1-1-2014

Alternative Nitrogen for Subsequent Southern Switchgrass (Panicum Virgatum L.) Production using Cool-Season Legumes

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Alternative nitrogen for subsequent southern switchgrass (*Panicum virgatum L.*)

production using cool-season legumes

By

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Masters of Science
in Agriculture
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

May 2014

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Pages in Study: 61

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Switchgrass (*Panicum virgatum* L.) has become an important bioenergy crop. Warm, winter temperatures in the southeastern USA allow for fall establishment and winter growth of cool-season legumes that may provide nitrogen to the spring perenniating crop of switchgrass. Data indicates variation due to year and location, but hairy vetch plots provided a greater nitrogen percentage in the subsequent biomass production of switchgrass. In 2011, switchgrass fertilized with 56 kg ha^{-1} N was greater than the control and in 2012 it was greater than the 28 kg ha^{-1} N treatment. Variation around the means prevented clear separation among other treatments. The data also showed that hairy vetch had the greatest volunteer frequency and cover percentage throughout the year. Data from the Dairy Farm showed no differences in yields due to a lack of field management the previous years and only ball clover increased its coverage over time.

ACKNOWLEDGEMENTS

The author would like to express his sincere gratefulness to all who assisted in this project. First, I would like to think my Lord and Savior, without him, nothing is possible. Phillippians 4:13 says, "I can do all things through Christ who strengthens me". This verse helped me get through the hard times during the writing and research of the thesis. Secondly, I would like to thank my loving wife Hannah Holmberg for her unconditional love. Thank you so much and for always standing by me and encouraging me to stand for what I believe in. Thirdly, I would like to thank my family. They put forth the effort to raise a Godly man that is honest and sincere and without them I would not be the man I am today. I would also like to sincerely thank my major professor Dr. Brian S. Baldwin for the opportunity to help further my education. He has made many sacrifices to help me further my knowledge, not only about academics, but day to day life. Likewise, I want to thank the members of my thesis committee, Dr. David J. Lang, Rocky W. Lemus, and Dr. Brian J. Rude , for their input and determination to help complete this thesis. I would also like to express a great appreciation to my fellow co-workers, Dr. J. Brett Rushing, David Russell, Jasper Cobb, Tyler Sandlin, Spencer Smith, and Jesse Morrison for their friendship and hard work helping me with this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The United States Department of Agriculture (USDA) and the Department of Energy (DoE) have been searching for ways to produce large quantities of biomass for bioenergy at a reduced cost, because of the increase in cost of petroleum based fuels. Bioenergy has been around for centuries, wood and other plant-based resources were burned and later used to make steam. The use of fossil fuels, including petroleum, are being depleted, and new resources are needed for energy. Herbaceous crops like switchgrass (*Panicum virgatum* L.) have been chosen, because of their fast growth, adaptability to marginal lands, and greater yield potential (McLaughlin and Walsh, 1998). These herbaceous crops are used as feedstocks to create bioenergy by: direct-firing, pyrolysis (fast, slow) or hydrolysis (acid, base, enzymatic) to create liquid biofuels (Badger, 2002). Production of these herbaceous crops offer environmental benefits such as; erosion control, enhanced water quality and provide wildlife habitat. Normally, switchgrass is thought of as a forage crop, but today the possibilities for its utilization have been broadened.

Finding ways to maintain greater yields with less inputs are crucial, when it comes to maximizing switchgrass biomass production. One of the greatest input costs for crop production is fertilizer, especially nitrogen. With an increase in petroleum cost, there is a corresponding increase in the cost of nitrogen fertilizer. Nitrogen is the most abundant element in the atmosphere, making up about 78%, but is considered the greatest limiting nutrient for plant growth. Nitrogen constantly cycles through the atmosphere, plants, organic matter, soil organisms and water, which makes for a complex biochemical transformation.

Warm winter temperatures in the southeastern USA, allow for establishment and growth of cool-season legumes. These legumes, through a symbiotic relationship with bacteria of the genus *Rhizobium*, have the potential to provide nitrogen to the subsequent spring crop of switchgrass, replacing or reducing the need for chemical nitrogen fertilizer application. The use of clovers (*Trifolium*), vetches (*Vicia*) and other cool-season legumes (*Medics, Medicagos, Meliotus*) planted with warm-season grasses in their dormant state (immediately after fall harvest) may provide nitrogen for the grass crop in the spring/summer. The objectives of this study are: (1) to evaluate biomass yield of switchgrass using nitrogen fertilizer, and cool-season legumes and (2) determine which legume species will provide sufficient nitrogen to maintain switchgrass yield and which legume species will reseed the following year.

CHAPTER II

LITERATURE REVIEW

Overview of Switchgrass

Switchgrass is a warm-season C_4 perennial grass that is native to the tall grass prairie of North America from Canada to Central Mexico. It can be found in company with other native grass species like; big blue stem (*Andropogon gerardii*), little blue stem (*Schizachyrium scoparium*), and indiangrass (*Sorghastrum nutans*). Being a North American native grass, it has resistance to most pest and plant diseases and requires limited amounts of fertilizer to produce greater yields (Mitchell and Bruce, 2008). Switchgrass grows between 0.5 to 3 meters tall and is comprised of two ecotypes, lowland and upland (Lewandowski et al., 2003). The lowland ecotypes are tall growing, resistant to rust, coarse-stemmed, adapted to wetter areas and typically have a longer growing season (Mooney et al., 2009). ‘Alamo' and ‘Kanlow' are among the best known lowland varieties. The upland ecotypes are typically shorter, fine-stemmed, adapted to drier areas and have a short growing season (Lewandowski et al., 2003). Better known upland varieties include: Trailblazer, Blackwell, Cave-in-Rock, Pathfinder, and Caddo (Karp and Shield, 2008). Choosing the correct variety for a specific location will help to maintain sustainable biomass production.

Switchgrass, once established, can produce greater yields. Cultivars grown in the Southeast such as 'Alamo' typically yield more than the upland ecotypes (Mooney et al., 2009). In Milan, Tennessee, first year yields ranged from 1.56 to 2.9 Mg ha^{-1}, while the second year ranged from 6.72 to11.2 Mg ha^{-1} and third year 11.2 to 22.4 Mg ha^{-1} (De Koff and Tyler, 2011). Yields in the third year of production have been reported in Alabama, Texas, Virginia, Tennessee, Kentucky and West Virginia that ranged from 15.2 to 16.8 Mg ha^{-1}, with maximum yields between 20.1 to 27.6 Mg ha^{-1} from more than 19 locations (Ferrell et al., 1995).

Switchgrass as a Biofuel Feedstock

With fossil fuels diminishing (82% in 2011 to 78% in 2040) and the price of petroleum on the increase, the federal renewable fuels standard states the renewable share of total energy use will grow from 9% in 2011 to 13% in 2040 (U.S. Energy Information Administration, 2013). In addition to the estimated depletion of the world's petroleum reserves in about 40 years, a drastic increase in global energy consumption and gasoline prices have sparked and continued to drive interest in renewable energy (DoE, 2006). Renewable energy refers to sources of energy that do not irreversibly exhaust or deplete resources, such as; wind, solar and bio-based fuels including: ethanol, biodiesel, and hydrogen (Vermerris et al., 2007).

In 1978 at the Oak Ridge National Laboratory, the Bioenergy Feedstock Program was initiated to select potential feedstocks for bioenergy. In 1991, switchgrass was one of the plant species selected (McLaughlin and Kszos, 2005). Switchgrass was chosen, because of its perennial growth habit, greater yield, broad area of adaptation, lesser nutrient use and lesser pesticide requirement. Using perennial cropping systems for energy production can benefit the economy. McLaughlin et al. (1999) stated perennial cropping systems can be compatible with conventional farming, reduce soil degradation, lesser reliance on foreign oil, and decrease greenhouse gases emissions.

With petroleum prices increasing, new energy sources are needed. Ethanol can be made from cellulose through microbial processes of fermentation. Fermentation of switchgrass has three steps before it is transformed to ethanol; the creation of solution of fermentable sugars (saccharification, the most difficult), the sugars fermented to ethanol, and distillation (Sanderson et al., 2006). When obtaining sugars from cellulose, there are three basic processes; acid hydrolysis, enzymatic hydrolysis and thermochemical (Badger, 2002). Acid hydrolysis is broken down into two forms; dilute acid and concentrated acid. When using dilute processes, it must be performed under extreme temperature and pressure to assist ongoing processing, which can take a few seconds to a few minutes. This process results in the continuous production of ethanol and results in a lesser sugar recovery efficiency (around 50%) (Badger, 2002). The concentrated acid process uses warm temperatures with some pressure for greater sugar competence and requires alkali (usually calcium hydroxide) to neutralize the acid.. This process takes longer, but has a sugar recovery of 90% or more (Hu and Wen, 2008). Hydrolysis is performed by incorporating starch or in this case, cellulose, with water and then heated to break the cell walls (Badger, 2002). There are specific enzymes that are added to break chemical bonds. During the enzymatic hydrolysis process, pretreatment processing is needed to fracture the structure of the biomass rendering the sugars from cellulose and hemicelluloses molecules available for fermentation (Badger, 2002). This process can take several days to accomplish.

There are two thermochemical processes. The first is a thermochemical coupled with a biological system in which cellulosic materials are gasified to carbon dioxide (CO_2), carbon monoxide (CO) and a methyl group derived from methane (CH_3) and the resulting gas is then fermented, or passed through a reactor containing catalysts that produces ethanol (C_2H_6O) (Badger, 2002). The second process converts the biomass to a bio oil that is de-oxygenated and distilled similar to petroleum.

One of the most common sources of ethanol is corn (*Zea mays*). A study by McLaughlin and Walsh (1998) determined an energy budget for corn and switchgrass. They found that the efficiency of energy production and carbon sequestration in a perennial grass system is 15 times greater and 20 to 30 times greater, respectively, than that of an annual row crop system. Pimentel (1991) conducted an analysis of ethanol production and determined that grain production ethanol does not improve energy security, it is uneconomical, it is not a renewable energy source and it negatively impacts the environment.

Woody biomass crops also play a role in energy production. Hall (2002) indicated, that forests provide about 14% of the worlds primary energy supply and they have the potential to meet up to 50% of the worlds energy demands. Also, 55% of the four billion cubic meters of wood is used annually by the world as fuel and charcoal for daily energy needs. Most energy from woody biomass is used to generate heat, steam, and electrical power. However, wood is harvested and transported wet (50% moisture or greater). This adds to the expense of transportation and compromises its energy value.

Perlack et al. (1992) indicated that on good agricultural land, herbaceous perennial crops and woody crops can yield between 15 to 20 dry Mg ha^{-1} yr^{-1} and 10 to 15 dry Mg ha^{-1} yr^{-1}, respectively.

Overview of Nitrogen

Nitrogen as an element was discovered in the late 18th Century by Carl Scheele, Daniel Rutherford and Antoine Lavoisier. It was later determined by Jean Baptiste Boussingault and Justus von Liebig that nitrogen can play a critical role in crop production. By the late 19th Century Hermann Hellriegel and Hermann Wilfarth discovered that some microorganisms could take non-reactive N_2 from the atmosphere and convert it to available forms of N for plant growth (Galloway and Cowling, 2002). This process is called biological nitrogen fixation (BNF).

The greatest cost input for agriculture is nitrogen (Peoples et al., 1995). Until around 1920, animal and vegetable waste were the main supplies of nitrogen for crop production. In the 1920's, the Haber-Bosch process, using natural gas to synthesize ammonia was developed. Since then, natural gas is the primary raw material to produce ammonia, accounting for 72 to 85% of the ammonia production cost (Huang, 2007). The FAO (2011) predicted that between the years 2011 and 2015, the demand for nitrogen fertilizer will increase at a rate of 1.7 percent annually until 2015, which is an increase of 6.9 million Mg (7.6 million tons). As petroleum prices increase, so will the price of nitrogen fertilizer. Table 1.1 shows the American average fertilizer. Figure 1.1 links the correlation between ammonium nitrate and gasoline prices.

Table 1 Average U.S. Farm Prices of Selected Fertilizers (USDA, ERS, 2013).

	Anhydrous Ammonia	Urea	Ammonium Nitrate	Sulfate of Ammonium	Diammonium phosphate
Year	US Dollars per Ton				
2000	227	200	194	167	240
2001	399	280	260	192	244
2002	250	191	195	187	227
2003	373	261	243	195	250
2004	379	276	263	205	276
2005	416	332	292	244	303
2006	521	362	366	266	337
2007	523	453	382	288	442
2008	755	552	509	391	850
2009	680	486	438	378	638
2010	499	448	398	326	508
2011	749	526	479	423	703
2012	783	554	506	451	726
2013	847	592	544	522	640

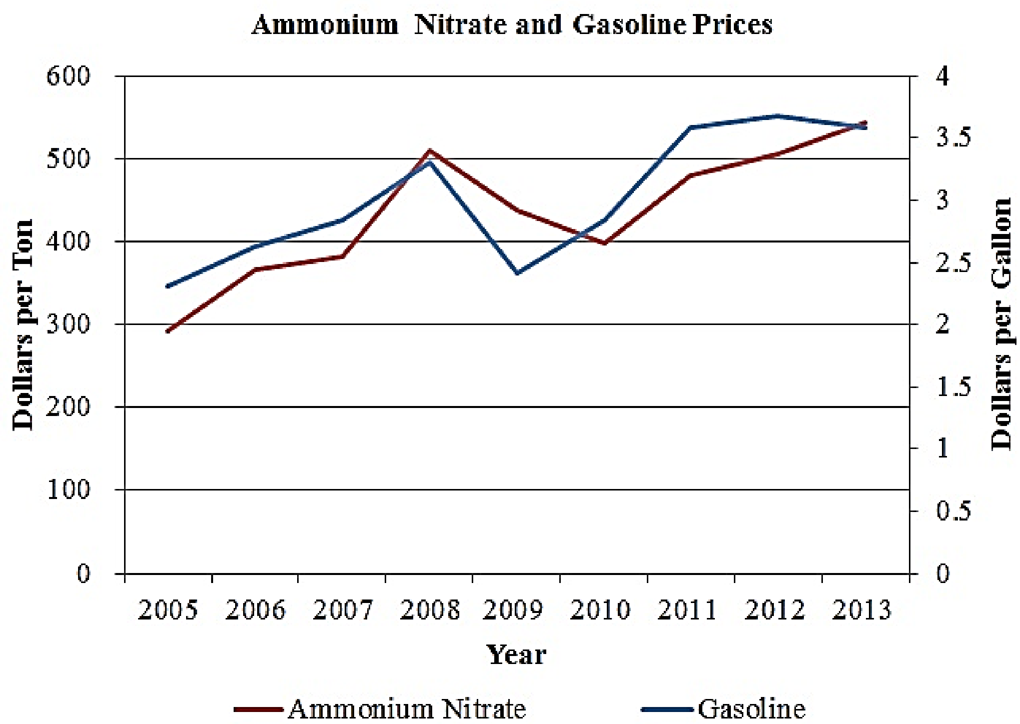

Figure 1 United States average price comparison between ammonium nitrate fertilizer and gasoline from 2005 to 2013 (U.S. EnergyInformation Administration, 2014 and USDA, ERS, 2013).

Nitrogen Fixation

The atmosphere is made up of about 78% percent nitrogen. Legumes form a symbiotic relationship with bacteria of the genus *Rhizobium*, which have the potential to fix "free" nitrogen or dinitrogen (N_2) and make it available to the living crop, replacing or reducing the need for soil or chemical nitrogen fertilizer. Nair et al. (2006) indicated other micronutrients that can be used during the nitrogen fixation process include; zinc, copper, molybdenum and cobalt. There are about 262,000 Mg ha^{-1} of nitrogen present in the atmosphere (Havlin et al., 2005). Nitrogen balance in a field depends on the dry matter yield potential, the environmental conditions and the how much N_2 the legume can fix. Havlin et al. (2005) indicated N_2 fixation by perennial legumes can range anywhere from 112 to 224 kg N ha^{-1} yr^{-1}, while annual legumes can fix between 56 to 112 kg N ha^{-1} yr^{-1}.

When manufacturing ammonia (NH_3) from elemental nitrogen (N_2) is comparable to about 1.7 kg glucose per kg of N and the cost for absorption of the nitrate in the soil is 1.9 kg glucose per kg of N for a total cost of 3.6 kg glucose per kg of N in the plant. A legume reduces N_2 to $-NH_2$ and it is comparable to 7.3 kg of glucose per kg of nitrogen, which makes nitrogen fixation by the plant more cost effective (Barnes et al., 1995).

Switchgrass Nitrogen Uses

Nitrogen content can fluctuate in switchgrass due to frequency and timing of harvest, soil mineralization rates, and plant biomass removal (McLaughlin and Kszos, 2005). Some studies suggest that the annual requirement of N for switchgrass is between 70 and 100 kg N ha^{-1} (McLaughlin and Walsh, 1998). A study conducted by Vogel and Masters (1998) at Mead, NE and Ames, IA compared harvest management and fertilization of Cave-in-Rock switchgrass. In this study six nitrogen fertilizers rates (0, 60 120, 180, 240 and 300 kg N ha^{-1}) of ammonium nitrate were used and harvesting dates were every seven weeks. They found that the greatest yields were at heading and harvesting during a four week period after heading. They also found that N rates of 120 to 180 kg N ha^{-1} are needed to maintain maximum yield, because N rates of 120 kg N ha^{-1} were cumulatively removed with the biomass at this harvest date.

In another study at Moody County (Wentworth-Egan soil), Marshall County (Buse soil) and Gregory County (Ree loam soil) South Dakota, harvest timing and N rates with switchgrass were conducted on Conservation Resource Program (CRP) land. The switchgrass cultivar is unknown. Five amounts of ammonium nitrate fertilizer (0, 56, 112, and 224 kg N ha^{-1}) with split applications were used, with one harvest each year. Harvest after the killing frost and application of 56 kg N ha^{-1} proved to be effective for biomass production. Fertilizer applied greater than 56 kg N ha^{-1} did not increase yield (Mulkey et al., 2006). A similar study by Vogel et al. (2002) at the University of Nebraska near Mead, NE (Sharpsburg silty clay loam) and Iowa State University, Ames, IA (Webster-Nicollet Complex) found Cave-in-Rock switchgrass harvested at full panicle (R3 to R5), fertilized with 120 kg N ha^{-1} of ammonium nitrate, yielded maximum biomass. The biomass yields obtained were 10.6 to 11.2 Mg ha^{-1} at Mead and 11.6 to 12.6 Mg ha^{-1} at Ames, respectively.

Lemus et al. (2008) tested the effect of N application on biomass dry matter yield of switchgrass from 1998 to 2002. This research was conducted in southern Iowa on a Yarmouth-Sangamon paleosol in Lucas and Wayne counties on two 10-year-old fields of Cave-in-Rock switchgrass with limited management. The fertility treatments 0, 56, 112, and 224 kg N ha^{-1} . In June 1998, urea fertilizer was used for the plots and in June1999, May 2000, July 2001, and May 2002 ammonium nitrate was used as the nitrogen source. The biomass yield increased from 1998 (3.6 Mg ha^{-1}) to 2002 (6.5 Mg ha^{-1}) with the greatest yield being 8.5 Mg ha^{-1} at the Lucas location in 2002 indicating a positive effect of N fertilizer over that period of time. The researchers determined that switchgrass harvested once between September and November that had been fertilized with N rates between 56 and 112 kg N ha^{-1} can produce greater sustainable yields.

It has been shown that the addition of nitrogen fertilizer to crops with legumes present can decrease the amount of N_2 fixation in the legume. In a study by Ledgard et al. (1996) near Hamilton, New Zealand on a Horotiu sandy loam, white clover (*Trifolium repens* L.) was grown with perennial ryegrass (*Lolium perenne* L.) and each site received urea fertilizer at either 0 or 390 kg N ha^{-1} yr^{-1}. Nitrogen fertilizer application increased the ryegrass yield from 12,830 kg ha^{-1} yr^{-1} to 16,010 kg ha^{-1} yr^{-1}, but it also decreased the amount of white clover biomass in the harvest from 3,600 to 2,970 kg ha^{-1} yr^{-1}. Since it decreased the amount of clover, it also decreased the amount N contribution from the clover from 111 to 47 kg N ha^{-1}. A similar study by Høgh-Jensen and Schjoerring (1997) outside of Copenhagen Denmark, where white clover was grown in combination with ryegrass, they also found that legume derived N can decrease with an increasing chemical N application. Over a three year period the total derived N measured in the harvested forage was 83, 71, 68 and 60 kg N ha^{-1} with treatments of 3, 24, 48, and 72 kg N ha^{-1}, respectively, indicating a negative relationship between N_2 fixation and chemical N fertilizer application.

Legume Species Used in This Study

The legume family, *Fabaceae*, has about 600 genera and 12,000 species. These legumes have a small range of adaptation and usually require better management inputs. Most legumes are outstanding forages and are known for their ability to fix atmospheric N_2 through a symbiotic association with members of the genus, *Rhizobium* (Barnes et al., 1995). Each legume/microbe combination produces different amounts of fixed nitrogen. Legumes can also be used extensively as cover crops. The southern United States has a distinct advantage when it comes to growing cool-season legumes. Mild winter and spring temperatures allow these legumes to grow and persist giving the potential to exploit them as a winter crop that can provide N to warm-seasons grasses.

Several species are well known in the southern United States. Many originated from the Mediterranean region. Arrowleaf clover (*T. vesiculosum* Savi) is a late season winter annual, that is one of the most frequently used pasture crops in the United States. It grows best on well-drained, fertile soils. Arrowleaf has the potential to volunteer, because most of the seed that it produces has a hard seedcoat. It has an appearance of arrow shaped leaflets, hence the name. The inflorescence is white and sometimes pink (Barnes et al., 1995). Literature indicates that the potential N fixed per year from arrowleaf clover is 56 to 168 kg ha^{-1} (Jennings, 2011) and Hancock (2009) estimates fixed N between 56 and 202 kg ha^{-1}.

Crimson clover (*T. incarnatum* L.) is a cool-season annual, that is one of the more important clover species. It can tolerate an extensive array of soil types and because of its early maturity, it may be interseeded with perennial grasses. It has the potential to reseed if the seed heads are allowed to ripen and there is no insect damage. It is a trifoliate with many secondary branches and hairy leaves and stem. The inflorescence is narrow with a bright red color (Barnes et al., 1995). The potential N fixed per year from crimson clover is 56 to 168 kg ha^{-1} (Jennings, 2011) and Hancock (2009) estimates fixed N between 34 and 168 kg ha^{-1}.

Ball clover (*T. nigrescens*) is a cool-season annual, that has a short growing season (Ball et al., 1991). It grows best on clay and loam soils, and it is tolerant to acidity and poor drainage. It is a good reseeder, because of its seed hardness and short prostrate growth. It has fragrant white flowers and sometimes is mistaken for white clover, but the inflorescence is smaller. (Barnes et al., 1995). The estimated fixed N of ball clover is between 56 and 140 kg ha^{-1} (Hancock, 2009).

Hairy vetch (*Vicia villosa* Roth.) is a viney plant, that has become an important winter annual cover crop for agriculture (Teasdale et al., 2004) because it is winter hardy for most of the United States. It can be grown as a summer annual in some northern regions that have cool summers, but in the South its growth is from December to May. Hairy vetch is a good reseeder, and can persist in the soil for many years, because of relatively large, hard seed. Its infloresence is a raceme that has a prostrate growth (climbs extensively if given support) with leaves that are hairy and pointed at both ends where tendrils form. When it flowers, there are numerous blooms with blue-violet florets. It has the ability to provide nitrogen and it is also a good cover crop (Barnes et al., 1995). The potential N fixed per year from hairy vetch is between 56 and 168 kg ha^{-1} (Jennings 2011; Hancock, 2009).

Common vetch (*V. sativa* L.) is a winter annual, that is best grown on well-drained fertile soils. It is related to hairy vetch. Common vetch volunteers well, because it also produces abundant hard seed. Its morphology has smooth, oblong leaves with a sharp point and smooth stems. It is a climbing plant with compound leaves that ends in tendrils. Its inflorescence can appear in pairs of flowers or it can be single in the leaf axils with reddish purple flowers (Barnes et al., 1995). The potential N fixed per year from common vetch is between 56 and 168 kg ha^{-1} (Hancock, 2009).

Jester Barrel medic (*Medicago truncatula*) is an important forage crop, and can improve soil fertility by nitrogen fixation (Nair et al., 2006). It is an annual cool-season legume that is autogamous (unusual for legumes). It is a raceme with one to five yellow flowers with hairy trifoliate leaves. Grows best on well drained sandy to clayey soils and is a good reseeder, because of its seed hardness (Gallardo et al., 2006). Barrel medic has the potential to fix between 56 and 168 kg ha^{-1} of N yr^{-1} (Hancock, 2009).

White clover is a cool-season perennial, that has naturalized in the southern United States. It persists longer in the Upper South and shorter lived in the Lower South (Ball et al., 1991). It grows best on moist, well drained soils, because of its minimal root system and it is very winter hardy. Usually used in permanent pastures and in combination with other forages and it is a prolific reseeder. Growth is a low, prostrate growth that spreads by stolons with smooth stems and white to pink flowers (Barnes et al., 1995). The potential N fixed per year from white clover is 84 to 168 kg ha^{-1} (Jennings, 2011) and Hancock (2009) estimates fixed N between 34 and 280 kg ha^{-1}.

In Mississippi from 1984 through 1988, Varco et al. (1991) reported yield and nitrogen content of legume cover crops. The total nitrogen content of the legumes were analyzed using the procedure of Nelson and Sommers (1973). Research was conducted at Brooksville, MS (1984 to 1985), Starkville, MS (1985 to 1987), and Sessums, MS (1987 to 1988). Each location had a different soil profile as follows: Okolona silty clay (Brooksville), Marietta fine sandy loam (Starkville), Leeper silty clay loam (Starkville), and Freestone fine sandy loam (Sessums), respectively. Some of the legumes they used were: hairy vetch; arrowleaf clover; 'Tibbee' crimson clover, and 'Chief' crimson clover. Over all years the legume with the greatest mean nitrogen content was Chief crimson clover (147 kg N ha^{-1}), followed by hairy vetch (132 kg N ha^{-1}) and Tibbee crimson clover (111 kg N ha^{-1}). Arrowleaf clover N content was lesser compared to the other three legumes (63 kg N ha^{-1}).

A study was conducted by Panciera and Sparrow (1995) in Fairbanks Alaska on a Tanana slit loam, to determine the amount of N_2 fixation a legume will produce with a "moderate" amount of N fertilizer. The study was conducted in two locations; Fairbanks on a neutral soil and Delta Junction on acid soil. Each plot was fertilized with ammonium nitrate at a rate of 90 kg N ha^{-1}. The legume species used were arrowleaf clover, crimson clover, barrel medic, and winter vetch (hairy vetch). The nitrogen yields for each legume in Fairbanks were: arrowleaf clover 45 kg N ha^{-1}, crimson clover 130 kg N ha^{-1}, barrel medic 155 kg N ha^{-1} and winter vetch 113 kg N ha^{-1}. These legumes responded positively to the neutral pH of the soil at this location. At the Delta Junction location, the yields were lower compared to the Fairbanks location, because the soil was more acidic: arrowleaf clover 27 kg N ha^{-1}, crimson clover 45 kg N ha^{-1}, barrel medic 49 kg N ha^{-1} and winter vetch 14 kg N ha^{-1}. The more acidic the soils are, the less nitrogen the legumes will fix.

In a study by Kumar and Goh (2000) at Lincoln University in New Zealand on a Templeton silt loam, white clover and field pea (*Pisum sativum* L.) were grown to test for nitrogen accumulation and N_2 fixation. Each field plot was fertilized with 200 kg ha^{-1} single superphosphate. In each plot was a randomly placed micro plot that was fertilized with a N-enriched ammonium sulphate applied at 3.65 kg N ha^{-1}. For dry matter yields, six random samples were taken from each plot. For the determination of N_2 fixation and N accumulation, plant tops and root samples were taken from the micro plots in each plot. The study shows that both crops accumulated similar amounts of dry matter, but the winter pea accumulated more N (427 kg N ha^{-1}) in the dry matter than white clover (387 kg N ha^{-1}). When they looked at N_2 fixation they found that white clover fixed more N (327 kg N ha^{-1}) than winter pea (286 kg N ha^{-1}). Also, white clover accumulated more N derived from the atmosphere (90%) than winter pea (69%). The net gain of N due to N fixation for both species ranged from 171 to 313 kg N ha^{-1} which may supply N benefits to subsequent crops.

Using Legumes as a Nitrogen Source for Switchgrass

The use of legumes as a nitrogen source for switchgrass can decrease the cost of inputs, while achieving/maintaining greater biomass yields. A study by Bow et al. (2008) near Stephenville, TX on a Windthorst fine sandy loam, compared cool-season legume integration with a 10 year old stand of Alamo switchgrass. Treatments consisted of no supplement or dairy manure compost applied at 30 Mg ha^{-1}. Dry matter yields of switchgrass ranged between 3022 and 6630 kg ha^{-1} yr^{-1}. Arrowleaf clover had the greatest production followed by common vetch and button medic (*Medicago orbicularis* L.). The compost did not affect seasonal yields until the second year, when grass-legume plots with compost, out yielded uncomposted plots. This work indicates that overseeding cool-season legumes into switchgrass did not decrease dry matter yields and yields could increase with time.

George et al. (1995) near Ames, IA on a Webster silty clay loam soil compared cool-season legume nitrification and nitrogen fertilizer with Cave-in-Rock switchgrass. Ten legumes were used: 'Polara' white-flowered sweetclover (*Melilotus alba* Medik.), 'Madrid' yellow-flowered sweetclover (*M. officinalis* Lam.), 'Norcen' birdsfoot trefoil (*Lotus corniculatus* L.), 'Fergus' birdsfoot trefoil, 'Apollo Supreme' alfalfa (*Medicago sativa* L.), 'Alfagraze' alfalfa, Mammoth red clover *(T. pretense* L.), 'Redland II' medium red clover, 'Emerald' crownvetch (*Coronilla varia* L.), common hairy vetch and the four inorganic nitrogen rates; 0, 60, 120, and 240 kg N ha^{-1}. During the first year of the study they found a nine percent increase in switchgrass yields, with legume renovation, compared to the 0 kg N ha^{-1} plots. During the second year, they found all legumes plots, except crownvetch, produced a greater switchgrass yield than switchgrass fertilized with 240 kg N ha^{-1} and a mixture of legumes (Mammoth red clover, birdsfoot trefoil, and trefoil-red clover) yielded, or exceeded 240 kg N ha^{-1}. They concluded that the second year of interseeded legumes provided adequate nitrogen amounts to the switchgrass to yield more than 120 or 240 kg N ha^{-1} inorganic N application. Grasses benefit in a grass-legume mixture if the legumes reach a sufficient stand density. Legumes and grasses grown in mixtures can either be: (1) compatible - avoid competition with each other, (2) competitive - make demands on the same resources, or (3) allelopathic - interact negatively with each other.

A study by Blanchet et al. (2005) was conducted in concurrence with the study of George et al. (1995) previously stated, but they evaluated the establishment and persistence of legumes interseeded into switchgrass. Legume density was assessed in early June of each seeding year to determine establishment and legume persistence was determined by stand counts in August and October each seeding year and again in June the second year. Mean legume plant density in June was 195 plants m-^{2} for 1991 and 163 plants m^{-2} for 1992. In June of the second year the mean legume persistence was almost 50% in 1991, but dropped to 30% in 1992 due to winter losses. In the second year, there was significant decline in grass stems noted for trefoil and the trefoil-red clover mixture. Minimal, to no effect, was observed for the sweetclovers, crown vetch and hairy vetch, and intermediate effects for alfalfa and red clover planted into switchgrass plots. In the Iowa studies, growth of switchgrass and cool-season legume over lap, substantially. Cold winter temperatures restrict legume growth to periods just before switchgrass growth, and cool springs cause competition between the legume and switchgrass. In the South, mild winters with daytime temperatures that range around 10° C, allow cool-season legumes to actively grow without competition from the switchgrass.

Legumes play a major role in agriculture, agroforestry and natural ecosystems, because of their ability to fix nitrogen and many other attributes. They are excellent colonizers for low nitrogen environments and have proven to be beneficial to warm season perennial grasses.

CHAPTER III

MATERIALS AND METHODS

Leveck Animal Research Center (2010 to 2013)

This study of alternative nitrogen for subsequent southern switchgrass production using cool-season legumes was conducted at the henry H. Leveck Animal Research Center (South Farm) (33° 26’ 20.91" N, 88° 47’ 49.35" W) on an established stand of Alamo switchgrass (8 years old). The soil makeup at this location is a Marietta fine sandy loam soil (Fine-loamy, siliceous, active, thermic Flucaquentic Eutrudepts), moderately well drained and permeable with a pH of 6.7. The mean average annual rainfall for Starkville, MS is 140.8 cm and the average temperature is 16.5°C. A randomized complete block design with four replications was used for this experiment. Each plot consisted of four rows of switchgrass on 50.8 cm centers. The plot size was 2.1 by 6.1 meters with 0.19 m alleyways. Legume treatments were: ‘Yuchi’ arrowleaf clover (*T. vesiculosum* Savi; ALC), ‘Dixie’ crimson clover (*T. incarnatum* L.; CC), ‘Patriot’ white clover (*T. repens* L.; WC), ball clover (*T. nigrescens;* BC), ‘Jester’ barrel medic (*M. truncatula*; BM), hairy vetch (*V. villosa* Roth.; HV), and common vetch (*V. sativa* L.; CV). Hairy vetch, CV, and BM were purchased from Hearne Seed Co. (King City, CA). The ALC, CC, WC, and BC were provided by Joel Douglas of the NRCS (Fort Worth, TX). Pure live seeding rates were as follows: ALC; 8.4 kg ha^{-1} (8.2 g/plot), CC; 28 kg ha^{-1} (27.3 g/plot), WC; 5 kg ha^{-1} (4.9 g/plot), BC; 2.8 kg ha^{-1} (2.7 g/plot), BM; 14 kg ha^{-1} (13.7 g/plot), HV; 25.2 kg ha^{-1} (24.6 g/plot), and CV; 33.6 kg ha^{-1} (32.8 g/plot). All legumes were inoculated with the appropriate strain of rhizobia before planting: HV and CV- Nitragin C (*Rhizobium leguminosarum* bv *viceae*), WC and BC- Nitragin AB *(R. meliloti* and *leguminosarum* bv *trifolii*), CC and ALC- Nitragin R/WR/O (R. *leguminosarum* and *R.* bv *trifolii*), and BM was pre-inoculated by the L.A. Hearne Seed Company. All inoculates, except BM, were purchased locally at the Oktibbeha County Co-op in Starkville, MS. Inoculates were stored under refrigeration (3°C) to ensure viability. Seed inoculation was performed by coating the seed with a carbonated soft drink, (Coca-Cola®), to insure inoculates adhesiveness. Then, inoculate powders were sprinkled over the top of the seed until the seed is covered and then dried. Seed was planted within 24 hours of inoculation.

Legumes were planted into the existing swtichgrass stand on 50.8 cm row spacings, between existing harvested switchgrass rows with a four row ALMACO cone planter. The center three rows of the four row plots were planted, on October 7, 2010. Shallow planting was used, less than a 0.635 cm, for a good legume stand. Ammonium nitrate (NH_4NO_3) was preweighed and hand-broadcasted on the fertilizer plots on April 14, 2011, again on the center of three rows at rates of 0, 28, 56, 84, and 112 kg ha^{-1}. Hand hoeing was used previously to keep legumes and weeds out of all plots and alleys before planting.

Stem and leaf nitrogen content from the switchgrass was taken every two months after switchgrass emergence, from March 15, 2011 until harvest. Random samples of 10 switchgrass tillers (stem and leaves), cut at 10.2 centimeters above the crown, were harvested from the center two rows of each plot. Each sample was weighed, dried and weighed again, for dry matter determination. Samples were dried at 60°C for 48 hours. All samples were evaluated by the Mississippi State Forage Testing Unit for Kjeldahl nitrogen analysis to determine the nitrogen percentage uptake of the switchgrass. The total nitrogen analysis data was calculated to determined the nitrogen use efficiency (NUE) percentage of the switchgrass by using the N difference procedure of Rahman et al. (2009). The weight of the samples that were harvested were be added back to the total biomass yield. Switchgrass plots were harvested after senescence, on October 25, 2011. Dry matter yield of each plot was determined by harvesting each of the four rows. A grab sample was taken from each plot to determine dry weight and final N amount in the switchgrass straw.

During the winter and early spring of 2012 (January to April), plots of the original test were scouted to determine a legumes volunteer frequency and percentage cover from the first year of planting. This was accomplished by using a quarter meter quadrat with 25 squares. The quarter meter quadrat was randomly placed three times in each plot and every time a square was occupied by a legume, the square was counted. The total number of squares occupied for each placement (three per plot) was accessed separately when statistical data was ran for a total of 84 observations.

A second year of data was collected for the South Farm. The same legume and fertilizer treatments were used, as previously mentioned. The legumes were not planted into the switchgrass. The hard seed from each species in the spring volunteered in the fall to supply nitrogen to the switchgrass for biomass yields. Ammonium nitrate

(NH_4NO_3) was preweighed and hand-broadcasted May 11, 2012 on the center of three rows of the four plots at 0, 28, 56, 84, and 112 kg ha^{-1}. Switchgrass tillers (stem and leaves) were harvested, 10.2 centimeters above crown, every two months from switchgrass emergence until harvest on the center two rows of the four row plots to determine the nitrogen percentage in the switchgrass. The total nitrogen analysis data was calculated to determine the nitrogen use efficiency percentage (NUE) of the switchgrass by using the N difference procedure of Rahman et al. (2009). Switchgrass was harvested on October 30, 2012 and the dry matter yield of each plot was determined by harvesting each row of the four row plots. A grab sample was taken from each plot to determine the dry weight and fixed N content of the switchgrass. During the winter and early spring of 2013 (January through April), plots of the original test were scouted to determine a legumes volunteer frequency and percentage cover from the first year of planting in 2011.

Joe Bearden Research Center (2011 to 2013)

The second year study was conducted at Joe Bearden Dairy Research Center (Dairy Farm) near Sessums, MS (33° 24’ 42.84" N, 88° 44’ 32.17" W). The soil at this location is a Freestone fine sandy loam (Fine-loamy, siliceous, semiactive, thermic Glossaquic) Paleudalfs, with 2 to 5 percent slopes. This soil is moderately well drained and permeable at a pH of 5.4. Mean annual precipitation of 134.6 to 157.5 cm per year and a mean air temperature of 16.1 to 18.9°C.

This study was initiated on established stand of Alamo switchgrass (6 years old). Recent studies on this plot were for forage and biomass yields. No legumes were present in the plot upon this study, due to prior herbicide application. The switchgrass plots were cut from a larger 2.57 ha production field to match the dimensions of the 2011 study at the South Farm. Each plot consisted of 4 rows on 50.8 cm centers of switchgrass and plot size was 2.1 by 6.1 meters with 0.19 m alleyways. The same legume species, seeding rates and inoculants were applied as previously mentioned (2010 to 2011). On October 26, 2011, the switchgrass was harvested and then legumes were planted on November 2, 2011 into the switchgrass on 50.8 cm row spacings with a cone planter in the center three rows of the 4 row plots. Pelletized lime was added on January 24, 2012 at a rate of 560 kg ha^{-1} to increase soil pH from 5.4 to 5.6. Ammonium nitrate (NH_4NO_3) was preweighed and hand-broadcasted May 11, 2012 on the center of three rows of the four plots at 0, 28, 56, 84, and 112 kg ha^{-1}. Nitrogen fertilizer plots were kept free of legumes by hoeing.

During the growing season switchgrass tillers (stem and leaves) were harvested every two months from switchgrass emergence until harvest on the center two rows of the four row plots to determine the switchgrass nitrogen percentage. The total nitrogen analysis data was calculated to determine the nitrogen use efficiency (NUE) percentage of the switchgrass by using the N difference procedure of Rahman et al. (2009). Plots were harvested on October 30, 2012. Dry matter yield of each plot was determined by harvesting each row of the four row plots. A grab sample was taken from each plot to determine the dry weight and final N content of the switchgrass. Each plot was scouted after harvest, January, 2013, until the end of April, 2013 to check for volunteer frequency and percentage cover of the legumes.

The experiment was a completely randomized block design. All data were analyzed by using SAS 9.2 as an analysis of variance (ANOVA). Tukey's LSD were determined by PROC GLM at the 5% level ($p \leq 0.05$) of significance. This LSD was used to separate mean treatment effects.

CHAPTER IV

RESULTS

Nitrogen Percentage

Switchgrass tiller (stem and leaves) nitrogen percentage analysis was conducted for two years of the South Farm plot. There was an interaction for nitrogen percentage data by harvest date for the South Farm plots in 2011 and 2012. In each plot, 10 tillers were collected from the center two rows at a cutting height of 10.2 centimeters. Tiller nitrogen percentage was determined for each entry using Kjeldahl nitrogen analysis (AOAC, 1990). The 2011 data indicated a significant difference in nitrogen in the tillers of the first harvest (May 16), the 84 kg ha^{-1} (0.89% N) and 112 kg ha^{-1} N (0.93% N) treatments were greater than control (0.72% N) (Table 2). Tillers from all other treatments sampled at the first harvest date were not significantly different than these from the control plot. For the second harvest (July 26), tillers from plots that were over seeded with barrel medic (0.48% N), crimson clover (0.53% N), hairy vetch (0.57% N), and white clover (0.49% N) were significantly greater in nitrogen than the control (0.37% N). No other treatments were significantly different than the control. The third harvest (September 23), tillers from the crimson clover (0.42% N) and hairy vetch (0.44% N) plots were significantly greater than the control (0.31% N). Tillers from other treatments were not significantly different from the control plot. The last harvest of 2011 (October

27) was taken as a grab sample during switchgrass harvest. At this harvest, the 112 kg ha^{-1} N (0.36% N), arrowleaf clover (0.41% N), common vetch (0.37% N) and crimson clover (0.36% N) plots had nitrogen percentages significantly worse than the control (0.46 % N). All other treatments were not significantly different from the control plot.

Table 2 Nitrogen percentage in the switchgrass tillers (stem and leaves) at the South Farm (first year) where ammonium nitrate was preweighed and hand-broadcasted on the fertilizer plots (April 14, 2011).

	May 16	Jul 26	Sep 23	Oct 27
Treatment	% Nitrogen			
0 kg ha^{-1} N	0.72 c†	0.37 de	0.31 c	0.46 ab
28 kg ha^{-1} N	0.75 bc	0.44 bcde	0.37 abc	0.42 bc
56 kg ha^{-1} N	0.83 abc	0.42 cde	0.38 abc	0.41 bc
84 kg ha^{-1} N	0.89 ab	0.44 bcde	0.32 c	0.41 bc
112 kg ha^{-1} N	0.93 a	0.34 e	0.34 bc	0.36 c
Arrowleaf clover	0.75 bc	0.47 abcd	0.37 abc	0.41 c
Ball clover	0.73 c	0.41 cde	0.33 c	0.42 bc
Barrel medic	0.72 c	0.48 abc	0.38 abc	0.40 bc
Common vetch	0.71 c	0.43 bcde	0.35 bc	0.37 c
Crimson clover	0.69 c	0.53 ab	0.42 ab	0.36 c
Hairy vetch	0.82 abc	0.57 a	0.44 a	0.54 a
White clover	0.69 c	0.49 abc	0.38 abc	0.40 bc
LSD	0.15	0.11	0.09	0.08

† Letter following mean denotes significant differences within column. Means separated by Tukey's LSD at $p < 0.05$.

Assessing switchgrass tiller (stem and leaves) nitrogen in 2012 (Table 3) at the South Farm (second year), the trends in the data were similar to the 2011 results. During the first harvest (June 11), only tillers from the 112 kg ha^{-1} N treatment were significantly greater than the control (1.02%N, 0.71% N), respectively. On this harvest date no other treatments were significantly different in percent N than control. For the second harvest (August 7), plots that were originally over seeded with hairy vetch (0.68% N) were the only treatment significantly greater than the control (0.64% N). No other treatments at this harvest date were significantly different than the control. Data from the third harvest (September 21), indicated no differences among any treatment compared to the control. On the last date (October 30) a grab sample was taken at switchgrass harvest. At this harvest the data indicated that tillers from plots over seeded with hairy vetch were significantly greater in N percentage (0.55% N) than the control (0.47% N). Also, plots seeded with white clover (0.40% N) were significantly lower in nitrogen than the control (0.47% N). All other treatments were not significantly different in nitrogen percentage over the control.

Table 3	Nitrogen percentage in the switchgrass tillers (stem and leaves) at the South Farm (second year) where ammonium nitrate was preweighed and hand-broadcasted on the fertilizer plots (May 11, 2012).

	Jun 11	Aug 7	Sep 21	Oct 30
Treatment		% Nitrogen		
0 kg ha^{-1} N	0.71 bc†	0.47 bcd	0.49 abc	0.47 b
28 kg ha^{-1} N	0.80 bc	0.46 cd	0.40 c	0.47 b
56 kg ha^{-1} N	0.81 b	0.47 bcd	0.50 abc	0.43 bc
84 kg ha^{-1} N	0.79 bc	0.42 cd	0.44 bc	0.43 bc
112 kg ha^{-1} N	1.02 a	0.38 d	0.42 bc	0.43 bc
Arrowleaf clover	0.70 bc	0.38 d	0.48 abc	0.44 bc
Ball clover	0.69 bc	0.51 bcd	0.50 abc	0.44 bc
Barrel medic	0.65 bc	0.61 ab	0.43 bc	0.45 bc
Common vetch	0.75 bc	0.54 bc	0.47 abc	0.42 bc
Crimson clover	0.72 bc	0.47 bcd	0.51 ab	0.47 b
Hairy vetch	0.78 bc	0.68 a	0.57 a	0.55 a
White clover	0.70 bc	0.53 bc	0.41 bc	0.40 c
LSD	0.16	0.15	0.11	0.07

† Letter following mean denotes significant differences within column.
Means separated by Tukey's LSD at $p \leq 0.05$.

As a follow up, switchgrass tiller (stem and leaves) nitrogen percentage analysis was conducted at a second location in 2012, at the Dairy Farm, as a second establishment year test. There was an interaction for tiller nitrogen percentage with the pooled data for the two locations, so as mentioned prior to the South Farm results, Dairy Farm data was evaluated separately (Table 4). The first harvest (June 11), tillers from the 56 kg ha^{-1} N and 112 kg N ha^{-1} treatment plots (0.82% N, 1.07% N), respectively, were significantly greater than the control (0.72% N). All other treatments at this harvest date were not significantly different than the control. For the second harvest (August 7), there was no significant differences observed between percentage nitrogen in the tillers from any treatment and the control (0.56% N). The third harvest (September 21), plots over seeded with hairy vetch were significantly greater in N percentage (0.52% N) than the control (0.37% N). No significant differences were observed for any other treatments at this date compared to the control. The last harvest date (October 30) was a grab sample taken at the end of season switchgrass harvest. At this harvest the data indicated that crimson clover was significantly lower in N percentage (0.40% N) than the control (0.48% N). All other treatments were not significantly different than the control.

Table 4 Nitrogen percentage in the switchgrass tillers (stem and leaves) at the Dairy Farm where ammonium nitrate was preweighed and hand-broadcasted on the fertilizer plots (May 11, 2012).

	Jun 11	Aug 7	Sep 21	Oct 30
Treatment		% Nitrogen		
0 kg N ha^{-1}	0.72 c†	0.56 a	0.37 b	0.48 a
28 kg N ha^{-1}	0.80 bc	0.55 a	0.43 b	0.45 ab
56 kg N ha^{-1}	0.92 ab	0.57 a	0.46 ab	0.41 ab
84 kg N ha^{-1}	0.81 bc	0.57 a	0.43 b	0.48 a
112 kg N ha^{-1}	1.07 a	0.62 a	0.42 b	0.43 ab
Arrowleaf clover	0.82 bc	0.53 a	0.44 b	0.43 ab
Ball clover	0.72 c	0.59 a	0.43 b	0.45 ab
Barrel medic	0.78 bc	0.61 a	0.44 ab	0.44 ab
Common vetch	0.77 bc	0.56 a	0.41 b	0.44 ab
Crimson clover	0.74 c	0.53 a	0.38 b	0.40 b
Hairy vetch	0.75 c	0.53 a	0.52 a	0.44 ab
White clover	0.78 bc	0.52 a	0.42 b	0.41 ab
LSD	0.16	0.11	0.08	0.08

† Letter following mean denotes significant differences within column. Means separated by Tukey's LSD at $p \leq 0.05$.

Switchgrass Yield

South Farm switchgrass yields were estimated on dry matter (DM) basis. The switchgrass yields were assessed for each entry by manually harvesting all four rows of each plot and correcting for percentage moisture (Table 5). For the 2011 dry matter yields, the data shows that the 56 kg ha^{-1} N (16.10 Mg ha^{-1}) treatment had a significantly greater yield than the control plot (10.92 Mg ha^{-1}). All other legume and fertilizer treatments showed no significant differences compared to the control. For the 2012 dry matter yields at the South Farm, the data indicated that the 56 kg N ha^{-1} (11.21 Mg ha-^{1}) treatment was significantly greater than 28 kg ha^{-1} N (7.92 Mg ha^{-1}) treatment, but no treatments were significantly greater than the control (8.14 Mg ha^{-1}).

Table 5 Switchgrass dry matter yield (Mg ha^{-1}) at the South Farm conducted in 2011 and 2012.

	2011	2012
Treatment	Yield (Mg ha^{-1})	
0 kg N ha^{-1}	10.92 b†	8.14 ab
28 kg N ha^{-1}	10.64 b	7.92 b
56 kg N ha^{-1}	16.10 a	11.21 a
84 kg N ha^{-1}	11.36 b	9.18 ab
112 kg N ha^{-1}	11.41 b	9.02 ab
Arrowleaf clover	13.79 ab	10.32 ab
Ball clover	12.68 ab	9.00 ab
Barrel medic	12.67 ab	8.98 ab
Common vetch	14.85 ab	10.57 ab
Crimson clover	13.63 ab	10.14 ab
Hairy vetch	13.48 ab	8.64 ab
White clover	12.57 ab	9.07 ab
LSD	4.67	3.1

† Letter following mean denotes significant differences within column.
Means separated by Tukey's LSD at $p \leq 0.05$.

Dairy Farm switchgrass yields the second establishment year, were also based on dry matter (DM) tonnage (Table 6). The switchgrass yields were assessed for each entry by manually harvesting all four rows of each plot and correcting for percentage moisture. The 2012 Dairy Farm switchgrass yields indicated that all legume and fertilizer treatments were not significantly different than the control (5.52 Mg ha^{-1}), nor each other.

Table 6 Switchgrass dry matter yield (Mg ha^{-1}) at the Dairy Farm conducted in 2012.

	2012
Treatment	Yield (Mg ha^{-1})
0 kg ha^{-1} N	5.52 a†
28 kg ha^{-1} N	5.96 a
56 kg ha^{-1} N	6.31 a
84 kg ha^{-1} N	6.53 a
112 kg ha^{-1} N	6.49 a
Arrowleaf clover	5.57 a
Ball clover	5.60 a
Barrel medic	5.57 a
Common vetch	5.64 a
Crimson clover	5.40 a
Hairy vetch	5.99 a
White clover	5.52 a
LSD	0.69

† Letter following mean denotes significant differences within column.
Means separated by Tukey's LSD at $p \leq 0.05$.

Legume Volunteer Frequency as Measured by Percentage Cover Over Time

Legumes have the ability to volunteer the following year eliminating the need for reseeding and reducing the need for chemical fertilizer application. For the 2012 South Farm (first year) legume volunteer frequency (Table 7, columns) were based on stand counts taken the first four months of the calendar year (January to April), after switchgrass harvest. A 0.25 m quadrat was used to obtain three counts of the volunteering legumes per treatment per replication for each month of testing. At the first count of 2012 (January 18), plots originally seeded to hairy vetch had the greatest initial volunteer frequency (94.67%), followed by barrel medic (49.00%) and crimson clover (43.67%). Common vetch (21.67%) and white clover (17.67 %) plots were not significantly different from arrowleaf clover plots (25.33%), having a significantly lower volunteer frequency than all other treatments except ball clover (34.00%). For the second assessment (February 23), plots originally hairy vetch had a significantly greater volunteer frequency (94.67%), than all other treatments; followed by crimson clover (41.33%), barrel medic (37.67%) and arrowleaf clover (35.33%). Common vetch (19.00%) and white clover (14.00%) plots were not significantly different from ball clover plots (25.00%), but they had a significantly lower volunteer frequency than all other treatments. The third (March 21) count showed that, plots originally seeded with hairy vetch had a significantly greater volunteer frequency (94.67%), than all other treatments; followed by arrowleaf clover (45.33%). Ball clover (10.33%) and white clover (12.67%) plots were not significantly different from common vetch (25.33%) plots, but they had a significantly lower volunteer frequency than all other treatments. For the final count (April 24), the same trend was observed, where plots originally seeded with hairy vetch (99.67%), were significantly greater than all other treatments; followed by arrowleaf clover (42.67 %) and crimson clover (31.33%). Common vetch (7.33%) plots were not significantly different from barrel medic (25.33%) plots, but common vetch plots had a significantly lower volunteer frequency than all other treatments. It should be noted that only hairy vetch reseeded at the rates greater than the test mean, and this occurred at every date.

The percentage cover overtime relative to the amount of ground cover within each individual species in each treatment per month were also compared (Table 7, rows). Plots seeded with arrowleaf clover increased coverage significantly from the first count (January 18) to the third count (March 21) 25.33, 35.33, 45.33%, respectively. Plots seeded with ball clover indicated no significant increase in coverage from the first count (January 18), second count (February 23) and the final count (April 24) 34.00, 25.00, 31.33%, respectively, but data indicated the third count (March 21) was significantly less (10.33%) than the other counting dates. Plots seeded with barrel medic showed a significant decrease in coverage from the first count (January 18) to the final count (April 24) 49.00, 37.67, 25.33, 17.33%, respectively. Plots seeded with common vetch showed no change in cover percentage from the first count (January 18) to the final count (April 24) (21.67, 19.00, 23.33, 7.33%), respectively. Crimson clover seeded plots also showed no significant change in cover percentage from the first count (January 18) to the final count (April 24) (43.67, 41.33, 31.33, 31.33%), respectively. Plots seeded with hairy vetch indicated a significant increase in coverage from the first count (January 18) to the final count (April 24) (94.67, 95.00, 97.67, 99.67%), respectively. Plots seeded with white clover indicated the final count (April 24) (23.67%) had a significantly greater cover percentage than the second (14.00%) and third count (12.67%), but was not significantly greater than the first count (January 18) (17.67%).

Table 7 Mean legume volunteer frequency as measured by percentage cover over time at the South Farm (first year) conducted in 2012.

	Jan 18	Feb 23	Mar 21	Apr 24	LSD
Treatment	Volunteer Frequency and % Coverage				
Arrowleaf clover	25.33 de† B£	35.33 bc AB	45.33 b A	42.67 b A	11.29
Ball clover	34.00 cd A	25.00 cd A	10.33 e B	31.33 cd A	11.29
Barrel medic	49.00 b A	37.67 bc AB	25.33 cd BC	17.33 de C	15.93
Common vetch	21.67 e A	19.00 d A	23.33 cde A	7.33 e A	18.11
Crimson clover	43.67 bc A	41.33 b A	31.33 c A	31.33 bc A	19.22
Hairy vetch	94.67 a B	95.00 a B	97.67 a AB	99.67 a A	3.87
White clover	17.67 e AB	14.00 d B	12.67 e B	23.67 cd A	9.26
Test Mean	40.86	38.19	35.14	36.19	
LSD	12.16	15.60	13.93	11.99	

† Lowercase letter following mean denotes differences in legume volunteer frequency within the column.
£ Uppercase letter following mean denotes differences in percent coverage within a species within row.
Means separated by Tukey's LSD at $p \leq 0.05$.

Planting at the Dairy Farm 2013 research is a repeat of the first year study at the South Farm (2012), so statistics were pooled to check for an interaction between years. An interaction occurred necessitating the evaluation of years (locations) separately. The 2013 Dairy Farm legume volunteering frequency (Table 8, columns) was based on stand counts after switchgrass harvest. As within the South Farm trial a 0.25 m quadrat was also used to determine the coverage of legumes that volunteered in the treatments. At the first count (January 7, 2013), plots seeded with crimson clover had a significantly greater volunteering frequency (5.33%) than ball clover, barrel medic, and white clover (0.33%, 0.33%, 0.00%), respectively. All other treatments (common vetch, hairy vetch, arrowleaf clover) were not significantly different from crimson clover. For the second count (February 5, 2013), plots seeded with hairy vetch had a significantly greater volunteering frequency (4.33%) than barrel medic and white clover (0.67%, 0.00%), respectively. All other treatments were not significantly different from hairy vetch nor barrel medic and white clover. At the third count (March 6, 2013), again plots seeded with hairy vetch had a significantly greater volunteering frequency (7.00%) than arrowleaf clover, barrel medic, common vetch, and white clover (0.33%, 0.33%, 0.00%, 0.00%), respectively. All other treatments (crimson and ball clover) were not significantly different from hairy vetch. For the fourth and final count (April 9, 2013) coverage assessments of the plots originally seeded with ball clover were significantly greater (9.33%) than barrel medic, common vetch, crimson clover and white clover (0.00%, 1.00%. 3.00%, 0.67%), respectively. All other treatments (hairy vetch and arrowleaf clover) were not significantly different from ball clover.

The relative amount of coverage between the South Farm and Dairy Farm showed a profound difference. There were fewer legumes (less coverage) at the Dairy Farm attributed to a decreased soil pH and a deficiency of nutrients impacting nitrogen fixation and legume establishment. The 2013 Dairy Farm percentage cover acts as a repeat of the first year at the South Farm were based on stand counts taken the first four months of the year, using the 0.25 m quadrat, after switchgrass harvest. The percentage cover (Table 8, rows) is relative to the amount of ground cover each individual species accumulates in each treatment per month. The 2013 Dairy Farm plots seeded with arrowleaf clover indicated no significant increase in coverage from the first count (January 7) to the final count (April 9) (1.67, 2.33, 0.33, 4.33%, respectively). Plots seeded with ball clover indicated a significant increase in coverage from the first count (January 7) to the final count (April 9) (0.33, 1.00, 5.00, 9.33%, respectively). Plots seeded with barrel medic were poor to establish, declined, and disappeared from the first count (January 7) to the final count (April 9) (0.33, 0.67, 0.33, 0.00%, respectively). Plots seeded with common vetch indicated no significant increase in coverage from the first count (January 7) to the final count (April 9) (2.67, 1.33, 0.00, 1.00%, respectively). Plots seeded with crimson clover indicated no significant increase in coverage from the first count (January 7) to the final count (April 9) (5.33, 1.67, 2.00, 3.00%, respectively). Plots seeded with hairy vetch indicated no significant increase in coverage from the first count (January7) to the final count (April 9) (2.33, 4.33, 7.00, 8.00%, respectively). Plots seeded with white clover indicated no significant increase in coverage from the first count (January 7) to the final count (April 9) (0.00, 0.00, 0.00, 0.67%, respectively).

Table 8 Mean legume volunteer frequency as measured by percentage cover over time at the Dairy Farm (first year) conducted in 2013.

	Jan 7	Feb 5	Mar 6	Apr 9	LSD
Treatment	Volunteer Frequency and % Coverage				
Arrowleaf clover	1.67 ab† A£	2.33 ab A	0.33 b A	4.33 abc A	4.2
Ball clover	0.33 b B	1.00 ab B	5.00 ab AB	9.33 a A	7.49
Barrel medic	0.33 b A	0.67 b A	0.33 b A	0.00 c A	0.93
Common vetch	2.67 ab A	1.33 ab A	0.00 b A	1.00 c A	4.2
Crimson clover	5.33 a A	1.67 ab A	2.00 ab A	3.00 bc A	4.73
Hairy vetch	2.33 ab A	4.33 a A	7.00 a A	8.00 ab A	6.36
White clover	0.00 b A	0.00 b A	0.00 b A	0.67 c A	0.95
Test Mean	1.81	1.62	2.09	3.76	
LSD	4.18	3.42	5.10	5.64	

† Lowercase letter following mean denotes differences in legume volunteer frequency within the column.
£ Uppercase letter following mean denotes differences in percent coverage within a species within row .
Means separated by Tukey's LSD at $p \leq 0.05$.

The 2013 South Farm (second year) volunteer frequency (Table 9, columns) were based on stand counts (January to April), three months after switchgrass harvest and during spring regrowth. The 0.25 m quadrat was used to determine the legume volunteering frequency within the treatments. The first count of 2013 (January 7), plots seeded with hairy vetch had a significantly greater volunteer frequency (20.33%) than all other plots, except white clover (11.67%). For the second count (February 5), plots seeded with hairy vetch had a significantly greater volunteering frequency (18.67%) than ball clover and common vetch (6.67%, 5.00%), respectively. All other treatments were not significantly different from hairy vetch. On the third count (March 6), plots seeded with hairy vetch had a significantly greater volunteering frequency (19.00%) than all other treatments. All other treatments showed no significant differences. For the fourth and final volunteer count (April 9), plots originally seeded to hairy vetch had a significantly greater volunteering frequency (36.33%) than all other treatments. Ball clover had a significantly worse volunteering frequency (4.00%) than arrowleaf clover and hairy vetch (17.00%, 36.33%), respectively, and ball clover was not significantly different than any other treatment. When looking at test mean for volunteer frequency, only hairy vetch significantly exceeded the test mean throughout all counting dates compared to all other treatments.

The 2013 South Farm cover within species were based on stand counts taken the first four months of the year, using the 0.25 m quadrat, after switchgrass harvest. The cover (Table 9 rows) for each individual species per treatment per month. Plots seeded with arrowleaf clover indicated no significant increase in coverage from the first count (January 7) to the second count (April 9) (10.00%, 11.33%, 10.33%, 17.00%), respectively. Plots seeded with ball clover indicated no significant increase in coverage from the first count (January 7) to the final count (April 9) (8.67%, 6.67%, 3.00%, 4.00%), respectively. Plots seeded with barrel medic indicated no significant increase in coverage from the first count (January 7) to the final count (April 9) (4.67%, 9.33%, 8.00%, 6.67%), respectively. Plots seeded with common vetch indicated no significant increase in coverage from the first count (January 7) to the final count (April 9) (9.67%, 5.00%, 4.33%, 6.67%), respectively. Plots seeded with crimson clover indicated no significant increase in coverage from the first count (January 19) to the final count (April 9) (8.33%, 12.67%, 4.33%, 9.67%), respectively. Plots seeded with hairy vetch were the only plots that showed a significant increase in coverage from the first count (January7) to the final count (April 24) (20.33%, 18.67%, 19.00%, 36.33%), respectively. Plots seeded with white clover indicated no significant increase in coverage from the first count (January 19) to the final count (April 24) (11.67%, 13.33%, 4.33%, 8.33%), respectively.

Table 9	Mean legume volunteer frequency as measured by percentage cover over time at the South Farm (second year) conducted in 2013.

	Jan 7	Feb 5	Mar 6	Apr 9	LSD
Treatment	Volunteer Frequency and % Coverage				
Arrowleaf clover	10.00 b† A£	11.33 ab A	10.33 b A	17.00 b A	10.38
Ball clover	8.67 b A	6.67 b A	3.00 b A	4.00 c A	6.89
Barrel medic	4.67 b A	9.33 ab A	8.00 b A	6.67 bc A	8.25
Common vetch	9.67 b A	5.00 b A	4.33 b A	6.67 bc A	8.55
Crimson clover	8.33 b A	12.67 ab A	4.33 b A	9.67 bc A	9.01
Hairy vetch	20.33 a B	18.67 a B	19.00 a B	36.33 a A	13.69
White clover	11.67 ab A	13.33 ab A	4.33 b A	8.33 bc A	12.17
Test Mean	10.48	11.00	7.62	12.67	
LSD	10.23	9.89	7.92	11.60	

† Lowercase letter following mean denotes differences in legume volunteer frequency within the column.
£ Uppercase letter following mean denotes differences in percent coverage within a species within row.
Means separated by Tukey's LSD at $p \leq 0.05$.

Switchgrass Nitrogen Use Efficiency

The nitrogen use efficiency (NUE) data was calculated as a percentage to determine the amount of nitrogen the switchgrass accumulated during the growing season by the legume or fertilizer treatments (Table 10). In 2011, at the South Farm, data shows that plots seeded with hairy vetch (22.59%) were significantly greater than the control (0.00%). All of the other treatments were not significantly different than the control. At the Dairy Farm in 2012, the data for NUE showed none of the treatments were significantly different than the control. The only significant difference the data showed was 112 kg ha^{-1} N was greater (9.15%) than crimson clover and white clover (-6.65%, -2.50%), respectively. The second year at the South Farm (2012) data showed that plots seeded with hairy vetch were significantly greater (17.52%) than the control (0.00%) with respect to NUE.

Table 10 Switchgrass nitrogen use efficiency percentage as calculated by the nitrogen difference procedure.

	2011 South Farm	2012 Dairy Farm	2012 South Farm
Treatment		% NUE	
0 kg ha^{-1} N	0.00 b†	0.00 ab	0.00 b
28 kg ha^{-1} N	5.02 b	2.49 ab	-5.96 b
56 kg ha^{-1} N	6.64 ab	4.93 ab	0.49 b
84 kg ha^{-1} N	3.66 b	5.55 ab	-4.45 b
112 kg ha^{-1} N	-4.63 b	9.15 a	-5.21 b
Arrowleaf clover	5.17 b	1.46 ab	-7.49 b
Ball clover	-3.90 b	1.24 ab	-0.11 b
Barrel medic	4.94 b	4.67 ab	-2.63 b
Common vetch	-2.93 b	1.87 ab	0.37 b
Crimson clover	5.76 ab	-6.65 b	1.81 b
Hairy vetch	22.59 a	3.17 ab	17.52 a
White clover	5.56 ab	-2.50 b	-7.74 b
LSD	17.32	14.24	12.44

† Lowercase letter following mean denotes differences within the column. Means separated by Tukey's LSD at $p \leq 0.05$.

CHAPTER V

DISCUSSION

Nitrogen Percentage

Kjeldahl N measures of percentage tiller (stem and leaves) nitrogen show percentage N to be the greatest at the first harvest and declined over the coarse of the growing season (Tables 2, 3, 4) in the nitrogen fertilizer treatments, which is expected. Lemus et al. (2008) has demonstrated that this is due to the retranslocation of N from the crown. Nitrogen fixation from the legumes tended to be reduced as the legume reaches full maturity. Reynolds et al. (2000) stated that nitrogen in the switchgrass tillers will decrease with the onset of senescence. The nitrogen will translocate to the crown of the plant where it will be stored for next year's growth. McKendrick et al. (1975) also found a decrease in tiller nitrogen with significant increases in rhizome nitrogen during the mid summer (July and August) in big bluestem and indiangrass, two native warm season grasses that are physiologically similar to switchgrass. In this study, nitrogen percentage of the switchgrass tillers, 112 kg ha^{-1} N treatment was greater than the control in all of the first cuttings (Tables 2, 3, 4). Also, 84 kg ha^{-1} N (Table 2) and 56 kg ha^{-1} N (Table 4) showed a greater nitrogen percentage in the switchgrass tillers than the control in the first cuttings. This is due to the fact that once the ammonium nitrate was applied, the ammonium and nitrate are forms of nitrogen the plant can take up and use immediately by switchgrass' extensive root system. During active growth, nitrogen is translocated to the upper parts of the plant. As the plant reaches full maturity (August), the nitrogen starts translocating the nutrient down to the crown for storage and next years growth.

Nitrogen derived from nitrogen fixation in the legume plots is not available until later in the season or the next growing season. USDA stated, about two-thirds of the N fixed by legumes becomes available the next growing season after a legume is in rotation. Legumes can supply approximately 36% of the N needs of the grass plants adjacent to them (USDA, 1998). As a legume decays, they slowly release nitrogen to the environment and therefore it is taken up by the switchgrass plant. This allows the switchgrass plant to translocate the nitrogen to the plant over a longer period than the chemical fertilizer. For the first year at the South Farm, data showed that crimson clover and hairy vetch (Table 2) were greater than the control for nitrogen percentage in the switchgrass tillers on the second and third harvest dates. Also, barrel medic and white clover were greater than the control for the second harvest. The second year at the South Farm showed that switchgrass tillers growing from plots seeded with hairy vetch had a greater nitrogen percentage than the control for the second and fourth harvest dates (Table 3), while all other legume treatments were not different from the control. This is in agreement with Hargrove (1986) where hairy vetch maintained a greater tissue N concentration than all other legumes in his study. The carbon to nitrogen ratio in the plant tissue is usually narrower for hairy vetch than crimson clover, which can result in a greater release of nitrogen during decomposition (Varco et al., 1991). This release of nitrogen is microbial mediated. The higher the C:N is, the harder it is for the microbes to digest, which can causes a decrease in the amount of nitrogen released (Havlin et al., 2005).

When data from both years at the South Farm (Tables 2 and 3) are evaluated, there was an increased amount of N in the switchgrass tillers (stems and leaves) from the first year, to the second year. The data shows an overall increase in the test means by harvest date; first year (0.77, 0.45, 0.36, 0.41), respectively, and the second year (0.76, 0.49, 0.47, 0.45), respectively. This could have been an environmental effect, but research has shown that nitrogen fixation of legumes usually manifested during the second year. As stated previously by USDA (1998), two-thirds of the N fixed will become available the second year after the legume is established.

The Dairy Farm nitrogen percentage in the switchgrass tillers data showed very little differences (Table 4). Lemus et al. (2008) stated, legumes must have a 30% to 40% stand for the legumes to benefit in nitrogen fixation. There was a dramatic decrease in legume stands for the Dairy Farm in 2013 (Table 8), which would be expected to lead to a decrease in nitrogen fixation. This is attributed to poor prior field management. The soil pH was considered low especially for legumes, at 5.6. On the first harvest date, 56 kg ha^{-1} N and 112 kg ha^{-1} N were the only treatments greater than the control. The only other treatment that was greater than the control for N percentage at the Dairy Farm, was hairy vetch on the third harvest date. Inconsistent results from the legume seeded plots are likely due to low pH. Ball et al. (1991) stated a soil with a low pH (less than 5.8) can quickly reduce the amount of *Rhizobium* bacteria present on the legume roots that is needed for nitrogen fixation. While all seed was inoculated, persistence of the microbe was likely limited. Hairy vetch is more drought tolerant than other vetch species and it can grow in acid soils where other clover species fail to establish (Hargrove, 1986).

Switchgrass Yield

Switchgrass yields were generally greater in 2011 at the South Farm than either South Farm or Dairy Farm in 2012. The yields at the Dairy Farm were roughly half those of the South Farm (Tables 5 and 6). Switchgrass biomass yields fluctuate from year to year depending on environmental conditions and management practices. A study by McLaughlin and Kszos (2005), stated that switchgrass needed approximately 50% less nitrogen to maintain yields than they originally thought. Field trials were conducted where nitrogen amounts ranged from 100 to 300 kg ha^{-1}. They found that 50 kg ha^{-1} N was adequate to maintain switchgrass yields. Bransby et al. (2002) found the same results. His experiment was conducted for three years in Alabama with an application of nitrogen at 122 kg ha^{-1}. Over the three years switchgrass yields averaged 15 Mg ha^{-1} yr^{-1}. The nitrogen applied increased the biomass yield/N of more than 150%, so the effective nitrogen application rate was estimated at 41 kg ha^{-1} yr^{-1}. These studies are commensurate with the results from the South Farm in 2011, switchgrass fertilized with 56 kg ha^{-1} N (16.10 Mg ha^{-1}) had a significantly greater biomass yield than the control plot and all other fertilizer treatments, but not significantly better than the legumes treatments. Interestingly greater rates of nitrogen did not increase switchgrass yields. In 2012 switchgrass fertilized with 56 kg ha^{-1} N (11.21 Mg ha^{-1}) had a significantly greater biomass yield than switchgrass fertilized with 28 kg ha^{-1} N , but not different than any other treatment including the control. Field variability would normally be suspected but, the 2012 yields at the Dairy Farm showed that no treatment was significantly different from the control. The switchgrass yields at the Dairy Farm were about half those at the South Farm. Lack of difference could be due to the environment (site location), land management, soil type and especially soil pH.

Legume Volunteer Frequency as Measured by Percentage Cover over Time

Differences in growth habit for each legume species which can lead to incompatibilities between the switchgrass and legumes when grown together. George et al. (1995) stated, because of incompatibilities, persistence of either species usually declines overtime as one component dominates. Some legumes are slower growing or more prostrate and were shaded out by the switchgrass before maturity, which reduced the legume's access to light and other resources (Moore et al., 1991). However, both these studies were conducted in the North where legume and switchgrass growth overlap. In this study, the majority of legume growth occurred in January and February, before switchgrass emergence. Hairy vetch competed well with emerging switchgrass the first year at the South Farm in 2012. It had the greatest volunteer frequency compared to all legume treatments and it significantly increased coverage throughout the year (Table 7). Owsley, (2011) stated hairy vetch is a climbing legume in which the stems can reach two to five feet in length. It is classified as a biennial/annual and can produce a lot of hard seeds that persist in the soil and volunteer. These hard seeds can last in the soil profile for five or more years. He also indicated that hairy vetch can have a negative environmental concern, because it has the ability to spread, climb into the canopy, and maintain a stand after establishment.

The volunteer frequency and percentage cover of the legumes at the Dairy Farm in 2013 (Table 8) showed a dramatic decrease from the initial seeding rates. At this location, the switchgrass stand was six years old and no additional fertilizer was added to the plot after it was established. The initial soil pH for the experimental field was 5.4. Lime was added (4.2 Mg/ha) which brought the final pH to 5.6. All legumes vary with in their sensitivity to soil pH. Caddel et al. (2011) stated that a successful pasture legume production depends on adequate amounts of phosphorus and potassium with a soil pH of at least 6.0. The only legume treatment that increased its coverage throughout the counting dates was ball clover (Table 8). Even though it significantly increased over time, ball clover was significantly lower than crimson clover on the first counting date and significantly better than barrel medic, common vetch and white clover on the final counting date. The second and third counting dates showed that ball clover was never significantly lower or greater than any other treatment. Ball et al. (1991) stated that ball clover grows best on clay and loam soils, and it is tolerant to acidity and poor drainage. When comparing ball clover to arrowleaf clover and hairy vetch, they all handled the acidic soils to a certain extent. When there is adequate nutrients and soil moisture, climbing legumes may shade out the grasses, but if soils have a lesser pH, this will generally favor the warm-season grasses compared to cool-season legumes, because of their deeper root development (Ball et al., 1991).

As stated previously, there is a difference in growth habit for each legume species which can lead to incompatibilities between the switchgrass and legumes when grown together. The South Farm second volunteer year data (Table 9) showed a decrease in legume re-establishment from the first year. The data from year two of the South Farm showed that hairy vetch had the greatest volunteer frequency for the last two counting dates, March 6th and April 9th and percentage cover significantly increased throughout the spring. Blanchet et al. (1995) found in their study that two sweetclovers 'Madrid' yellow-flowered (*Melilotus officinalis* Lam.) and 'Polara' white-flowered (*Meliltous alba* Medik.) and hairy vetch had less stem densities and a lesser percentage of legume coverage, because of the biennial/annual life cycles of the legumes. They also stated that these legumes naturally decline the second year unless natural reseeding occurs.

While all legumes in this study senesced and shed seed, the decline in the amount of legume volunteering was likely due to predation, seed loss due to flash flooding, and physical damage. These legume species that produced seed in April to June had to persist under field conditions until the following winter. Most of the legumes used were annuals that had to handle adverse conditions in the Southern United States with hot temperatures and drought conditions. As stated throughout the research, there are some legumes that can handle adverse conditions, like hairy vetch.

Switchgrass Nitrogen Use Efficiency

One of the greatest inputs for crop production in nitrogen fertilizer. Legumes are commonly used in these cropping systems as a source of nitrogen for subsequent crops. Nitrogen use efficiency is used to determine the amount of nitrogen the crop uses over a growing season. In this study, data shows that hairy vetch provided a significantly greater amount of nitrogen than the control for both years at the South Farm as measured by NUE. Rahman et al. (2009) studied nitrogen use efficiency and recovery from N fertilizer and found that nitrogen derived from broad bean and hairy vetch is readily available and can be used efficiently by a wetland rice crop. Both legumes have the potential to be substituted for chemical nitrogen fertilizer. At the Dairy Farm, no treatment was significantly greater than the control with respect to NUE. This could be attributed to a low soil pH, which can impact nitrogen fixation of the legumes. Also there could have been some associative nitrogen fixation. Rao et al. (1998) stated that associative bacteria can contribute 10 to 80 kg N ha^{-1} depending on the environment, cultural practices and rice variety grown. Another study by Brejda et al. (1994) stated that eastern gamagrass (*Tripsacum dactyloides*) is capable of associative nitrogen fixation and the degree of nitrogenase activity in gamagrass is higher than in switchgrass. Brejda et al. (1994) also stated that low annual inputs by associative nitrogen fixation could have contributed considerable amounts of nitrogen to prairie soils over time. This may be an explanation why some legumes showed a negative NUE percentage for all locations and testing years.

CHAPTER VI

SUMMARY AND CONCLUSIONS

There are no clear conclusions regarding the effect of cool-season legumes on the yield of the subsequent switchgrass. There are many legumes to choose from that have the ability to fix and supply nitrogen to adjacent plants, which may off set the need for inorganic nitrogen application. Some factors need to be considered when choosing the right legume for alternative nitrogen in switchgrass production like nitrogen production potential, winter hardiness, competition, ease of establishment and geography. The legume that supplied the most nitrogen to the switchgrass tillers (stem and leaves) in this study was hairy vetch. Out of twelve tiller harvest dates, hairy vetch was significantly greater than the control five times, with all of them being in the second, third and fourth harvest dates. The only other treatment that came close was 112 kg ha^{-1} N with three harvest dates that were greater than the control. Hairy vetch is a legume that can supply nitrogen to the switchgrass longer, because of its later senescence. Hairy vetch was also greater than the control for nutrient use efficiency percentage for both years at the South Farm, but not the Dairy Farm. Switchgrass yields varied from year to year, because of environmental conditions and former field management practices. The lack of significant differences in yield for most treatments, for both locations, could have indicated by an adequate amount of nitrogen reserves in the switchgrass crown and root system or the roots could have extracted nutrients from a deeper soil profile. Also, the lack of significance in yields could indicate that all legume treatments supplied enough nitrogen to maintain yields, except for crimson clover at the Dairy Farm in 2012. All of the other legume treatments caused switchgrass yields to fall between 0 kg ha^{-1} N and 112 kg ha^{-1} N. Using 0 kg ha^{-1} N would not be recommended, because switchgrass needs nitrogen to maintain greater yields, especially in the early years of production. In this study, it was found that hairy vetch had the greatest volunteer frequency and percentage cover at the South Farm for both years. Out of eight volunteer counts at the South Farm, hairy vetch was significantly greater than all other treatments six times. At the Dairy Farm, no legumes were different when comparing volunteer frequency as measured by coverage. This was due to the fact that the soil for the legumes was not properly managed. Greater quantities of lime were necessary to increase the pH to the appropriate amount, but were impractical given the time frame of this study.

The research and results in this thesis show that the locations, and legume and fertilizer treatments, play a major role in the patterns of switchgrass yields. With that being said, more research is needed to successfully say that inorganic chemical fertilizer can be replaced by the nitrogen fixation of certain legumes to provide sufficient nitrogen to maintain switchgrass biomass yields. Even though hairy vetch, overall, supplied the most nitrogen to switchgrass tillers and had the best percentage cover, there was no trend observed in the switchgrass yields.

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