al. (2007) reported that successful overwintering of winter canola depends directly on planting date, cultivar and weather conditions. Researchers in the southern Great Plains have reported that earlier planted canola (15 Aug. to 1 Sept.) was more vigorous than later planted canola (1 Oct. to 15 Oct.) going into winter (Holman et al., 2011; 2015; Balodis et al., 2010). An earlier planting date increased root diameter, plant and root mass, and taproot length (Balodis et al 2010). The earlier canola is planted, the greater the chance of the crop surviving the winter; however, if above ground biomass accumulates in excess, it could result in more severe cold injury and consequently, stand loss (Boyles et al., 2004). Early planting can also lead to problems with pests and/or create an excess growth with increased risk for foliar disease (Alford, 2003).

Fall development of canola leaves may affect not only their overwintering and subsequent vegetative regrowth in the spring, but also yield (Becka et al., 2004; Boyles et al., 2004; Sidlauskas, 1999). In the southern Great Plains, Assefa et al. (2014) reported that canola planted 1 Sept. yielded higher (2,245 kg ha^{-1}) than canola planted 15 Aug. (1,908 kg ha^{-1}) or 15 Sept. (1,908 kg ha^{-1}) and that yield decreased by nearly 75% when planting dates in the southern Great Plains were delayed until 15 Sept. or later. Holman et al. (2015) reported that yield was most affected by winter survival. Low yields are generally attributed to winterkill, severe weather, or poor stand establishment (Holman et al., 2011).

Manipulation of plant growth during fall may allow producers to improve plant readiness for winter. Plant growth regulators (PGRs) are not widely used on winter canola in the United States, however, there is potential to manipulate crop physiology and plant structure through the use of PGRs (Alford, 2003). Based on previous research, canola responds well to the application of PGRs but, little research has been done to investigate fall applications to improve winter hardiness of canola (Becka et al., 2004; Kirkland, 1992; Pits et al., 2008; Morrison et al., 1992; Child et al., 1993; Berry and Spink, 2009).

The chemical properties of PGRs that cause inhibition of plant growth and cell elongation may influence the overwintering capability of some canola cultivars as well as, cause inhibition of stem and leaf growth that leads to alterations in the canopy, improve the synchrony of pod ripening, and reduce lodging (Morrison et al., 1992; Scarisbrick et al., 1985). Paclobutrazol applied at 50 L ha^{-1} in spring reduced plant height by 35% (Hua et al., 2014). Traiapenthol applied at 50 L ha^{-1} to canola in the bud stage increased yield, reduce height, and increase branching (Kirkland, 1992).

Triazole fungicides have been reported to cause similar effects on the growth and physiology of winter canola when applied in the fall (Armstrong et al., 1991; Berry and Spink, 2009; Hua et al., 2014). Metconazole applied at in fall at 0.6 L ha^{-1} or in spring at 1.2 L ha^{-1} was found to lower the height of the crown, improve root growth, and reduce the canopy height of canola resulting in greater winter survival (Balodis et al., 2010; Berry and Spink, 2009; Setia et al., 1995). Paclobutrazol was shown to increase root biomass when applied to *Brassica carinata* L. (Setia et al., 1995).

The objectives of this research are to evaluate the biological effects of fall-applied plant growth regulators on winter canola in Oklahoma and evaluate the effects of five planting dates, each being treated with fall-applied plant growth regulators on stand establishment, winter

hardiness, yield, oil and protein content. We hypothesize that PGRs can improve winter survival of canola in Oklahoma by improving freeze tolerance and winter hardiness. PGRs will be evaluated on winter canola to assess fall growth control to permit canola to be more freeze tolerant as they enter the winter season. Coupled with a range of planting dates, the use of PGRs have the potential to expand the planting window for canola and help prevent excessive winter kill. With the use of PGRs and an earlier planting date we hope to improve root development while limiting above-ground biomass accumulation in winter canola. Less crop loss through the winter months could increase yield resulting in benefits to the local economy and improvement of the wheat-canola rotation in Oklahoma and possibly entire the southern Great Plains.

Materials and Methods

In order to evaluate the effects of different plant growth regulators and planting timings on winter canola in Oklahoma, two cultivars (DKW 44-10 and Mercedes) were planted. DKW 44-10 is a glyphosate resistant cultivar and Mercedes is a conventional cultivar. Experiments were conducted at four (DKW 44-10) and two (Mercedes) field locations across Oklahoma in the 2015-2016 cropping season. Additionally, a greenhouse experiment which was repeated, measured only plant growth parameters.

Field Methodology

A strip block design with a factorial treatment structure was used at all field sites. For the all experiments, the main factor was planting date (5 DKW 44-10; 2 Mercedes) and the sub-factor was 9 plant growth regulator treatment. All plots were seeded at 2 kg ha^{-1} with DKW 44-10 or Mercedes using a small plot research drill. Treatments included a control plot for each planting date that received no PGR treatment, and eight PGRs: 61.5 g a.i. ha^{-1} tebuconazole, 123.1 g a.i. ha^{-1} tebuconazole, 11.9 g a.i. ha^{-1} mepiquat chloride, 23.9 g a.i. ha^{-1} mepiquat chloride, 58.1 g a.i ha^{-1} mepiquat pentaborate, 136.3 g a.i. ha^{-1} prohexadione-calcium, 51.3 g a.i. ha^{-1}

metconazole, and 2.3 g a.i. ha^{-1} kinetin + 0.8 g a.i. ha^{-1} gibberellic acid + 1.1 g a.i. ha^{-1} indole butyric acid. Plots were 1.8 meters wide and 7.6 meters long and a 1.5 meter alley between each replication. PGR treatments were applied to plots as each planting dates reached the 4 to 6 leaf growth stage. Applications were made using a CO_2 propelled backpack sprayer and a TeeJet® VS 11003 nozzle delivering 274 L ha^{-1}.

In the 2015-2016 growing season, planting dates varied according to location (Table 1). The first three planting dates of Mercedes at all sites failed, however, planting dates 4 and 5 used seed from a different lot. Therefore, data for Mercedes were only collected from the last two planting dates. Nitrogen fertilizer in the form of urea (46-0-0) was applied at each location: 80 kg ha^{-1} N in fall and 70 kg ha^{-1} N in spring. At all sites, weeds and insects were controlled using commercially available pesticides as needed.

Growing degree days (GDD) were calculated from crop emergence to 4 wk after treatment and averaged across locations by using a base temperature of 0° C (Begna & Angadi, 2016). Population density was taken in a 1 m^2 area at the time of PGR application and again at 4 wk after treatment. Plant height was recorded and plant biomass samples were collected from each plot at 4 wk after treatment. To measure plant height, three random plants were selected and heights averaged. To collect biomass, three random plants were cut at the soil level, fresh weights recorded, then placed in a dryer at 49 °C for 72 hours, removed and dry weights were recorded.

Percent winter kill was assessed using the Canopeo application (Patrignani and Ochsner 2015). To estimate winter kill, Canopeo was used to estimate green canopy cover throughout the winter. Green canopy cover was estimated before (4 Nov.) the first hard freeze and again after (18 Nov.) the first hard freeze and at spring dormancy break (4 Feb.).

Four locations were harvested for yield in the 2015-2016 growing season with a Wintersteiger Classic small plot combine. Seed moisture, protein, and oil content were measured using a PerTen® DA 7200 NIR analyzer. For yield determinations, seed moisture was adjusted to 10% for all grain. DKW 44-10 plantings were not harvested at Lake Carl Blackwell.

Greenhouse Methodology

The greenhouse experiment was conducted using a randomized complete block design with 13 treatments, 4 replications and was repeated. Two cultivars were tested independently of one another: DKW 44-10 and Mercedes.

Seed were planted into 3.8 L pots containing professional potting mix and later thinned to one plant per pot. Canola was treated at carrier volume of 280 L ha^{-1} in a DeVries Generation II Research Track Sprayer at 4 to 6 leaf stage. Treatments included a control that received no PGR treatment and PGRs: 61.5 g a.i. ha^{-1} tebuconazole, 123.1 g a.i. ha^{-1} tebuconazole, 1.9 g a.i. ha^{-1} mepiquat chloride; 23.9 g a.i. ha^{-1} mepiquat chloride, 58.1g a.i. ha^{-1} mepiquat pentaborate, 116.3 g a.i. ha^{-1} mepiquat pentaborate, 136.3 g a.i. ha^{-1} prohexadione-calcium, 272.6 g a.i. ha^{-1} prohexadione-calcium, 51.3 g a.i. ha^{-1} metconazole, 102.6 g a.i. ha^{-1} metconazole, 2.3 kinetin + 0.8 g a.i. ha^{-1} gibberellic acid + 1.1 g a.i. ha^{-1} indole butyric acid, and 4.6 g a.i. ha^{-1} kinetin + 1.6 g a.i. ha^{-1} gibberellic acid + 2.2 g a.i. ha^{-1} indole butyric acid.

The greenhouse was maintained with a 16/8 hour day/night photoperiod and maintained at 17 °C ± 2. Plant height, plant diameter, leaf number and crown diameter were taken weekly for 4 wk after treatment. At 4 wk after treatment above ground biomass and root biomass were collected. To collect biomass data, each pot was soaked with a 5% solution of sodium hexametaphosphate for 12 hr rinsed with tap water, dried for 72 hr in an oven at 49 ° C and then weighed (Van Noordwijk 1993).

Statistical Analysis

SAS software 9.4 was used to analyze all data. Linear mixed models were used to analyze data, including repeated measures. Means were compared using Tukey's pairwise comparisons at $\alpha = 0.05$. Predictive models were used for simple linear regression. Generalized mixed models (Poisson) were used to analyze leaf count of the greenhouse data.

Results and Discussion

Field Experiment

Two cultivars (DKW 44-10 and Mercedes) were field tested and analyzed separately. There was no planting date by treatment interaction for any variable tested at any location at $\alpha = 0.05$. However, for each variable tested, at least one of the experimental factors did have an effect at $\alpha = 0.05$ and each are discussed individually below.

Biomass

The earlier winter canola was planted, the greater the accumulated biomass (Table 2). At planting date 5, DKW 44-10 had lower biomass accumulation than all other planting dates. Accumulated GDD were averaged across each location from emergence to 4 wk after treatment (Table 2). There was a linear correlation (biomass = - 19.087 + 0.0355 GDD) between biomass and accumulated GDD for DKW 44-10 ($R^2 = 0.95$ and p value <0.0001). This correlation was not apparent for Mercedes because of the limited data set. Mercedes planting date 4 had the least amount of biomass accumulation at 4 wk after treatment. The relationship between planting date or GDD and biomass was also reported by Begna & Angadi (2016), where it was reported that it was reported that the number of GDD was significantly affected by planting date in two cropping seasons. There was no biomass response to any PGR treatment at any planting date and no response by cultivar.

Population Density

For both cultivars tested, population density increased as planting dates were delayed into the fall season (Table 2). Soil temperature decreased with each planting date (Appendix table 1). There was a linear correlation of population density to soil temperature (stand count = 719.63 -24.162 soil temperature) with an R^2 of 0.95 and p value of <0.0001. Similar results were found by Benga & Angadi (2016), with higher population densities in early-October planting dates (133 plants m^{-2}) in comparison to mid-Sept. planting dates (128 plants m^{-2}). There was no population density response to any PGR treatment at any planting date and no cultivar response.

Height

Height was affected by planting date and PGR treatment. DKW 44-10 planting dates 2, 3, and 5 were shorter than planting dates 1 and 4. Metconazole reduced plant height of DKW 44-10 and Mercedes by 15% and 25% respectively, when compared to the non-treated. These results are consistent with that of other research in which metconazole applications reduced plant height (Berry and Spink, 2009; Setia et al., 1995)

Winter Hardiness

DKW 44-10 did exhibit differences in canopy cover throughout the winter (Table 4). Planting date 3 had the highest green canopy cover (64%) going into winter but did have green canopy loss reported at spring green-up. Planting dates 1 and 2 had the highest reported green cover at spring green-up at 31 and 32%, respectively; however, there were no significant differences at $\alpha = 0.05$. In the southern Great Plains, similar research has shown that earlier planted canola resulted in larger plants with greater fall vigor and greater winter survival (Holman et al., 2011, Holman et al., 2015, Assefa et al., 2014). Due to the mild winter of the 2015-2016 growing season, there was an increase in green canopy recorded after the first freeze. This freeze

event likely did not cool soil temperatures enough for the canola crop to experience a damaging freeze; therefore, the crop to continued to grow.

Yield, Protein, Oil

Planting date affected yield, protein and oil content of DKW 44-10 (Table 5). Planting dates 3 (2489 kg ha^{-1}), 4 (2523 kg ha^{-1}), and 5(2417 kg ha^{-1}), which are within the recommended planting timing for the region, resulted in higher yields than earlier planted canola. Planting date 5 resulted in the highest protein content (20.4%) and planting date 1 resulted in the highest oil content (44.1%). DKW 44-10 yield, protein or oil content showed no response to PGR treatment. Planting date affected yield of Mercedes (Table 5). Planting date 4 (3185 kg $ha^{-1)}$ resulted in higher yields. Mercedes protein and oil content were not affected by planting date or PGR treatment.

These results are consistent with other research in the region. Holman et al. (2015) reported higher canola yields when winter canola was planted 1 Sept. (2246 kg ha^{-1}) compared to plantings of 25 Aug. (1909 kg ha^{-1}). Begna and Angadi (2016) reported highest seed yields when winter canola was planted in mid-Sept. (2634 kg ha^{-1}) compared to an early-Oct. planting date (1944 kg ha^{-1}). Planting winter canola during the first 3 wk of Sept. provided the most consistent yield response in this research as well as in other previous work (Assefa et al., 2013; Begna & Angadi, 2016; Holman et al., 2011, 2015)

Greenhouse experiment

In the greenhouse experiments, time after planting had an effect on crown diameter, plant height, and leaf number (Appendix Tables 2-4), meaning the plants continued to grow and the plant growth regulator treatments exhibited no effect on the canola.

Conclusion

Based on the results of this experiment, winter canola was produced with higher yields, shorter plants, and less biomass accumulation when planted from September 8 to September 28. Earlier fall planted canola accumulated more biomass, resulting in taller plants, lower population density, and lower winter canola yields. Metconazole applied at 51.3 g a.i. ha^{-1} resulted in shorter plants. Oklahoma had a mild winter in 2015-2016 cropping season; therefore, the winter survival component of this experiment was inconclusive and more research should be done to further investigate the effects of PGRs and planting date on winter survival of winter canola.

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Table 1. Planting dates of DKW 44-10 and Mercedes by location[1]

Planting Date	Cultivar	Location				Days between planting among all locations
		Lamont	Chickasha	Perkins	Lake Carl Blackwell	
1	DKW 44-10	25 Aug.	26 Aug.	28 Aug.	28 Aug.	3
2	DKW 44-10	31 Aug.	3 Sept.	4 Sept.	4 Sept.	5
3	DKW 44-10	7 Sept.	11 Sept.	10 Sept.	10 Sept.	4
4	DKW 44-10	23 Sept.	25 Sept.	24 Sept.	23 Sept.	2
5	DKW 44-10	28 Sept.	30 Sept.	29 Sept.	29 Sept.	2
4	Mercedes	—	25 Sept.	—	23 Sept.	2
5	Mercedes	—	30 Sept.	—	29 Sept.	1

[1] Mercedes planting dates 1, 2, and 3 failed at all locations.

Table 2. The effects of planting dates on winter canola biomass, height, and stand count at five Oklahoma locations.[1,3]

Planting date	Cultivar	Accumulated growing degree days[2]	Biomass	Height	Stand count
			g	cm	m^{-2}
1	DKW 44-10	1493	33^{A}	21^{A}	17^{B}
2	DKW 44-10	1086	21^{B}	19^{BC}	7^{C}
3	DKW 44-10	1018	17^{C}	18^{C}	20^{B}
4	DKW 44-10	857	14^{C}	21^{BA}	63^{A}
5	DKW 44-10	837	7^{D}	19^{BC}	68^{A}
4	Mercedes	857	2^{b}	7^{b}	29^{b}
5	Mercedes	837	2^{a}	8^{a}	34^{a}
p-value	DKW 44-10	-	<0.0001	<0.0001	<0.0001
	Mercedes	-	0.0049	<0.0001	0.0003

[1] Mercedes planting dates 1, 2, and 3 failed at all locations.

[2] Growing degree days accumulated from emergence to 4 wk after treatment using base temp 0 degrees C

[3] Data collected 4 wk after treatment

[4] Means within the same column followed by the same letter were not significantly different as determined by Tukey's t test at $\alpha = 0.05$. Capital letters represent differences for DKW 44-10 and lowercase letters represent differences for Mercedes.

Table 3. Effect of plant growth regulator treatment at 4 weeks after treatment on winter canola height, planting dates pooled[1].

Treatment	Rate	DKW 44-10[2]	Mercedes[2]
	g a.i. ha^{-1}	cm	
Non-treated		51^{A}	21^{a}
Tebuconazole	61.5	50^{AB}	20^{ba}
Tebuconazole	123.1	50^{AB}	20^{ba}
Mepiquat chloride	11.9	50^{AB}	21^{a}
Mepiquat chloride	23.9	50^{AB}	19^{ba}
Mepiquat pentaborate	58.1	51^{A}	19^{ba}
Prohexadione-calcium	136.3	51^{A}	22^{ba}
Metconazole	51.3	43^{B}	16^{b}
kinetin + gibberellic acid + indole butyric acid	2.3 + 0.8 + 1.1	52^{A}	22^{a}
p-value	—	0.024	0.024

[1] Planting dates: week of 25 Aug., 31 Aug, 7 Sept., 23 Sept., and 28 Sept.

[2] Means within the same column followed by the same letter were not significantly different as determined by Tukey's t test at $\alpha = 0.05$. Capital letters represent differences for DKW 44-10 and lowercase letter represent differences for Mercedes.

Table 4. Effects of planting date on percent green canopy of winter canola in Oklahoma[1, 2]

		Green canopy		
Planting date	Cultivar	Before freeze	After freeze	Spring green-up
		%		
1	DKW 44-10	56^{CB}	55^{C}	31^{A}
2	DKW 44-10	55^{CB}	56^{C}	32^{A}
3	DKW 44-10	64^{A}	62^{B}	28^{A}
4	DKW 44-10	56^{B}	67^{A}	29^{A}
5	DKW 44-10	52^{C}	67^{A}	30^{A}
4	Mercedes	43^{a}	61^{a}	29^{a}
5	Mercedes	41^{a}	61^{a}	30^{a}
p-value	DKW 44-10	<0.0001	<0.0001	0.4445
	Mercedes	0.0673	0.6643	0.4446

[1] Mercedes planting dates 1, 2, and 3 failed at all locations.

[2] Means within the same column followed by the same letter were not significantly different as determined by Tukey's t test at $\alpha = 0.05$. Capital letters represent differences for DKW 44-10 and lowercase letter represent differences for Mercedes.

Table 5. Effects of planting date on winter canola yield, protein and oil content, four Oklahoma Locations.[1, 2]

Planting date	Cultivar	Yield	Protein	Oil
		kg ha^{-1}	%	
1	DKW 44-10	2299^{B}	20.1^{BC}	44.1^{A}
2	DKW 44-10	2156^{B}	20.1^{C}	43.7^{B}
3	DKW 44-10	2489^{A}	20.1^{BC}	43.2^{B}
4	DKW 44-10	2523^{A}	20.3^{AB}	42.6^{D}
5	DKW 44-10	2417^{A}	20.4^{A}	42.5^{D}
4	Mercedes	3185^{a}	19.5^{a}	41.8^{a}
5	Mercedes	3056^{b}	19.4^{a}	41.8^{a}
p-value	DKW 44-10	<0.0001	<0.0001	<0.0001
	Mercedes	0.0401	0.411	0.8171

[1] Mercedes planting dates 1, 2, and 3 failed at all locations.
[2] Means within the same column followed by the same letter were not significantly different as determined by Tukey's t test at $\alpha = 0.05$. Capital letters represent differences for DKW 44-10 and lowercase letter represent differences for Mercedes.

CHAPTER II

GIBBERELLIC ACID SEED TREATMENT ON WINTER CANOLA IN THE SOUTHERN GREAT PLAINS

Running title: GA_3 Seed treatment on Winter Canola

ABSTRACT

Winter canola (*Brassica napus* L.) yield in the southern Great Plains is limited by crop establishment and fall growth. A greenhouse rate titration was conducted on two cultivars of winter canola (DKW 44-10 and Mercedes) to determine biomass and root growth as well as, a field experiment to evaluate the effects of Gibberellic Acid (GA_3) seed treatments paired with foliar plant growth regulator (PGR) treatments on winter canola growth, yield, oil and protein content. Both cultivars tested had the highest amount of growth at 45 days after planting when 300 ppm GA_3 was applied to canola seed, therefore, this rate was used for seed treatments in the field experiments. The synergistic effect of GA_3 seed treatment with foliar PGR applications on winter canola was investigated at two sites in Oklahoma. GA_3 (300 ppm) seed treatments increased biomass (54%) and root production (40%). Foliar applications of metconazole (51.3 g a.i. ha^{-1}) increased root growth by 40%. The increase in winter canola fall growth did not result in an increase in final yield, protein or oil content.

Introduction

Winter canola (*Brassica napus* L.) production of in the southern Great Plains can be limited by crop establishment and winter survival (Holman et al., 2015; Conley et al., 2004). Establishing a winter canola crop in the southern Great Plains is difficult without irrigation due to the dry soil conditions and shallow planting requirement of winter canola (Holman et al., 2015). The time from planting to seedling establishment is of considerable importance in crop production and has major impacts on plant growth, final yield, and post-harvest seed quality (Wurr and Fellows, 1985).

Germination is the most sensitive stage for water deficiency stress and gibberellic acid (GA_3) has been shown to induce drought tolerance of various crops (Chakrabarti and Mukherji, 2003, Z Li. et al. 2010, W. Zhang and L.V. Gusta 2010, R. Roychowdhury et al., 2012, and D. Tsai and R.N. Arteca 1985.) Compared with no GA_3 seed treatment, canola seed treated with 300 ppm Ga_3 showed significant increases in drought tolerance (88%), seedling fresh weight (43 %), and hypocotyl length (73 %) (Z Li. et al., 2010). Priming seed with growth substances not only enhances drought tolerance, germination, and emergence but also improves plant growth and final yield under non-stressed and salt-stressed conditions (Ashraf and Foolad 2005). For example, grain yield of pearl millet (*Pennisetum glaucum* L.) was improved under saline conditions by treating seed with 50 mg l^{-1} GA and plant growth of bhendi (*Abelmoschus esculentus*) was improved under sodic soil conditions by treating seed with GA (Vijayaraghavan, 1999).

Successful overwintering of winter canola depends mainly on fall growth. Before the winter, canola should create sufficient above ground biomass (6 to 8 leaves) and root mass, but on the other hand should not be overgrown (Holman et al., 2011; Balodis and Gaile, 2010; Conley et al., 2004). If above ground biomass accumulates in excess, it could result in severe cold injury or winter kill (Boyles et al., 2004). The potential for vigorous fall growth of canola increases in the southern Great Plains and could result in greater susceptibility to winter kill or premature bolting in the fall season (Alford, 2003; Assefa et al., 2014; Boyles et al., 2004). The exposed growing point of canola seedlings makes the crop more susceptible to freeze damage; however, Boyles et al. (2004) reported that canola plants with three to four leaves are very tolerant to freezing conditions.

Manipulation of plant growth during fall may allow producers to improve plant hardiness for winter. Plant growth regulators (PGRs) are not widely used, and in some countries they are not registered for use on canola; however, there is potential to manipulate the crop physiology and plant structure through the use of plant growth regulators (Alford, 2003). Based on previous research, canola responds well to the application of PGRs (Becka et al., 2004; Kirkland, 1992; Pits et al., 2008; Morrison et al., 1992; Child et al., 1993; Berry and Spink, 2009). Many previous studies have been conducted on the use of PGRs in canola for crop uniformity but little research has been done investigating fall applications to improve winter hardiness of canola (Becka et al., 2004; Kirkland, 1992; Pits et al., 2008; Morrison et al., 1992; Child et al., 1993; Berry and Spink, 2009). In these experiments, we evaluated the field effect of exogenously applied GA_3 on winter canola germination, and investigated the appropriate seed treatment concentration for winter canola.

The objectives of this research are to evaluate the biological effects of GA_3 seed treatments alone on winter canola in Oklahoma and the effects of GA_3 seed treatments paired with fall-applied foliar plant growth regulator treatments on plant growth, winter hardiness, and yield. We hypothesize that the GA_3 seed treatments paired with PGRs can improve the winter survival of canola in Oklahoma by improving crop establishment and early fall growth. The use of GA_3 seed treatments and PGRs may improve root development while limiting above ground biomass accumulation in winter canola.

Materials and Methods

Rate Titration Methodology

An experiment was conducted in a greenhouse to determine the optimal GA_3 concentration for seed treatment on two canola cultivars: DKW 44-10 and Mercedes. A

randomized complete block design with six treatments and four replications was used and the experiment was repeated. For seed treatments, seed were hydrated with a five weight/volume (w/v) of GA_3 solution and placed in a tumbler for 30 min. The seed were then dried on absorbent paper for 8 hr at 23 ± 1 °C. Treatments include a non-treated check that received no GA_3 seed treatment, and GA_3 concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm. Seed were planted in 3 L pots with professional potting media. A growth scale was used to rate plants weekly for 6 wk (Table 1). At the end of 6 wk, plants were harvested. Root and shoot length, crown diameter, and leaf number were collected. Plants were harvested by washing roots in a 5% sodium hexametaphosphate solution, rinsed with tap water, separated at the crown, and placed in a dryer at 49 °C for 72 hr (Van Noordwijk 1993). When removed from the dryer, dry weights were recorded.

Field Methodology

An experiment was conducted at two sites in Oklahoma: Chickasha and Perkins. A split block design with a 2 X 5 factorial treatment structure was used at all sites. Blocks were split planted with GA_3 treated seed and non-treated seed. The main factor was seed treatment (2) and the subfactor was PGR treatment (5). Treatments included a non-treated check that received no foliar PGR treatment, tebuconazole at 123.1 g a.i. ha^{-1}, prohexadione-calcium at136.3 g a.i. ha^{-1}, metconazole at 51.3 g a.i. ha^{-1}, and kinetin + gibberellic acid + indole butyric acid at 2.3 + 0.8 + 1.1 g a.i. ha^{-1}.

Winter canola cultivar DKW 44-10 seed was treated with GA_3 at a concentration of 300 ppm. Seeds were surface washed with tap water for 5 min and then thoroughly rinsed with distilled water. For seed treatments, seeds were hydrated with a five weight/volume (w/v) of GA_3 solution and placed in a rotary drum tumbler for 30 min the seed was then dried on absorbent

paper for 8 hr at 23 ± 1 °C. Seed were planted on 24 Sept. in 3 by 8 m plots at a seeding rate of 2 kg ha^{-1}. A foliar PGR treatment was applied to each plot at the 4 to 6 leaf growth stage. Applications were made using a CO_2 propelled backpack sprayer with a TEEJET® 11003 nozzle applying 274 L ha^{-1}. Population density was taken 4 wk after emergence. At approximate partitioning timing, three random plants per plot were excavated. Roots were washed in the field using tap water and plants were separated at the crown and dried in a drying oven at 49 °C for 72 hr. When removed from the dryer, dry weights were recorded.

Experiments were direct harvested for yield with a Wintersteiger Classic small plot combine. Seed moisture, protein, and oil content were analyzed using a PerTen® DA 7200 NIR analyzer. Due to environmental conditions at harvest, only the Chickasha site was harvested. For yield determinations, all grain moisture was adjusted to 10% moisture.

Statistical Analysis

SAS software 9.4 was used to analyze all data. Linear mixed models were used to analyze data, including repeated measures. Means were compared using Tukey's pairwise comparisons at $\alpha = 0.05$. Generalized mixed models (Poisson) were used to analyze leaf count of the greenhouse data.

Results and Discussion

Rate Titration

An experiment was performed to correctly identify the most effective concentration of GA_3 applied to the seed of two different winter canola cultivars adapted to Oklahoma, DKW 44-10 and Mercedes. A rate titration of GA_3 concentrations was performed on both cultivars and each was analyzed independently of one another.

DKW 44-10 and Mercedes plants responded to the GA_3 seed treatments, and resulted in the most growth when treated with 300 ppm (Figures 3 and 4), which corresponds to rates used in other work (Li et al., 2010). Higher rates of GA_3 resulted in a reduction of plant growth for both cultivars (Figures 3 and 4). These results were not expected and believed to be due to a malfunction in greenhouse maintenance software, resulting in inadequate lighting and temperature.

Field Experiment

GA_3 seed treatment resulted in a 54% increase of canola biomass production (Figure 1). GA_3 seed treatment resulted in a 40% increase of root production (Figure 1). Winter canola treated with metconazole resulted in a 40% increase in root production when compared to all other treatments, including the non-treated check (Figure 2). Berry and Spink (2009) also reported similar results with a 25% increase in root production when metconazole was applied at 50 L ha^{-1}.

Winter canola biomass exhibited no response to foliar PGR treatment at $\alpha = 0.05$. GA_3 seed treatment and foliar PGR treatment did no improve winter canola yield, protein content, and oil content at $\alpha = 0.05$ (Table 3). These results were not expected as previous research has reported foliar PGR treatments altering canopy height, as well as, resulting in yield increases (Balodis et al., 2011; Berry and Spink, 2009; Morrison et al., 1992; Scarisbrick et al., 1985; Setia et al., 1995).

Conclusion

Due to the mild winter in Oklahoma in the 2015-2016 cropping season it is not known how these seed and foliar treatments will effect winter survival of winter canola in Oklahoma. The results of this study suggest that there is a benefit from GA_3 seed treatments for successful

crop establishment and that fall foliar applications of metconazole can increase root growth of winter canola. More research should be conducted to identify if there is a yield benefit to these applications and if there could be added winter hardiness.

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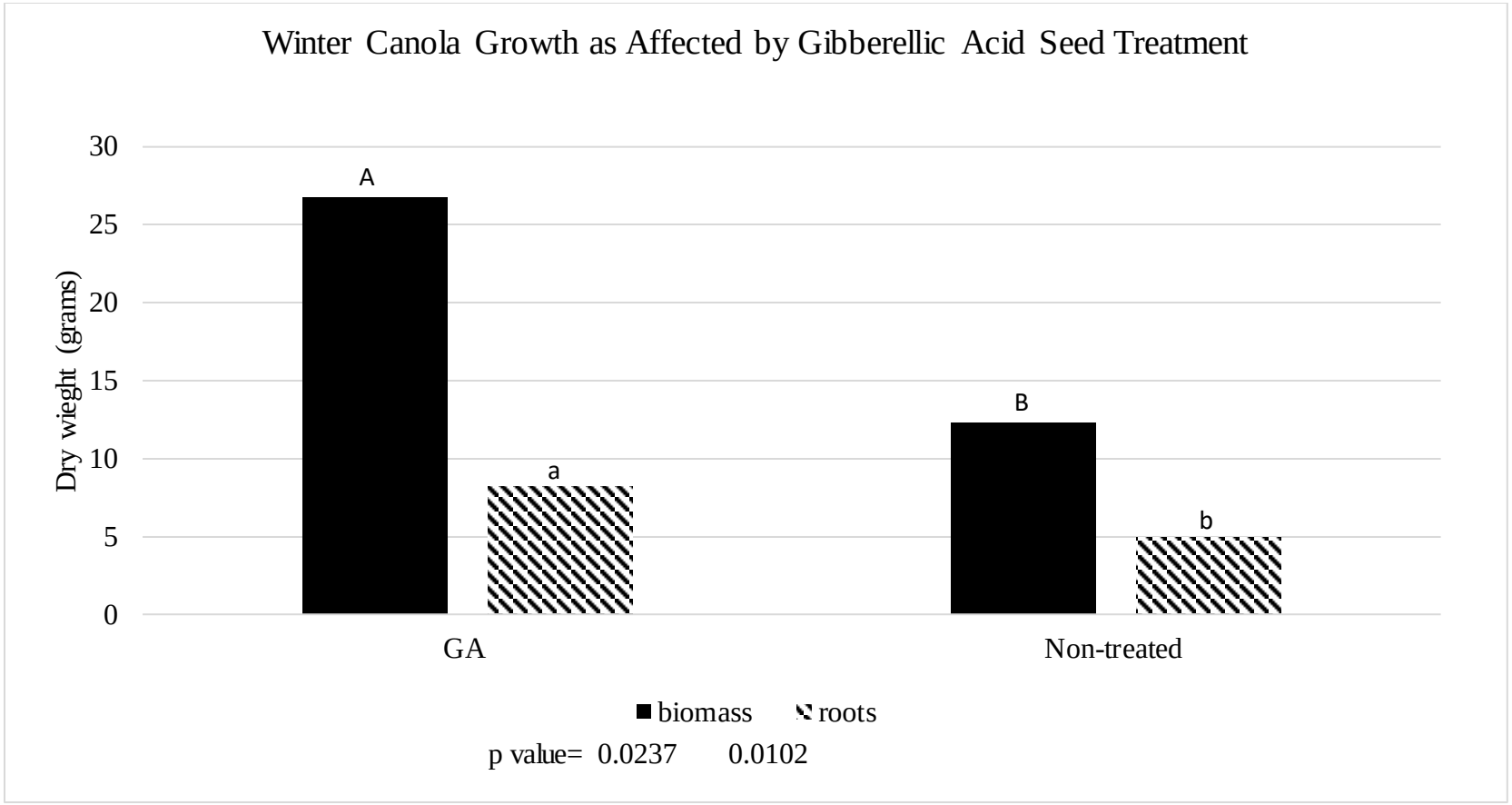

Figure 1. Effects of gibberellic acid seed treatments on winter canola growth.

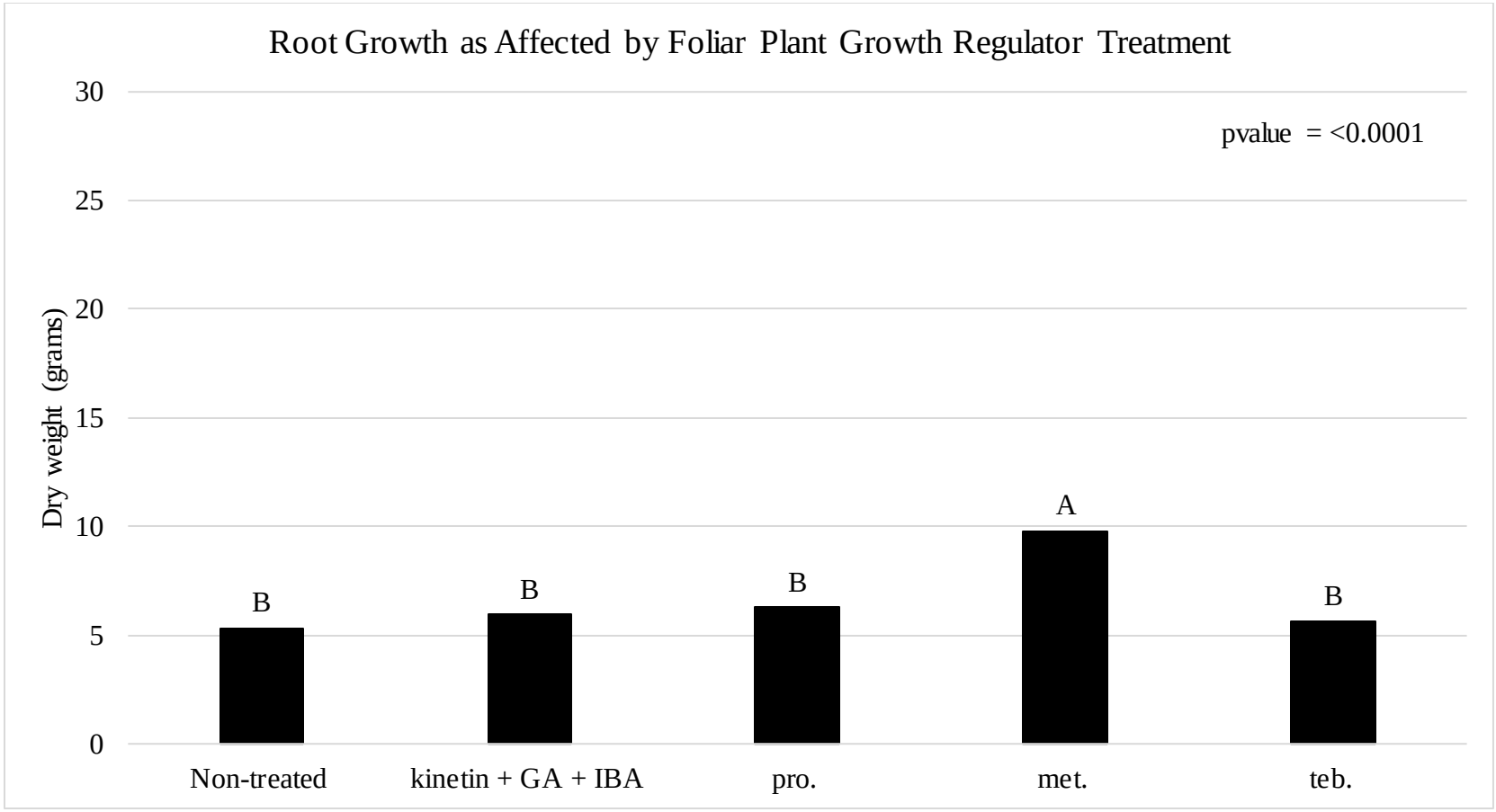

Figure 2. Effects of fall-applied PGRs on winter canola root growth.

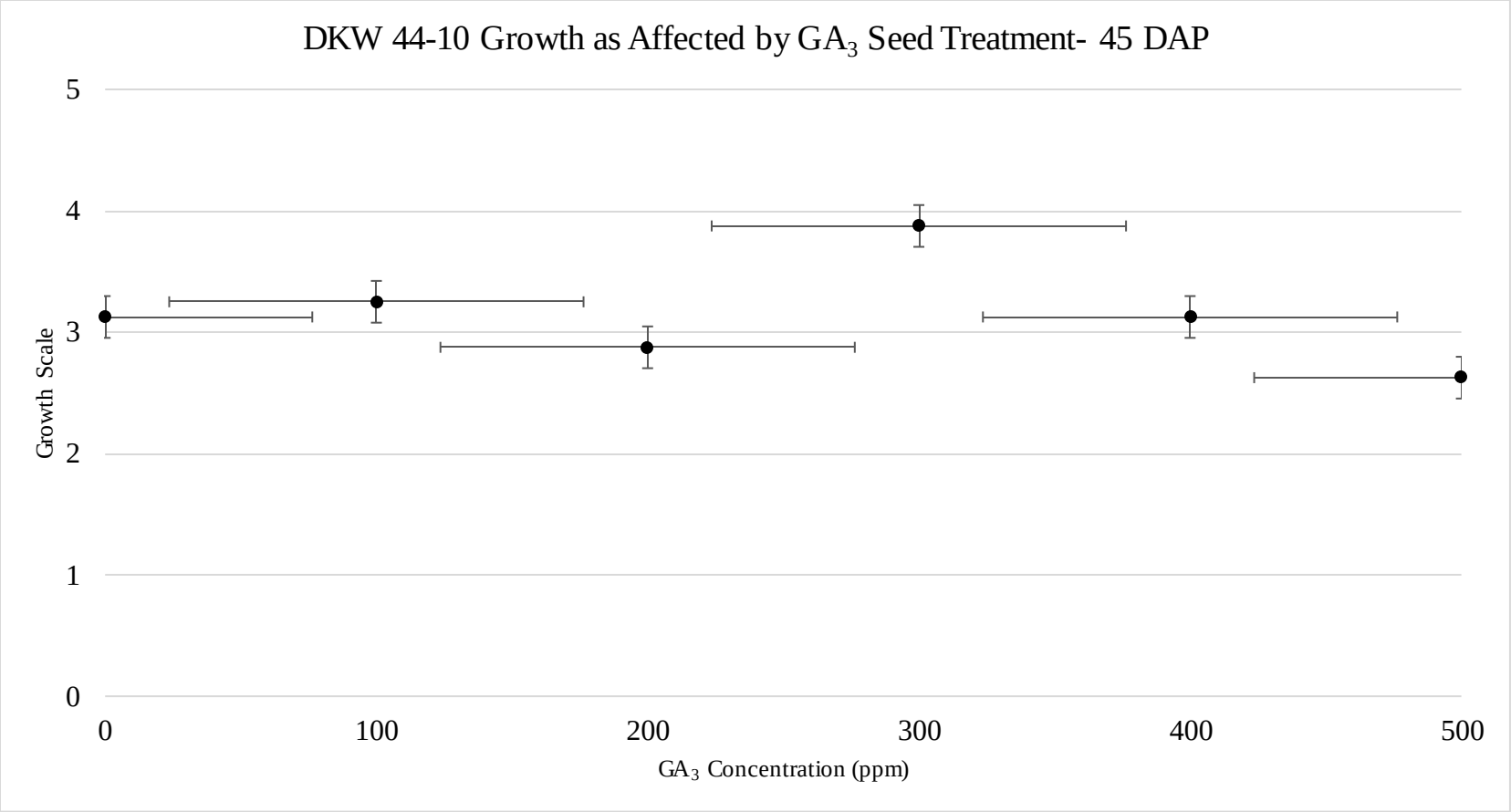

Figure 3. DKW 44-10 response to GA_3 seed treatments.

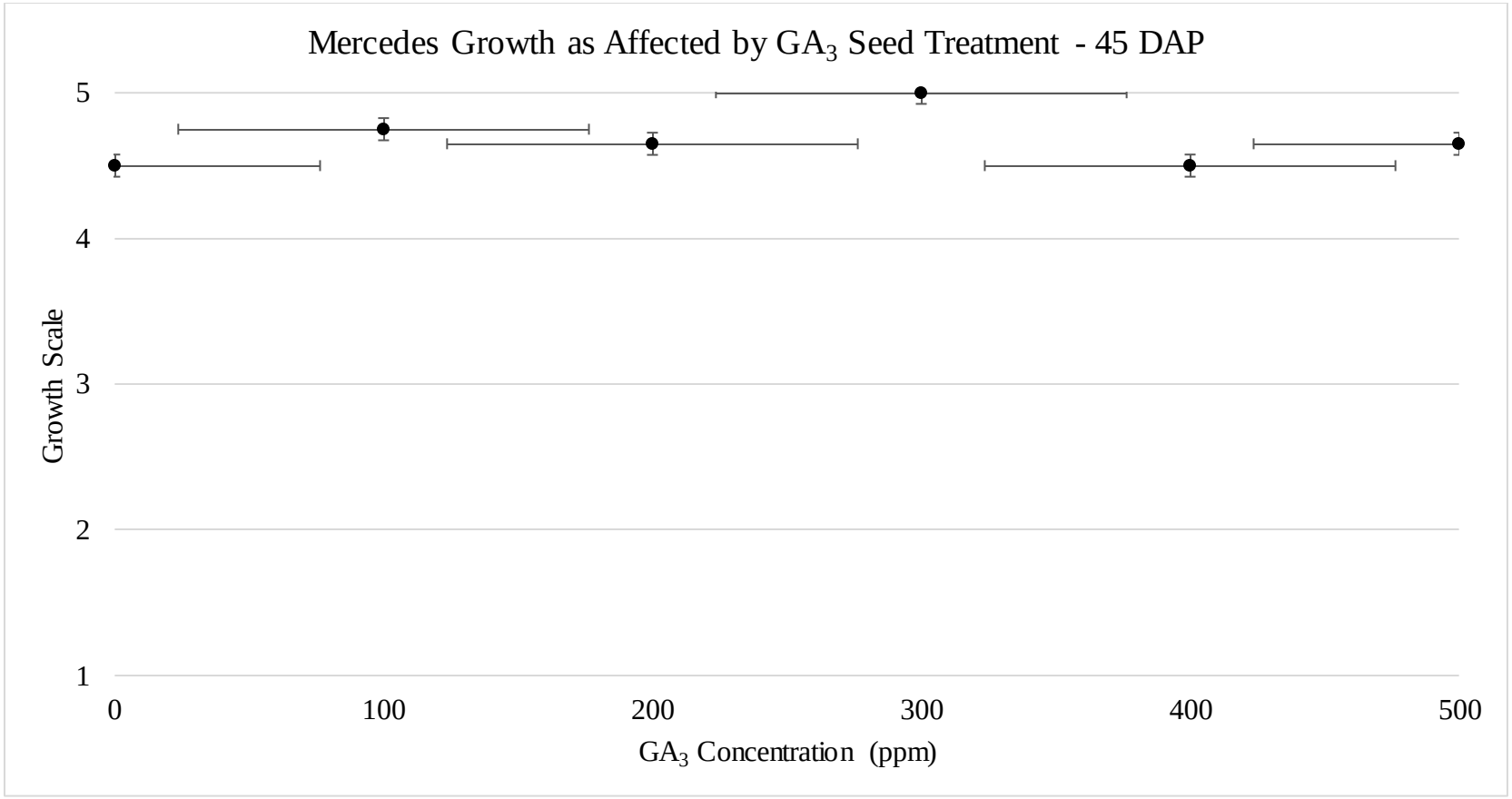

Figure 4. Mercedes response to GA_3 seed treatments.

Table 1. Rating scale and growth stages

rating	growth stage
0	not germinated
1	germinated
2	cotyledons fully expanded
3	1-2 leaf
4	3-5 leaf
5	6-8 leaf

Table 2. Effects of fall-applied PGRs on winter canola root production.[1]

Foliar Treatment	Yield	Protein	Oil
	kg ha^{-1}	%	
Non-treated	2348^{A}	18.5^{A}	46.2^{A}
Tebuconazole	2236^{A}	18.5^{A}	46.2^{A}
Prohexadione-calcium	2241^{A}	18.2^{A}	46.5^{A}
Metconazole	2451^{A}	18.3^{A}	46.8^{A}
kinetin + gibberellic acid + indole butyric acid	2269^{A}	18.4^{A}	46.7^{A}
p-value	0.3502	0.2098	0.2375

[1] Means within the same column followed by the same letter were not significantly different as determined by Tukey's t test at $\alpha = 0.0$.

Table 3. Effects of GA_3 seed treatment on winter canola yield, protein, and oil content.[1]

Seed treatment	Yield	Protein	Oil
	kg ha-1	%	
GA_3	2395[A]	18.3[A]	46.5[A]
Non-treated	2323[A]	18.4[A]	46.4[A]
p-value	0.6193	0.3221	0.2030

[1] Means within the same column followed by the same letter were not significantly different as determined by Tukey's t test at $\alpha = 0.0$.

APPENDICES

Table 1. Soil temperature and population density of winter canola by planting dates

Planting Date	Population Density	Soil Temperature
	m^2	°C
1	17	29
2	7	30
3	20	29
4	63	27
5	68	27

Table. 2. Effects of PGR treatments on DKW 44-10 winter canola crown diameter, height, leaf number, root and biomass growth in the greenhouse.

Treatment	rate	Crown diameter	Height	Leaf	Root (dw)	Biomass (dw)
	g a.i. ha^{-1}	cm		count	g	
non-treated		1	23	9	6	13
tebuconazole	61.5	1	21	10	4	13
tebuconazole	123.1	1	20	9	3	11
mepiquat-chloride	1.9	1	22	10	6	13
mepiquat-chloride	23.9	1	21	10	5	12
mepiquat-pentaborate	58.1	1	22	9	3	13
mepiquat-pentaborate	116.3	1	23	11	5	13
prohexadione-calcium	136.3	1	23	11	5	15
prohexadione-calcium	272.6	1	22	10	4	13
metconazole	51.3	1	21	10	4	12
metconazole	102.6	1	23	11	6	12
kinetin + gibberellic acid + indole butyric acid	2.3 + 0.8 + 1.1	1	20	13	5	15
kinetin + gibberellic acid + indole butyric acid	4.6 + 1.6 + 2.2	1	20	12	4	12
p-value	-	0.4983	0.2008	0.2379	0.3030	0.4572

Table 3. Effects of PGR treatments on Mercedes winter canola crown diameter, height, leaf number, root and biomass growth in the greenhouse.

Treatment	Rate	Crown diameter	Height	Leaf	Root (dw)	Biomass (dw)
	g a.i. ha^{-1}	cm		count	g	
non-treated		1	24	8	7	8
tebuconazole	61.5	1	27	8	5	7
tebuconazole	123.1	1	23	8	8	9
mepiquat-chloride	1.9	1	23	8	7	7
mepiquat-chloride	23.9	1	24	7	6	6
mepiquat-pentaborate	58.1	1	24	8	7	8
mepiquat-pentaborate	116.3	1	23	8	7	8
prohexadione-calcium	136.3	1	25	8	6	7
prohexadione-calcium	272.6	1	26	8	8	9
metconazole	51.3	1	22	8	7	9
metconazole	102.6	1	25	9	7	7
kinetin + gibberellic acid + indole butyric acid	2.3 + 0.8 + 1.1	1	24	8	8	9
kinetin + gibberellic acid + indole butyric acid	4.6 + 1.6 + 2.2	1	25	8	8	8
p-value	-	0.1415	0.4818	0.1712	0.1548	0.1855

Table 4. Effect of time on winter crown diameter, height, and leaf number in the greenhouse[1].

Week after treatment	Cultivar	Crown diameter	Height	Leaf
		cm		count
1	DKW 44-10	1^{D}	9.^{D}	2^{C}
2	DKW 44-10	1^{C}	18^{C}	3^{B}
3	DKW 44-10	1^{B}	27^{B}	3^{B}
4	DKW 44-10	2^{A}	32 ^{A}	4^{A}
1	Mercedes	1^{c}	21^{b}	2^{d}
2	Mercedes	1^{b}	21^{b}	3^{c}
3	Mercedes	1^{b}	21^{b}	3^{b}
4	Mercedes	2^{a}	36^{a}	4a
p-value	DKW 44-10	<0.001	<0.001	<0.001
	Mercedes	<0.001	<0.001	<0.001

[1] Means within the same column followed by the same letter were not significantly different as determined by Tukey's t test at $\alpha = 0.05$. Capital letters represent differences for DKW 44-10 and lowercase letter represent differences for Mercedes.

VITA

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