

PHYTOREMEDIATION OF SOIL PHOSPHOROUS  
WITH CRABGRASS

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PHYTOREMEDIATION OF SOIL PHOSPHOROUS WITH  
CRABGRASS

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## CHAPTER I

### PHYTOREMEDIATION OF SOIL PHOSPHOROUS WITH CRABGRASS

#### ABSTRACT

Nutrient buildup in pastures from repeated animal manure application may increase soil phosphorus (P) and contribute to eutrophication and water quality deterioration. Options to remove excess nutrients in pastures have been limited in the past. The objective of this 2-yr study was to evaluate the potential of crabgrass (*Digitaria ciliaris*) to remove excess soil P from nutrient loaded soils. Red river crabgrass was planted in boxes containing Dennis, Richfield, and Kirkland soils in a greenhouse in 2010 and under ambient conditions in 2011. Ten years before this experiment, each soil received four different rates of commercial P fertilizer to raise soil test P to elevated levels. Average Mehlich-3 phosphorus (M3P) at the beginning of this experiment ranged from 57.0 to 836 mg kg<sup>-1</sup>. The experiment was a randomized block design with 12 treatments (three soils and four P levels) and three replications repeated for two growing seasons. The average biomass yield of crabgrass ranged from 9.90 to 14.2 Mg ha<sup>-1</sup> with an overall average of 12.2 Mg ha<sup>-1</sup> in 2010 and from 6.8 to 13.4 Mg ha<sup>-1</sup> with an overall average of 10.7 Mg ha<sup>-1</sup> in 2011. The harvested biomass contained an average of 0.45% P and 2.26% nitrogen (N) in 2010 and 0.37% P and 1.45% N in 2011. Therefore, the

crabgrass removed an average of 49.1 kg P ha<sup>-1</sup> and 237 kg N ha<sup>-1</sup> per year. In addition, the concentration of P in the grass and P removed from the soil increased as STP increased although the biomass was not affected by STP. Crabgrass can serve as a good quality hay and as an effective plant for removing nutrients from soils.



## INTRODUCTION

In areas of intensive livestock and poultry production, soils may be enriched in phosphorus (P) due to long-term application and, in some cases, over-application of animal manure. Historically, the rates of manure application were based on the nitrogen (N) requirement of the crop. The amount of P in the soil or the requirement of P by plants was typically not considered when deciding manure application rates. This practice has resulted in a significant increase in soil test phosphorus (STP) since the N/P ratio of manure is typically lower than what is required by plants (Pote, et al, 1996). For example, the N/P ratio of poultry litter is about 2:1, but the N/P ratio in bermudagrass is about 4:1. Hence, fertilizing bermudagrass with poultry litter based on the agronomic N need will result in over applying P. Over time, this practice may saturate the soil's P sorption capacity near the soil surface, causing an increase in STP which has been shown to increase dissolved P in runoff (Kingery et al., 1994; Sharpley et al., 1994; Pautler and Sims, 2000). According to the U.S. Environmental Protection Agency (U.S. EPA, 2000), agriculture is the leading source of impairment to rivers and streams in the United States, and nutrients are the highest percentage of pollutants contributing to water quality deterioration. Soil P is transported to sensitive rivers and streams as particulate P and dissolved P during runoff events. Particulate P includes P sorbed to soil particles and contributes to 75-95% of P lost from cultivated land (Sharpley et al., 1994). Dissolved P is mostly readily available for biological use in aquatic ecosystems (Nurnberg and Peters, 1984) and is the predominant form of P lost in runoff from pastures. When introduced to aquatic ecosystems, excess nutrients, especially nitrogen and phosphorus, accelerate the eutrophication process. The influx of nutrients stimulates algae and aquatic plant growth,

resulting in low dissolved oxygen when these organisms die and are decomposed by microorganisms. This hypoxic condition has occurred in the Gulf of Mexico and many other aquatic systems. Eutrophication impairs water quality for recreational activities, drinking water, and industry costing cities money and resulting in other environmental problems. Therefore, studies have been conducted and better management practices have been implemented for reducing the amount of non-point source (NPS) pollutants from agricultural sources.

Available best management practices (BMPs) that are effective for reducing NPS pollutants, that are both financially feasible and easily integrated into existing production systems by agricultural managers, are very limited. According to Logan (1990), BMPs can be classified as structural, cultural or managerial practices. Structural practices are aimed at reducing runoff and erosion through terraces, filter strips, water retention basins, etc. Cultural practices include protecting the soil surface by including cover crops, reduced or no tillage operations, and stream bank protection. Even under these practices agricultural fields with high soil P may continue to lose excess P (Mueller et al., 1984; Zeimen et al., 2006). Thus, management practices that include fertilizer, manure, and pesticide application and storage plans are a critical component of BMPs. In 2008, the U.S. EPA passed regulations that require the majority of animal feeding operations in the U.S. to implement comprehensive nutrient management plans (CNMPs) (U.S. EPA, 2008). Such plans typically include soil, forage, and manure testing, and BMP implementation to ensure that the application of fertilizer and/or manure will be economical for the producer, while also avoiding unnecessary nutrient application. Nutrient management plans benefit the producer by ensuring the amount of nutrients

applied is adequate for plant growth, while also protecting water resources. However, implementing some of these practices (e.g., buffer strips, riparian zone protection) may decrease the amount of land available for crop production and increase the cost of implementation, thus negatively impacting the producer. Research has also been conducted on reducing the loss of nutrients from manure by incorporating, rather than surface applying, manure and using soil amendments that have a high capacity to absorb P. Amending broiler litter with alum (aluminum sulfate) has been shown to reduce the amount of soluble P in runoff (Moore and Miller, 1994; Peters and Basta, 1996; Moore et al., 2000). These practices are intended to reduce the effects of NPS agricultural pollution on the environment; however, cases may exist where the soil is highly elevated in nutrient levels and most practices are not cost effective.

Another option is to remove excess nutrients from the soil with plants in order to minimize the nutrient loss potential. This process, commonly referred to as phytoremediation, reduces the source of the contaminants. Selected plants are used to take up nutrients from the soil; the above ground part is then harvested and removed from the site. If managed properly, this practice can be a cost effective and environmentally sound method for conserving soil and water resources as well as providing farmers with needed hay for livestock. Phytoremediation is often associated with the uptake of heavy metals from soils and is being utilized more readily because of its low cost relative to other remediation technologies such as soil removal (Salt et.al 1995; Ryan, 2006; Cook et. al, 2009). Recently, phytoremediation has been used to alleviate soils of excess nutrients that could be problematic to the environment. Delorme et al. (2000) assessed the ability of 12 different plants to accrue P from the soil. The plant species that removed

the most P from the soil was whole corn plants (*Zea Mays*) and Indian mustard (*Brassica juncea*) with P uptake of 114 and 108 kg ha<sup>-1</sup> under favorable conditions, respectively.

Since harvesting whole corn plants occurs only once a season, research has been conducted on forage grasses to determine the amount of P uptake. The P removed is dependent on the amount of biomass produced (Eilers, 1998). Studies have shown that forage grasses have a good potential to remove P from P enriched soils due to high biomass yields, multiple harvests, and high tissue P concentrations (Evers, 2002; Read et al., 2007). Brink et al. (2004) reported that hybrid bermudagrass (*Cynodon dactylon*) could remove 50 to 60 kg P ha<sup>-1</sup> yr<sup>-1</sup> depending on the yield and tissue P concentration. However, a study by Evers (2002) showed that ryegrass (*Lolium*) contained almost twice the tissue P content compared to that of bermudagrass. Sistani (2003) studied the potential of ryegrass and crabgrass P uptake from the soil. Their study evaluated 5 different cultivars of ryegrass grown in the winter followed by Red River crabgrass grown on a Ruston silt loam with a STP > 700 mg kg<sup>-1</sup>. The results of their study indicated that P removal by crabgrass was considerably higher than the removal by ryegrass with P uptakes ranging from approximately 32 to 41 kg ha<sup>-1</sup> yr<sup>-1</sup>. Thus, crabgrass has a great potential to lower soil P as well as serve as a high quality forage crop. However, the potential of using crabgrass needs to be studied in different soils with varying soil test P levels

Crabgrass is an annual grass and often considered to be a noxious weed in many farming systems and lawns; however it has superior nutritive quality to some other warm season perennial grasses. Crabgrass must be planted each season or supported from volunteer plants. It can be easily managed to produce volunteer plants to emulate

perennial forage by leaving seed strips to allow seed drop. Crabgrass provides high quality summer forage, superior protein content, and palatability. Dalrymple et al. (1999) found that on average crabgrass had crude protein of 15.2% and with 70% of harvests having 14% crude protein or higher. Crabgrass is highly responsive to N fertilizer; but nitrate toxicity ( $>5000$  ppm  $\text{NO}_3$ ) may occur if nitrogen fertilization is not managed properly (Teutsch et al., 2005). Crabgrass can be easily incorporated into both double cropping and mixed forage systems and is often used for soil conservation and environmental management (Ball, et al., 2002). The objective for this study was to determine the ability of crabgrass to remove P from three different soils with different soil test P levels.

## MATERIAL AND METHODS

Prior to this greenhouse study, three soils were collected for a rainfall simulation study in June 2000 (Davis et al., 2005). The soils were collected from three different agricultural research stations located in eastern, central, and western Oklahoma. The three soil series are representative of different climates, geographical regions, and major land use area of Oklahoma (USDA-NRCS, 2010). The soils included: Richfield series (Fine, smectitic, mesic Aridic Argiustolls) collected from Goodwell, Dennis series (Fine, mixed, active, thermic Aquic Argiudolls) collected from Haskell, and Kirkland series (Fine, mixed, superactive, thermic Udertic Paleustolls) collected from Stillwater, Oklahoma (Figure 1.1). After collection from the surface (0 to 15 cm) in 2000, the field moist soil was crushed with a shovel, sieved through a 19 mm sieve and homogenized using an industrial mortar mixer. The soils were mixed with four rates of diammonium phosphate (DAP) fertilizer to raise soil test P (STP) to four different levels (Davis et al., 2005). The soils went through several wet and drying cycles and were used for a rainfall simulation study in 2001. After the study, the soils were stored in a greenhouse for nine years until the start of this experiment in the spring of 2010.

For this phosphorus removal study, the soils were placed in 48 x 96 x 25 (WxLxH) cm plastic boxes with drain holes in the bottom. The 36 boxes consisted of the three soils with four levels of increasing STP of each soil and three replications. The treatments, soil and STP, were randomly arranged with three replications (in three rows) (Figure 1.2). Initial soil tests showed variable amounts of nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and ammonium nitrogen ( $\text{NH}_4\text{-N}$ ); therefore, the boxes were saturated with water in order to

leach out the excess amounts of nitrogen (N) in some boxes so that all boxes would start with a similar available N content.

### *Soil Analysis*

Before planting crabgrass, a soil test was performed for each box. Six cores at a depth of 15 cm, or the actual depth of the soil if it was less than 15 cm were removed from each box and mixed in a bucket to form a composite sample. Samples were dried overnight at 65°C and ground to pass through a 2 mm sieve. The samples were analyzed for pH, extractable P, K and N, and water soluble P. The Mehlich-3 extractable P (M3P) and K were measured by extracting a 2.0 g sample with 20 mL of M3 solution (0.015 M  $\text{NH}_4\text{F}$ , 0.2 M  $\text{CH}_3\text{COOH}$ , 0.25 M  $\text{NH}_4\text{NO}_3$ , 0.013 M  $\text{HNO}_3$ , 0.001 M EDTA) and shaking for 5 min (Mehlich, 1984). The P and K in the extracts were analyzed by an inductively coupled plasma-atomic emission spectroscopy (ICP-AES). The soil pH was measured by using a combination electrode in a 1:1 soil to water suspension (Thomas, 1996). Nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) and ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) were measured using 2.5 g of soil and 10 mL of 1.0 M KCl and shaking for 15 min. Then  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  in the extracts were determined using a Lachat Quickchem 8000 automated flow-injection analyzer (Mulvaney, 1996). After completion of the last crabgrass harvest, the soils were sampled again in the same manner as pre-plant. In addition to the above analyses, water soluble P (WSP) was also determined by adding 20 mL of 0.01 M  $\text{CaCl}_2$  to 2 g of soil and shaking for 30 min (Self-Davis et al., 2000). The extracts were filtered (Whatman #42) and analyzed for P using ICP-AES. Soil texture was determined by the hydrometer method (Gee and Baude, 1986)

### *Soil Preparation, Planting, and Harvesting*

The boxes containing soils with pH of 5.0 or less were limed at a rate of 2223 kg ha<sup>-1</sup> ECCE (effective calcium carbonate equivalent) (i.e. 240.6 g/box) to establish a suitable pH range for crabgrass. According to Dalrymple et al. (1999) an ideal soil pH for crabgrass production is between 6.0 to 7.2. Although the soils were leached once, different amounts of available N existed among boxes and were credited so that the sum of pre-plant residual N and N fertilizer equaled 168 kg ha<sup>-1</sup>. Nitrogen fertilizer was applied at planting and after each harvest. Red River Crabgrass was planted in April 2010 and May 2011 at approximately 5.56 kg seed ha<sup>-1</sup> (0.35 g per box) by broadcasting then mixing to a depth of about 1.25 cm. The crabgrass was irrigated as needed and each box was entirely harvested four times in 2010 and three times in 2011 using hand scissors at approximately five cm above soil surface.

After every harvest the crabgrass was dried at 85°C for at least 24 h for biomass calculation. Representative subsamples were ground using a mechanical grinder for chemical analyses.

### *Forage Sample Analysis*

The forage samples were analyzed for total N and mineral contents. The mineral analysis was achieved by acid digestion (Jones and Case, 1990), in which 10.0 mL of concentrated trace metal grade HNO<sub>3</sub> was added to 0.50 g of plant sample and pre-digested for one hour in the HotBlock™ Environmental Express block digester. The sample was then heated to 115°C for 2 h and 15 min. The digests were analyzed for P and other minerals by ICP-AES. To ensure quality, one control sample with known



ranges was included for every 20 plant samples. In addition, a standard reference material (SRM 1515 - Apple Leaves, National Institute of Standards and Technology) was also included to ensure data quality. The total N content of the plant sample was analyzed using a LECO TruSpec Carbon and Nitrogen Analyzer (Jones and Case, 1990).

### *Statistical Analysis*

Statistical analysis was performed using PC SAS v. 9.2 (SAS Institute, 2009). Simple effects were analyzed with the PROC MIXED procedure. The LSMEANS statement and the SLICE option were used to analyze the effects of one factor for a fixed level of year on various factors. Linear contrasts were used for the numeric rate variable for P levels. Treatment effects were reported significant at an alpha value of 0.05.

## RESULTS AND DISCUSSION

### *Mehlich 3 P and Water Extractable P*

Soil samples were collected from each box before planting and after the last harvest in 2010 and 2011. The initial average M3P ranged from 57.0 to 836 mg kg<sup>-1</sup> in 2010 and from 31.0 to 488 mg kg<sup>-1</sup> in 2011. The decrease in M3P from 2010 to 2011 may have been a consequence of leaching in addition to crabgrass P removal. Phosphorus leaching has often been considered insignificant in both the agriculture and environmental sectors, because of the capability of subsoil to fix P (Sims et al. 1998). However, there are exceptions, such as soils containing high amounts of organic matter (Fox and Kamprath, 1971), saturated soils (Khalid et al., 1976), and also acidic sandy soils that are frequently fertilized (Breeuwsma and Silva, 1992). Also, P leaching has been based on extractable P as a function of depth. Since the soils in this study were shallow, the vertical P movement may have been more substantial when compared to field research. Additionally, clay soils that undergo shrink-swell processes can exhibit significant P leaching (Stamm et al., 1998; Jensen et al., 2000). Both the Richfield and Kirkland soil series underwent shrink-swell processes due to wetting and drying cycles that may have increased P leaching.

This two year study, showed that as the M3P of each soil increased there was also an increase in WSP (Figure 1.3). There were also differences in slopes ( $p > 0.05$ ) among the three soils (Figure 1.3) probably due to the difference in soil texture and other soil properties (Table 1.1). A strong correlation between water soluble P and STP has been previously established (Sharpley et al., 1994; Pote et al., 1996; Hooda et al., 2000 Davis et al., 2005). Several studies have shown that when there is an elevated amount of P in

the surface soil, there is an increase in concentration of P in runoff (Pote et al., 1996; Sibbesen and Sharpley, 1997). The relationship between surface P runoff and STP is dependent on soil type and sorption capacities (Sharpley, 1995; Pote et al., 1999; Cox and Hendricks, 2000; Davis et al., 2005). Agricultural runoff contributes considerable amounts of P to surface water, which is a main factor that causes eutrophication (U.S. EPA, 2004). Therefore, minimizing excess STP may reduce the concentration of P in runoff, potentially reducing eutrophication of surface waters. Understanding the relationship of STP and water soluble P associated with soils that have different textures and soil properties may aid management decisions regarding reducing STP in the future.

#### *Crabgrass Biomass*

The dry biomass ranged from 9.90 to 14.2 Mg ha<sup>-1</sup> in 2010 and from 6.86 to 13.4 Mg ha<sup>-1</sup> in 2011 (Table 1.2). The average dry biomass in 2010 was not significantly different ( $p > 0.05$ ) among different soil test P levels of each soil. However, the dry biomass from the Dennis and Richfield soils did significantly increase ( $p < 0.05$ ) with the increase of soil test P levels in 2011 (Table 1.2). In addition, the crabgrass grown in the Dennis and Richfield soil was slower to germinate with poorer stand prior to the first harvest, than the crabgrass grown in the Kirkland soil in 2011. The poor stand establishment in the Dennis and Richfield soils was most likely due to a hard seed bed or soil crusting which reduced germination and emergence. The lack of biomass response to increasing P level in 2010 and for the Kirkland soil in 2011 should be expected, since all the M3P was close to or above the adequate levels for all crops in Oklahoma ( $>33 \text{ mg kg}^{-1}$ ) (Zhang and Raun, 2006). The linear response to STP of crabgrass grown in the

Richfield and Dennis soil series in 2011 is not adequately understood. The average dry biomass for this greenhouse study agrees with previous field studies. Ryan (2006) showed that crabgrass (*Digitaria sanguinalis*) grown over three years had an average yield of 12.2 Mg ha<sup>-1</sup>. For this study, the average biomass over two years was 11.5 Mg ha<sup>-1</sup>. According to Dalrymple (1999), crabgrass in Oklahoma produced 9.0 to 11.0 Mg DM ha<sup>-1</sup>, which was lower than the average bermudagrass yield. Under optimal precipitation and nutrient availability, modern bermudagrass cultivars yield an average of 13.4 Mg ha<sup>-1</sup>, depending on the location in Oklahoma (Redfearn et al., 2010). When determining if a grass is useful for phytoremediation, other factors such as tissue P content, protein content, and digestibility should be considered along with yield. Even though bermudagrass has slightly higher average yields, the average tissue P content is lower. In a study conducted by Ryan (2006) it was found that crabgrass had significantly higher tissue P concentration than bahiagrass (*Paspalum notatum* Flugge.), common bermudagrass (*Cynodon dactylon* (L.) Pers.), and switchgrass (*Panicum virgatum* L.). The elevated tissue P content in crabgrass compensates for its lower yield in terms of the total amount of P removed.

#### *Tissue Phosphorus Content*

The tissue P content of the crabgrass increased with the increased level of P for the crabgrass grown in all 3 soils (Table 1.2). Similarly, Vervoort et al. (1998) showed that N and P uptake increased with an increase in poultry litter applications on bluegrass (*Poa pratensis* L.), tall fescue (*Festuca arundinacea*) and bermudagrass/tall fescue pastures. The tissue P sufficiency ranges for crabgrass are not available; however, the

ranges for other forage and hay crops such as coastal bermuda (*Cynodon dactylon* L.), tall fescue (*Festuca arundinacea*), orchardgrass (*Dactylis glomerata* L.), and smooth brome (*Bromus inermis* Leyss) have been determined and range from 0.20-0.40% (Plank and Campbell, 2000; Lessman and Thom, 2000; Donohue and Savoy, 2000). The average tissue P for all samples from 2010 were in this range, but the average tissue P for the crabgrass grown in the Dennis and Richfield soils with the lowest soil test P level in 2011 was below the low limit of the sufficiency range (Table 1.2). This may be due to the harvest time since the tissue P content of plants generally declines with plant age (Fageria, 2009). The crabgrass in 2011 was harvested after longer periods of time, allowing it to mature for an extended period of time. Since there was a longer interval between harvests the overall average tissue P in 2011 was lower compared to 2010. Mostly, the tissue P content averages were around the upper range or exceeded the tissue P sufficiency range. According to Pant et al. (2004), luxury uptake of P by forages is not well determined and Rhoads et al. (1997) found that bahiagrass tissue P content did not respond to a P application  $> 84 \text{ kg P ha}^{-1} \text{ yr}^{-1}$ . However, the increase in crabgrass tissue P content could be considered luxury uptake, since the soil P was more than adequate. Similar luxury uptake has been reported in some bahiagrass and rangeland grasses (Burton et al., 1997; Islam and Adams, 1999). Luxury uptake of soil P is crucial for phytoremediation of excess P. Since all of the treatments over two years showed a linear relationship of tissue P to M3P, it can be assumed that crabgrass may uptake excess P from the soil making it useful for phytoremediation.

### *Phosphorus Removal*

The P removed by the harvested forage was calculated as the product of biomass and tissue P content and ranged from 31.5 to 74.4 kg P ha<sup>-1</sup> with an average of 56.4 kg P ha<sup>-1</sup> in 2010. Phosphorus removal ranged from 10.5 to 70.9 kg P ha<sup>-1</sup> with an average of 41.7 kg P ha<sup>-1</sup> in 2011. In 2010, P removed was significantly related to M3P ( $p < 0.001$ ) for the Dennis soil ( $r^2 = 0.40$ ) and the Richfield soil ( $r^2 = 0.55$ ) (Figure 1.4). However, possibly due to high treatment variability, P removed was not significantly ( $p > 0.05$ ) related to M3P in the Kirkland soil in 2010. In 2011, P removed was significantly related ( $p < 0.001$ ) to M3P in all three soils ( $r^2$  values ranging from 0.79 to 0.85). The tissue P and P removed for both years was significantly affected by the treatment level (Table 1.2). In addition, the treatment  $\times$  soil interaction was significant for tissue P and P removed in 2011 but not in 2010 (Table 1.2). The crabgrass grown in the Richfield soil removed the most P for both years with an average of 59 and 46 kg P ha<sup>-1</sup> for 2010 and 2011, respectively (Table 1.2). The average P removed in 2010 was higher than in 2011. This difference may have been due to overall higher P levels available for uptake, a difference in harvest intervals since P content typically declines with plant age (Fageria, 2009) and/or growing conditions. There was no significant difference in P removal between similar soil P levels in 2010 (Table 1.3). However, in 2011 there was a significant difference between the P removed for the crabgrass grown in the Dennis soil series compared to the other two soil series for the second STP level. The significantly lower value appears to be a consequence of the low tissue P content for the crabgrass grown in the Dennis soil for the second STP level; however, the reason for the low value is not known. A review of forage phosphorus phytoremediation conducted by Pant et al.

(2004) found annual removal of P by forage could range from 14.6 kg ha<sup>-1</sup> by bluegrass (*Poa annua* L.) to 83 kg ha<sup>-1</sup> by johnsongrass (*Sorghum halepense* L.). However, johnsongrass is considered a noxious weed in some states.

### *Crabgrass Hay Quality and Nitrogen Removal*

Since the P removed depends on the forage yield (Adeli and Varco, 2001), N fertility may improve yield and increase P uptake. In our study, the average N removed was 280 and 154 kg N ha<sup>-1</sup> in 2010 and 2011, respectively. The amount removed for 2010 was higher than in 2011 most likely due to harvest timing. In 2010, the crabgrass was harvested four times and had an average tissue N content of 2.26%, while in 2011 the average N content was 1.45%. In a study conducted by Evers (2002) it was found that when N was applied to Rygrass (*Lolium multiflorum* L.) the grass removed 32 to 34 kg P ha<sup>-1</sup> compared to 26 to 29 kg P ha<sup>-1</sup> when N was not applied during the growing season. In our study, N was supplied before or shortly after planting, and after each harvest to ensure adequate supply to the crabgrass. The average N:P uptake for the crabgrass was about 5:1. An adequate N supply is essential for P removal since the hay yield is largely determined by N application rate. Crabgrass is considered a high quality hay due to digestibility and high protein content compared to many other hay crops. In this study, the crabgrass had an average crude protein of 14.0% in 2010 and 9.0% in 2011 (Table 1.2). The difference between the two years is most likely due to the difference in harvest intervals and plant maturity. Since the crabgrass harvested in 2011 matured for a slightly longer period of time the protein content was lower. With the exception of the crabgrass grown in the Dennis soil series in 2010, a linear relationship between protein and M3P

did not exist ( $p > 0.05$ ). The reason for this incongruent response is not known. Nitrogen fertilization has been shown to increase the crude protein of crabgrass (Teutsch et al., 2005), and is highly correlated with crude protein content in forage (Olson and Kurtz, 1982). In this study, the average nitrate ( $\text{NO}_3\text{-N}$ ) content was  $3065 \text{ mg kg}^{-1}$  in 2010 and  $425 \text{ mg kg}^{-1}$  in 2011. As with the protein content, the  $\text{NO}_3\text{-N}$  content varies depending on plant maturity and was influenced by harvest timing. Nitrate accumulation increases with high levels of nitrogen fertilizer, especially under water stress (Teutsch and Tilson, 2004). This accumulation can become toxic to livestock and the concentration in forage that causes toxicity depends on the amount ingested, forage type, adaptation, and health of the animal (Teutsch and Tilson, 2004). Additionally, the study by Teutsch and Tilson (2004) showed that nitrate concentrations in crabgrass were in the generally safe range ( $<5000 \text{ mg kg}^{-1} \text{ NO}_3\text{-N}$ ) when  $56 \text{ kg N ha}^{-1}$  was applied one time. This should be kept in mind when trying to maximize crabgrass yields with N fertilizer to increase P removal.



## CONCLUSIONS

This two year P removal study was conducted using Dennis, Kirkland, and Richfield soil series with four increasing STP levels. Red River Crabgrass was grown to determine the prospective for P phytoremediation from the three soil series. On average, crabgrass removed 49.1 kg P ha<sup>-1</sup> over two years. The average tissue P content of crabgrass grown in all three soils over 2 years was 0.41% and had a linear response to increasing STP for each soil. The luxury P uptake demonstrated by the crabgrass in this study is beneficial when it is used for phytoremediation. Phytoremediation using forage species has the benefit of complete harvest removal many times in a season, unlike crops where only the grain is harvested once a season. Using crabgrass for P phytoremediation has potential if it is managed as a hay crop and is completely removed from the site. Crabgrass had a high protein content compared to many other types of forages; therefore, it can be a good quality hay as well as an effective N and P remover. Additional research utilizing a double cropping system with crabgrass and a cool season forage should be conducted to determine the potential benefits under field conditions.

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## TABLES

Table 1.1. Chemical and physical characteristics of the three soils series used for this study.

Soil Series	Organic Matter	Clay	Treatment	pH <sup>‡</sup>	M3P <sup>§</sup>
	———— % ————				-mg kg <sup>-1</sup> -
Richfield	1.8	34	1	7.2	45
			2	6.8	163
			3	6.4	366
			4	5.9	536
Dennis	2.0	11	1	7.3	57
			2	7.3	137
			3	6.4	339
			4	6.0	681
Kirkland	2.4	17	1	5.7	77
			2	5.5	187
			3	5.6	480
			4	5.1	786

‡ Average pH of both years.

§ Average Mehlich 3 phosphorus of both years.



Table 1.2. Average crabgrass biomass, tissue phosphorus (P) content, amount of phosphorus removed, and crabgrass forage protein content for 2010 and 2011 as affected by three soil series and four treatment levels.

Soil	Treatment	2010				2011			
		Biomass	Tissue P	P removed	Protein	Biomass	Tissue P	P removed	Protein
		Mg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	%	Mg ha <sup>-1</sup>	%	kg ha <sup>-1</sup>	%
Dennis	1	9.90a <sup>‡</sup>	0.30a	31.5a	13.3a	6.86a	0.16a	10.5a	9.46a
	2	13.1a	0.39a	51.8ab	13.7a	8.52ab	0.22b	19.2a	8.57ab
	3	11.5a	0.54b	61.5b	15.1a	10.7bc	0.44c	47.7b	8.16b
	4	13.0a	0.53b	68.7b	15.3a	12.2c	0.51c	60.6b	9.11ab
	Linear	NS	***	***	**	**	***	***	NS
Kirkland	1	12.2a	0.36a	47.5a	14.1ab	11.1ab	0.25a	28.6a	9.81a
	2	11.5a	0.41b	50.9a	13.6ab	11.8b	0.35b	40.0b	9.40a
	3	12.4a	0.55c	69.9a	15.3a	10.1a	0.48c	48.5c	10.01a
	4	12.1a	0.51d	60.5a	12.9b	10.7ab	0.57d	60.7d	10.57a
	Linear	NS	***	NS	NS	NS	***	***	NS
Richfield	1	10.7a	0.30a	32.6a	14.3a	7.94a	0.14a	11.4a	9.47a
	2	12.2a	0.44b	64.3b	13.7a	12.3b	0.33b	41.1b	7.75b
	3	14.0a	0.51b	63.6b	15.2a	13.1b	0.48c	61.7c	8.21b
	4	14.2a	0.51b	74.4b	12.7a	13.4b	0.53d	70.9c	8.39b
	Linear	NS	***	***	NS	***	***	***	NS
Source of Variation	DF								
Treatment	3	*	***	***	*	***	***	***	**
Soil	2	NS	NS	NS	NS	***	***	**	***
Block × Treatment	6	**	NS	NS	NS	NS	NS	NS	NS
Soil × Treatment	6	NS	NS	NS	NS	***	**	*	NS
Block × Soil	4	**	NS	NS	NS	NS	NS	NS	NS

<sup>‡</sup>Means followed by a different letter are significantly different at the 0.05 level within a column and for each soil series.

DF, Degrees of Freedom

NS, not significant

\*Significant at the 0.05 alpha level

\*\* Significant at the 0.01 alpha level.

\*\*\* Significant at the 0.001 alpha level.

Table 1.3. Average crabgrass biomass, tissue phosphorus (P) content, amount of P removed, and protein for 2010 and 2011 organized by treatment as affected by three soil series and four treatment levels.

Soil	Treatment	2010				2011			
		Biomass Mg ha <sup>-1</sup>	Tissue P %	P removed kg ha <sup>-1</sup>	Protein %	Biomass Mg ha <sup>-1</sup>	Tissue P %	P removed kg ha <sup>-1</sup>	Protein %
Dennis	1	9.90a <sup>§</sup>	0.30a	31.5a	13.3a	6.86a	0.16a	10.5a	9.46a
Kirkland	1	12.2a	0.36b	47.5a	14.1a	11.1b	0.25b	28.6a	9.81a
Richfield	1	10.7a	0.30a	32.6a	14.3a	7.94a	0.14a	11.4a	9.47a
Dennis	2	13.1a	0.39a	51.8a	13.7a	8.52a	0.22a	19.2a	8.57a
Kirkland	2	11.5a	0.41a	50.9a	13.6a	11.8b	0.35b	40.0b	9.40a
Richfield	2	12.2a	0.44a	64.3a	13.7a	12.3b	0.33b	41.1b	7.75a
Dennis	3	11.5a	0.54a	61.5a	15.1a	10.7a	0.44a	47.7a	8.17a
Kirkland	3	12.4a	0.55a	69.9a	15.3a	10.1a	0.48a	48.5a	10.0b
Richfield	3	14.0a	0.51a	63.6a	15.2a	13.1a	0.48a	61.7a	8.21a
Dennis	4	13.0a	0.53a	68.7a	15.3a	12.2a	0.51a	60.6a	9.11ab
Kirkland	4	12.1a	0.51a	60.5a	12.9a	10.7a	0.57a	60.7a	10.6a
Richfield	4	14.2a	0.51a	74.4a	12.7a	13.4a	0.53a	70.9a	8.39b

<sup>§</sup>Means followed by a different letter are significantly different at the 0.05 alpha level within a column and for each treatment.



Figure 1.1. Locations of soil samples collected for the previous rainfall simulation study and used for the phosphorus removal experiment.



Figure 1.2. Plot layouts for 2010 (top) and 2011 (bottom). Each of the three replications (rows) contains Kirkland, Dennis, and Richfield soils with four levels of increasing soil test phosphorus (STP). The treatments, soil and STP, were randomly arranged within each replication.

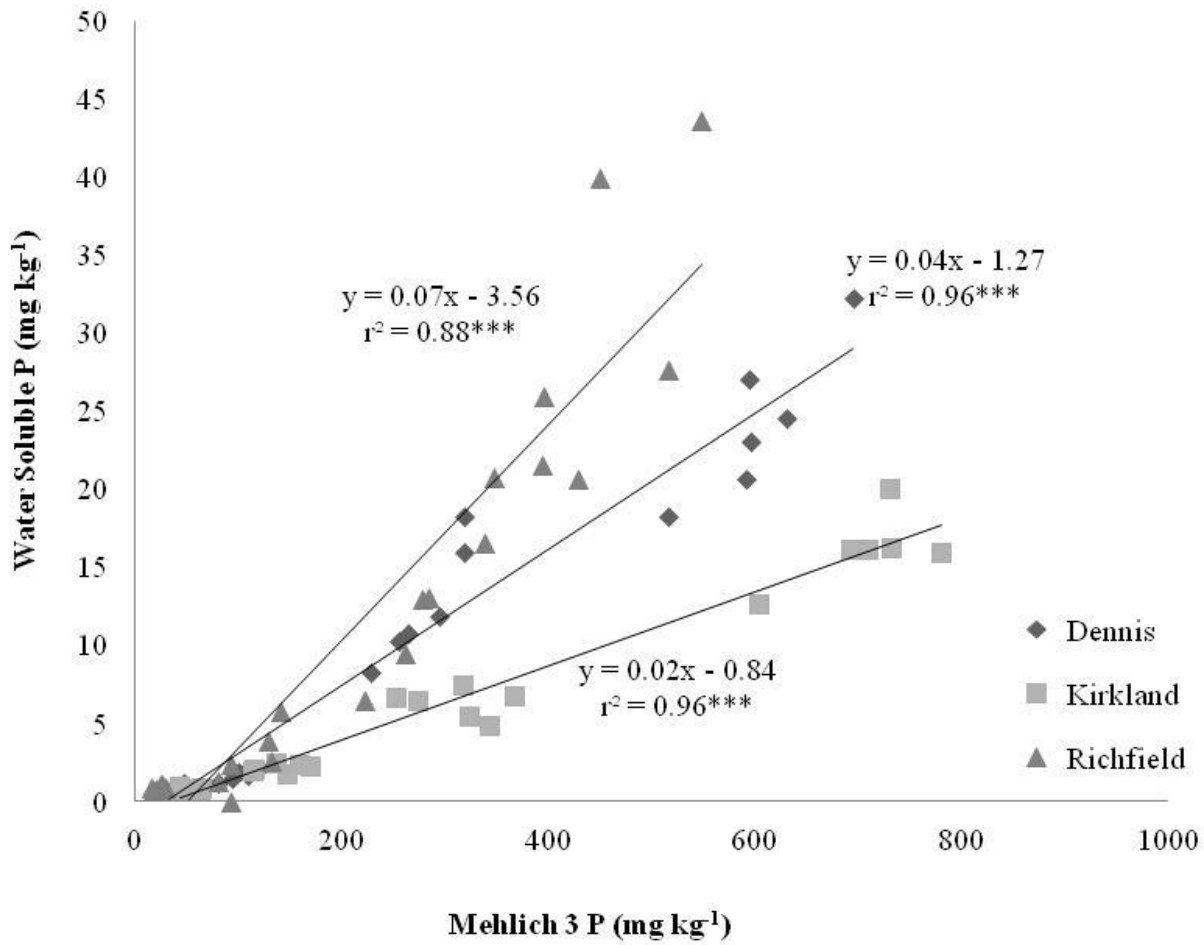


Figure 1.3. Relationship between soil Mehlich 3 P and water soluble P for the Dennis, Kirkland, and Richfield soil series for 2010 and 2011.  
 \*\*\*Significant at the 0.001 alpha level.

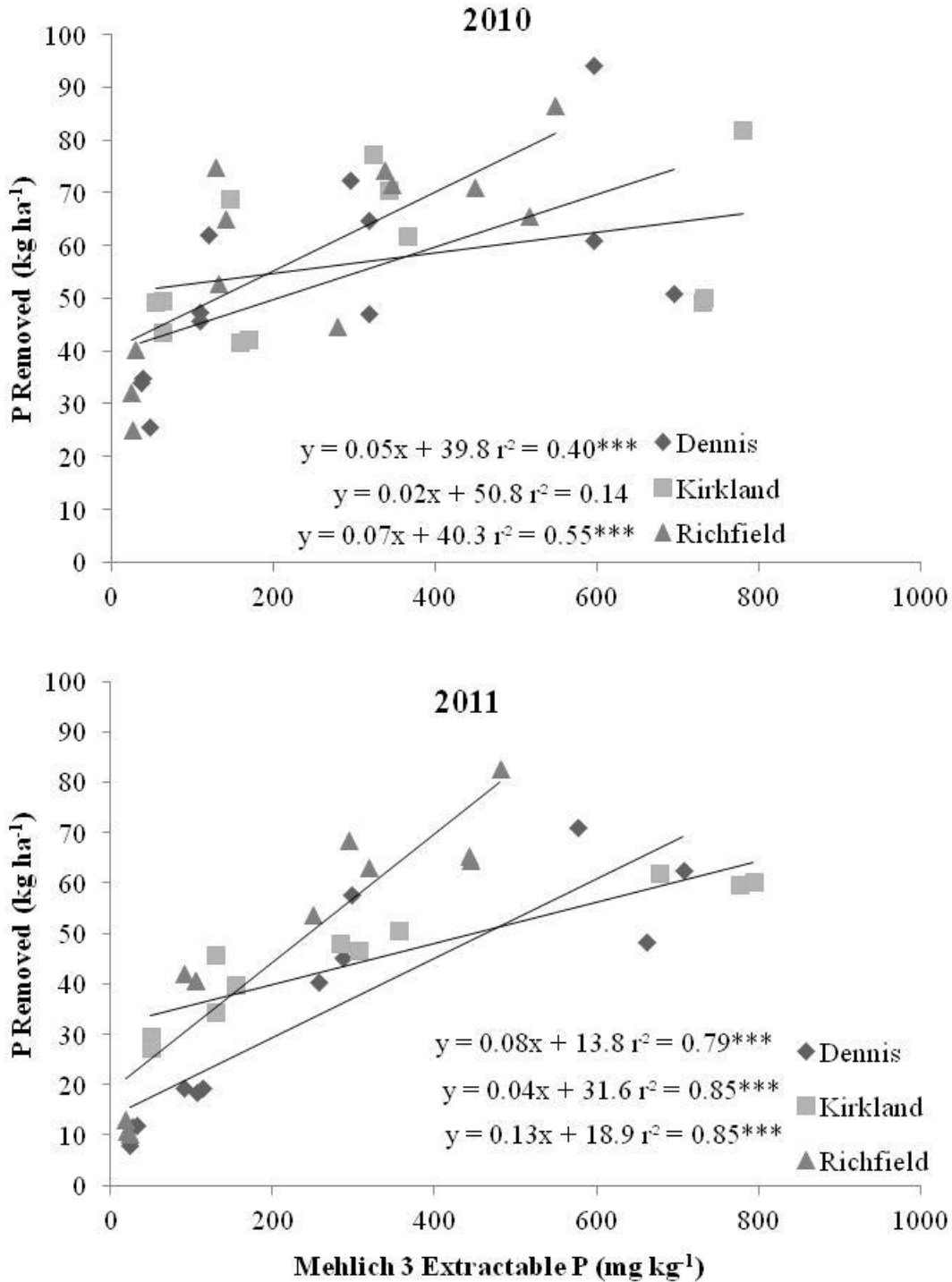


Figure 1.4. Phosphorus removed by crabgrass grown in Dennis, Kirkland, and Richfield soil series with four levels of increasing Mehlich 3 P for 2010 and 2011. \*\*\*Significant at the 0.001 alpha level.

## CHAPTER II

### WINTER CANOLA YIELD, OIL CONTENT AND TISSUE NUTRIENT CONTENTS AS AFFECTED BY SULFUR SOURCES AND RATES

#### ABSTRACT

Canola (*Brassica napus*) production in Oklahoma has sharply increased in recent years due to canola's benefits as a winter rotation crop for continuous wheat producers. However, limited research exists about sulfur (S) requirements of canola in the Southern Great Plains and producers are being encouraged to apply S regardless of soil test results. The objectives of this two-year study were to evaluate the effect of two sources of S and four application rates of 0, 11.2, 22.4, and 33.6 kg ha<sup>-1</sup> on yield, seed oil content, and tissue nitrogen (N) and S concentrations of two canola cultivars and to determine the effect of foliar applied boron (B) on canola yield. Field experiments were conducted during the 2009-2010 and 2010-2011 growing seasons in Lahoma and Perkins, Oklahoma. Surface (0-15 cm) and subsurface (15-46 cm) soil samples were collected before planting at each site. In 2009, the combined average total soil sulfate sulfur for the experimental site was 48 kg ha<sup>-1</sup> at Perkins and 51 kg ha<sup>-1</sup> at Lahoma. In 2010, the average at Perkins was 63 kg ha<sup>-1</sup> and 108 kg ha<sup>-1</sup> at Lahoma. The two canola cultivars used were Hyclass 154 and DKW 47-15, and the two sources of S were elemental sulfur and ammonium sulfate. An application of B was foliar applied to additional plots in each

replication and for both varieties receiving 33.6 kg ha<sup>-1</sup> S from ammonium sulfate in March of 2009 and 2010. In 2011, tissue samples for each plot were collected at the bolting stage and analyzed for S and other nutrients. There were no statistical yield differences between S sources and among rates for either canola cultivar over both growing seasons although plant tissue S content was increased by ammonium sulfate application. The application of B also had no effect on canola yield or oil content. The lack of canola yield and oil content response to applied S was probably due to the adequate supply of sulfate sulfur in the soil. Therefore, it is important to take soil samples before deciding if S fertilizer is needed.



## INTRODUCTION

Sulfur (S) is an essential element for plant growth and development. It is a component of the amino acids methionine and cysteine, which are critical for the production of protein. Compared to cereal crops, canola has a high demand for S due to its high proportion of cysteine and methionine (Anderson, 1975). Soil S includes many inorganic and organic forms whose availability varies throughout the season due to decomposition of organic matter, additions of fertilizer, leaching, microbial activity, plant uptake, and atmospheric deposition (Anderson et al., 1992). Sulfate ( $\text{SO}_4^{2-}$ ) is the most important inorganic form of S for plant growth and development because it is the major form taken in by plant roots. Crop yield and quality may be negatively impacted if a soil cannot supply the needed S. Sulfur deficiencies in canola have become more common in Canada and some parts of the United States due to environmental laws restricting sulfur dioxide emissions from industrial sources, the use of more concentrated fertilizers that do not contain sulfur impurities, the increase in yields resulting from genetic and technological advances, and a decrease in application of pesticides and fungicides containing sulfur (Blair, 2002).

In many parts of the world S has been identified as a limiting factor in crop production. Some studies have shown an increase in crop quality and yield associated with S fertilization on deficient soils (Janzen and Bettany, 1984; Nuttall et al., 1987; Ahmad et al., 2007). However, several of these studies reflect results from Canada and the northwestern United States where historically deposition of S has been low (National Atmospheric Deposition Program, 2007) due to distance from industrialization and slow rates of S mineralization. Because of these reasons, S fertilization need is highly dependent on location and climates. A review of S fertilization studies on canola from

1957-1998 in Michigan showed that there were only three cases of yield response to applied S out of more than 50 location-year experiments and a survey of 176 fields showed that there was only one case where S tissue concentration was below the critical level. In that same study they showed that there was not a significant yield response of canola over two years (Christenson, 1998). Michigan soils receive S from precipitation and the author concluded that S fertilizer additions were not recommended for field crops since response is rare. A study conducted in Oklahoma by Girma et al. (2005) using gypsum and application rates of zero 56, 112, and 224 kg S ha<sup>-1</sup> showed over seven years and two locations that winter wheat (*Triticum aestivum*) only had a linear response to increasing rates of S in four of 14 site-years. However, canola has a higher requirement for S than winter wheat (Anderson, 1975). Even so, in Arkansas Slaton et al. (2008) found that canola yields were not significantly responsive to an application of ammonium sulfate at a rate of 112 kg S ha<sup>-1</sup> on a Dewitt silt loam, further showing that S fertilization is dependent on climate and location. Additionally, Zhang (2000) showed that the average SO<sub>4</sub>-S level from 0-60 cm was 144 kg ha<sup>-1</sup> from over 200 sets of soil samples in 17 counties in Oklahoma. The Zhang (2000) study also reported that SO<sub>4</sub>-S in their data set ranged from 45 to 827 kg ha<sup>-1</sup>. Nevertheless, producers in Oklahoma are being encouraged to fertilize with sulfur regardless of soil test results (Boyles et al., 2007), which may be economically unsound because many Oklahoma soils are still able to supply needed S. Since there is not a strong correlation between soil test SO<sub>4</sub>-S and crop response, many recommendations use an available N to available S ratio as a guide for S management in soil (Janzen and Bettany, 1984). For canola, Janzen and Bettany (1984) suggested a 7:1 N/S ratio as a guideline to supply an adequate amount S. Currently the

7:1 N/S ratio is the recommended rate in Oklahoma or to apply 22.4 kg ha<sup>-1</sup> regardless of soil test levels (Boyles et al., 2007)

A typical soil test is taken at a depth of 0-15 cm. However, Bole and Pittman (1984) found that canola is capable of using SO<sub>4</sub>-S from depths of 54 cm or more. Therefore, a surface soil test (0-15 cm) may not adequately serve as an indicator and may underestimate soil S supply available for canola growth resulting in an erroneous S recommendation. A subsoil SO<sub>4</sub>-S test (15-45 cm) is needed to better assess soil S supply for canola production and to make more appropriate S fertilizer recommendations. For canola, Jackson (1999) recommended a soil test critical level of 45 mg kg<sup>-1</sup> from 0-30 cm. In the Great Plains region of the United States, soils with less than 22.4 kg S ha<sup>-1</sup> from 0-60 cm are considered deficient (Boyles et al., 2007).

In the United States, canola oil consumption has increased due to health benefits, availability, and price of the oil relative to some other types of oils. Approximately 5.75 x 10<sup>5</sup> ha of canola was harvested in the United States in 2010 (USDA National Agricultural Statistics Service, 2012). In Oklahoma, canola hectares harvested increased from about 923 ha in 2007 to 22,662 ha in 2010. Winter canola has been recognized as a beneficial break crop for cereal producers due to improved wheat quality and yields the following year. Benefits include: reducing wheat pathogens, increasing subsoil water and N extraction, and controlling some weedy species (Angus et al., 1994; Angus et al. 1991; Blackshaw, 1994; Kirkegaard, et al., 1994).

Canola is a relatively new crop in Oklahoma and research relating the effects of sulfur on canola seed quality and yield is needed to aid producers in making agronomically and economically sound decisions. Additionally, research regarding boron

(B) and its influence on canola yield and quality is lacking in Oklahoma. Boron is a micronutrient important for cell division and development and is active in sugar translocation and the synthesis of nucleic acids and plant hormones. Boron exists in soil solution as neutral boric acid ( $\text{H}_3\text{BO}_3$  or  $\text{B}(\text{OH})_3$ ) which is taken up by plants in this form. Like S, soil organic matter is a major source of B and is released through mineralization. The range in the soil that can cause a deficiency or lead to toxicity is smaller for B than for any other essential plant nutrient (Bohn et. al., 2001). Dicotyledonous plants, like canola, usually require more B than monocotyledonous plants during the vegetative and reproductive stages (Gupta, 1993). If canola experiences B deficiency, root growth may be hindered, so that the amount of soil surface area contacted for nutrient and water uptake are severely affected and new leaves are stunted and may curl in. Few studies have been done regarding the application of B on canola. In Canada, Karamanos et al. (2003) found no response of either oil content or canola seed yield to an increase in either soil or foliar applications of B, even with soils that contained  $<0.15 \text{ mg kg}^{-1}$  of hot-water extractable B which is normally considered deficient.

Plant tissue analysis may provide a better understanding of B and other nutrient status in the plant at a particular growth stage of canola. At flowering, B content in canola was reported to be between 20 to 30 ppm (McKenzie, 1992). According to Plank and Tucker (2000), the sufficiency range for B in canola (mature leaves prior to flowering) is 25 to 54 ppm. However, the foliar application of B should be done in the vegetative to early flowering stage to increase yield if there is a B deficiency. More precise sampling time and tissue test interpretation are needed to make top dress recommendation based on tissue testing results.

The objectives of this study were to determine the impact of sulfur fertilization rates on canola yields, oil content, and S and N tissue contents and to evaluate the effect of spring foliar application of B on canola yield.

## MATERIAL AND METHODS

### *Experimental Site and Treatments*

The sulfur fertilization field trials were conducted at Oklahoma State University's Cimarron Valley Research Station one mile north of Perkins, Oklahoma and at the North Central Research Station one mile west of Lahoma, Oklahoma in the 2009-2010, and 2010-2011 growing seasons. The canola field plots located in Perkins were established on a Teller loam (Fine-loamy, mixed, active, thermic Udic Argiustolls) and the plots located in Lahoma were established on a Grant silt loam (Fine-silty, mixed, superactive, thermic Udic Argiustolls). The Cimarron Valley Research Station has an average winter temperature of 5°C, average summer temperature of 33°C, and annual precipitation of 94 cm. The North Central Research Station has an average winter temperature of 3°C, summer temperature of 34°C, and annual precipitation of 82 cm. The 2.4 x 7.6 m plots were established in October of 2009 and 2010 in a randomized complete block design with 4 replications. The 2009 and 2010 sites were in adjacent but not identical locations. The treatments were designed to determine if two different sources of sulfur fertilizers and four S rates had an effect on yield and seed quality of two different cultivars. The two canola cultivars were hybrid HyClass 154W and open pollinated DKW 47-15. The two sulfur fertilizers used were ammonium sulfate (21-0-0 24% S) and elemental sulfur (TigerSul 90 CR 90% S). The four rates of S were 0, 11.2, 22.4, and 33.6 kg S ha<sup>-1</sup>. Both S fertilizers were broadcast and incorporated with a cultivator before planting. One additional treatment was an application of 1 kg ha<sup>-1</sup> B at the bolting stage. The B treated plots received 33.6 kg ha<sup>-1</sup> S as well.

### *Soil Collection and Analysis*

Before planting in both growing seasons, composite soil surface (0-15 cm) and subsurface (15-45 cm) samples consisting of 15-25 cores were collected from each replication. Samples were dried overnight at 65°C and ground to pass through a 2.0-mm sieve. The samples were analyzed for pH, extractable P, K, NO<sub>3</sub>-N, NH<sub>4</sub>-N, SO<sub>4</sub>-S, and B. The Mehlich 3 (M3) extractable P and K were measured by extracting a 2.0-g sample with 20 mL of M3 solution (0.015 M NH<sub>4</sub>F, 0.2 M CH<sub>3</sub>COOH, 0.25 M NH<sub>4</sub>NO<sub>3</sub>, 0.013 M HNO<sub>3</sub>, 0.001 M EDTA) and shaking for 5 min (Mehlich, 1984). The P and K in the extracts were analyzed by an inductively coupled plasma spectrometer (ICP). The soil pH was measured by using a combination electrode in a 1:1 soil to water suspension (Thomas, 1996). Nitrate nitrogen (NO<sub>3</sub>-N) and ammonium nitrogen (NH<sub>4</sub>-N) were measured using 2.5 g of soil and 10 mL of 1.0 M KCl and shaking for 15 min. Then NO<sub>3</sub>-N and NH<sub>4</sub>-N in the extracts were determined using a Lachat Quickchem 8000™ automated flow-injection analyzer (Mulvaney, 1996). The SO<sub>4</sub>-S was measured by extracting a 10.0 g sample with 25 ml of 0.008 M calcium phosphate, shaking for 30 min followed by analysis with ICP (Schulte and Eik, 1988). The soil B was measured by extracting a 10.0 g sample with 20 ml of DTPA-Sorbitol (0.005 M DTPA, 0.1 M triethanolamine (TEA), 0.01 M CaCl<sub>2</sub>, and sorbitol) and shaking for 2 h. The extracts were then analyzed by ICP for B (Lindsay and Norvell, 1978).

### *Fertilization and Plot Preparation*

In October 2009 and September 2010, urea ammonium nitrate (28-0-0) (about 1/3 of the total N) was applied with a CO<sub>2</sub> backpack sprayer the same day as planting based

on yield goal, soil tests, and the N added from the ammonium sulfate. Both sulfur fertilizer treatments, ammonium sulfate and elemental sulfur, were applied using a Gandy™ drop spreader to the predetermined plots. After fertilization, plots were tilled using a field cultivator to a depth of 8-10 cm. Canola was seeded at 5.5 kg ha<sup>-1</sup> using a small plot grain drill to a depth of about 1.3 cm. In March of 2010 and 2011, the remaining N was applied to each plot to a total of 168 kg N ha<sup>-1</sup>. In November and March of both years the canola plots were sprayed with Roundup PowerMax® at 1.18 L ha<sup>-1</sup> to control weeds.

#### *Tissue Sampling and Analysis*

Canola tissue samples were collected at the bolting stage to diagnose nutrient status. Thirty to 35 first mature canola leaves from the growth point were picked in each plot (one leaf from each random healthy plant), dried overnight at 75°C to destroy enzymes that decompose plant material (Tauber, 1949), and ground to pass a 1 mm sieve. The mineral analysis was completed by acid digestion followed by ICP quantification. In the digestion, 10.0 mL of concentrated trace metal grade HNO<sub>3</sub> was added to 0.50 g of plant sample and pre-digested for one h. The sample was then heated to 115°C for 2.5 h in the HotBlock™ Environmental Express block digester (Jones and Case, 1990). To ensure quality, a control sample with known elemental concentrations was included for every 20 plant samples. In addition, a standard reference material (SRM 1515 - Apple Leaves, National Institute of Standards and Technology) was included to ensure reliable results. The total N content of the tissue sample was analyzed using a LECO TruSpec Carbon and Nitrogen Analyzer (Jones and Case, 1990).



### *Harvest and Oil Content Analysis*

Plots were harvested with a small plot combine (Wintersteiger Classic, Wintersteiger Inc, Salt Lake City, UT 84116) in June 2010 and 2011. The harvested seeds were weighed and cleaned using a commercial cleaner with a #8 round hole top screen and a 1/50.8 cm round hole bottom screen to remove canola pod fragments, vetch, chaff, hulls and other impurities. A Grain Analysis Computer (GAC 2000, Dickey–john Corporation, Auburn, IL 62615) was used to determine the test weight and moisture content. Canola yields were adjusted to a 10% moisture basis. The harvested seeds were analyzed for protein and oil using a Near Infrared Spectrometer (NIR, DA 7200 model Perten Instruments, Kungens Kurva, Sweden) at the Food and Agricultural Product Center at Oklahoma State University.

### *Statistical Analysis*

Statistical analysis was performed using PC SAS v. 9.2 (SAS Institute, Cary, NC). Simple effects were analyzed with the PROC MIXED procedure. The growing seasons and locations were considered as blocking factors. Selected contrasts were used to determine differences between treatment means. Linear and quadratic contrasts were used for the numeric rate variable for sulfur sources and canola varieties. Treatment effects were reported significant at an alpha value of 0.05.

## RESULTS AND DISCUSSION

### *Effects of S rates and sources on canola yield and oil content*

The average soil test results for the experimental site showed sufficient levels of soil test phosphorus (P) and potassium (K) with a range of 43.3 to 70.8 kg ha<sup>-1</sup> and 303 to 567 kg ha<sup>-1</sup>, respectively (Table 2.1). Soil pH was moderately acidic and ranged from 5.6 to 6.1 for the experimental sites (Table 2.1). The average yields over two years and across two locations ranged from 2,253 to 2,447 kg ha<sup>-1</sup> (Table 2.2). However, canola did not display a significant yield response ( $p > 0.05$ ) to increasing S rates of either ammonium sulfate or elemental sulfur at either location across both years (Table 2.2). Both canola cultivars had similar effects by S rate and source treatment. The findings from this study differ from results of previous studies that showed an increase in yield with an increase in sulfur application rates (Jackson, 2000; Malhi and Gill, 2002; Malhi et al., 2007). The lack of response for this study is most likely due to an adequate soil sulfur supply at both locations, which according to Zhang (2000) may be representative of other locations in Oklahoma. For canola, Janzen and Bettany (1984) suggested an N/S ratio of 7:1; therefore, if the total required N is 168 kg ha<sup>-1</sup> (residual and applied) then 24 kg S ha<sup>-1</sup> is sufficient. The average extractable SO<sub>4</sub>-S (0-45 cm sample) at Lahoma and Perkins was 51 kg ha<sup>-1</sup> and 48 kg ha<sup>-1</sup> in 2009 and 108 kg ha<sup>-1</sup> and 63 kg ha<sup>-1</sup> in 2010 (Table 2.1). According to Boyles et al. (2007), soils in the Great Plains region of the United States with greater than 22.4 kg S ha<sup>-1</sup> are considered sufficient (Boyles et al., 2007). Thus, in our study, the extractable SO<sub>4</sub>-S was adequate for the yield goal. Approximately 62 to 70% of the total extractable S was available in the subsurface soils (15-45 cm). Because canola has a taproot and is capable of removing soil S from subsurface soils, soil samples

from both 0-15 cm and 15-45 cm should be considered when assessing the availability of S. Brennan and Bolland (2006) showed that S deficiency of canola in the rosette stage occurred but did not affect yield, because as canola grows the roots are able to uptake S. Also, S can be supplied by organic mineralization over the growing season and by dry and wet deposition (Eriksen, 2009). Previous studies have shown a response to S fertilization; however, a majority of those studies have been conducted in Canada and the northwestern United States; where historically, deposition of S is low (National Atmospheric Deposition Program, 2007) due to distance from industrialization. Also, because of low temperatures in Canada and the northwestern United States, mineralization of soil S occurs at a slower rate, potentially reducing the amount available for plant growth.

Average oil content of HyClass 154 over two years and across two locations ranged from 42.2 to 42.6% and was significantly different ( $p < 0.05$ ) from the corresponding treatments for DKW 47-15 which ranged from 43.4 to 43.7% (Table 2.1). Oil content of HyClass 154 followed a quadratic expression for the elemental sulfur treatment, and was narrowly significant ( $p=0.04$ ) (Table 2.1). However, the ammonium sulfate treatment did not significantly increase oil content for either of the cultivars. Likewise, the elemental sulfur treatment did not significantly increase oil content in the DKW 47-15 cultivar.

#### *Effects of S rates and sources on canola tissue sulfur and nitrogen content*

The average tissue S and N concentrations over two years and across two locations ranged from 0.70 to 0.94% and from 3.65 to 4.24%, respectively (Table 3.1).

Tissue S concentrations of all samples were in the sufficiency range of 0.65-0.90% as described by Plank and Tucker (2000) (Table 2.3). The critical S value at 90% relative yield for canola is 0.47% (Plank and Tucker, 2000). Tissue S at the bolting stage had a significant linear response to S application rates for both cultivars treated with the ammonium sulfate (Table 2.3, Figure 2.1). Conversely, neither cultivar had a significant response to elemental sulfur treatments (Table 2.3). Our results are similar to those reported by Khalid et al. (2009) who showed that an increase in ammonium sulfate significantly increased the concentration of S in canola biomass. Ammonium sulfate is immediately available for plant uptake and sulfates can accumulate more than what is needed by the plant (Oenema and Postma, 2003). On the other hand, elemental sulfur is not soluble and is slowly converted to plant available sulfate by microbially mediated S, which is contingent upon temperature, moisture, and particle size, making sulfate sulfur gradually available throughout the season and possibly the following season (Eriksen, 2009). The lack of tissue S response to the application of elemental sulfur could be due to the delay in availability of sulfate sulfur. Since S and N are crucial for protein synthesis, the interaction between them can significantly affect oil seed quality and yield of canola if one is not adequate (Grant and Bailey, 1993; Ahmad and Abdin, 1999; Jackson, 2000). However, the tissue N concentration was also not significantly affected by either the S rate or S source (Table 2.3). Tissue testing should be used in addition to soil testing to help ensure an adequate supply and uptake of S.

### *Effects of B foliar application on canola yield and quality*

Canola yield and oil content were not increased ( $p > 0.05$ ) by a single foliar application rate of B for both cultivars (Figure 2.2). Yield and yield component results from previous studies from the application of B are inconsistent. Karamanos et al. (2003) found no response of yield or oil content of canola seed with both foliar and soil applications of B on Canadian prairie soils that contained between 0.1 and 0.8 mg kg<sup>-1</sup> hot-water soluble B. However, Yang et al. (2009) found a significant increase of yield after application of B on deficient (<0.06 mg kg<sup>-1</sup> hot-water soluble B), sandy soils in Wuhan, Huibei Province in China. The soil test B for our study ranged from 0.1 to 0.4 mg kg<sup>-1</sup>. This value is considered low for the production of peanuts and alfalfa which require a soil B concentration of 0.5 mg kg<sup>-1</sup>. Even though this value is considered low, there was not a significant yield increase ( $p < 0.05$ ) with an application of B (Figure 2.2). The available B in the subsoil may also play an important role although we did not analyze subsoil B. The average tissue B content for no application and 1 kg ha<sup>-1</sup> foliar applied B over both years and locations for HyClass 154 cultivar was 48.8 and 55.1 mg kg<sup>-1</sup> and 42.7 and 53.2 mg kg<sup>-1</sup> for DKW 47-15. The application of B did significantly affect the tissue B concentration; however, tissue B concentrations of all samples were in the sufficiency range of 25 to 54 mg kg<sup>-1</sup> (Plank and Tucker, 2000).

## CONCLUSIONS

This study evaluated the effect of four sulfur application rates and two sulfur sources on canola yield, seed oil content, and N and S tissue content. There was an additional treatment of 1 kg ha<sup>-1</sup> foliar application of B to plots receiving 33.6 kg ha<sup>-1</sup> ammonium sulfate. The results indicate that the application of S as ammonium sulfate or elemental sulfur had no significant effects on yield of HyClass 154 or DKW 47-15. Even on a sandy textured soil (the Perkins site) where leaching processes can decrease the amount of available sulfate-S, no significant yield response to S fertilization occurred. The lack of response to S fertilizer applications is most likely due to an adequate soil supply of sulfate-S. The experimental sites were chosen due to the representative properties of the soils chemical and physical properties in different locations across Oklahoma. However, sulfate-S may vary from site to site depending on management practices, making tissue and soil testing critical in fertilizing application decisions. Increasing rates of elemental sulfur did produce a slight quadratic effect for oil content of HyClass 154. HyClass 154 had lower oil content than DKW 47-15 due to different flowering and ripening periods; however, a linear response did not occur for either cultivar. The tissue S did increase with an increasing application of ammonium sulfate; however, it did not influence the uptake of N or the seed yield of canola. Application of B did not affect yield or oil content of canola, due to an adequate soil B supply. However, tissue content of B did increase with a foliar application, which is expected since excess B tends to accumulate in plant tissue. All tissue samples were in the B sufficiency range (Plank and Tucker, 2000). Sulfur and B deficiency in Oklahoma are rare and soil and tissue testing should be utilized to decide if S and B fertilizers should be applied.

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TABLES

Table 2.1. Average soil pH, surface and subsurface nitrite nitrogen (NO<sub>3</sub>-N), soil test P and K (STP and STK), surface and subsurface sulfate sulfur (SO<sub>4</sub>-S), and DTPA-sorbitol extractable boron (B) for the entire experimental plot at Lahoma and Perkins before canola planting in 2009 and 2010.

Location	pH	Surface <sup>†</sup> NO <sub>3</sub> -N	Subsurface <sup>‡</sup> NO <sub>3</sub> -N	Total NO <sub>3</sub> -N	STP	STK	Surface <sup>†</sup> SO <sub>4</sub> -S	Subsurface <sup>‡</sup> SO <sub>4</sub> -S	Total SO <sub>4</sub> -S	B
-----kg ha <sup>-1</sup> -----										mg kg <sup>-1</sup>
<b>2009</b>										
Lahoma	6.1	22.0	22.4	44.4	43.3	567	19.0	32.1	51.1	0.30
Perkins	5.8	11.5	10.0	21.6	70.8	310	14.6	33.0	47.6	0.21
<b>2010</b>										
Lahoma	5.7	39.8	40.3	80.1	59.9	549	40.6	67.8	108	0.37
Perkins	5.6	7.3	10.5	17.8	70.5	303	18.5	44.5	63.0	0.13

<sup>†</sup> Surface soil samples were 0-15 cm.

<sup>‡</sup> Subsurface soil samples were 15-45 cm.

Table 2.2. Average canola seed yield and oil content across Lahoma and Perkins for the 2009-10 and 2010-11 growing seasons of two *Brassica* cultivars as affected by four rates and two sources of sulfur fertilizer.

<i>Brassica</i> Cultivar	S Rate	S Source	Seed Yield	Oil Content
	--kg ha <sup>-1</sup> --		--kg ha <sup>-1</sup> --	--%--
HyClass 154	0	None	2349	<b>42.52</b> <sup>†</sup>
	11.2	Elem Sulfur <sup>‡</sup>	2279	<b>42.26</b>
	22.4	Elem Sulfur	2447	<b>42.38</b>
	33.6	Elem Sulfur	2417	<b>42.55</b>
Linear			NS	NS
Quadratic			NS	*
	0	None	2349	<b>42.52</b>
	11.2	A. Sulfate <sup>§</sup>	2374	<b>42.64</b>
	22.4	A. Sulfate	2446	<b>42.19</b>
	33.6	A. Sulfate	2321	<b>42.31</b>
Linear			NS	NS
Quadratic			NS	NS
DKW 47-15	0	None	2313	43.57
	11.2	Elem Sulfur	2253	43.72
	22.4	Elem Sulfur	2333	43.55
	33.6	Elem Sulfur	2280	43.72
Linear			NS	NS
Quadratic			NS	NS
	0	None	2313	43.57
	11.2	A. Sulfate	2375	43.55
	22.4	A. Sulfate	2309	43.64
	33.6	A. Sulfate	2253	43.41
Linear			NS	NS
Quadratic			NS	NS

NS, not significant.

<sup>‡</sup> Elem Sulfur, elemental sulfur.

<sup>§</sup> A. Sulfate, ammonium sulfate.

\* Significant at the 0.05 alpha level.

<sup>†</sup> Mean values that are bolded are significantly different from those that are not bolded. for corresponding treatments for each column at the 0.05 alpha level.

Table 2.3. Average canola tissue nitrogen and sulfur content (%) across Lahoma and Perkins for the 2009-10 and 2010-11 growing seasons of two *Brassica* cultivars as affected by four sulfur rates and two sulfur sources.

<i>Brassica</i> Cultivar	S Rate	S Source	Nitrogen Content	Sulfur Content	
	--kg ha <sup>-1</sup> --		-----%-----		
HyClass 154	0	None	<b>4.18<sup>±</sup></b>	<b>0.72<sup>±</sup></b>	
	11.2	Elem Sulfur <sup>‡</sup>	<b>4.09</b>	<b>0.73</b>	
	22.4	Elem Sulfur	<b>4.24</b>	<b>0.75</b>	
	33.6	Elem Sulfur	<b>4.16</b>	<b>0.75</b>	
	Linear		NS	NS	
	Quadratic		NS	NS	
	0	None	<b>4.18</b>	<b>0.72</b>	
	11.2	A. Sulfate <sup>§</sup>	<b>4.13</b>	0.82	
	22.4	A. Sulfate	<b>4.04</b>	0.85	
	33.6	A. Sulfate	<b>4.16</b>	0.85	
	Linear		NS	***	
	Quadratic		NS	NS	
	DKW 47-15	0	None	3.65	<b>0.75</b>
		11.2	Elem Sulfur	3.7	<b>0.73</b>
22.4		Elem Sulfur	3.83	<b>0.71</b>	
33.6		Elem Sulfur	3.74	<b>0.7</b>	
Linear			NS	NS	
Quadratic			NS	NS	
0		None	3.65	<b>0.75</b>	
11.2		A. Sulfate	3.8	0.87	
22.4		A. Sulfate	3.87	0.89	
33.6		A. Sulfate	3.72	0.94	
Linear			NS	***	
Quadratic			NS	NS	

NS, not significant.

<sup>‡</sup> Elem Sulfur, elemental sulfur.

<sup>§</sup> A. Sulfate, ammonium sulfate.

\*\*\* Significant at the 0.001 alpha level.

<sup>±</sup> Mean values that are bolded are significantly different from those that are not bolded for corresponding treatments for each column at the 0.05 alpha level.

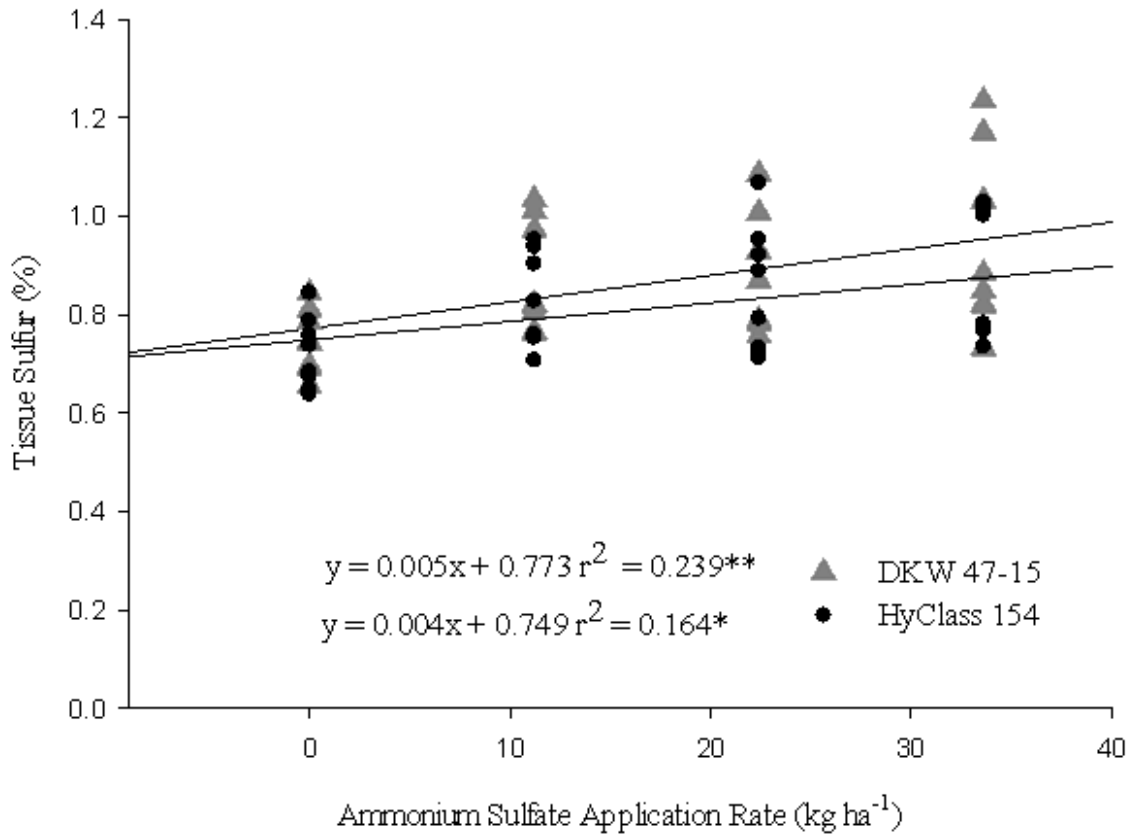


Figure 2.1. Average canola tissue sulfur content (%) across Lahoma and Perkins for the 2009-10 and 2010-11 growing seasons of two *Brassica* cultivars as affected by increasing ammonium sulfate application rates on tissue sulfur content for DKW 47-15 and HyClass 154 cultivars at the bolting stage.

\*Significant at the 0.05 alpha level.

\*\*Significant at the 0.01 alpha level.

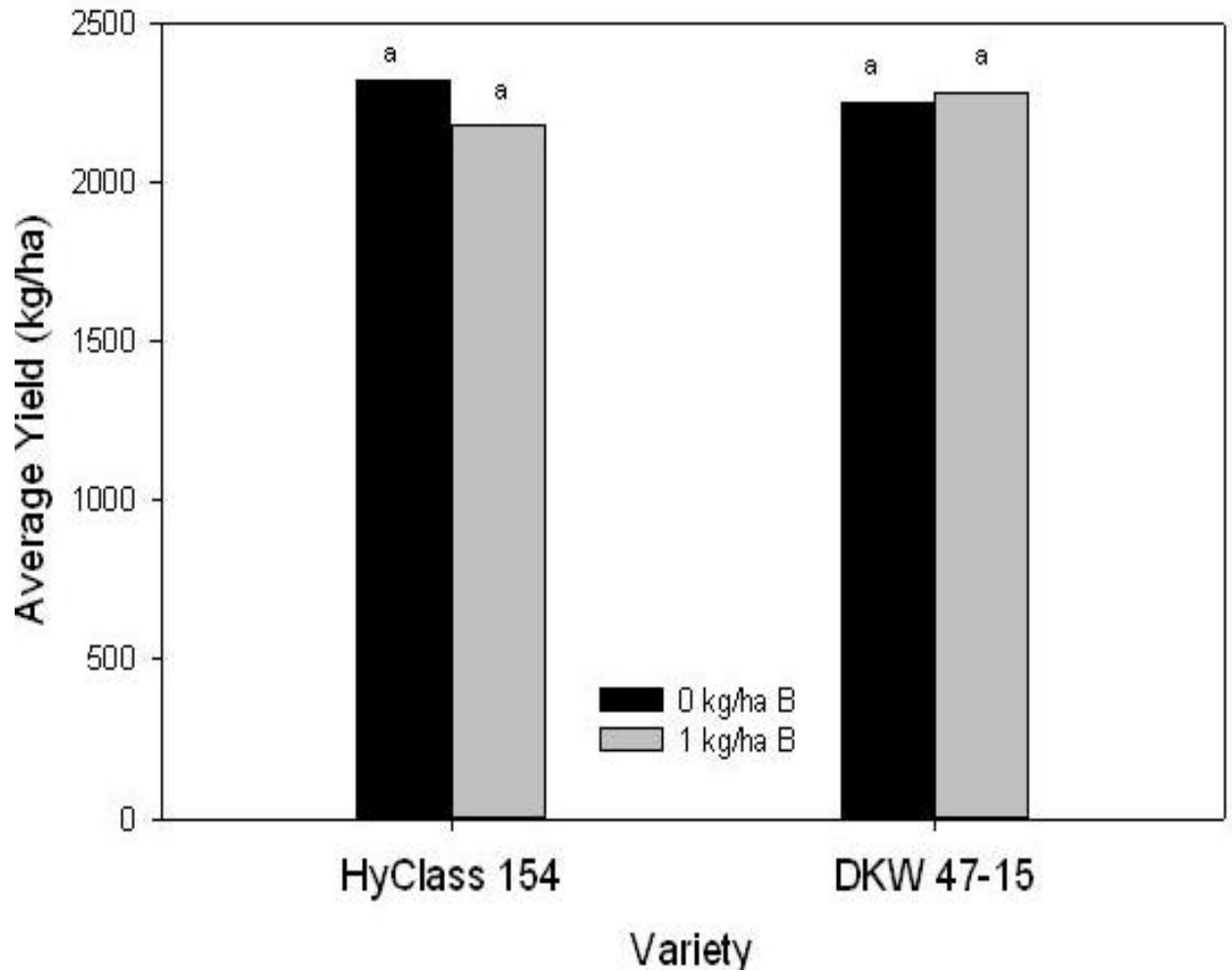


Figure 2.2. Combined average canola seed yield of HyClass and DKW 47-15 from Lahoma and Perkins for the 2009-10 and 2010-11 growing seasons as affected by the foliar application of 1 kg B ha<sup>-1</sup>. Bars with the same letter are not significantly different at an alpha level of 0.05.

APPENDIX

Appendix A. Change in Mehlich 3 P (M3P) from November 2000 to August 2011 for three soil types with four rates of phosphorus.

Soil Series	P Rate	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Aug	Oct	Apr	Mar	Sept	May	Aug
		<u>2000</u>	<u>2000</u>	<u>2001</u>	<u>2001</u>	<u>2001</u>	<u>2001</u>	<u>2001</u>	<u>2001</u>	<u>2001</u>	<u>2001</u>	<u>2001</u>	<u>2008</u>	<u>2010</u>	<u>2010</u>	<u>2011</u>
mg kg <sup>-1</sup>																
Richfield	0	32	35	35	26	26	34	33	28	23	24	41	57	27	31	18
	150	285	255	235	228	156	186	184	172	187	184	176	195	134	118	89
	350	670	650	635	682	443	453	441	428	418	410	388	409	322	296	257
	600	1295	1105	1195	1325	831	925	920	817	817	759	678	585	505	640	407
	<b>Average</b>	<b>570</b>	<b>511</b>	<b>525</b>	<b>565</b>	<b>364</b>	<b>400</b>	<b>394</b>	<b>361</b>	<b>361</b>	<b>344</b>	<b>321</b>	<b>311</b>	<b>247</b>	<b>271</b>	<b>193</b>
Dennis	0	35	36	35	30	32	36	36	35	32	32	43	82	41	63	25
	150	185	170	155	134	128	142	139	144	148	140	137	156	113	153	92
	350	545	535	535	606	451	433	433	411	385	383	352	382	311	347	250
	600	970	935	1030	1192	765	827	842	925	774	739	678	723	629	737	580
	<b>Average</b>	<b>434</b>	<b>419</b>	<b>439</b>	<b>490</b>	<b>344</b>	<b>359</b>	<b>363</b>	<b>379</b>	<b>335</b>	<b>323</b>	<b>302</b>	<b>336</b>	<b>273</b>	<b>325</b>	<b>237</b>
Kirkland	0	58	64	64	57	54	53	52	56	59	58	72	92	61	32	44
	150	210	205	190	192	164	170	166	169	185	176	179	221	159	131	123
	350	580	540	540	530	451	473	467	478	449	447	433	613	362	323	282
	600	1045	920	980	872	765	910	906	919	847	875	764	836	748	488	669
	<b>Average</b>	<b>473</b>	<b>432</b>	<b>443</b>	<b>413</b>	<b>358</b>	<b>402</b>	<b>398</b>	<b>405</b>	<b>385</b>	<b>389</b>	<b>362</b>	<b>440</b>	<b>332</b>	<b>244</b>	<b>279</b>



VITA

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Scope and Method of Study: Nutrient buildup in pastures from repeated animal manure application may increase soil phosphorus (P) and contribute to eutrophication and water quality deterioration. Options to remove excess nutrients in pastures have been limited in the past. The objective of this 2 year study was to evaluate the potential of using crabgrass (*Digitaria ciliaris*) to remove excess soil P from nutrient loaded soils. Red river crabgrass was planted in boxes containing Dennis, Richfield, and Kirkland soils in a greenhouse in 2010 and under ambient conditions in 2011. Average Mehlich3 phosphorus (M3P) at the beginning of this experiment ranged from 57.0 to 836 mg kg<sup>-1</sup>. The experiment with 12 treatments (three soils and four soil test P levels) was a randomized block design with three replications and repeated two growing seasons. Crabgrass was watered when necessary, and harvested four times from May to August, 2010 and three times in 2011. The harvested biomass was analyzed for concentrations of P and other nutrients.

Findings and Conclusions: The average biomass yield of crabgrass ranged from 9.90 to 14.2 Mg ha<sup>-1</sup> with an overall average of 12.2 Mg ha<sup>-1</sup> in 2010 and from 6.86 to 13.4 Mg ha<sup>-1</sup> with an overall average of 10.7 Mg ha<sup>-1</sup> in 2011. The harvested biomass contained an average of 0.45% P and 2.26% nitrogen (N) in 2010 and 0.37% P and 1.45% N in 2011. Therefore, the crabgrass removed an average of 49.1 kg P ha<sup>-1</sup> and 237 kg N ha<sup>-1</sup> per year. In addition, the concentration of P in the grass and P removed from the soil increased as soil test P (STP) increased although the biomass was not affected by STP. Crabgrass can serve as a good quality hay and as an effective plant for removing nutrients from soils.

ADVISER'S APPROVAL: Dr. Hailin Zhang

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