# EFFECT OF DIFFERENT WINTER LEGUMES AS NITROGEN SOURCES FOR SWITCHGRASS (*Panicum virgatum* L.) GROWN FOR CELLULOSIC ETHANOL, AND EFFECT OF FOLIAR-APPLIED PHOSPHITE ON GROWTH AND YIELD OF CORN (*Zea mays* L.)

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Abstract:

*Scope and Method of Study:*

Increasing fertilizer price has motivated scientists to find alternative sources of nitrogen to reduce cost of crop production. Because winter legumes may provide significant amount of nitrogen while conserving soil quality, the role of legumes intercropped with switchgrass (*Panicum virgatum* L.) warrants renewed attention. The objectives of this study were to investigate the effects of different winter legumes as a source of nitrogen on switchgrass dry matter yield, forage quality for biofuel production, and soil fertility status. A field experiment was carried out in which six winter legumes and four rates (0, 56, 112, and 168 kg $ha^{-1}$) of fertilizer nitrogen were studied over a 3-year period. The second study was conducted to determine the effect of foliar-applied phosphite on growth and yield of corn (*Zea mays* L.). One major problem that affects the efficiency of foliar fertilization is the form of phosphorus. Phosphite is more reactive than widely used phosphate. A field study and a greenhouse study were conducted over a 2-year period in which the effect of foliar-applied phophite, soil-applied nitrogen and phosphorus were investigated on corn biomass yield, grain yield; grain, stem, and leaf P concentrations.

*Findings and Conclusions:*

Winter legumes did not increase switchgrass dry matter yield, cellulose, lignin, and hemicellulose content. The rate of 112 kg nitrogen $ha^{-1}$ was required to achieve highest dry matter yield. Soil nitrate nitrogen, pH, and mineral content were largely unaffected. Soil organic matter depleted up to 33% after three years. As a replacement of fertilizer nitrogen, and to conserve soil productivity, winter legume-switchgrass intercropping system may not be recommended. In the field studies where phosphorus status was not significantly limited, foliar phosphite increased corn grain and biomass yield with soil-applied nitrogen fertilizer. No treatment effects were found in the greenhouse where initial soil phosphorus status was very low. In both studies, grain, stem, and leaf phosphorus concentration were largely unaffected by foliar phosphite. We concluded that, phosphite may be used as a partial supplement; however, may not be recommended when the soil phosphorus status is very low.

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# CHAPTER I

## EFFECT OF DIFFERENT WINTER LEGUMES AS NITROGEN SOURCES FOR SWITCHGRASS (*Panicum virgatum* L.) GROWN FOR CELLULOSIC ETHANOL

### ABSTRACT

The opportunity to include legumes in crop rotations has been enhanced in the Southern Great Plains over the decades; however, feasibility of winter legumes intercropped with switchgrass as a source of nitrogen has not been evaluated. This study was conducted to determine whether winter legumes could supply adequate nitrogen for ‘Alamo’ switchgrass (*Panicum virgatum* L.) and increase soil productivity. The effect of winter legumes on switchgrass dry matter, forage quality for biofuel production, soil nitrate nitrogen, soil organic matter, soil pH, and soil mineral content were investigated at two locations (Perkins and Maysville) in Oklahoma. Six winter legumes (arrowleaf clover, *Trifolium vesiculosum* S.; Austrian winter pea, *Pisum sativum* L.; button medic, *Medicago orbicularis* L.; crimson clover, *Trifolium incarnatum* L.; hairy vetch, *Vicia villosa* R.; and red clover, *Trifolium pratenser* L.) and four rates (0, 56, 112, and 168 kg ha$^{-1}$) of fertilizer nitrogen were arranged in a randomized complete block design at both locations. In terms of legume dry matter production, hairy vetch was the superior at Perkins. At Maysville, Austrian winter pea was the best winter legume in switchgrass stand. No legume treatments increased switchgrass dry matter yield regardless of years and sites. The two highest N rates (112 kg N ha$^{-1}$ and 168 kg N ha$^{-1}$) produced the greatest dry matter yield in all locations. Neither winter legumes nor fertilizer nitrogen increased cellulose, lignin, and hemicellulose content which are important component of biofuel. In some instances, winter legumes decreased biofuel quality by increasing mineral content in the dry matter. Soil nitrate nitrogen, pH, and mineral content status were largely unaffected. Soil organic matter depleted up to 33% at the end of the experiment when compared to initial values. As a replacement of fertilizer nitrogen and to conserve soil productivity, winter legume-switchgrass intercropping system is not feasible in arid environmental condition where drought and excessive heat are common.

# INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a warm-season, perennial C4 plant native to the North American tall-grass prairie. The grass is drought and flood tolerant and well adapted to the wide range of soil types and pH ranges. Low nutrient requirement, pest and disease resistance, high biomass yield, multi-harvest, and high cellulose content make this grass a potential best plant for cellulosic ethanol production. According to the Energy Independence and Security Act of 2007, a total of 21 billion gallons of ethanol requires to be obtained from cellulosic and other advanced feedstock. As renewable energy becomes a rational solution to the energy crisis, federal and state government are focusing on this crop for biofuel production. To sustain high biomass yield, switchgrass requires an adequate supply of nitrogen (N). With the persistent fluctuation of N fertilizer prices and the need to reduce production costs, it is essential to utilize alternative N sources when possible.

The value of legumes in crop rotations was recognized in the Southern United States in early 1900's by many researchers. Many documented that, N requirements by the subsequent non-legume crops can be replaced by some or all of the N nutrient carryover by previous winter legumes (Ebelhar et al., 1984; Touchtone et al., 1984). Use of winter legumes as a cover crop for corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), and grain sorghum (*Sorghum bicolor* L.) were evaluated, however, there is limited knowledge on legume N contribution for the switchgrass under specific soil and environmental conditions of the Southern Great Plains. We hypothesized that, winter legumes can be grown in established switchgrass stands to provide N and replace the need for commercial inorganic N without any economic loss of yield.

Leguminous cover crops are known to enhance soil quality by adding organic matter, recycling minerals, and increasing water infiltration in cereal crop production systems. However, the effects of legumes to soil are unknown on a single-harvest-monoculture switchgrass production system. As switchgrass is considered as the best potential plant for cellulosic ethanol production, producers are growing switchgrass commercially in a large scale. Therefore, it is required to understand the effect of legumes on soil quality to protect it from further degradation.

# REVIEW OF LITERATURE

Switchgrass (*Panicum virgatum* L.) is a perennial grass native to North America (Smith, 1979). The species adapts well in a wide variety of growing conditions. Two genetically and phenotypically different ecotypes are found from Mexico to Canada. The lowland ecotype has evolved in the southern habitats and upland variety is found mostly in the northern latitudes (Gunderson et al., 2008). Lowland varieties are larger and bushier than upland varieties when the environmental differences are eliminated (Porter, 1966). Compared to upland ecotype, low land ecotype 'Alamo' has the higher biomass yield and broader adaptability (Sanderson et al., 1996). Once established, roots of lowland ecotype can grow to a depth of 3 m creates a strong rooting system that allows switchgrass to uptake nutrients and water from deep in the soil. However, essential nutrients such as N is the most limiting factor for plant growth due to slow weathering of rocks and minerals which contain about 98% of earth's N (Coyne, 1999). There are many pathways by which N can be depleted from soil. Low rainfall and high temperature in the Southern Great Plains accelerate N loss through volatilization. Leaching is another cause of N loss. A laboratory study has showed that mineral N concentration had reached its highest level at the beginning of the rainy season and progressively decreased due to increase of leaching (Holloway et al., 2001).

Molecular N is abundant in the atmosphere, however, most plants cannot use N directly for growth and reproduction (Hubbell and Kidder, 1978). The triple bond between two N atoms is very stable. Oxidation of molecular N to $NO_3$-N requires a large amount of energy. Therefore, N is limiting for most soils and required to apply for higher yields.

Switchgrass can grow in less fertile soils. However, N fertilization increases above ground dry matter yield and quality (Nikkiema et al., 2011; Hall et al., 1982; Smith, 1979). Positive response was reported to N fertilizer up to 168 kg $ha^{-1}$ in low fertility and low organic matter soils (Muir et al., 2001). The optimum fertilization rate for 'Cave-in Rock' switchgrass was estimated as 120 kg N $ha^{-1}$ for dry matter production. Similar dry matter yields were obtained from two other locations. In this study, 10.6-11.2 Mg $ha^{-1}$ dry matter yield was obtained in Mead, NE, and 11.6-12.6 Mg $ha^{-1}$ in Ames, IA. The amount of N removed at this fertilization rate was approximately the same as the amount applied suggesting that 10 to 12 kg N $ha^{-1}$ is required for each mega gram per hectare of dry matter yield. Therefore, from economic viewpoint, to maximize dry matter yield, it is recommended to apply additional N.

Forage legumes could provide biologically fixed N for switchgrass production. As legume seeds germinate, if *Rhizobia* bacteria are present in the soil, they penetrate through the root hairs and move into the root. Bacteria multiply inside the root causing swellings which are termed as nodule. The bacteria then produce ammonia using N from the soil air and hydrogen from carbohydrate in the plants. The ammonia provides a source of N for the plants to grow (Sylvia et al., 2004). This symbiotic relationship allows legumes to produce high protein forage and seed. Nitrogen addition to soil by a legume cover crop occurs when the plant parts decompose. Soil microorganisms use organic matter as a source of N for metabolism. These microorganisms also release N to the soil when they die. Badaruddin and Meyer (1989) reported an increase of soil $NO_3$-N following legume cover crops. Other effects of legume cover crops on soil fertility status include a redistribution of potassium (K) to the soil surface from deeper in the soil profile and a lower C/N ratio in the soil organic matter (Hargrove, 1986).

The value of legumes in crop rotations was recognized in the southern United States in the early 1900's by many researchers. Ebelhar et al. (1984) reported that, winter annual legume cover crops added significant amount of N to no-tillage corn. In this study, hairy vetch (*Vicia vollosa* R.) produced highest dry matter with a higher N percentage, which resulted in a higher N concentration in corn plants following big flower vetch (*Vicia grandiflora* W. Koch var. *Kitailbeliuna*) and crimson clover (*Triflium incarnatum* L.). Average five-year grain yields following hairy vetch cover crop with no fertilizer applied were about 2.5 Mg ha$^{-1}$. Approximately, 90-100 kg N ha$^{-1}$ was supplied by hairy vetch annually. Furthermore, legumes provide a wide range of soil quality benefits. Increased water-stable aggregates were reported following cover crops in the 0 to 0.025 m depth (McVay et al., 1989). Greatest infiltration rates were found following hairy vetch than following wheat. Zeng et al. (2008) reported that forage legume increase water content in soil layers. Water use efficiency increased up to 70% in the erect milk vetch (*Astragalus adsurgens* P.) plots. Total organic carbon content was also increased in the plots where legumes were planted after a fallow period.

Crimson clover was tested on many prominent crops as an alternative source of nitrogen. In a study of no-tillage cotton (*Gossypium hirsutum* L.), Touchton et al. (1984)

reported that crimson clover can provide the N needs in sandy soils naturally low in N. In a different study, crimson clover was seeded along N fertilizer annually to no-till corn (Myers and Wagger, 1991). Combined data analysis after three years shows that, corn grain yields increased significantly under cover crop management with inorganic N fertilizer than fallow and only N fertilizer. A similar result was reported for grain sorghum production. Nitrogen produced by crimson clover was sufficient for a grain yield of 7,098 kg $ha^{-1}$ without supplemental applications of inorganic N; which was 438 kg $ha^{-1}$ higher than those non treated plots (Touchton et al., 1982). Smith et al. (1996) reported that crimson clover and hairy vetch mixture can be used as a cover crop to meet the N requirement of pecan (*Carya illinoinensis* Wangenh. K. Koch). The legume mixture supplied the equivalent of 101 kg N $ha^{-1}$ to 159 kg N $ha^{-1}$.

Winter legumes must produce abundant biomass prior to growth termination to be considered a successful cover crop. Boquet and Dabney (1991) reported that arrowleaf clover (*Trifolium vasiculosum* S.) produced 6,300 kg $ha^{-1}$ dry matter with a total N of 203 kg $ha^{-1}$ which is theoretically sufficient to meet the requirements of grain sorghum production. Fleming et al. (1981) found that corn yield was 272% higher following arrowleaf clover than no N was applied.

Research has indicated that, legume cover crops have great potential for reducing fertilizer cost. In a 2-year-long rotation study, legumes were used as a source of N for oat (*Avena fatua* L.) and corn. Red clover (*Trifolium pretense* L.) and hairy vetch yielded an equivalent to 132 kg N $ha^{-1}$ (Stute and Posner, 1995). Corn grain yield was up to 10,230 kg $ha^{-1}$ following red clover. Red clover was less effective in terms of supplying N for pecan trees. However, together with white clover (*Trifolium repens* L.) the mixture supplied up to 132 kg N $ha^{-1}$ (Smith et al., 1996).

High dry matter yield and N concentration of the legumes correspond to high level inorganic N and water soluble N in the soil. Kou et al. (1997) reported that Austrian winter pea (*Pisum sativum* L.) is a potential legume cover crop due to its high dry matter yield and rapid degradation. The study showed that, net nitrogen mineralization four weeks after residue incorporation was 17.9 g N $kg^{-1}$, and had a higher winter survival and spring growth when compared to crimson clover and hairy vetch (Holderbaum et al., 1990). There is very limited knowledge on the effect of button medic (*Medicago orbicularis* L.) as a cover crop. However, a study, where button medic was over-seeded with switchgrass showed no effect on dry matter and nitrogen yield (Butler et al., 2012).

Seeding success, persistence, and impact of cool-season forage legumes on established 'Cave-in-Rock' switchgrass were evaluated at Ames, IA (Blanchet et al., 1995). Ten forage legumes and a legume mixture were no-till interseeded in April for two years. Excellent legume establishment was reported after 2.5 months of interseedings. Mean legume plant-density was up to 195 plants $m^{-2}$. Average legume persistence was as high as 50%. Switchgrass stem density was not affected by most of the legumes during establishment. Considerable variations in canopy types were observed among legumes, which contributed in competitive ability with switchgrass. Blanchet et al. (1995) concluded that, cool-season legumes can be successfully established into switchgrass and can be maintained into the subsequent years unless severe winter conditions reduce legume survival.

Lignocellulose is the primary constituent of plant cell walls (Kumar et al., 2009). Other components include hemicellulose and smaller amounts of pectin, protein, sugars, nitrogenous materials, chlorophyll, and waxes. The quality of dry matter for biofuel depends on the concentration of the cell wall components, particularly cellulose, hemicellulose, and lignin (Oasmaa and Czernik, 1999). High concentration of these components present in the cell wall meaning a better quality biomass. However, presence of ash, chloride, potassium, sodium, and silicon dioxide affect the quality of dry matter (Lemus et al., 2002; Miles et al., 1996). Typically, grasses contain about 25% to 40% cellulose, 35% to 50% hemicellulose, and 10% to 30% lignin (Kumar et al., 2009). The composition of these constituents can vary on different growth conditions. Kering et al. (2011) reported a significant reduction of acid detergent fiber (ADF) and neutral detergent fiber (NDF) in bermudagrass (*Cynodon dactylon* L.) treated with inorganic nitrogen fertilizer. Lemus et al. (2008) reported no trend on biofuel quality due to N fertilization on switchgrass over years. They concluded that, nitrogen fertilization can substantially increase dry matter yield without affecting the quality of the feedstock. However, effects of legumes in a legume-grass intercropping system on biofuel quality of forage are not well known.

# OBJECTIVES

This project was designed with the general objectives of evaluating the effects of six selected winter legumes on dry matter production of switchgrass and soil quality. We hypothesize that, decomposition of legumes will provide enough N for switchgrass throughout the growing season and thus may increase dry matter yield and quality for biofuel production.

The specific objectives were to:

1. Determine the N contribution from the six winter legumes in a switchgrass stand,
2. Determine the influence of winter legumes on soil nutrient conditions and organic matter and,
3. Evaluate the effects of legumes on yield and quality of switchgrass grown for cellulosic ethanol production.

# MATERIALS AND METHODS

### *Study Sites*

Field experiments were established at the Cimarron Valley Research Station at Perkins, OK and at the Noble Foundation research farm in Maysville, OK. Both studies were initiated in fall 2009. Rainfall and air temperature data were collected using Oklahoma Mesonet (Brock et al., 1995) monthly weather report.

The Cimarron Valley Research Station is located approximately 274 m above sea level (35º 59′ 23.86″ N, 97 º 2′ 41.94″ W) along the Cimarron River (Oklahoma Mesonet). The soil at Perkins is a Teller sandy loam (fine-loamy, mixed thermic, Udic Argiustoll) with 3% to 5% slope. The soils are deep, well drained and moderately permeable. The Konawa series (fine-loamy, mixed, active, thermic Ultic Haplustalfs) is found where the slope ranges from 1% to 8%. Soil pH ranges from 5.1 to 6.5. The annual average temperature is 926 mm. Average summers' high temperature is 32.8°C; average winter temperature is -3.3°C. The soils are highly productive with appropriate fertilization. Monthly rainfall and temperature during the three years study are shown in Figure 1.1. Initial soil samples were collected before establishment of the experiment to correct P, K, and lime deficiencies. The initial soil chemical properties of the experimental plots (0 to 15 cm depth) are given in Table 1.1.

The soil at Maysville (34º 48′ 26.46″N, 97º 28′ 54.47″W) is McLain silty clay loam (fine, mixed, superactive, thermic Pachic Argiustolls) consist of very deep, moderately well drained, slowly permeable soil that formed from material weathered from clay and loamy alluvium. The site has a slope of 0% to 1% and is rarely flooded. The elevation is approximately 292 m above sea level. Monthly temperature and rainfall are shown in Figure 1.2. Initial soil test report (0 to 15 cm depth) is given in Table 1.1. Phosphorus (P) was deficient in the experimental plots and was corrected by broadcasting 67 kg P ha$^{-1}$ as triple super phosphate.

### *Experimental Design and Treatment Structure*

The experimental design was a randomized complete block with three replications. Each plot was 3.04 m long and 1.52 m wide. Treatments were a check, six winter legumes, and four rates of inorganic N fertilizer. Treatments structure is presented in Table 1.2.

A lowland switchgrass variety ‘Alamo’ was planted in both locations. In Maysville, switchgrass was planted on June 25, 2008 and April 2009 in Perkins. The variety was chosen because of its high dry matter yield potential. A maximum of 22.5 Mg ha$^{-1}$ dry matter yield was reported when 168 kg ha$^{-1}$ N was applied (Muir et al., 2001). Plots were seeded at the rate of 6 kg ha$^{-1}$ pure live seed (PLS) with row spacing of 36 cm. Switchgrass seeds were planted in a well-prepared seedbed with a John Deere 1590 no-till drill to a depth of 1.5 cm. At least one growing season was allowed for both locations to ensure complete establishment. Switchgrass was cut with a sickle mower in October 2009 before planting legume treatments. The six winter legumes used were, Yuchi arrowleaf clover, Austrian winter pea, button medic, crimson clover, hairy vetch, and red clover. Legume seeds were inoculated with appropriate commercial inoculants to enhance symbiotic nitrogen fixation. At Perkins, furrows were made with a hand hoe between two switchgrass rows. Legumes seeds were then hand planted in the furrows each year in the fall. In Maysville, instead of hand planting, a conventional drill was used to plant legume seeds every year. Seed rates and corresponding bacterial inoculants are presented in Table 1.3.

Four rates of N (0, 56, 112, and 168 kg N $ha^{-1}$) were used to create a response curve to determine legume contribution to switchgrass and soil properties. Inorganic N treatments were applied in the spring at all locations. In 2010 and 2011, urea (46-0-0) was used as a source of inorganic N. In 2012, a liquid N fertilizer urea ammonium nitrate (28-0-0) was applied in both locations.

### ***Harvesting and Sample Collection***

Dry matter production of legumes was measured by hand harvesting above-ground plant parts from 1 m length of a row at maturity. The dry weights of legumes are then multiplied by three to obtain dry matter from 1 $m^2$ area. These weights were then converted to kilogram per hectare. The remaining portion of legumes was terminated with DuPont™ Cimarron®MAX (Metsulfuron Methyl 0.75%, Dimethylamine salt of Dicamba 12.25%, Dimethylamine salt of 2, 4-D 35.25%, and 51.75% inert ingredients) and left in the field to decompose. Harvesting dates of legumes are presented in Table 1.4.

Switchgrass forage was hand harvested with a hand sickle at Perkins every fall. A 1 m by 1 m strip was clipped to 8 cm stubble height from the center of each plot. The plots at Maysville were harvested with a carter harvester in 2010. A Wintersteiger harvester was used for harvesting in 2011 and 2012. The switchgrass harvesting dates are presented in Table 1.4.

Immediately after switchgrass harvest, soil samples were collected each year from two depths, 0 to 15 cm, and 15 to 30 cm. A 30 cm-long hand probe was used to collect 15 to 20 cores from each plot. Then the cores were mixed well in a plastic bucket. A subsample was taken from the mixture to get a representative composite sample.

### ***Sample Processing and Laboratory Testing***

Soil samples were oven dried at 60ºC overnight and ground to pass a 2-mm sieve. The samples were analyzed for pH, organic matter and nitrate N. Extractable phosphorus (P) and potassium (K) were also analyzed. A 1:1 soil-water suspension and glass electrode pH meter was used to measure soil pH and buffer index (Thomas, 1996; Sims, 1996; Sikora, 2006). A five gram of soil sample was extracted with 25 ml of 1 *M* KCl solution to determine $NO_3$-N and was quantified using a flow-injection Autoanalyzer (LACHAT, 1994). Mehlich III solution was used to extract P and K (Mehlich, 1984); and the amount were quantified using a Spectro CirOs inductively coupled plasma spectrometer (Soltanpour et al., 1996). Percent soil organic carbon was determined using a LECO TruSpec® dry combustion carbon analyzer (Nelson and Sommers, 1996). Percent organic matter was calculated as OM (%) = organic carbon (%) * 1.724.

For laboratory analysis of tissue samples, a sub-sample of each legumes and switchgrass was ground after oven-drying for three days at 65°C to pass through a 425-μm sieve. Percent N in the forage was determined using a LECO TruSpec® Elemental Analyzer (LECO Corporation, 2008). ANKOM 200 Fiber analyzer was used to determine acid detergent fiber (ADF) and neutral detergent fiber (NDF). Acid detergent solution was prepared by adding 20 g cetyl trimethylammonium bromide to 1 L 1 $N$ $H_2SO_4$ which was used to determine ADF (ANKOM Technology, 2010). Neutral detergent solution was prepared by adding 30.0 g sodium dodecyl sulfate USP, 18.61 g ethylenediaminetetraacetic disodium salt dehydrate, 6.81 g sodium borate, 4.56 g sodium phosphate dibasic anhydrous, and 10.0 ml triethylene glycol in 1 L distilled water. The solution was check for a pH range of 6.9 to 7.1. The solution was used to determine NDF (ANKOM Technology, 2010). Acid detergent fiber is the amount of cellulose and lignin together (Linn et al., 1999). Percent hemicellulose was calculated as follows (Lemus et al., 2002):

$$\text{Hemicellulose (\%)} = \text{Neutral detergent fiber (\%)} - \text{Acid detergent fiber (\%)}$$

All forage analyses were conducted in Agricultural Testing Services at The Samuel Roberts Noble Foundation, Ardmore, Oklahoma. Soil samples were analyzed in the Soil, Water and Forage analytical Laboratory at Oklahoma State University.

### *Statistical Analyses*

SAS 9.3 computer software (SAS Institute Inc.) was used for statistical analyses. Data were subjected to Analysis of Variance using General Linear Model (GLM) procedure. Mean separations were performed using Duncan's multiple range tests at 0.05 level of significance.

## RESULTS AND DISCUSSION

The effects of winter legumes and N fertilizer on switchgrass forage parameters and soil quality are presented in Tables 1.5-1.19.

### *Weather Conditions During Growing Periods*

Legumes seeds were dormant and did not germinate until significant temperature and the presence of moisture increase in March each year. Before termination of growth in May, legumes at Perkins received 293 mm rainfall in 2010, 198 mm rainfall in 2011, and 272 mm rainfall in 2012. Maysville received 205 mm rainfall in 2010, 193 mm in 2011, and 321 mm rainfall in 2012, respectively (Figure 1). In the basis of total rainfall, 2011 growing season was the driest year for both locations. The day time average monthly air temperature during these growing seasons was near 30°C in both locations. Monthly highest average temperature was 34°C in May 2011 at Perkins. Switchgrass is considered to be adapted to hot and dry conditions. However, during its growing season, from March to September each year at Perkins, total rainfall was 649 mm, 682, and 472 mm in 2010, 2011, and 2012, respectively. At Maysville, total rainfall was 565, 266, and 591 mm in 2010, 2011, and 2012 (Figure 2). The growing season precipitation was very low in 2011. In this dry and variable rainfall region, air temperature can rise in the summer to more than 40ºC regularly. In both 2011 and 2012, Oklahoma has experienced consecutive high temperature in July and August with a high temperature of 46°C at Perkins in August 2012.

### *Dry Matter, N Concentration and, Aboveground Total N of Legumes*

#### *Perkins*

Total dry matter production, N concentration, and above ground total N of the legumes for all three years are presented in Table 1.5. Legume stand varied from year to year. In terms of dry matter production, 2012 was a good year for all legumes at Perkins. Arrowleaf clover produced highest 3052 kg ha$^{-1}$ dry matter in 2012 followed by hairy vetch (2235 kg ha$^{-1}$). Arrowleaf clover had 73% lower dry matter yield in 2011 compared to dry matter yield in 2012. However, the species was the best in 2011 which produced 819 kg ha$^{-1}$ dry matter. In 2010, hairy vetch produced the greatest dry matter which was 2046 kg ha$^{-1}$. By adding three years data, hairy vetch produced a total of 4863 kg ha$^{-1}$ dry matter with an average dry matter yield of 1621 kg ha$^{-1}$ yr$^{-1}$. Among six legumes intercropped with switchgrass, button medic had the poorest stand followed by red clover in terms of dry matter production. In this three-year-long study, the average dry matter production of button medic was 423 kg ha$^{-1}$ yr$^{-1}$ and red clover was 485 kg ha$^{-1}$ yr$^{-1}$.

Dry matter of legumes in 2011 was very low for all legumes largely attributed to the exceptionally dry weather (198 mm rainfall) during the spring months of 2011. This dry condition is confounded by the fact that following winter dormancy, switchgrass grows very fast in the initial months of spring competing for limited moisture with the legumes.  Deep rooted switchgrass was able to take available water from that was not replenished by spring precipitation, leaving little water for legumes.

Nitrogen concentration of each legume varied over the two years in which data were collected (Table 1.5). Except for Austrian winter pea which had a N concentration of 23.57 g kg$^{-1}$, other legumes had 29% lower N concentration in 2012 compared to 2010 followed by a drought year of 2011. On the three years average, N concentration of hairy vetch was the greatest (26.71 g kg$^{-1}$) among other five legumes.

Severe environmental conditions could affect *Rhizobium*-legume symbiotic system. Stress factors such as heat and drought suppress the growth and symbiotic characteristics of most *Rhiozobia* (Zahran, 1999). The years of 2011 and 2012 were extremely dry year with high temperature which may have prevented *Rhizobia* from developing a symbiotic system with legumes resulting low N concentration in those two years.

Legume tissue N concentrations were significant among treatments probably due to the differences in N production (Touchtone et al., 1984). All legumes were harvested in a same day. Therefore, maturity status of different legumes was different resulting different N concentrations.

In 2010, hairy vetch had the greatest aboveground total N due to significantly higher biomass production (Table 1.5). However, in 2012, hairy vetch, arrowleaf clover, and Austrian winter pea appeared as the best legumes in term of total above ground N.

By combining three years data, hairy vetch was the best legume which produced an average of 56 kg $ha^{-1}$ $yr^{-1}$ aboveground total N.

Hairy vetch appeared to be the superior legume in terms of dry matter yield, N concentration, and above ground total N when compared to other legumes. In this study we only analyzed total N for aboveground plant parts. Mitchell and Teel (1977) reported that above ground portion of winter legumes contain two-third of total N and roots contain one-third. Therefore, hairy vetch root contains a calculated amount of 18 kg $ha^{-1}$ $yr^{-1}$. By adding aboveground and belowground total N, at most, hairy vetch had an average of 74 kg total N $ha^{-1}$ $yr^{-1}$.

***Maysville***

No significant difference between arrowleaf clover, Austrian winter pea and hairy vetch was found in terms of dry matter production and aboveground total N in 2012 at Maysville (Table 1.6). By numerical value, Austrian winter pea was the best legume in this location and environmental conditions which had produced 2535 kg $ha^{-1}$ aboveground dry matter and had a total aboveground N content of 40 kg $ha^{-1}$. Dry matter yield of arrowleaf clover (2380 kg $ha^{-1}$), crimson clover (1865 kg $ha^{-1}$), and hairy vetch (1801 kg $ha^{-1}$) did not significantly differ with the highest yield of Austrian winter pea.

Red clover had a higher tissue N concentration (20.80 g $kg^{-1}$) followed by hairy vetch (17.22 g $kg^{-1}$). However, red clover produced the least total dry matter (721 kg $ha^{-1}$) thus resulting significantly lower aboveground total N which is 14 kg $ha^{-1}$. Austrian winter pea produced the highest 40 kg $ha^{-1}$ aboveground total N largely due to high dry matter production. Tissue N concentration of Austrian winter pea was 16 g kg$^{-1}$ which is significantly lower than red clover. Unlike at Perkins, button medic showed a good establishment (1192 kg ha$^{-1}$) with switchgrass at Maysville.

Arrowleaf clover, Austrian winter pea, crimson clover, and hairy vetch appeared to be the best legumes in this location. Like Perkins, red clover and button medic were not well suited to compete or growth with switchgrass. Our results at this location also showed that, button medic and red clover were less drought tolerant than other legumes.

### *Dry Matter, N Concentration, and Aboveground Total N of Switchgrass*

#### *Perkins*

Influence of legume treatments and N rates on switchgrass dry matter yield is presented in Table 1.7. None of the legumes had any effect in terms of switchgrass dry matter production in this three-year-long study. Addition of inorganic N increased dry matter yield linearly (Figure 1.5). However, no significant differences were found between two highest rates of N fertilizer. The rate of 56 kg N ha$^{-1}$ had no positive response to dry matter yield compared to non-fertilized plots.

The soil at Perkins in a switchgrass stand was very dry during the spring urea application. Lack of moisture in the soil may have caused urea volatilization and left no N for switchgrass to take.

There was no significant difference in switchgrass yield among legume treatments in 2010. However, button medic treatment produced the greatest switchgrass dry matter yield in 2011 and 2012, while hairy vetch and red clover treatments produced numerically lowest yields. By adding three years average, 168 kg N $ha^{-1}$ of inorganic N appeared to be the superior in dry matter production (Figure 1.3). A total of 54,940 kg $ha^{-1}$ dry matter produced in the 168 kg N $ha^{-1}$ plots; where, 18,313 kg ha-1 dry matter produced in the 112 kg N $ha^{-1}$ plots. Among legume treatments, arrowleaf clover produced the highest switchgrass dry matter (11,478 kg $ha^{-1}$ $yr^{-1}$); however, not significantly higher than the check which produced an average of 13,478 kg $ha^{-1}$ $yr^{-1}$.

It may take several months to several years for the legumes to add N to the soil. Nitrogen contribution by the legumes was not expected in the first growing season. Decomposition of some legumes may take eight weeks (Carmen et al., 2000) when the conditions are ideal. The process takes longer when soil moisture is low. Switchgrass grows faster in spring than summer and fall; growth slows down as temperature is increased in the months of July and August. Lack of proper level of nutrients in the spring may have affected vegetative growth. No response was observed in the subsequent years was likely due to severe drought. Despite the good stand of hairy vetch with switchgrass, the treatment had the lowest switchgrass dry matter yield (11,909 kg $ha^{-1}$ $yr^{-1}$). The reason for the reduced dry matter was due to the external morphology of hairy vetch. Long vine type hairy vetch was competitive with switchgrass for water, nutrients, and light. Additionally, hairy vetch wrapped down switchgrass, which prevented the grass from its normal growth; resulting in lower dry matter yield.

Nitrogen concentration in the above ground biomass did not vary among legume and inorganic N fertilizer treatments in 2010 and 2011. However, N concentration was relatively higher in switchgrass dry matter when 112 kg N ha$^{-1}$ and 168 kg N ha$^{-1}$ were applied. In 2012, dry matter produced from button medic and hairy vetch treatments had the highest N concentration similar to the rate of 168 kg N ha$^{-1}$. Nitrogen concentration in the dry matter was not affected by other legume treatments.

Applying 168 kg N ha$^{-1}$ yr$^{-1}$ increased above ground total N of switchgrass dry matter (Figure 1.7). Average switchgrass aboveground N content was 108 kg ha$^{-1}$ yr$^{-1}$. In 2010, Austrian winter pea and hairy vetch treatments had equivalent N content level compared to 112 kg N ha$^{-1}$ yr$^{-1}$ and 168 kg N ha$^{-1}$ yr$^{-1}$ due to higher dry matter yield. Neither the application of N fertilizer to switchgrass plots nor the legumes had any effects on switchgrass total aboveground N content in 2011. In this year, treatment effects were masked due severe heat and drought. Interseeded legumes did not increase total N in switchgrass compared to check plot in 2012. The only case in which switchgrass total N was greater than the control plots was when the plots were fertilized with 112 kg N ha$^{-1}$ and 168 kg N ha$^{-1}$.

### ***Maysville***

Inorganic N fertilizer created the greatest response to switchgrass dry matter production (Table 1.8; Figure 1.4). Like Perkins, yield response to N rates was linearly related (Figure 1.6). The effects of legume treatments were not apparent on switchgrass dry matter yield for any of the years. In 2010 the rate of 112 kg N ha$^{-1}$ 20,055 kg ha$^{-1}$ of biomass while, while 168 kg N ha$^{-1}$ had the highest dry matter yield in 2011 and 2012. Combining three years average, the rate of 168 kg N ha$^{-1}$ yr$^{-1}$ produced 59, 164 kg switchgrass dry matter with an average of 19,721 kg ha$^{-1}$ yr$^{-1}$ followed by the rate of 112 kg N $ha^{-1}$ $yr^{-1}$ with 16,721 kg $ha^{-1}$ $yr^{-1}$ dry matter. However, the two inorganic N treatments were not significantly different. Like Perkins, hairy vetch treatment had the lowest switchgrass dry matter yield among all treatments at 10,022 kg $ha^{-1}$ $yr^{-1}$ due to the same reasons described earlier.

Neither fertilizer N nor legumes had significant impact on N concentration in 2010. In 2011, those treatments fertilized with urea had significantly lower N concentration when compared to check plots (Table 1.8). Austrian winter pea had numerically highest N concentration. However, not significantly form check plots. Similar results were obtained in 2012. Except red clover and crimson clover, all other legume treatments had higher N concentration when compared to inorganic N treatments. Hairy vetch had the highest N concentration.

There were no significant treatment effects in 2010 in terms of aboveground total N. Except red clover, total N was low for all other legume treatments in 2011 compared to check plots. Inorganic fertilizer treatments did not increase dry matter total N. In 2012, arrowleaf clover and Austrian winter pea had 63.59 kg $ha^{-1}$ and 62.41 kg $ha^{-1}$ above ground total N which was significantly higher than check plot. However, this increase was not significant from other legumes and N treatments. Aboveground total N increase was largely due to the high N concentration in the dry matter. The rate of 168 kg N $ha^{-1}$ $yr^{-1}$ yielded highest above ground total N when three years data were summed. The average above ground total N was 58.61 kg $ha^{-1}$ $yr^{-1}$. Compared to check plot (33.18 kg N $ha^{-1}$ $yr^{-1}$), all legume treatments had higher average aboveground total N in which arrowleaf clover was the best (50.44 kg N $ha^{-1}$ $yr^{-1}$) followed by Austrian winter pea.

### *Biofuel Quality of Switchgrass*

#### *2010 Growing Season*

The effect of legumes and inorganic N rates on swithcgrass biofuel quality at Perkins and Maysville in 2010 is presented in Table 1.9. Cellulose, hemicellulose, and lignin content are very important for biofuel quality. High amount of these components in the cell wall store more energy. High amount of minerals such as P, K, Mg, and Ca, may result in biomass quality reduction during ethanol production through pyrolysis. None of the treatments increased cellulose, hemicelluloses, and lignin content at either site when compared to check. Fertilizer N and legumes were likely decrease hemicellulose content. At Perkins, dry matter quality for biofuel decreased regardless of legumes and fertilizer treatments. Mineral uptake was higher for all treatments compared to check plots. No significant treatment effects were observed at Maysville in terms of mineral content in switchgrass dry matter.

#### *2011 Growing Season*

No treatment effects were found on cellulose and lignin content in switchgrass at Perkins (Table 1.10). Inorganic N fertilizer did not impact hemicelluloses content. Arrowleaf clover, Austrian winter pea, and hairy vetch decreased hemicelluloses content in switchgrass dry matter; while other legumes had no effect when compared to the check. In terms of mineral content, there were no significant treatment effects on Ca. However, hairy vetch increased Mg content and arrowleaf clover increased K uptake.

Except button medic and crimson clover, other legumes increased P uptake resulting low biofuel quality. No N treatments had any impact on P Uptake.

Applying 168 kg N ha$^{-1}$ increased cellulose and lignin content at Maysville in 2011 (Table 1.10). The amount of cellulose and lignin was 427 g kg$^{-1}$. However, the treatment produced less cellulose and lignin content compared to other sites and years. In the same location in 2010, highest cellulose and lignin content was 514 g kg$^{-1}$. Arrowleaf clover decreased hemicelluloses, while other treatments had no impact. Plots fertilized with 168 kg N ha$^{-1}$ improved biofuel quality by decreasing P and K uptake. Arrowleaf clover and Austrian winter pea decreased quality as Ca and Mg uptake increased.

### ***2012 Growing season***

Cell wall components were not measured in the laboratory for Perkins location in the final year due to budget constraints and lack of treatment response in the previous years (Table 1.11). At Perkins, red clover increased biofuel quality as the treatment had lowest P uptake. Phosphorus concentration of red clover treatments was 0.80 g kg$^{-1}$ which was significantly lower than the non-treated check plot. There were no impact on K and Ca content when compared to check. Austiran winter pea, hairy vetch, and 168 kg N ha$^{-1}$ had the highest Mg uptake thus decrease forage quality.

At Maysville, an increased lignin and cellulose content was obtained when 168 kg N ha$^{-1}$ was applied (Table 1.11). None of the treatments increased hemicelluloses. Arrowleaf clover and crimson clover significantly decreased hemicelluloses content when compared to check plot. Crimson clover and 112 kg N ha$^{-1}$ improved forage quality in terms of biofuel production as the treatments had lowest P uptake. While, arrowleaf clover and hairy vetch decreased quality due to higher Ca and Mg content in the dry matter. No treatments had any impact on K content.

In this study, inorganic N fertilizer increased dry matter yield. However, cell wall components and mineral concentration did not follow any trend regardless of inorganic N and legume treatments. These results suggest that N fertilization and legumes as a source of N had no meaningful effect on biofuel quality. Lemus et al. (2008) in their study of switchgrass in southern Iowa reported similar effect of N fertilization on biofuel quality.

### ***Influence of Winter Legumes and Fertilizer N on Soil Organic Matter (OM), and Nitrate Nitrogen***

#### ***Perkins***

Legumes can provide organic matter (OM) and N, P, and K through residue decomposition. Nitrogen mineralization from legume residue is a slow process. Considerable amount of N from the mineralization of legume residue could be available the following year (Njunie et al., 2004). Therefore, changes in soil characteristics are unlikely after one year and could take several years. Soil samples were collected from 0 to 15 cm and 15 to 30 cm depth and analyzed. Neither inorganic N nor legumes increased organic matter concentration regardless of year or depths (Table 1.12). Soil nitrate N content was very low in all years and depths. Switchgrass utilized all available N in the soil. In 2010, arrowleaf clover treatment had the highest nitrate N in the surface soil which was 4.1 kg ha$^{-1}$ compare to check (1.86 kg ha$^{-1}$). The rate of 112 kg N ha$^{-1}$ had the highest surface nitrate N (1.82 kg ha$^{-1}$) in 2011. There was no treatment impact on surface nitrate N levels in 2012. None of the treatments increased sub-surface N content in this three-year-long study. Surface nitrate N was higher than sub-surface N content. A slight increase of nitrate N concentration was noticed in both depths compared to initial values in the final year.

Overall, sub-surface organic matter content was higher than the surface organic matter content regardless legume and N rates. Organic matter content in two depths decreased over years. Compared to organic matter content in 2010, up to a 20% organic matter reduction in the top soil was measured at the end of the study in those plots with arrowleaf clover and 112 kg N ha$^{-1}$ yr$^{-1}$. Among other legumes, button medic added about 19% organic matter.

A calculated of 33% organic matter loss occurred in the sub-surface soil at the conclusion of the study at Perkins in the non-treated plots. Of the legume treatments, Austrian winter pea and crimson clover had the greatest loss of 24% organic matter content in the soil.

***Maysville***

In the conclusion of this study in 2012, soil was taken for analysis from hairy vetch and crimson clover as these were greatest dry matter producer to see if there were any differences compared to check plots. No differences were found either in 2010 or in 2012 (Table 1.13). However, like Perkins, OM decreased over years consistently in all treatments. There were 33% organic matter loss by crimson clover treatment at the end of the study when compared to first year's organic matter content.

The $NO_3$-N concentrations in the soil surface were less in all legumes and N treatments than the initial values suggesting that there was no N accumulation in soil. Treatment difference were not apparent may be due to short interval between legume senescence and switchgrass growth.

### *Effect of Winter Legumes and Fertilizer N on Soil Fertility*

The influence of legumes and fertilizer on soil fertility status is presented in Table 1.14 to Table 1.19. Mineral accumulation in the soil did not occur in the plots where legumes were planted. Switchgrass may have absorbed most minerals during the growing season that were deposited in the soil due to legume decomposition. At Perkins, pH was largely unaffected in all three years in both depths. In 2011, soil slightly acidified under red clover treatments in the sub-surface soil. There were no treatment effects at Maysville in terms of pH in 2010. Soil pH significantly decreased under legume treatments in 2012 at 0 to 15 cm depth. McVay et al. (1989) had found similar results where soil pH decreased following crimson clover and hairy vetch. They have concluded that this may have happened due to the differences in exchangeable ions. Concentration of Al and Mn following crimson clover were found greater than other cover crop treatments. In addition, concentration of magnesium and calcium was lower in the top soil. However, in this study, zinc concentration was not affected by legume treatments. Sub-surface soil was unaffected by both legumes and N treatments.

Compared to check plots, P and K content in the surface and sub-surface soil at Perkins did not differ due to treatments. However, in 2010, 112 kg N $ha^{-1}$ had greater P content over the 56 kg N $ha^{-1}$. P was deficient in Maysville at the initiation of the experiments. Phosphorus fertilizer was applied to correct deficiency. Those plots where button medic was grown in 2010 and crimson clover in 2012 had higher extractable soil P than any other treatments. Soil P was likely redistributed in the surface soil perhaps due to rapid decomposition of these legumes. P status in the subsurface soil was fairly unaffected. No significant treatment effects were found for extractable K. Overall, the amount of K decreased over years presumably due to uptake by switchgrass.

# CONCLUSIONS

Hairy vetch produced greatest dry matter following arrowleaf clover at Perkins. These two legumes also had highest N concentration. At Maysville, Austrian winter pea had highest dry matter yield. Using winter legumes as a source of N intercropped with switchgrass is questionable as none of the six legumes used in this experiment increased switchgrass dry matter yield when compared to non-fertilized check. The soil at both locations had very low in nitrate N content. Low rate of N treatment had no impact on switchgrass yield possibly due to N loss. A rate of 112 kg N ha$^{-1}$ was required to achieve greater dry matter yield. Winter legumes may not be a potential source of N for switchgrass when N requirement is high in this arid environmental condition in Oklahoma where drought and excessive heat are common. Winter legumes and inorganic nitrogen treatments generally did not enhance cell wall components. However, in some instances, winter legumes decreased biofuel quality by increasing mineral concentration in the dry matter. No sizeable increase of nitrate N was detected in the soil regardless of depths by any treatments. This is due to all available N was taken up by switchgrass. There was a decreasing trend of organic matter depletion at both locations also indicate a reduction of total N in the soil. At Perkins, organic matter slightly reduced in the sub-surface soil. At Maysville, in the top 15 cm soil, on average, organic matter depleted by 33% in the final year when compared to first year's organic matter content. No considerable change occurred in soil pH at Perkins. In this location, soil P and K status decrease in the subsequent years. Similar results were found in Maysville except crimson clover and hairy vetch significantly increase soil acidity. This is may be due to increase of Al and Mn concentration in the soil. To reduce input cost as a replacement of inorganic fertilizer N and to conserve soil quality, winter legumes-switchgrass intercropping is not recommended in Oklahoma.

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Table 1.1. Chemical properties of soils (0 to 15 cm) of the experimental plots. Samples were taken before legume planting in 2009.

| Location | pH | $NO_3$-N | Mehlich III P | Mehlich III K |
|---|---|---|---|---|
| | | ------------------------------ | ppm | ------------------------------ |
| Perkins | 6.5 | 0.5 | 5 | 132 |
| Maysville | 6.4 | 1 | 67 | 138 |

Table 1.2. Treatments and descriptions that used in the study for both Perkins and Maysville locations.

| **Trt no.** | **Treatments** | **Description** |
|---|---|---|
| 1 | 0 kg N $ha^{-1}$ | Check |
| 2 | 56 kg N $ha^{-1}$ | Applied in spring |
| 3 | 112 kg N $ha^{-1}$ | Applied in spring |
| 4 | 168 kg N $ha^{-1}$ | Applied in spring |
| 5 | Arrowleaf clover | No-till planted in fall |
| 6 | Austrian winter pea | No-till planted in fall |
| 7 | Button medic | No-till planted in fall |
| 8 | Crimson clover | No-till planted in fall |
| 9 | Hairy vetch | No-till planted in fall |
| 10 | Red clover | No-till planted in fall |

Table 1.3. Seed rates and inoculant types of corresponding legumes species. Seeds were inoculated before planting.

| Legumes | Scientific Name | Seed Rate [kg ha$^{-1}$] | Inoculant |
|---|---|---|---|
| Arrowleaf clover | *Trifolium vasiculosum* S. | 6 | *Rhizobium leguminosarum* |
| Austrian winter pea | *Pisum sativum* L. | 55 | *Rhizobium leguminosarum* |
| Button medic | *Medicago orbicularis* L. | 10 | *Sinorhizobium meliloti* |
| Crimson clover | *Trifolium incarnatum* L. | 22 | *Rhizobium leguminosarum* |
| Hairy vetch | *Vicia vollosa* R. | 45 | *Rhizobium leguminosarum* |
| Red clover | *Trifolium pretense* L. | 9 | *Rhizobium leguminosarum* |

Table 1.4. Dates of planting and harvesting of legumes and switchgrass. Legume growth was terminated after harvesting.

| Year | Legumes | | Switchgrass forage harvest |
|---|---|---|---|
| | Planting date | Termination/harvesting date | |
| | | Perkins, Oklahoma | |
| 2009 to 2010 | 11/30/2009 | 05/16/2010 | 10/14/2010 |
| 2010 to 2011 | 10/25/2010 | 06/14/2011 | 10/26/2011 |
| 2011 to 2012 | 10/28/2011 | 05/25/2012 | 09/16/2012 |
| | | Maysville, Oklahoma | |
| 2009 to 2010 | 11/10/2009 | 05/15/2010 | 10/22/2010 |
| 2010 to 2011 | 10/08/2010 | 06/14/2011 | 10/17/2011 |
| 2011 to 2012 | 11/02/2011 | 05/25/2012 | 09/14/2012 |

Table 1.5. Dry matter, N concentration, and above ground total N by year of the six legumes grown at Cimarron Valley Research Station near Perkins, Oklahoma.

| Legume | Dry matter | | | N concentration | | | Aboveground total N | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2010 | 2011 | 2012 | 2010 | 2011 | 2012 | 2010 | 2011 | 2012 |
| | ------------kg $ha^{-1}$----------- | | | --------------g $kg^{-1}$----------- | | | ------------kg $ha^{-1}$------------ | | |
| Arrowleaf clover | 912bc* | 819a | 3052a | 25.97bc | - | 16.48c | 23.79bc | - | 50.24a |
| Austrian winter pea | 1091b | 344bc | 1964b | 25.25bc | - | 23.57a | 27.60b | - | 45.77a |
| Button medic | 268cd | 205c | 798d | 30.77a | - | 19.36bc | 7.92cd | - | 15.69b |
| Crimson clover | 1156b | 287bc | 1843bc | 27.32b | - | 11.46d | 31.63b | - | 21.45b |
| Hairy vetch | 2046a | 582ab | 2235b | 31.89a | - | 21.54ab | 63.59a | - | 47.97a |
| Red clover | 74d | 198c | 1183cd | 23.02c | - | 23.14a | 1.69d | - | 27.34b |
| CV (%) | 61.38 | 33.24 | 21.57 | 10.53 | - | 9.27 | 58.13 | - | 22.73 |

*Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's multiple range test.

Table 1.6. Dry matter, N concentration, and aboveground total N by year of six legumes grown at Maysville in 2012.

| Legume | Dry matter | N concentration | Aboveground total N |
|---|---|---|---|
| | [kg $ha^{-1}$] | [g $kg^{-1}$] | [kg $ha^{-1}$] |
| Arrowleaf clover | 2380a* | 14.49c | 34.35ab |
| Austrian winter pea | 2535a | 16.04bc | 40.30a |
| Button medic | 1192bc | 15.78bc | 18.23cd |
| Crimson clover | 1865ab | 10.81d | 20.89bcd |
| Hairy vetch | 1801ab | 17.22b | 31.28abc |
| Red clover | 721c | 20.80a | 14.71d |
| CV (%) | 40.21 | 10.64 | 40.40 |

*Means within a column followed by the same letter are not significantly different at the 0.05 probabiity level using Duncan’s multiple range test.

Table 1.7. Dry matter, N concentration, and aboveground total N of switchgrass fertilized with N fertilizer or interseeded with legumes at Perkins averaged across the growing seasons.

| Treatments | Yield | | | N concentration | | | Aboveground total N | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2010 | 2011 | 2012 | 2010 | 2011 | 2012 | 2010 | 2011 | 2012 |
| | ---------------kg ha$^{-1}$-------------- | | | ------------g kg$^{-1}$---------- | | | ------------kg ha$^{-1}$-------------- | | |
| Arrowleaf clover | 13275bc* | 7800b | 13360cde | 4.30a | 4.68a | 5.44abcd | 56.14b | 34.88a | 72.65c |
| Austrian winter pea | 15183bc | 9667ab | 16627cd | 5.02a | 5.71a | 5.17bcd | 75.42ab | 55.12a | 84.43bc |
| Button medic | 14150bc | 13983a | 14347cde | 4.52a | 5.09a | 5.92ab | 65.08b | 80.19a | 84.75bc |
| Crimson clover | 14367bc | 8250b | 13497cde | 4.54a | 3.85a | 4.80d | 61.36b | 34.01a | 64.03c |
| Hairy vetch | 13867bc | 10783ab | 11077e | 5.78a | 6.19a | 6.18ab | 77.42ab | 66.51a | 68.80c |
| Red clover | 11892c | 10300ab | 11910de | 4.73a | 3.83a | 5.17bcd | 57.30b | 39.96a | 59.30c |
| 0 kg N ha$^{-1}$ | 14967bc | 11200ab | 14267cde | 3.63a | 3.62a | 4.90cd | 55.67b | 41.92a | 70.56c |
| 56 kg N ha$^{-1}$ | 13133bc | 9750ab | 17587bc | 3.94a | 3.97a | 4.58d | 51.79b | 38.64a | 80.89bc |
| 112 kg N ha$^{-1}$ | 18133ab | 13350a | 22610a | 5.16a | 5.16a | 4.58d | 99.02ab | 68.75a | 104.07b |
| 168 kg N ha$^{-1}$ | 22100a | 11000ab | 21840ab | 5.22a | 5.22a | 6.50a | 119.82a | 64.06a | 142.25a |
| CV (%) | 24.45 | 21.44 | 16.10 | 29.22 | 34.19 | 12.09 | 44.03 | 50.45 | 16.25 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's multiple range test.

Table 1.8. Dry matter, N concentration, and aboveground total N of switchgrass fertilized with N fertilizer or interseeded with legumes at Maysville averaged across the growing seasons.

| Treatments | Yield | | | N concentration | | | Aboveground total N | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2010 | 2011 | 2012 | 2010 | 2011 | 2012 | 2010 | 2011 | 2012 |
| | ----------------kg ha$^{-1}$------------- | | | --------------g kg$^{-1}$------------ | | | -------------kg ha$^{-1}$----------- | | |
| Arrowleaf clover | 14452cd* | 7061cd | 11756bc | 3.68a | 4.98abc | 5.27ab | 52.19a | 35.54b | 63.59a |
| Austrian winter pea | 13787d | 6056d | 11957bc | 3.50a | 6.26a | 5.37ab | 45.58a | 37.12b | 62.41a |
| Button medic | 15155bcd | 6270d | 9422bc | 3.38a | 5.36abc | 3.29bd | 49.38a | 34.19b | 33.86ab |
| Crimson clover | 13913d | 7550cd | 11582bc | 3.77a | 4.06abc | 4.90abc | 54.21a | 30.42b | 57.86ab |
| Hairy vetch | 13560d | 7877cd | 8631bc | 4.19a | 4.08abc | 5.81a | 56.50a | 31.96b | 53.70ab |
| Red clover | 14578cd | 7384cd | 9165bc | 3.40a | 5.70ab | 3.76abcd | 47.21a | 43.03ab | 35.31ab |
| 0 kg N ha$^{-1}$ | 14913bdc | 11617bcd | 6552c | 2.59a | 3.69abc | 3.55abcd | 38.84a | 42.33ab | 18.37b |
| 56 kg N ha$^{-1}$ | 19157ab | 13953bc | 12194bc | 3.10a | 3.28bc | 3.20bdc | 58.52a | 39.73b | 43.60ab |
| 112 kg N ha$^{-1}$ | 20055a | 16347ab | 14292ab | 2.58a | 2.93c | 1.77d | 51.35a | 47.19ab | 23.50ab |
| 168 kg N ha$^{-1}$ | 18815abc | 22324a | 18025a | 3.29a | 3.09bc | 2.82cd | 62.13a | 61.98a | 51.73ab |
| CV (%) | 18.89 | 34.27 | 33.44 | 35.69 | 32.31 | 36.84 | 34.74 | 28.78 | 56.07 |

* Means within a column followed by the same letter are not significantly different at the 0.05 level using Duncan's multiple range test.

Table 1.9. Effect of winter legumes and inorganic N fertilizer on biofuel quality of switchgrass forage in 2010 growing season.

| Treatments | Cell wall component [g kg$^{-1}$] | | Mineral component [g kg$^{-1}$] | | | |
|---|---|---|---|---|---|---|
| | Cellulose+lignin | Hemicellulose | P | K | Ca | Mg |
| | | Perkins | | | | |
| Arrowleaf clover | 450a* | 345ab | 1.63a | 10.11ab | 1.63ab | 2.11ab |
| Austrian winter pea | 445a | 345ab | 1.65a | 10.10ab | 1.90ab | 2.16ab |
| Button medic | 453a | 344ab | 1.63a | 10.36ab | 1.78ab | 2.15ab |
| Crimson clover | 450a | 349ab | 1.65a | 10.33ab | 1.61ab | 2.06ab |
| Hairy vetch | 451a | 338b | 1.71a | 10.55a | 2.31a | 2.43a |
| Red clover | 445a | 344ab | 1.66a | 10.00ab | 1.75ab | 2.06ab |
| 0 kg N ha$^{-1}$ | 459a | 360a | 1.50b | 8.86b | 1.23b | 1.70b |
| 56 kg N ha$^{-1}$ | 457a | 339b | 1.60ab | 9.83ab | 1.63ab | 2.00ab |
| 112 kg N ha$^{-1}$ | 449a | 349ab | 1.62ab | 10.40ab | 2.00ab | 2.10ab |
| 168 kg N ha$^{-1}$ | 460a | 346ab | 1.61ab | 10.56a | 1.90ab | 2.06ab |
| CV (%) | 4.60 | 2.49 | 4.78 | 9.69 | 34.58 | 17.31 |
| | | Maysville | | | | |
| Arrowleaf clover | 484a | 291abc | 0.63a | 6.01a | 2.85a | 1.40a |
| Austrian winter pea | 471a | 286bc | 0.56a | 6.81a | 2.88a | 1.41a |
| Button medic | 474a | 289abc | 0.60a | 7.10a | 3.23a | 1.48a |
| Crimson clover | 476a | 290abc | 0.55a | 5.78a | 3.28a | 1.46a |
| Hairy vetch | 485a | 280c | 0.60a | 7.45a | 3.75a | 1.56a |
| Red clover | 477a | 293abc | 0.56a | 5.75a | 2.78a | 1.38a |
| 0 kg N ha$^{-1}$ | 487a | 298ab | 0.50a | 4.26a | 2.53a | 1.36a |
| 56 kg N ha$^{-1}$ | 496a | 296ab | 0.40a | 4.70a | 2.23a | 1.16a |
| 112 kg N ha$^{-1}$ | 502a | 304a | 0.46a | 4.83a | 2.06a | 1.03a |
| 168 kg N ha$^{-1}$ | 514a | 302a | 0.50a | 5.66a | 2.40a | 1.20a |
| CV (%) | 6.24 | 3.31 | 36.67 | 35.50 | 44.30 | 25.39 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan’s multiple range test.

Table 1.10. Effect of winter legumes and inorganic N fertilizer on biofuel quality of switchgrass forage in 2011 growing season.

| Treatments | Cell wall component [g kg$^{-1}$] | | Mineral component [g kg$^{-1}$] | | | |
|---|---|---|---|---|---|---|
| | Cellulose+lignin | Hemicellulose | P | K | Ca | Mg |
| | | Perkins | | | | |
| Arrowleaf clover | 446a* | 340bc | 1.66ab | 11.03a | 1.83a | 2.26abc |
| Austrian winter pea | 439a | 337bc | 1.66ab | 9.80ab | 2.33a | 2.40ab |
| Button medic | 453a | 345abc | 1.63abc | 10.56ab | 1.96a | 2.30ab |
| Crimson clover | 459a | 345abc | 1.60bc | 10.13ab | 1.43a | 1.90ab |
| Hairy vetch | 445a | 332c | 1.76a | 10.70ab | 2.40a | 2.56a |
| Red clover | 459a | 349abc | 1.70ab | 9.96ab | 1.36a | 1.90ab |
| 0 kg N ha$^{-1}$ | 459a | 360a | 1.50c | 8.86b | 1.23a | 1.70b |
| 56 kg N ha$^{-1}$ | 457a | 339bc | 1.60bc | 9.83ab | 1.63a | 2.00ab |
| 112 kg N ha$^{-1}$ | 449a | 349ab | 1.60bc | 10.40ab | 2.00a | 2.10ab |
| 168 kg N ha$^{-1}$ | 460a | 346abc | 1.60bc | 10.56ab | 1.90a | 2.06ab |
| CV (%) | 4.60 | 2.49 | 4.78 | 9.69 | 34.58 | 17.31 |
| | | Maysville | | | | |
| Arrowleaf clover | 409ab | 325c | 1.13abc | 6.43a | 2.33a | 2.53ab |
| Austrian winter pea | 370b | 343bc | 1.23ab | 6.90a | 1.96ab | 2.56a |
| Button medic | 373b | 342bc | 1.20ab | 6.90a | 1.63abc | 2.26abc |
| Crimson clover | 379b | 340bc | 1.23ab | 7.80a | 1.36bc | 2.23abc |
| Hairy vetch | 380b | 347b | 1.10bc | 5.93a | 1.53abc | 2.23abc |
| Red clover | 375b | 353ab | 1.26a | 7.73a | 1.56abc | 2.46abc |
| 0 kg N ha$^{-1}$ | 387b | 350ab | 1.20ab | 6.86a | 1.16bc | 2.23abc |
| 56 kg N ha$^{-1}$ | 400ab | 349ab | 1.13abc | 6.16a | 1.20bc | 2.16abc |
| 112 kg N ha$^{-1}$ | 392ab | 359ab | 1.03cd | 6.16a | 1.33bc | 2.10c |
| 168 kg N ha$^{-1}$ | 427a | 369a | 0.93d | 3.56b | 0.76c | 2.13bc |
| CV (%) | 5.23 | 3.19 | 6.94 | 15.50 | 33.08 | 9.44 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan’s multiple range test.

Table 1.11. Effect of winter legumes and inorganic N fertilizer on biofuel quality of switchgrass forage in 2012 growing season.

| Treatments | Cell wall component [g kg$^{-1}$] | | Mineral component [g kg$^{-1}$] | | | |
|---|---|---|---|---|---|---|
| | Cellulose+lignin | Hemicellulose | P | K | Ca | Mg |
| | | Perkins | | | | |
| Arrowleaf clover | - | - | 1.06ab* | 6.80ab | 2.30ab | 2.30ab |
| Austrian winter pea | - | - | 1.03ab | 7.10ab | 2.20ab | 2.60a |
| Button medic | - | - | 1.20a | 7.46ab | 2.50ab | 2.46ab |
| Crimson clover | - | - | 1.13a | 5.93b | 2.23ab | 2.16ab |
| Hairy vetch | - | - | 1.03ab | 6.76ab | 2.90a | 2.66a |
| Red clover | - | - | 0.80b | 6.23ab | 2.46ab | 2.33ab |
| 0 kg N ha$^{-1}$ | - | - | 1.13a | 7.20ab | 2.30ab | 2.03b |
| 56 kg N ha$^{-1}$ | - | - | 1.00ab | 6.06ab | 2.00b | 2.20ab |
| 112 kg N ha$^{-1}$ | - | - | 0.90ab | 5.73b | 2.30ab | 2.30ab |
| 168 kg N ha$^{-1}$ | - | - | 0.96ab | 7.20ab | 2.26ab | 2.70a |
| CV (%) | - | - | 15.76 | 13.47 | 15.25 | 11.96 |
| | | Maysville | | | | |
| Arrowleaf clover | 430ab | 313d | 1.10abc | 7.83ab | 2.69a | 2.57ab |
| Austrian winter pea | 419ab | 339abc | 1.08abc | 7.85ab | 2.32ab | 2.28b |
| Button medic | 419ab | 347a | 1.19ab | 8.93a | 1.38bc | 2.47ab |
| Crimson clover | 430ab | 325cd | 1.08bc | 7.47b | 2.25ab | 2.22b |
| Hairy vetch | 434ab | 327bcd | 1.17ab | 7.85ab | 2.09abc | 2.77a |
| Red clover | 404b | 350a | 1.13abc | 7.96ab | 1.58bc | 2.24b |
| 0 kg N ha$^{-1}$ | 407b | 344ab | 1.21a | 8.70ab | 1.34bc | 2.18b |
| 56 kg N ha$^{-1}$ | 420ab | 349a | 1.13abc | 8.75ab | 1.53bc | 2.34ab |
| 112 kg N ha$^{-1}$ | 430ab | 352a | 1.02c | 8.09ab | 1.20c | 2.18b |
| 168 kg N ha$^{-1}$ | 445a | 339abc | 1.10abc | 8.27ab | 1.11c | 2.36ab |
| CV (%) | 4.41 | 3.68 | 7.1 | 10.19 | 35.31 | 11.90 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan’s multiple range test.

Table 1.12. Effect of winter legumes and inorganic N fertilizer on soil organic matter and NO$_3$-N across the growing season at Perkins.

| Treatments | 2010 | | 2011 | | 2012 | |
|---|---|---|---|---|---|---|
| | OM | NO$_3$-N | OM | NO$_3$-N | OM | NO$_3$-N |
| | g kg$^{-1}$ | kg ha$^{-1}$ | g kg$^{-1}$ | kg ha$^{-1}$ | g kg$^{-1}$ | kg ha$^{-1}$ |
| | | | 0-15 cm depth | | | |
| Arrowleaf clover | 7.83a* | 4.10a | 8.00a | 0.96abc | 6.30a | 5.23a |
| Austrian winter pea | 7.00a | 3.54ab | 7.66a | 0.75bc | 6.43a | 3.73a |
| Button medic | 7.16a | 3.17abc | 8.33a | 1.18abc | 8.53a | 2.98a |
| Crimson clover | 8.00a | 1.49c | 8.33a | 0.53c | 7.40a | 3.36a |
| Hairy vetch | 7.66a | 3.73ab | 8.66a | 1.07abc | 7.60a | 5.97a |
| Red clover | 7.83a | 2.24abcd | 7.66a | 1.18abc | 7.73a | 4.48a |
| 0 kg N ha$^{-1}$ | 8.33a | 1.86bcd | 8.33a | 0.64bc | 8.76a | 3.36a |
| 56 kg N ha$^{-1}$ | 7.33a | 2.98abcd | 7.66a | 1.07abc | 7.76a | 3.36a |
| 112 kg N ha$^{-1}$ | 8.66a | 1.12d | 8.33a | 1.82a | 6.96a | 3.36a |
| 168 kg N ha$^{-1}$ | 7.00a | 2.24abcd | 8.66a | 1.50ab | 7.06a | 4.11a |
| CV (%) | 13.72 | 43.65 | 11.37 | 43.16 | 25.43 | 38.57 |
| | | | 15-30cm depth | | | |
| Arrowleaf clover | 10.16a | 0.37a | 10.33a | 0.96ab | 8.26a | 4.11a |
| Austrian winter pea | 10.80a | 0.89a | 9.66a | 0.43ab | 8.16a | 2.61a |
| Button medic | 9.50a | 0.37a | 8.33a | 0.53ab | 7.93a | 3.36a |
| Crimson clover | 10.50a | 0.18a | 8.66a | 0.43ab | 7.90a | 2.98a |
| Hairy vetch | 10.00a | 0.74a | 10.33a | 1.29ab | 7.70a | 2.61a |
| Red clover | 9.66a | 0.18a | 9.66a | 1.39ab | 8.63a | 2.24a |
| 0 kg N ha$^{-1}$ | 11.33a | 0.37a | 9.00a | 1.50a | 7.53a | 4.48a |
| 56 kg N ha$^{-1}$ | 10.66a | 0.37a | 9.33a | 0.32b | 8.20a | 2.24a |
| 112 kg N ha$^{-1}$ | 8.66a | 0.74a | 9.00a | 0.75ab | 7.70a | 2.61a |
| 168 kg N ha$^{-1}$ | 10.66a | 0.00a | 10.00a | 0.53ab | 8.63a | 2.98a |
| CV (%) | 22.00 | 141.33 | 15.64 | 67.22 | 10.26 | 44.51 |
| Depth | *** | *** | *** | * | ns | * |
| Treatments*Depth | ns | * | ns | * | ns | ns |

*Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's multiple range test.

ns, **, and *** denote non-significant or significant at P≤0.05, P≤0.01, and P≤0.001 respectively.

Table 1.13. Effect of winter legumes and inorganic N fertilizer on soil organic matter and $NO_3$-N (0-15 cm depth) across the growing season at Maysville.

| Treatments | 2010 | | 2011 | | 2012 | |
|---|---|---|---|---|---|---|
| | OM | $NO_3$-N | OM | $NO_3$-N | OM | $NO_3$-N |
| | g $kg^{-1}$ | kg $ha^{-1}$ | g $kg^{-1}$ | kg $ha^{-1}$ | g $kg^{-1}$ | kg $ha^{-1}$ |
| Arrowleaf clover | 25.66a* | 0.74a | 20.99 | 0.00 | - | - |
| Austrian winter pea | 26.50a | 1.30a | 18.99 | 0.00 | - | - |
| Button medic | 26.50a | 1.86a | 20.99 | 0.00 | - | - |
| Crimson clover | 27.50a | 0.93a | 22.00 | 0.00 | 18.33a | 1.12a |
| Hairy vetch | 25.66a | 0.93a | 20.99 | 1.12 | 18.66a | 1.12a |
| Red clover | 26.66a | 1.12a | 20.99 | 1.12 | - | - |
| 0 kg N $ha^{-1}$ | 25.66a | 1.12a | 20.00 | 3.36 | 18.33a | 1.12a |
| 56 kg N $ha^{-1}$ | 25.66a | 1.86a | 18.99 | 1.12 | 18.00a | 1.12a |
| 112 kg N $ha^{-1}$ | 28.00a | 0.74a | 22.00 | 1.12 | - | - |
| 168 kg N $ha^{-1}$ | 28.33a | 1.12a | 24.00 | 0.00 | 20.33a | 1.12a |
| CV (%) | 8.97 | 77.08 | - | - | 10.69 | 0.00 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's multiple range test. Absence of letters within a column indicates mean separation procedure was unable to carry out due to zero degrees of freedom.

Table 1.14. Effect of winter legumes and inorganic N fertilizer on soil fertility status at Perkins in 2010.

| Treatments | 0 to 15 cm depth | | | 15 to 30 cm depth | | |
|---|---|---|---|---|---|---|
| | pH | P | K | pH | P | K |
| | | -------kg ha$^{-1}$---- | | | ------kg ha-$^{1}$----- | |
| Arrowleaf clover | 6.2a* | 114a | 233a | 6.2a | 129ab | 201a |
| Austrian winter pea | 6.3a | 113a | 248a | 6.4a | 105ab | 194a |
| Button medic | 6.2a | 119a | 272a | 6.3a | 118ab | 203a |
| Crimson clover | 6.1ab | 128a | 214a | 6.2a | 125ab | 196a |
| Hairy vetch | 6.2a | 109a | 243a | 6.3a | 107ab | 221a |
| Red clover | 6.2a | 120a | 218a | 6.3a | 127ab | 203a |
| 0 kg N ha$^{-1}$ | 6.3a | 113a | 227a | 6.5a | 105ab | 198a |
| 56 kg N ha$^{-1}$ | 6.3a | 106a | 247a | 6.5a | 94b | 209a |
| 112 kg N ha$^{-1}$ | 6.3a | 127a | 207a | 6.2a | 178a | 206a |
| 168 kg N ha$^{-1}$ | 5.8b | 119a | 188a | 6.1a | 115ab | 204a |
| CV (%) | 3.29 | 19.33 | 22.72 | 4.19 | 37.79 | 17.70 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's multiple range test.

Table 1.15. Effect of winter legumes and inorganic N fertilizer on soil fertility status at Perkins in 2011.

| Treatments | 0 to 15 cm depth |  |  | 15 to 30 cm depth |  |  |
|---|---|---|---|---|---|---|
|  | pH | P | K | pH | P | K |
|  |  | ----- kg ha$^{-1}$---- |  |  | ----- kg ha$^{-1}$---- |  |
| Arrowleaf clover | 6.1a* | 103a | 274a | 6.0ab | 103a | 224a |
| Austrian winter pea | 6.4a | 100a | 256a | 6.4ab | 97a | 224a |
| Button medic | 6.2a | 116a | 274a | 5.9ab | 94a | 225a |
| Crimson clover | 6.2a | 105a | 270a | 6.0ab | 125a | 213a |
| Hairy vetch | 6.3a | 84a | 253a | 5.9ab | 100a | 207a |
| Red clover | 6.1a | 107a | 253a | 5.8b | 118a | 204a |
| 0 kg N ha$^{-1}$ | 6.2a | 94a | 254a | 6.2ab | 106a | 203a |
| 56 kg N ha$^{-1}$ | 6.4a | 85a | 251a | 6.5a | 82a | 210a |
| 112 kg N ha$^{-1}$ | 6.3a | 109a | 264a | 6.2ab | 124a | 210a |
| 168 kg N ha$^{-1}$ | 6.1a | 102a | 275a | 5.9ab | 107a | 216a |
| CV (%) | 2.85 | 19.75 | 11.36 | 5.63 | 37.64 | 11.65 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's multiple range test.

Table 1.16. Effect of winter legumes and inorganic N fertilizer on soil fertility status at Perkins in 2012.

| Treatments | 0 to 15 cm depth |  |  | 15 to 30 cm depth |  |  |
|---|---|---|---|---|---|---|
|  | pH | P | K | pH | P | K |
|  |  | ---- kg ha$^{-1}$------ |  |  | ---- kg ha$^{-1}$----- |  |
| Arrowleaf clover | 6.0ab* | 118a | 239ab | 6.2a | 91a | 232a |
| Austrian winter pea | 6.2a | 96a | 250ab | 6.4a | 68a | 236a |
| Button medic | 6.1ab | 108a | 239ab | 6.1a | 144a | 239a |
| Crimson clover | 6.1ab | 109a | 272a | 6.2a | 109a | 228a |
| Hairy vetch | 6.1ab | 107a | 242ab | 6.2a | 100a | 225a |
| Red clover | 6.0ab | 114a | 248ab | 6.1a | 127a | 235a |
| 0 kg N ha$^{-1}$ | 6.2ab | 100a | 236ab | 6.4a | 89a | 230a |
| 56 kg N ha$^{-1}$ | 6.1ab | 77a | 209b | 6.5a | 78a | 230a |
| 112 kg N ha$^{-1}$ | 6.0ab | 115a | 239ab | 6.2a | 120a | 232a |
| 168 kg N ha$^{-1}$ | 5.9b | 122a | 244ab | 6.0a | 109a | 234a |
| CV (%) | 2.70 | 28.05 | 9.18 | 4.16 | 37.31 | 5.93 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's multiple range test.

Table 1.17. Effect of winter legumes and inorganic N fertilizer on soil fertility status at Maysville in 2010.

| Treatments | 0 to 15 cm depth | | |
|---|---|---|---|
| | pH | P | K |
| | | -------------------- kg ha$^{-1}$ -------------------- | |
| Arrowleaf clover | 6.0a* | 12ab | 198a |
| Austrian winter pea | 5.8a | 15ab | 226a |
| Button medic | 6.0a | 20a | 238a |
| Crimson clover | 6.1a | 15ab | 230a |
| Hairy vetch | 6.0a | 13ab | 203a |
| Red clover | 5.9a | 15ab | 208a |
| 0 kg N ha$^{-1}$ | 6.0a | 12ab | 199a |
| 56 kg N ha$^{-1}$ | 5.7a | 19a | 224a |
| 112 kg N ha$^{-1}$ | 5.7a | 10b | 203a |
| 168 kg N ha$^{-1}$ | 5.7a | 12ab | 209a |
| CV (%) | 5.53 | 34.73 | 16.25 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan's multiple range test.

Table 1.18. Effect of winter legumes and inorganic N fertilizer on soil fertility status at Maysville in 2011.

| Treatments | 0 to 15 cm depth | | | 15 to 30 cm depth | | |
|---|---|---|---|---|---|---|
| | pH | P | K | pH | P | K |
| | | ----- kg ha$^{-1}$----- | | | ----- kg ha$^{-1}$------ | |
| Arrowleaf clover | 6.9 | 16 | 215 | 7.7 | 11 | 182 |
| Austrian winter pea | 6.3 | 11 | 208 | 7.3 | 9 | 182 |
| Button medic | 6.8 | 13 | 213 | 7.7 | 11 | 229 |
| Crimson clover | 6.9 | 13 | 240 | 7.3 | 9 | 168 |
| Hairy vetch | 7.0 | 13 | 217 | 8.0 | 11 | 204 |
| Red clover | 6.9 | 18 | 211 | 8.2 | 13 | 226 |
| 0 kg N ha$^{-1}$ | 6.8 | 13 | 220 | 7.6 | 11 | 177 |
| 56 kg N ha$^{-1}$ | 6.2 | 13 | 222 | 8.1 | 13 | 217 |
| 112 kg N ha$^{-1}$ | 6.3 | 13 | 226 | 8.0 | 11 | 200 |
| 168 kg N ha$^{-1}$ | 6.1 | 11 | 204 | 8.0 | 16 | 220 |
| CV (%) | 4.58 | 17.99 | 5.51 | 3.45 | 14.99 | 9.09 |

Note: Mean separation procedure was unable to carry out due to zero degrees of freedom.

Table 1.19. Effect of winter legumes and inorganic N fertilizer on soil fertility status at Maysville in 2012.

| Treatments* | 0 to 15 cm depth | | | 15 to 30 cm depth | | |
|---|---|---|---|---|---|---|
| | pH | P | K | pH | P | K |
| | | ----- kg ha$^{-1}$---- | | | ----- kg ha$^{-1}$----- | |
| Crimson clover | 5.5b* | 41a | 200a | 7.3ab | 11a | 171a |
| Hairy vetch | 5.7b | 23b | 250a | 7.7ab | 12a | 181a |
| 0 kg N ha$^{-1}$ | 6.7a | 20b | 248a | 7.1b | 14a | 170a |
| 56 kg N ha$^{-1}$ | 5.9ab | 16b | 300a | 8.2a | 12a | 201a |
| 168 kg N ha$^{-1}$ | 6.1ab | 31ab | 212a | 7.9ab | 13a | 187a |
| CV (%) | 7.10 | 31.31 | 28.81 | 6.65 | 45.4 | 13.80 |

* Means within a column followed by the same letter are not significantly different at the 0.05 probability level using Duncan’s multiple range test.

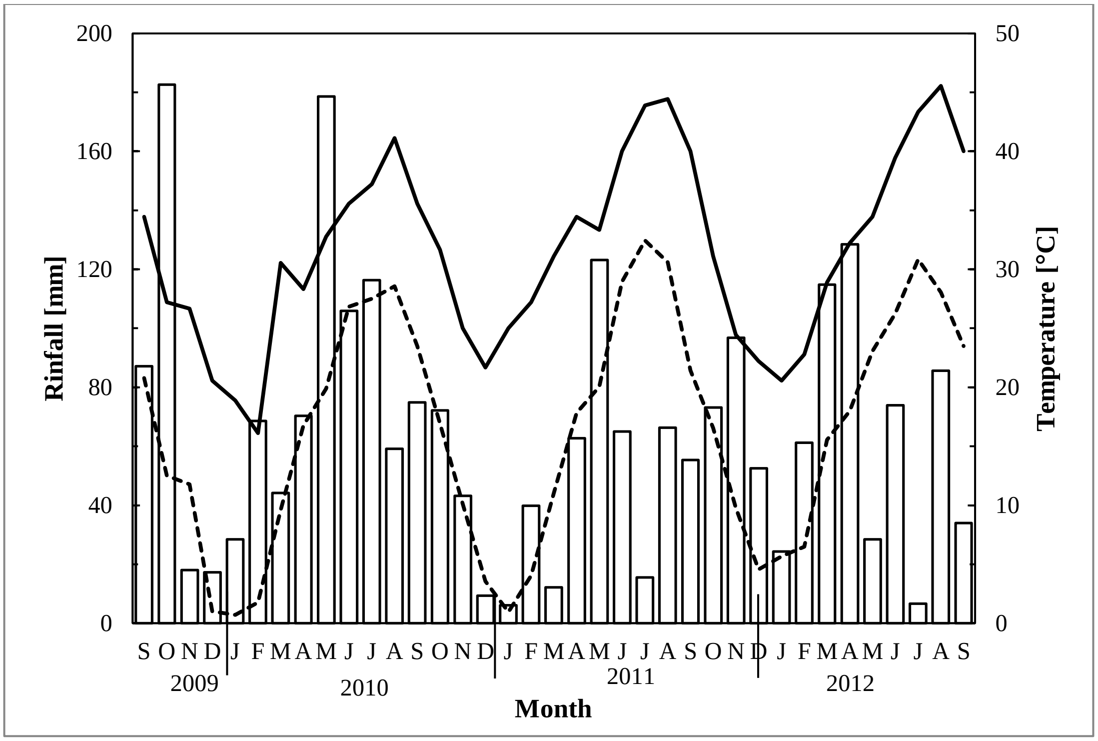

Figure 1.1. Monthly rainfall and air temperature during three years study at Cimarron Valley Research Station near Perkins, Oklahoma. Bars represent mean rainfall [mm], the solid line represents maximum air temperature, and the broken line represents average air temperature.

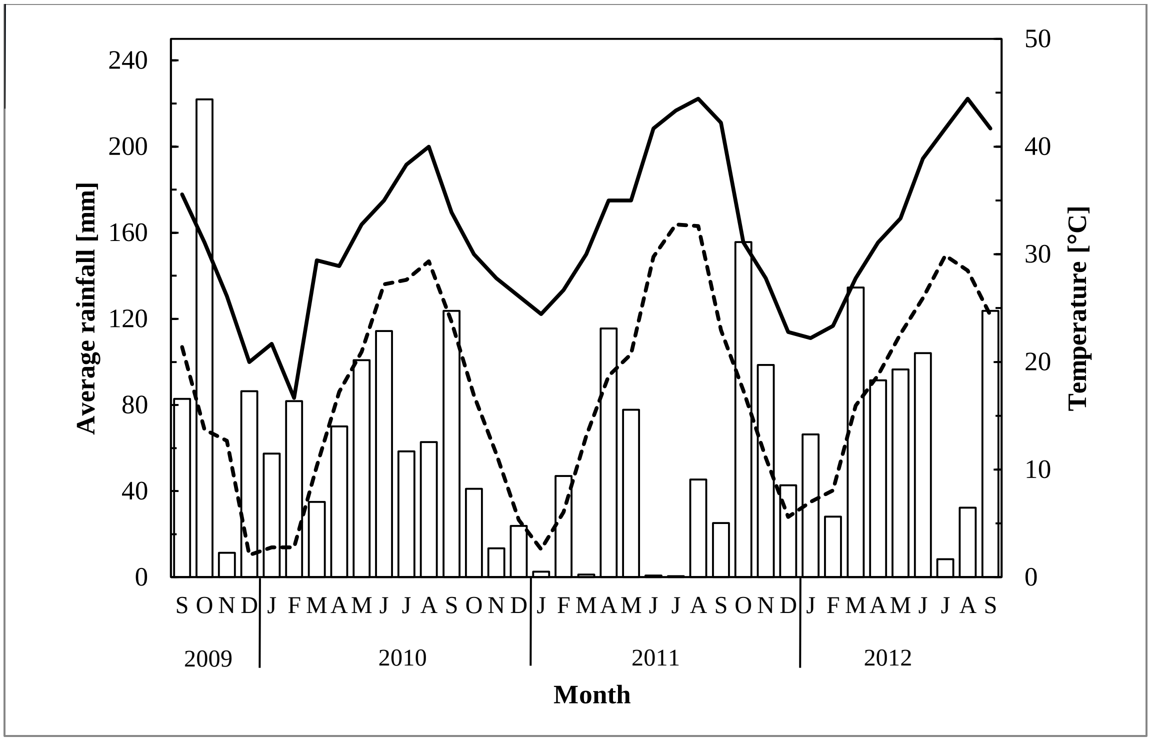

Figure 1.2. Monthly rainfall and air temperature during three years study at Maysville research station. Bars represent mean rainfall [mm], the solid and broken lines represent maximum and minimum air temperatures, respectively.

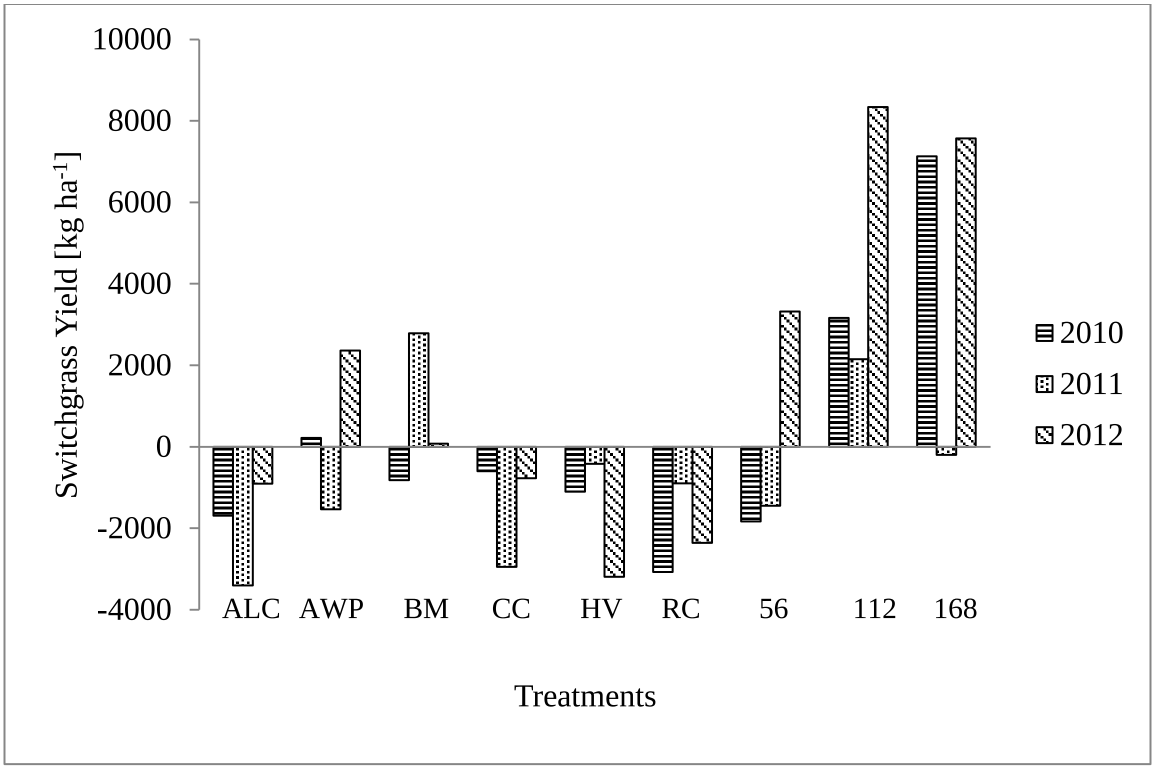

Figure 1.3. Yield of switchgrass increased/decreased when compared to check plot for legumes and inorganic N treatments at Perkins. ALC = Arrow Leaf Clover, AWP = Austrian Winter Pea, BM = Button Medic, CC = Crimson Clover, HV = Hairy Vetch, RC = Red Clover, 56 = 56 kg N ha$^{-1}$, 112 = 112 kg N ha$^{-1}$, 168 = 168 kg N ha$^{-1}$.

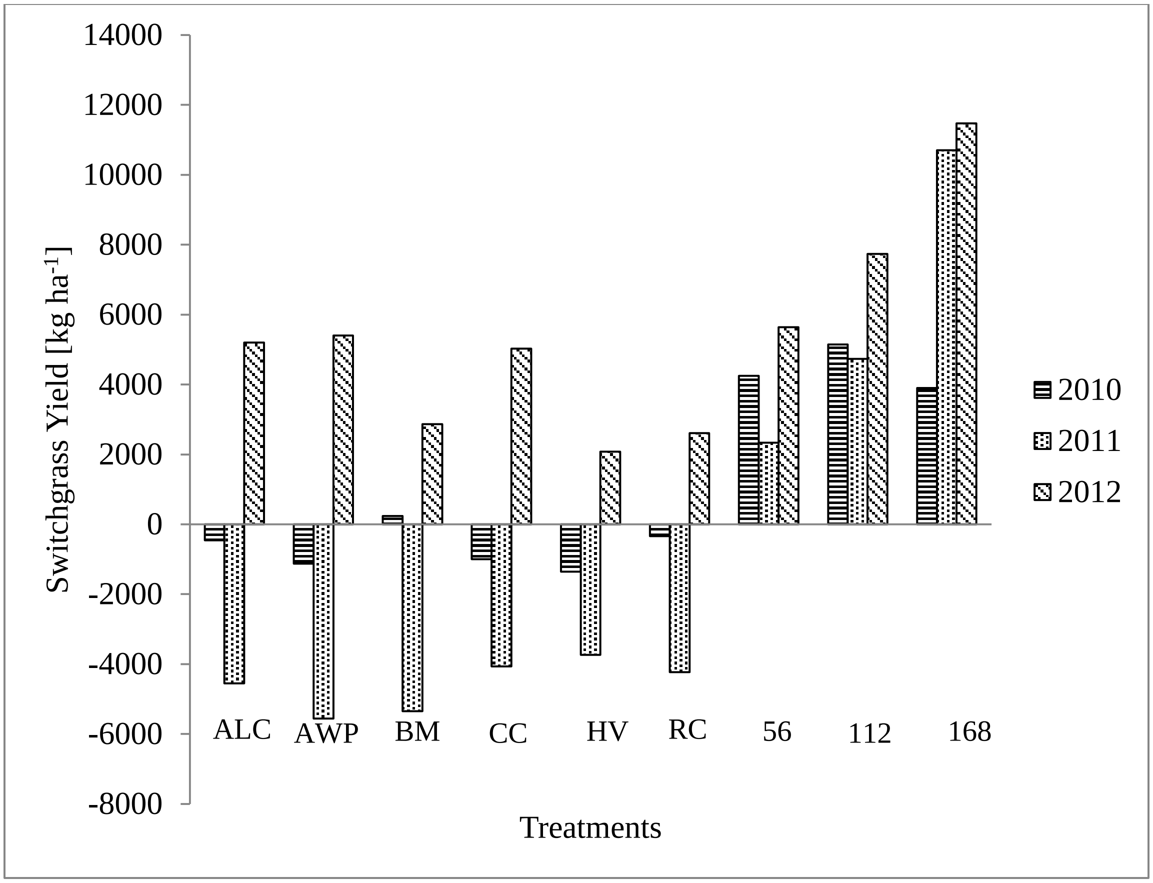

Figure 1.4. Yield of switchgrass increased/decreased when compared to check plot for legume and inorganic N treatments at Maysville. ALC = Arrow Leaf Clover, AWP = Austrian Winter Pea, BM = Button Medic, CC = Crimson Clover, HV = Hairy Vetch, RC = Red Clover, 56 = 56 kg N ha$^{-1}$, 112 = 112 kg N ha$^{-1}$, 168 = 168 kg N ha$^{-1}$.

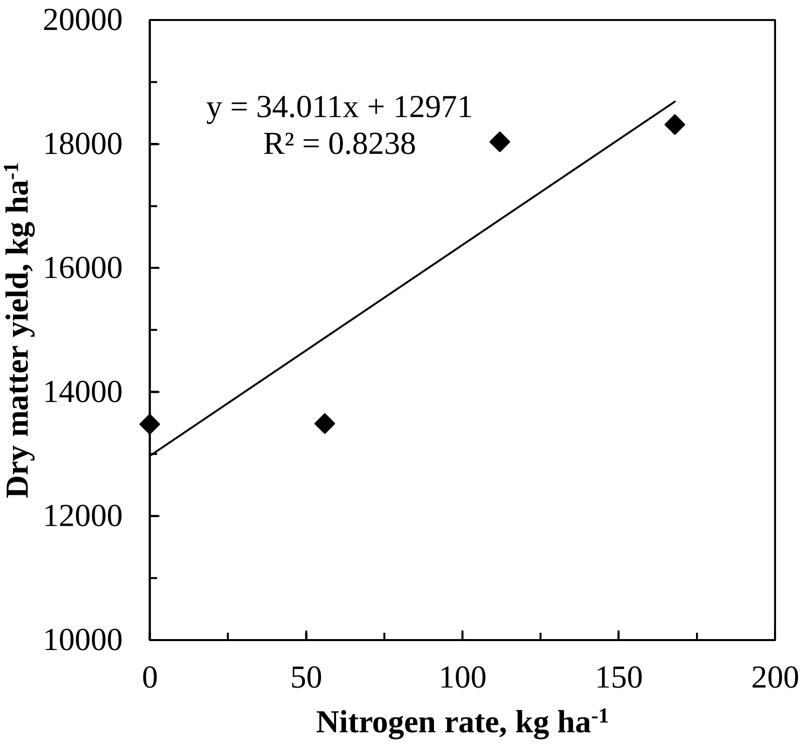

Figure 1.5. Dry matter yield as a function of inorganic nitrogen rate at Perkins. Data points are three years average.

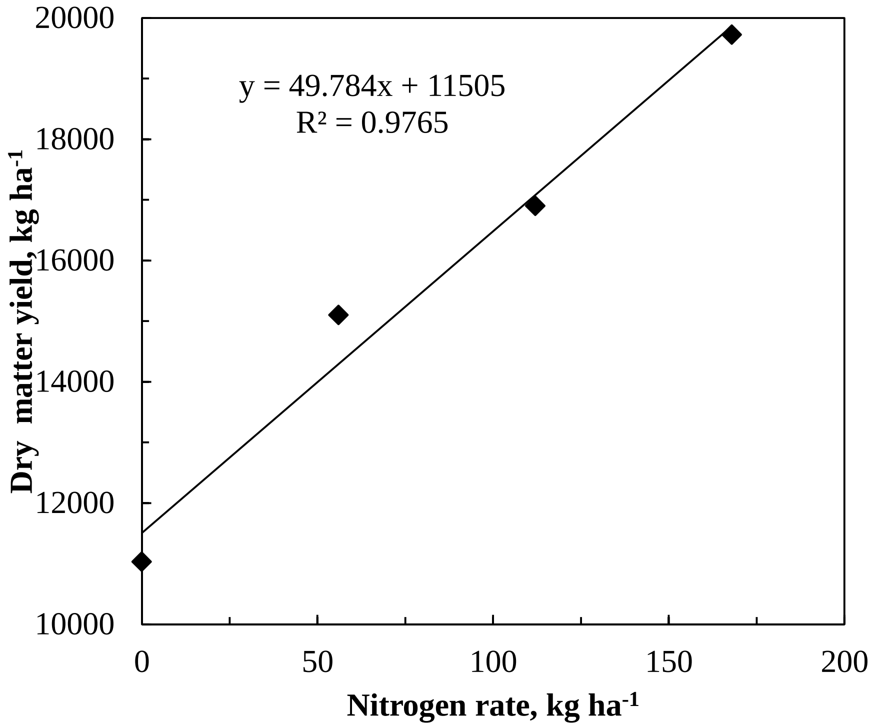

Figure 1.6: Dry matter yield as a function of inorganic nitrogen rate at Maysville. Data points are three years average.

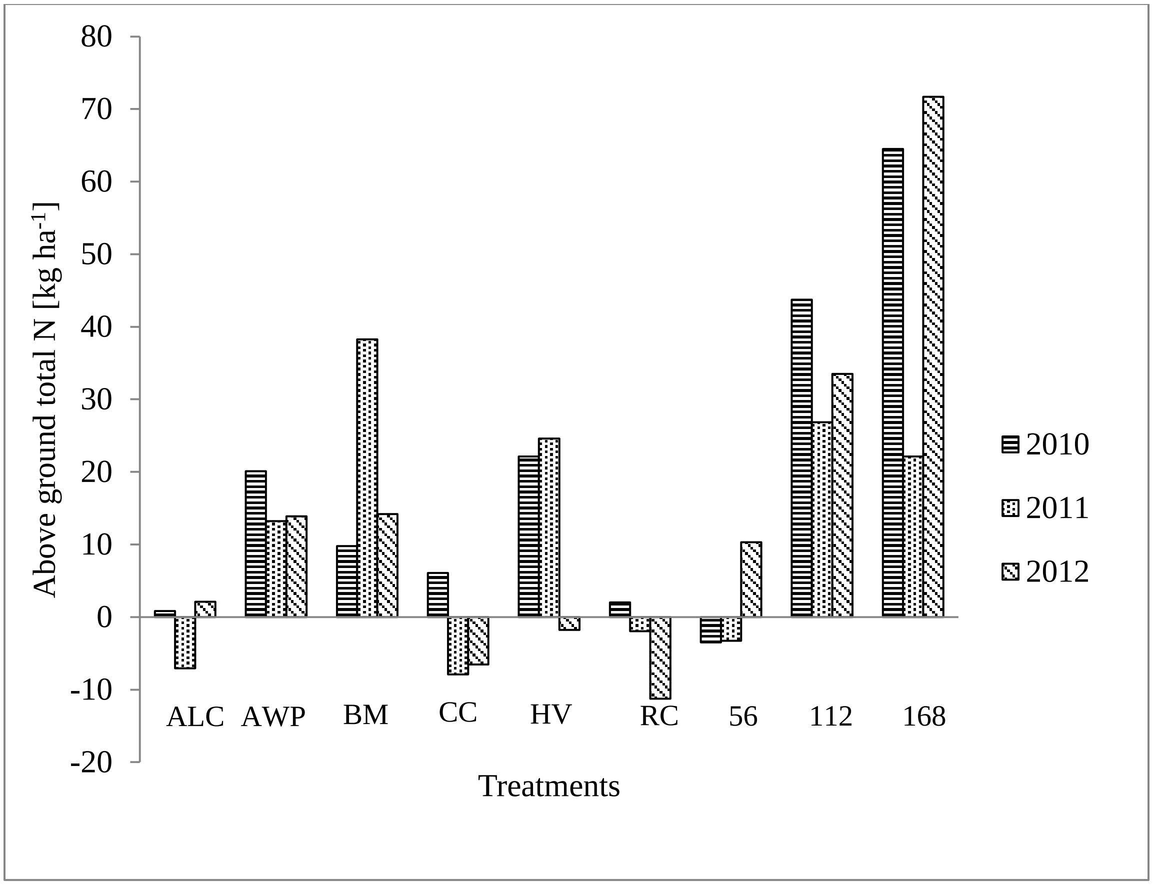

Figure 1.7. Nitrogen gain/loss of switchgrass when compared to check plot for legumes and inorganic N treatments at Perkins. ALC = Arrow Leaf Clover, AWP = Austrian Winter Pea, BM = Button Medic, CC = Crimson Clover, HV = Hairy Vetch, RC = Red Clover, 56 = 56 kg N $ha^{-1}$, 112 = 112 kg N $ha^{-1}$, 168 = 168 kg N $ha^{-1}$.

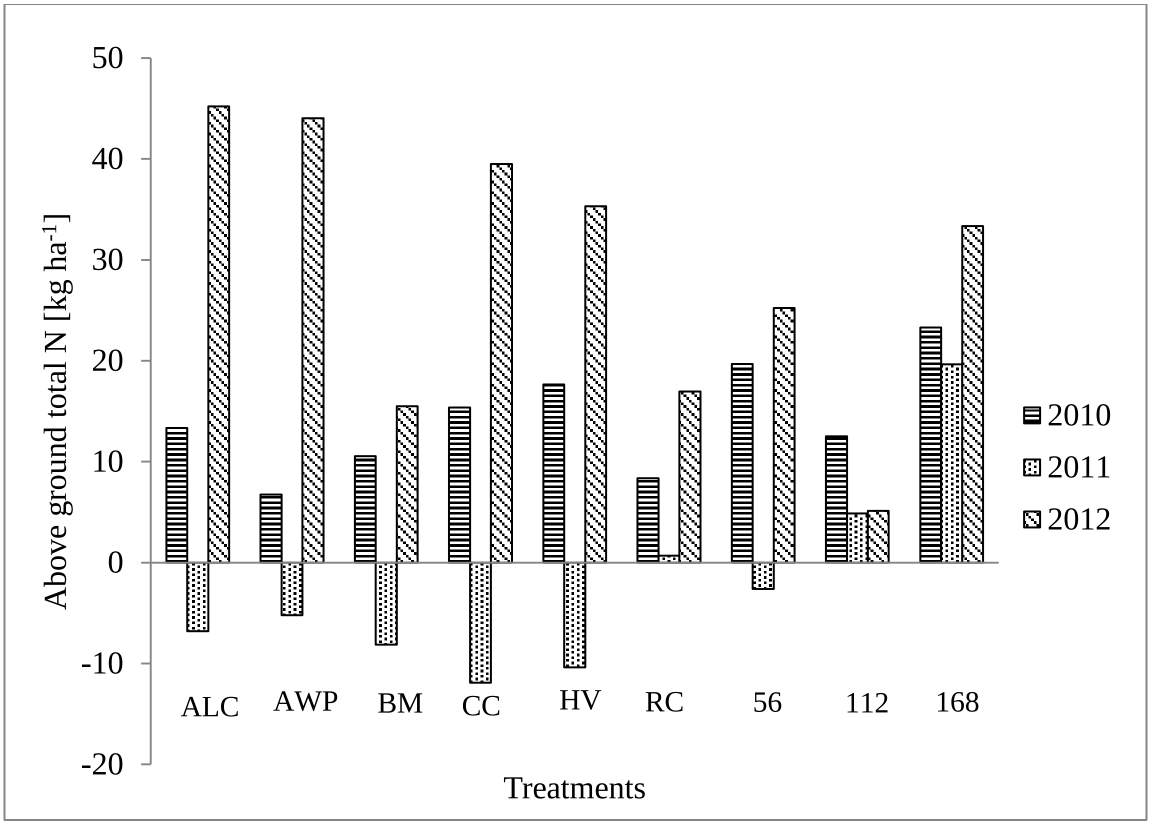

Figure 1.8. Nitrogen gain/loss of switchgrass when compared to check plot for legumes and inorganic N treatments at Maysville. ALC = Arrow Leaf Clover, AWP = Austrian Winter Pea, BM = Button Medic, CC = Crimson Clover, HV = Hairy Vetch, RC = Red Clover, 56 = 56 kg N ha$^{-1}$, 112 = 112 kg N ha$^{-1}$, 168 = 168 kg N ha$^{-1}$.

# CHAPTER II

## EFFECT OF FOLIAR-APPLIED PHOSPHITE ON GROWTH AND YIELD OF CORN (*Zea mays* L.)

## ABSTRACT

Grain yield response to foliar fertilization (N, P, K, and S solution) of corn (*Zea mays* L.) has stimulated interest in the use of similar material such as phosphate as a source of phosphorus (P) as an economic and efficient way of nutrient supply. Phosphite could potentially improve P absorption through leaves because oxidation state makes this molecule slightly more reactive than phosphate. This study was conducted to determine the effect of foliar-applied phosphite ($PO_3^{3-}$) which is chemically more reactive than phosphate on growth and yield of corn. The effects of foliar application of phosphite compared to soil applied P were evaluated on corn biomass yield, stem and leaf P concentration, grain yield, and P concentration in the grain. Two field studies and two greenhouse studies were conducted in Oklahoma. One field study was at the Lake Carl Blackwell (LCB) near Stillwater; other field study was at Cimarron Valley Research Station (CVR) at Perkins. Greenhouse studies (GH2011 and GH2012) were conducted at Oklahoma State University. A commercial phosphite solution which contains 20% P was sprayed at the rate of 2.34 L ha$^{-1}$ at sixth-leaf stage for one treatment. Sixth-leaf stage and tasseling stage were selected for a multi-application treatment. In 2011, both one and two

application of foliar phosphite increased biomass yield at LCB. Grain yield also was increased in the field studies compared to non-fertilized treatment P was not deficient. In the greenhouse studies, where soil P status were 11 ppm and 24 ppm did not increase biomass and grain yield. Phosphorus concentration in the grain, stem, and leaf were largely unaffected by foliar phosphite regardless of number of applications. The concentration of the phosphite solution could not be increased due to leaf damage. Results obtained in this study suggested that, phosphite may be used as partial supplement of P for corn. However, foliar application of phosphite was not an adequate method of phosphorus fertilization when soil test P was low.

# INTRODUCTION

Phosphorus (P) is a component of adenosine diphosphate (ADP) and adenosine triphosphate (ATP) which regulates most biological processes in plants including photosynthesis, respiration, and nucleic acid synthesis. Nucleic acids are important in cell division and tissue development. In addition, during early growth stages, root development depends on the availability of P in the soil. A healthy root system is very important as mineral uptake depends upon it for vegetative growth. In P deficient condition, root growth is often stunned causing nutrient uptake difficulties in plants. Consequently, vegetative growth and seed production decrease significantly. Power et al., (1963) reported that vegetative growth can be maximized over a wide range of temperatures when P supply is adequate.

Phosphorus use efficiency remains very low in cereal crop production. A large amount of P fixation occurs with iron (Fe) and aluminum (Al) to form products that are very insoluble. Moreover, negatively charged soil colloids repel negatively charged $H_2PO^{4-}$ and $HPO^{4-}$ which are the most available form of P for plant uptake. Fertilizer use efficiency is as low as 5.5% when P is applied as broadcast in winter wheat (Sander et al., 1990). Various methods of improved management such as banded and seed application may increase use efficiency, however, may also increase overhead and equipment cost significantly which could be an issue for the producers.

The solubility of P in the soil solution depends on soil pH (Bohn et al., 2001). Phosphorus is not soluble in either acidic or alkaline soils and fertilizer P will be rapidly insoluble. The optimum soil pH range for maximum P solubility is 6.0 to 7.0. This is a challenge for crop production where soils are acidic or alkaline. In acidic condition, P fertilizers become unavailable after they are applied. Due to increase of available Fe and Al, increasing pH with lime will be costly and may take long period of time to take effect.

In this regard, foliar fertilization of P may be an effective measure to supply P and increase use efficiency. The absorption of materials by leaf and stem involves absorption through stomata, surface absorption, passive penetration through cuticle, and active absorption by the cell beneath the leaf cell (Haynes et al., 1977). Therefore, the rate of foliar absorption depends on the morphological and anatomical structure of leaf and stem. Stems and leaves surfaces are covered by cuticle. Cuticle is a non-cellular structure which consists of several layers. The outer layer consists of wax and cutin. The inner layer consists of cellulose and cutin together. In some species, a third layer exists which consists of cellulose and pectine. These layers are very hard and thick and are not designed for absorption of water and nutrients. The primary functions of these layers are to protect plants from losing water during transpiration. Thus the terrestrial plants are adapted with thick and less permeable cuticular layers (Schonherr and Bukovac, 1972). However, there are numerous canals and pores that exist in the cuticular layers. Permeability of water and nutrients through these canals and pores primarily follows the law of diffusion.

Foliar application has been shown by many studies to be an effective tool of fertilizer application when the crop is in need of additional nutrients. Also, mid-season foliar application is a convenient method to supply nutrients in standing crop fields. Physiological activity such as photosynthesis could reduce due to depletion of nutrients in the leaves during grain filling period because of poor nutrient uptake from the soil. Foliar application of nutrients could be very effective to meet such deficiency in leaves and translocate the nutrient elements in the developing seeds. During seed filling period of soybean (*Glycine max* L.), foliar application of nitrogen (N), phosphorus (P) potassium (K), and sulphur (S) increased soybean yield (Garcia and Hanway, 1976). Yield increases were not due to increase in size of seeds but due to the increase in the number of seeds. Similar results were reported by Harder et al. (1982) that foliar application of N, P, and K after silking stage of corn (*Zea mays* L.) significantly increased grain protein content and percent grain P at harvest. Increased wheat (*Triticum aestivum* L.) grain yield and quality were also reported when soil P was supplemented with foliar P fertilizers (Mosali., 2006; Ling and Silberbush, 2002. Another study has showed that dilute foliar spray of potassium dihydrogen phosphate ($KH_2PO_4$) which contains 52% P increased wheat grain yield in china (Sherchand and Paulsen, 1985). Increased cell sugar content was also notable.

Most foliar fertilizers are in liquid state and thus formulation potentially affects the fertilization efficiency. Jyung and Wittwer (1964) reported that carriers of foliar fertilizers play an important role in uptake. Also, penetration of molecules depends on their chemical structure, properties, and size. Several P fertilizers including di-ammonium phosphate (DAP), Triple Super Phosphate (TSP), and monoammonium phosphate (MAP) were evaluated in corn and wheat (Girma et al., 2007). Success was limited because some of these molecules (e.g. ammonium ion) were not small enough for entry through leaf and the formulation dried quickly (Mosali et al., 2006).

To overcome problems associated with absorption of P through leaf tissues, phosphite ($PO_3$) may be potentially used as it is comparatively smaller molecule than traditional phosphate ($H_2PO_4^{1-}$ and $HPO_4^{2-}$) and more reactive. Phosphite is a derivative of phosphorus acid consists of a phosphorus atom chemically bound to three oxygen atoms. Due to oxidation state, $PO_3^{3-}$ is slightly more reactive than $PO_4^{3-}$. Based on the information above, we hypothesized that; foliar application of phosphite will increase P absorption through plant tissues and thus improve growth and yield of corn. Therefore, the main objective of this study was to evaluate the effect of foliar applied phosphite on growth and yield of corn.

## MATERIALS AND METHODS

The study was initiated in 2011 at Lake Carl Blackwell (LCB) near Stillwater Oklahoma and continued for the second year in 2012 at Cimarron Valley Research (CVR) Station at Perkins Oklahoma. In addition to field studies, treatments were replicated in the greenhouse for two years. Soils for the greenhouse study were collected from Lahoma and Ardmore Oklahoma for 2011 and 2012 respectively. Soil series, initial soil characteristics and climatic conditions for the sites are presented in Table 2.1 to Table 2.3. Rainfall and temperature data were obtained from Oklahoma Mesonet weather station located in Lake Carl Blackwell and Perkins. Lake Carl Blackwell was under conventional tillage and irrigated until lake water level dropped below critical level. Irrigation stopped on June 15th due to lack of water in the lake. Corn was planted no-till at Perkins and was not irrigated. The experiments were arranged in a randomized complete block design with three replications. At Lake Carl Blackwell, plot size was 6.09 m x 3.04 m with 1.52 m alley between replicates. Width of the plots at Perkins was 4.57 m; other dimensions were same as Lake Carl Blackwell. Treatments include application of foliar phosphite, soil applied N and P, and a combination of phosphite and soil applied N and P fertilizers. Urea (46-0-0) and TSP (0-46-0) were used for N and P application. A commercial brand of phosphite solution named Nutri-Phite Take Off™ P foliar MZ was used as a source of foliar P. Nutri-Phite is described by the company as a concentrated nutrient solution containing certain elements in highly soluble form that are beneficial to plant growth. The complete analysis of this product is as follows: total nitrogen 3%, phosphorus 20%, soluble potash 7%, manganese 0.5%, and zinc 0.5%. Nitrogen derived from ammonium phosphite; phosphorus derived from phosphorus acid; potash derived from potassium hydroxide. Chelated manganese derived from manganese EDTA and zinc from zinc EDTA. This product was manufactured by Biagro Western Sales, Inc., Visalia, CA, USA. A total of 10 treatment combinations were tested in this study. A detail treatment structure is presented in Table 2.4.

Soil samples were collected at the beginning of the experiment to calculate fertilizer requirements. Composite samples from four locations, each averaged from at least 12 soil cores were collected with a bucket auger from 0 to 15 cm depth prior to planting. Samples were oven dried at 60°C and ground to pass a 2 mm sieve. A 1:1 soil-water suspension and glass electrode pH meter was used to measure soil pH and buffer index (Thomas, 1996; Sims, 1996; Sikora, 2006). Five gram soil was extracted with 25 ml 1M KCl solution to determine $NO_3$-N and was quantified using a flow-injection Autoanalyzer (LACHAT, 1994). Mehlich III solution was used to extract P and K. The amount was then quantified using a Spectro CirOs inductively coupled plasma spectrometer (Soltanpour et al., 1996).

Pioneer corn hybrid ‘P0902XR’ at a rate of 79000 seeds ha$^{-1}$ was planted with a John Deere Max Emergence planter in the field studies. Two seeds of the same variety were planted in the greenhouse study. Plots with N and P treatments were fertilized for a 12550 kg ha$^{-1}$ expected corn yield. One-third urea was broadcasted before planting and the rest was broadcasted at sixth-leaf stage. All TSP were broadcasted before planting.

Soil was moderately acidic in the second greenhouse study in 2012. Lime was applied accordingly to raise the soil pH of 6.4. Phosphite rate was determined based on manufacturer's recommendation. The nutrient solution at a rate of 2.34 L ha$^{-1}$ was mixed with 150 L ha$^{-1}$ water to make the concentration less than 2%. A backpack sprayer was used to spray the nutrient-water mixture on the upper surface of the leaves at walking speed. The first application was performed at sixth leaf stage (V6), and the second application applied at tasseling (VT) followed by the treatment structure.

Whole plants from the two center rows were harvested at maturity to obtain dry matter and grain yield. The samples were then dried in a forced air oven at 30°C for 10 days and weighed. Plant samples were then divided into grain, stem and leaf. Grain samples were measured to calculate grain yield for all treatments. Instead of measuring total yield, relative grain and dry matter yield was calculated for greenhouse experiments. The weights of these samples were divided by the highest yields to obtain relative yields. All forage and grain samples were ground to pass a 140-mesh sieve (100 mμ) for chemical analysis. Phosphorus concentrations of the samples were analyzed by a Spectro CirOs ICP following wet digestion (NFTA, 1993).

SAS 9.3 computer software (SAS Institute Inc.) was used for statistical analyses. Data were subjected to Analysis of variance using General Linear Model (GLM) procedure. Fisher's protected LSD (Least Significant Difference) were used to compare treatment means when appropriate.

## RESULTS

### *Biomass yield*

In general, application of N increased dry matter production in all sites and years Table 2.6). In 2011 at LCB, N-only treatment yielded 24886 kg $ha^{-1}$ dry biomass which is not significantly different than N along with any form of P. All the treatments without N had lower biomass accumulation except for P+V6 treatment at LCB and at the greenhouse in 2011. Soil applied P and foliar phosphite at sixth-leaf stage yielded 28072 kg $ha^{-1}$ dry biomass similar to N and P treatments. Both soil applied P and foliar P along with soil applied N increased biomass yield significantly compared to non-fertilized plots. However, phophite applied either once or twice within the season did not significantly differ from each other. These result suggested that second application of foliar phophite was not equivalent to first application. In the greenhouse, N treatments increased yield significantly compared to non-treated plots. Neither foliar nor soil-applied P had any impact on biomass production. In 2012, there were no treatment effects at the Cimarron Valley Research Station at Perkins (Table 2.6). Similar results were obtained in the greenhouse. No significant difference was found between soil applied P and foliar phosphite treatments when applied with N.

### *Stem P concentration*

Effect of the nutrient sources, application method, and timing on stem P concentration at all locations and year are presented in Table 2.7. Foliar P at sixth-leaf stage with soil-applied P had the highest P concentration of 2743 mg $kg^{-1}$ at LCB in 2011. However, the treatment did not significantly increase stem P concentration. Stem P concentration was significantly lower (1980 mg $kg^{-1}$) than check when N was applied alone and N was applied with two foliar sprays. This year in the greenhouse, soil-applied P with soil-applied N significantly increased stem P concentration. Soil-applied P and one application of foliar phosphite increased stem P concentration in the greenhouse in 2012. In 2012, the field results showed no response from either soil or foliar applied P due to the environmental stress.

Overall, treatments with soil-applied N and P and foliar P with soil-applied N had higher grain yield, however, exhibited low P concentration in the stem. This is presumably due to the fact that P was likely translocated to grain from stem during grain filling period as it is mobile in the vascular system. Therefore, one and two foliar application of phosphite has similar effect on stem P concentration.

### *Leaf P concentration*

Treatment effect in terms of leaf P concentration was not significant in the field studies. Foliar P applied at sixth-leaf stage had 2573 mg $kg^{-1}$ P which is the highest value among other treatments at LCB in 2011. Overall, P concentration in the leaf at CVR in 2012 was 52% lower. This site was not irrigate and was in serious water stress. In the greenhouse in 2011, only soil-applied N with foliar spray at sixth leaf stage had highest P concentration of 2424 mg kg$^{-1}$. Other treatments were significant when compared to check. Data obtained from the greenhouse in 2012 showed that foliar P application with soil applied N, and soil-applied N significantly decreased P concentration compared to check. Other treatments had no effect on leaf P concentration. Soil applied P accumulated numerically highest 2933 mg kg$^{-1}$ P in the leaf.

***Grain P concentration and grain total P***

Grain P concentration did not increase significantly at LCB in 2011 (Table 2.9). At CVR in 2012, grain P concentration (4366 mg kg$^{-1}$) significantly increased compared to check (3566 mg kg$^{-1}$) on those treatments where soil P and two application of foliar phophite (4500 mg kg$^{-1}$) were performed along with soil applied N. However, these treatments did not differ each other. In this location, phosphite sprayed at V6 and VT growth stages yielded higher P concentration (4500 mg kg$^{-1}$) compared to one foliar application (4033 mg kg$^{-1}$). Other treatments had no influence on grain P concentration. In the greenhouse, grain was obtained from three treatments out of ten treatments.

Total grain P was increased significantly when soil and foliar P applied with N at LCB in 2011 (Table 2.9). Two applications of foliar phosphite yielded the greatest among all treatments with a total grain P of 12.64 kg ha$^{-1}$. Nitrogen also increased total grain P (9.29 kg ha$^{-1}$) by 61% compared check (3.55 kg ha$^{-1}$). However, this increase is statistically lower than two application of foliar P. Foliar application in sixth-leaf stage decrease total grain P. Similar results were obtained from CVR in 2012 except there were no differences between one and two foliar applications.

### *Grain yield*

Grain yield differed among treatments in all locations and years (Table 2.10). Foliar phosphite increased grain yields compared to check plots at LCB in 2011. The highest corn grain yield was 2808 kg ha$^{-1}$ when foliar Phosphite applied at V6 and VT growth stages with N fertilizer. Soil applied P and N, and N treatments yielded 2680 kg ha$^{-1}$ and 2465 kg ha$^{-1}$ grain yield, respectively. However, no significant differences were found between N treatments and N and P treatments. These results suggested that application of N promotes soil or foliar P uptake resulting increased grain yield. However, two applications of foliar spray at V6 and VT stages with N produced significantly greater grain yield than one foliar application at V6 stage.

Soil P level was very low (11 ppm) in the greenhouse study in 2011 (Table 2.3). This means, soil was 80% P sufficient, based on the Oklahoma State University research (Raun and Zhang, 2006). Grain was obtained from the plots where N was applied. Soil applied N and P treatment produced greater relative grain yield followed by N and N with foliar phosphite application at V6 growth stage. Phosphite applied at V6 growth state with N yielded 20% grain when compared to soil applied N and soil applied P treatments. Relative data showed that the foliar application of phosphite at V6 yielded 80% less grain compared to N+P treatments. When no P was applied, yield loss was calculated as 60%. Those plots that received two foliar phosphite applications (V6 and VT) did not produce grain at all.

Corn grain yields were 25% higher at CVR in 2012 than LCB in 2011 (Table 2.6). At this location (CVR), both soil and foliar applied P with N increased yield. A highest grain yield of 3595 kg ha$^{-1}$ was obtained from soil applied N and P treatment. One application of foliar P with N and two application of foliar P with N yielded 2999 kg ha$^{-1}$ and 3494 kg ha$^{-1}$, respectively; however, no significant differences suggest that second application of foliar P did not increase grain yield. Treatments with soil applied preplant P or foliar phosphite without N increased grain yield numerically compared to check but was not significantly.

# DISCUSSION

In 2011 and 2012 growing season, Oklahoma had experienced a historic heat and drought. During the tasselling and grain filling period of the crop at LCB in June and July, 79 mm rainfall was recorded. Good management of water is helpful at post anthesis stage for improving yield and grain quality. The LCB experiment site was irrigated until water was unavailable. The lack of water with excessive heat caused poor grain yield regardless of treatments. Especially, heat during tasseling period largely affected grain yield. Research plots at Cimarron Valley Research Station was not irrigated. This site also experienced severe drought in 2012 during tasseling and grain filling period.

Foliar absorption of nutrients occurs when the environmental conditions are favorable. There are many factors that interfere with foliar absorption. In extreme heat and dry condition, stomatal and cuticular absorption do not occur and stomata remain closed. The dissolved nutrients dry very quickly preventing absorption. Stomata remain closed when it is very hot. However, those plot treated with full N fertilizer had relatively better vegetative growth. This may have resulted in higher P absorption through foliar and root systems.

Soil P status at the study sites was reasonable for the plant growth. Based on the initial soil test report, at both sites soil P status was around 95% sufficient of crop need. Corn plants have extended root system which increases its contact with soil (Girma et al., 2007). Therefore, P uptake by the root system was adequate for the plants to reach maximum yield. This may be one of the possible reasons for lack of differences in biomass production between N treatments and treatments fertilized with foliar or basal P. However, biomass production in those plots treated with N at LCB was higher than the check plot. This is due to the effect of N application which enhanced vegetative growth when water was not limited. This location was not irrigated and received low rainfall during vegetative growth stage. N volatilization was greater due to low rate of hydrolysis of urea fertilizer. The granule of urea was seen even after one week of application.

The phosphite solution sprayed as a source of P contains 20% P by volume. The amount is too low that, it is suspected, one or two foliar spray will not deliver adequate amount of P when soil deficiency is high. This could prevent us to get statistically detectable yield difference among treatments. In this experiment phosphite was sprayed at the rate of 2L ha$^{-1}$ in 150 L water ha$^{-1}$. This means treatments where phosphite sprayed once at sixth leaf stage as foliar received 0.4 kg P ha$^{-1}$; and treatments where phosphite sprayed twice received 0.8 kg P ha$^{-1}$. The amount was too low and was not able to provide sufficient P through leaves even when the soil was P deficient. The concentration of phosphite solution could not be increased due to potential leaf damage. A preliminary study was abandoned at Haskell Oklahoma in 2010 for excessive leaf damage due to higher rate of phosphite application. The phosphite solution was sprayed at the rate of 4L ha$^{-1}$.

In the greenhouse study, soil P concentration was very low (11 ppm) in the first year study. Soil applied N and P yielded highest biomass. In the second year greenhouse study, N+P plot numerically yielded highest biomass but not significantly different from foliar treatments as the soil was not P deficient which may have masked the treatment effects. However, in 2011, plots treated with foliar phosphite yielded less grain and biomass yield than soil applied P. These results suggest that the amount of foliar P was not sufficient to meet P requirement. Another potential reason may be the high reactivity of phosphite molecule. The comparatively more reactive phosphite molecule may have damaged leaf tissues and resulted in reduced photosynthesis. In the case when grain yield increased by foliar application may be partially compensate P requirement due to insufficient uptake by the roots (Mosali et at., 2006; Ling and Silberbush, 2002).

# CONCLUSION

The responses in biomass and grain yields and grain, stem and leaf P concentrations obtained from foliar and soil applied P were largely affected by severe heat and drought during the growing seasons. In the greenhouse studies, pots may not had enough soils to provide available P for the plants even if P level were not that low. On average, each pot contains about 18 kg soil. That means, in 2011 each pot had 198 mg P and in 2011, each pot had 431 mg P. In 2011, foliar application of phosphite increased biomass yield in the field and in the greenhouse compared to the non-fertilized treatment. One application of foliar did not significantly differ with two foliar applications. In 2012, neither foliar nor soil-applied P increased biomass yield. However, grain yield increased in the field studies by applying foliar phosphite. Two applications of foliar phosphite increased grain yield than one application. In the greenhouse, where soil P status was very low, foliar application did not increase grain yield, yet, soil applied P did. Foliar phosphite application did not increase grain P concentration at in either in the fields or in the greenhouse study. Neither soil applied P increased grain P concentration this year. In 2012, two applications of foliar phosphite with fertilizer N increased grain P concentration compared to non-fertilizer check. Stem P concentration was largely unaffected in the field studies. However, stem P concentrations were greatest in the greenhouse when P was applied in the soil.  Leaf P concentration was significantly. Further field study is required to determine treatments effects in those soils that are low in P. Soils collected for the greenhouse experiment were low in P did not increase grain yield and biomass. Our results indicate that, one or two application of phosphite anion as a source of P may be used as a partial supplement for corn. However, using phosphite is not recommended when the soil is low in P to avoid multiple applications.

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Table 2.1. Maximum, minimum, and mean air temperature; total monthly rainfall from April to August at Lake Carl Blackwell (LCB) and Cimarron Valley Research Station (CVR), Oklahoma.

| Month | Air temperature [°C] | | | | | | Total rainfall [mm] | |
|---|---|---|---|---|---|---|---|---|
| | LCB, 2011 | | | CVR, 2012 | | | | |
| | Max | Min | Mean | Max | Min | Mean | LCB, 2011 | CVR, 2012 |
| April | 24.4 | 8.3 | 17.2 | 23.3 | 12.2 | 18.0 | 43 | 128 |
| May | 26.6 | 13.3 | 19.9 | 29.4 | 16.6 | 23.0 | 115 | 28 |
| June | 35.5 | 20.5 | 28.5 | 32.7 | 20.0 | 26.2 | 73 | 74 |
| July | 40.0 | 23.8 | 31.8 | 38.3 | 23.3 | 30.8 | 6 | 7 |
| August | 39.4 | 22.2 | 30.6 | 35.5 | 21.1 | 28.0 | 16 | 85 |

Table 2.2. Soil series and description at the experiment sites.

| Location | Series name |
|---|---|
| LCB | Pulaski (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents) |
| CVR | Konawa (Fine-loamy, mixed, active, thermic Ultic Haplustalfs)<br>Teller (Fine-loamy, mixed, active, thermic Udic Argiustolls) |
| GH2011 | Grant (Fine-silty, mixed, superactive, thermic Udic Argiustolls) |
| GH2012 | Normangee ( Fine, smectite, thermic Udertic Haplustalfs) |

LCB = Lake Carl Blackwell near Stillwater, Oklahoma; CVR = Cimarron Valley Research Station near Perkins, Oklahoma; GH2011 = Greenhouse study in 2011; GH2012 = Greenhouse Study in 2012. The notations associated with the series name have been used throughout the discussion to identify a particular treatment effect.

Table 2.3. Initial surface (0-15 cm) soil test characteristics at LCB, CVR and the soil collected for greenhouse experiment.

| Location | pH [1:1] | $NO_3$-N | Mehlich III P [ppm] | Sufficiency* [%] | Mehlich III K [ppm] |
|---|---|---|---|---|---|
| LCB | 7.1 | 5 | 20 | 95 | 149 |
| CVR | 6.7 | 2 | 22 | 95+ | 122 |
| GH2011 | 6.8 | 5 | 11 | 80 | 316 |
| GH2012 | 4.8 | 24 | 24 | 95+ | 167 |

* Phosphorus sufficiency was determined based on the soil test report and Oklahoma State University research results.

Table 2.4. Treatment structure of the study and their description. Same treatment structure was used in the field and greenhouse study.

| **No.** | Notations | Treatment Descriptions |
|---|---|---|
| 1 | Control | No fertilizers used |
| 2 | V6 | Phosphite sprayed at sixth-leaf growth stage |
| 3 | V6+VT | Phosphite sprayed at both sixth-leaf and tasseling growth stages |
| 4 | N | 1/3 urea (46-0-0) applied as preplant and 2/3 urea applied at sixth-leaf growth stage |
| 5 | N+V6 | 1/3 urea applied as preplant and 2/3 urea applied at sixth-leaf growth stage; phosphite sprayed at sixth-leaf growth stage |
| 6 | N+V6+VT | 1/3 urea applied as preplant and 2/3 urea applied at sixth-leaf growth stage; phosphite sprayed at both sixth-leaf and tasseling growth stages |
| 7 | P | Triple Super Phosphate (TSP) applied as preplant |
| 8 | P+V6 | Triple Super Phosphate (TSP) applied as preplant; phosphite sprayed at sixth-leaf growth stage |
| 9 | P+V6+VT | Triple Super Phosphate (TSP) applied as preplant; phosphite sprayed at both sixth-leaf and tasseling growth stages |
| 10 | N+P | 1/3 urea applied as preplant and 2/3 urea applied at sixth-leaf growth stage; Triple Super Phosphate (TSP) applied as preplant |

* The notations associated with the treatment description have been used throughout the discussion to identify a particular treatment effect.

Table 2.5. Planting and harvesting date at all locations and year.

| Location | 2011 | | 2012 | |
|---|---|---|---|---|
| | Planting | Harvesting | Planting | Harvesting |
| LCB | 11-Apr | 15-Aug | - | - |
| CVR | - | - | 23-Apr | 27-Aug |
| Greenhouse | 5-Aug | 16-Dec | 4-May | 7-Sep |

LCB = Lake Carl Blackwell near Stillwater, Oklahoma; CVR = Cimarron Valley Research Station near Perkins, Oklahoma.

Table 2.6. Effect of different P treatments on biomass yield at all locations and year.

| | 2011 | | 2012 | |
|---|---|---|---|---|
| Treatments | LCB | GH2011 | CVR | GH2012 |
| | kg $ha^{-1}$ | Relative yield | kg $ha^{-1}$ | Relative yield |
| Control | 18399de* | 0.34d | 20866 | 0.68abc |
| N | 24886abcd | 0.71b | 21041 | 0.60bc |
| P | 23221bdc | 0.33d | 16492 | 0.71abc |
| N+P | 29364ab | 0.93a | 22660 | 0.85a |
| V6 | 14007e | 0.31d | 17148 | 0.57c |
| V6+VT | 20294cde | 0.32d | 16885 | 0.61bc |
| N+V6 | 25575abc | 0.63c | 22528 | 0.70abc |
| P+V6 | 28072ab | 0.33d | 20604 | 0.70abc |
| N+V6+VT | 31775a | 0.62c | 19335 | 0.71abc |
| P+V6+VT | 20207cde | 0.33d | 19291 | 0.79ab |
| Mean | 23580 | 0.49 | 19685 | 0.69 |
| C. V. (%) | 17.18 | 9.74 | 20.06 | 16.93 |
| LSD (α = 0.05) | 6953 | 0.08 | NS | 0.20 |

* Means with the same letters are not significantly different at α = 0.05; NS = Non-significant.

LCB = Lake Carl Blackwell near Stillwater, Oklahoma; CVR = Cimarron Valley Research Station near Perkins, Oklahoma; GH2011 = Greenhouse study in 2011; GH2012 = Greenhouse Study in 2012.

Table 2.7. Effect of different P treatments on stem P concentration at all locations and year.

| | 2011 | | 2012 | |
|---|---|---|---|---|
| Treatments | LCB | GH2011 | CVR | GH2012 |
| | mg kg$^{-1}$ | | mg kg$^{-1}$ | |
| Control | 2373ab* | 1058bc | 1966abc | 1833bc |
| N | 1980b | 1453ab | 1766bcd | 1100d |
| P | 2676a | 1206b | 2400a | 2566a |
| N+P | 2200ab | 2007a | 1200d | 1366cd |
| V6 | 2756a | 867bc | 2133ab | 1966b |
| V6+VT | 2730a | 1051bc | 1566bcd | 2200ab |
| N+V6 | 2610a | 1011bc | 1200d | 1066d |
| P+V6 | 2743a | 1439ab | 1466cd | 2633a |
| N+V6+VT | 1946b | 317c | 1600bcd | 1366cd |
| P+V6+VT | 2336ab | 1018bc | 1866abc | 2300ab |
| Mean | 2435 | 1143 | 1716 | 1840 |
| C. V. (%) | 14.76 | 38.09 | 20.26 | 15.86 |
| LSD (α = 0.05) | 616 | 746 | 596 | 500 |

* Means with the same letters are not significantly different at α = 0.05; NS = Non-significant.

LCB = Lake Carl Blackwell near Stillwater, Oklahoma; CVR = Cimarron Valley Research Station near Perkins, Oklahoma; GH2011 = Greenhouse study in 2011; GH2012 = Greenhouse Study in 2012.

Table 2.8. Effect of different P treatments on leaf P concentration at all locations and year.

| | 2011 | | 2012 | |
|---|---|---|---|---|
| Treatments | LCB | GH2011 | CVR | GH2012 |
| | mg kg$^{-1}$ | | mg kg$^{-1}$ | |
| Control | 2330 | 2114b* | 966 | 2433abc |
| N | 2253 | 2167ab | 1400 | 1466de |
| P | 2283 | 2286ab | 1300 | 2933a |
| N+P | 2526 | 2295ab | 1266 | 1700cde |
| V6 | 2573 | 2191ab | 1033 | 1933bcde |
| V6+VT | 2340 | 2115b | 1000 | 2300abcd |
| N+V6 | 2540 | 2424a | 1266 | 1400e |
| P+V6 | 2220 | 2042b | 900 | 2566ab |
| N+V6+VT | 2703 | 2278ab | 1366 | 2000bcde |
| P+V6+VT | 2350 | 2254ab | 1000 | 2700ab |
| Mean | 2412 | 2217 | 1150 | 2143 |
| C. V. (%) | 11.83 | 8.08 | 30.88 | 23.16 |
| LSD (α = 0.05) | NS | 307 | NS | 851 |

* Means with the same letters are not significantly different at α = 0.05; NS = Non-significant.

LCB = Lake Carl Blackwell near Stillwater, Oklahoma; CVR = Cimarron Valley Research Station near Perkins, Oklahoma; GH2011 = Greenhouse study in 2011; GH2012 = Greenhouse Study in 2012.

Table 2.9. Effect of different P treatments on grain P concentration and total grain P content at all locations and years.

| Treatments | LCB 2011 | | | CVR 2012 | |
|---|---|---|---|---|---|
| | P conc. | Total grain P | GH2011 | P conc. | Total grain P |
| | mg kg$^{-1}$ | kg ha$^{-1}$ | | mg kg$^{-1}$ | kg ha$^{-1}$ |
| Control | 3876abc* | 3.55ef | - | 3566cd | 4.16b |
| N | 3766ab | 9.29bc | 1866 | 4366ab | 14.52a |
| P | 3993abc | 2.85fg | - | 3433d | 6.37b |
| N+P | 4146ab | 11.11ab | 2066 | 4300ab | 15.29a |
| V6 | 3983abc | 1.06g | - | 3433d | 5.97b |
| V6+VT | 3703bc | 3.82ef | - | 3466d | 7.09b |
| N+V6 | 4153ab | 7.31cd | 1433 | 4033abcd | 12.54a |
| P+V6 | 3710bc | 5.95de | - | 3666bcd | 7.25b |
| N+V6+VT | 4510a | 12.64a | - | 4500a | 15.67a |
| P+V6+VT | 3336c | 2.34fg | - | 3466d | 6.12b |
| Mean | 3918 | 5.99 | 1788 | 3823 | 9.50 |
| C. V. (%) | 10.25 | 23.83 | 38.43 | 11.55 | 28.50 |
| LSD($\alpha$=0.05) | 689 | 2.45 | NS | 757 | 4.64 |

* Means with the same letters are not significantly different at $\alpha$ = 0.05; NS = Non-significant.

LCB = Lake Carl Blackwell near Stillwater, Oklahoma; CVR = Cimarron Valley Research Station near Perkins, Oklahoma; GH2011 = Greenhouse study in 2011; GH2012 = Greenhouse Study in 2012.

Table 2.10. Effect of different nutrient sources on grain yield at all locations and year.

| | 2011 | | 2012 |
|---|---|---|---|
| Treatments | LCB | GH2011 | CVR |
| | kg ha$^{-1}$ | Relative yield | kg ha$^{-1}$ |
| Control | 932d* | 0.0d | 1169b |
| N | 2465a | 0.4b | 3322a |
| P | 719ed | 0.0d | 1786b |
| N+P | 2680a | 1.0a | 3595a |
| V6 | 270e | 0.0d | 1725b |
| V6+VT | 1024cd | 0.0d | 2041b |
| N+V6 | 1772b | 0.2c | 2999a |
| P+V6 | 1611bc | 0.0d | 1975b |
| N+V6+VT | 2808a | 0.0d | 3494a |
| P+V6+VT | 751ed | 0.0d | 1765b |
| Mean | 1503 | 0.15 | 2387 |
| C. V. (%) | 25.13 | 49.38 | 22.70 |
| LSD ($\alpha$ = 0.05) | 648 | 0.13 | 930 |

* Means with the same letters are not significantly different at $\alpha$ = 0.05; NS = Non-significant.

LCB = Lake Carl Blackwell near Stillwater, Oklahoma; CVR = Cimarron Valley Research Station near Perkins, Oklahoma; GH2011 = Greenhouse study in 2011; GH2012 = Greenhouse Study in 2012.

Figure 2.1: Phosphorus deficiency symptoms in the greenhouse study in 2012.

# VITA

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