To the Graduate Council:

I am submitting herewith a thesis written by Kara Lee Warwick entitled “Establishment and persistence of legumes in switchgrass biomass and forage/biomass production systems.” I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

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Establishment and Persistence of Legumes in Switchgrass Biomass and Forage/biomass Production Systems

A Thesis
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Kara Lee Warwick
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Abstract

Switchgrass (*Panicum virgatum*) is being developed as an economically and ecologically sustainable biomass crop. Nitrogen is considered one of the most limiting inputs of switchgrass. Alternatives to synthetic nitrogen fertilization may be nitrogen-fixing legumes interseeded into switchgrass. The objectives of this research were: (1) develop efficient legume management strategies for switchgrass production systems, (2) evaluate and identify cool and warm-season legumes that can be grown compatibly with switchgrass, (3) determine whether switchgrass yields are increased by legume N-fixation, and (4) determine N-fixation of common (*Vicia sativa*) and hairy vetch (*Vicia villosa*).

This study examined the establishment and persistence of ten different legume species in ‘Alamo’, a lowland variety of switchgrass in two switchgrass production systems: a one-cut biomass harvest and a two-cut forage/biomass harvest. Cool-season legumes were alfalfa (*Medicago sativa)*, arrowleaf clover (*Trifolium vesiculosum*), common vetch, crown vetch (*Securigera varia*), red clover (*Trifolium pretense*), hairy vetch, and crimson clover (*Trifolium incarnatum*). Warm-season legumes were Illinois bundle flower (*Desmanthus illinoensis*), trailing wild bean (*Strophostyles helvula*), and partridge pea (*Chamaechrista fasciculata*). Red clover showed the highest plant densities with the potential to increase switchgrass yields when interseeded into existing switchgrass stands in both harvest systems. Crude protein levels were highest in the 135 kg N ha^{-1} treatment in the forage cut of the two-cut harvest system. Arrowleaf clover, crimson clover, and red clover had high stand densities with annual reseeding. A combination of cool-season legumes, crimson clover and common vetch, in combination with warm-season partridge pea, were established in existing switchgrass stands after one year.

Common vetch was evaluated for its nitrogen fixing capacity, seed germination, establishment, and effects on yield of switchgrass. Scarification by sulfuric acid had higher seed germination than other scarification treatments, except 100 grit sandpaper treatment for one minute at 0.7 kg of pressure. Common and hairy vetch nitrogen contributions were 59.3 and 43.3 kg N ha^{-1} respectively at seeding rates of 6.7 kg PLS ha^{-1}. Switchgrass yields might increase with common and hairy vetch seeding rates of 7.6 and 10.4 kg PLS ha^{-1} to achieve 67 kg N ha^{-1}, the recommended rate of N-fertilization for switchgrass stands.

TABLE OF CONTENTS

Chapter **Page**

Part I: Establishment of cool and warm-season legumes into existing stands of switchgrass for biomass and forage/biomass harvests

Chapter **Page**

Part II: Establishment and reseeding of cool and warm-season legumes into existing stands of switchgrass

Chapter **Page**

Part III: Estimation of nitrogen fixation rates of common versus hairy vetch and seeding rates needed for interseeding into established switchgrass stands.

LIST OF TABLES

Table **Page**

Part II: Establishment and reseeding of cool and warm-season legumes into existing stands of switchgrass

Part III: Establishment and nitrogen fixation rates of common versus hairy vetch in established stands of switchgrass

Table **Page**

Appendix D

LIST OF FIGURES

Figure **Page**

Part I: Establishment of cool and warm-season legumes into existing stands of switchgrass for biomass and forage/biomass harvests

Introduction

Switchgrass (*Panicum virgatum*) is currently undergoing intense research for carbon fiber and biofuel development. The Department of Energy began investigating the possibilities of switchgrass and other warm-season grasses for bioenergy after the 1970's oil embargo. Interest in switchgrass research waned when oil prices declined, but due to the Renewable Fuel Standard (RFS) program in 2005 and the State of the Union of Address by President George W. Bush in 2006, switchgrass was again at the forefront of biofuel research. The objectives of this research were (1) develop efficient legume management strategies for switchgrass biomass and forage production systems, (2) evaluate and identify selected cool and warm-season legumes that can be grown compatibly with switchgrass, (3) determine whether switchgrass yields are increased by compatible legume nitrogen fixation, and (4) determine N-fixation of common (*Vicia sativa*) and hairy vetch (*Vicia villosa*).

Switchgrass is a warm-season perennial grass with excellent potential for producing biomass in the warm, dry summer months due to its C4 photosynthetic pathway (Cherney et al. 1991). Modest inputs are required once switchgrass has been established. Nitrogen is one input that has increased the productivity of switchgrass after the first year of establishment. Nitrogen fixing legumes can help fulfill the N requirement for switchgrass production.

For companion species to work together effectively, the two species must be able to grow simultaneously, not be eliminated from the stand, and take advantage of their companion's dormancy or semi-dormancy growth patterns (Cherney et al., 1991). Grass-legume mixtures benefit switchgrass by taking advantage of the different annual growth patterns of the legumes that can improve seasonal distribution of forage. Many studies have been done on legumes interseeded into warm-season grasses to increase forage yields. In this experiment, legume species were chosen for their different growth habits and nitrogen-fixing abilities that could positively affect switchgrass yields.

Two different harvest systems were used in this experiment. The first harvest system was a single-cut biomass only system that is typically harvested in the fall after switchgrass has matured and become dormant. The second harvest system was a two-cut forage/biomass harvest system. This system was cut once in early summer for forage use and a second time in late fall after switchgrass matured for biomass production. One-cut harvest systems typically remove less nitrogen than the two-cut harvest systems (Reynolds et al., 2000**)**. Harvest systems allow for comparison between biomass yields, forage and biomass yields, and increased nitrogen potential and forage quality from legumes. Major potential benefits of interseeding legumes into switchgrass are nitrogen contribution, enhanced mixture quality, and increased yields.

Several cool and warm-season legume species were selected for their ability to grow in switchgrass stands with annual seeding. Combinations of legumes, including both cool and warm-season legumes, were sown together in switchgrass plots to take advantage of dormant and early switchgrass growth periods. This study did not measure legume biomass yield, it focused on the beneficial aspects (nitrogen fixation, forage quality, and species diversity) of companion planting legumes into switchgrass.

Establishment and persistence of legumes is dependent on the growth habits and cycles of the individual legume species and how they are managed when interseeded into switchgrass. Legumes may substantially reduce switchgrass growth early in its growing cycle if proper legume persistence, maturity, and seasonal growth habits of the companion legume are not considered (Blanchet et al., 1995; Posler et al., 1993). Alfalfa and hairy vetch persistence were both over sixty percent when seeded into “Cave-In-Rock” switchgrass, an upland variety (Blanchet et al., 1995). This study determined the viability of establishment and persistence of ten different cool and warm-season legume species into ‘Alamo’ switchgrass, a lowland variety. The cool-season legumes selected were alfalfa (*Medicago sativa* cv “Evermore”), arrowleaf clover (*Trifolium vesiculosum* cv “Apache”), common vetch, crown vetch (*Securigera varia* cv “Penngift”), red clover (*Trifolium pretense* cv “Cinnamon Plus”), hairy vetch, and crimson clover (*Trifolium incarnatum*). The warm-season legumes included were Illinois bundle flower (*Desmanthus illinoensis*), trailing wild bean (*Strophostyles helvula* cv “Tamu-H”), and partridge pea (*Chamaechrista fasciculata*). The legumes covered a wide range of growth habits and cycles that helped determine the best legume species interseeded into switchgrass.

A compatible, persistent warm-season switchgrass and legume combination could significantly increase overall switchgrass yield and forage quality early in the season (George et al., 1995; Posler et al., 1993). The two-cut forage/biomass system provides early season forage for livestock and/or hay and a secondary cut for biomass production. The forage harvest is taken when switchgrass is predominately leafy vegetation and contains large amounts of plant nitrogen. The two-cut harvest system has been shown to remove twice the plant nitrogen compared to a one-cut harvest system (Fike et al., 2006; Lemus et al., 2009; Reynolds et al., 2000; Yang et al., 2009). Legumes could be added into these systems to increase available N in the soil and protein content of switchgrass, while maintaining increased switchgrass yields and sustainable conservation practices.

Legumes have the potential to increase the crude-protein content in grass forage (Barnett and Posler, 1983; Posler et al., 1993), improve nitrogen availability, and enhance switchgrass yields. The addition of legumes into cool-season pasture systems can consistently improve animal performance by 25% to 50% (Allen et al., 1992) and can improve overall total forage quality. In vitro digestible dry matter (IVDDM) concentrations improved in switchgrass forage when interseeded with legumes, but legume reseeding was necessary for continued improvement of IVDDM concentrations after consecutive years of legume growth (Posler et al., 1993).

Legumes may be agronomically beneficial to switchgrass because they fix nitrogen through a symbiotic relationship with rhizobia (soil bacteria). Rhizobia form nodules on the plant root that convert dinitrogen (N_2) from the atmosphere into ammonium (NH_4^+), a form that plants can take up through the roots (Graham, 2005). The plant supplies the bacteria with nutrients that it needs to carry out growth and N-fixation. It is this relationship that enhances the soil quality and may reduce the amount of synthetic nitrogen fertilizer applied to switchgrass crops.

Nitrogen fertilization of switchgrass has been documented to increase yields and is recommended in production systems at a rate of 67 kg ha^{-1} (Garland et al, 2008), equal to about one half of the rate recommended for corn production (Sanderson et al. 1996). Nitrogen is considered one of the most limiting nutrients in switchgrass. Annual switchgrass biomass production averages in the upper Southeast are 15.9 Mg ha^{-1} (Lemus et al., 2009). Experiments have shown that alfalfa and birdsfoot trefoil may transfer a high proportion of N to a companion grass stand (Brophy et al., 1987). Crimson clover and hairy vetch can supply N to a successive crop (Holderbaum at al., 1990); while alfalfa may fix 82 to 254kg N ha^{-1} when grown with a companion grass (Heichel and Henjum, 1991). Legume-switchgrass mixtures were shown to produce more total-season upper canopy yield than a monoculture switchgrass field fertilized with 240 kg N ha^{-1} (George et al., 1995). According to Mallarino et al. (1990), average N derived from legumes in tall fescue (*Festuca arundinacea* Schreb) increased from 20% in the first year after seeding to 45% -60% N in the following year. As seen above, when properly established and maintained legumes can enhance switchgrass yields by adding N into the soil environment.

Biological nitrogen fixation (BNF) is directly affected by the amount of nitrogen in the soil. Inorganic (synthetic) nitrogen in the soil reduces BNF during dry conditions and fertilizer use. In the long term, BNF leads to accumulation of soil N, grass dominance, and then reduced BNF as nitrogen in the soil increases (Ledgard and Steele, 1992). Cool-season legume N fixation can be affected by weather and available nutrients when decay of legumes is not in sync with the peak demand of N by main crop (Larson et al., 2001).

Nodulation of legumes by species-specific rhizobia is critical for optimal nitrogen fixation to take place. Legumes must be inoculated with the correct strain of species-specific rhizobia when first established into a new field to ensure proper nodulation and fixation of nitrogen (Graham, 2005). An effective symbiotic relationship and interaction with the soil N environment measured by plant dry matter yields will determine the rate of nitrogen fixation by the legume (Unkovich and Pate, 2000). Many methods are used to determine biological nitrogen fixation including N-balance, N-difference, acetylene reduction, hydrogen evolution, and ^{15}N isotope techniques.

Research into maximizing switchgrass production is still in its beginning stages. Established legumes will fix and supply enough nitrogen to the switchgrass to reduce the amount of nitrogen fertilizer applied to increase biomass or forage growth. Legumes may also increase forage quality of switchgrass and increase biodiversity, in an otherwise, singular plant community which in turn helps reduce disease and pests.

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Part I

Establishment of cool- and warm-season legumes into existing stands of switchgrass for biomass and forage/biomass harvests.

Abstract

The major recurring input for switchgrass (*Panicum virgatum*) being produced as a biofuel feedstock is nitrogen. It has been hypothesized that legumes may be interseeded into switchgrass to increase available nitrogen in the soil, thereby reducing fertilizer costs, enhancing switchgrass yields, and, if being managed as a dual-use crop, forage quality. The objective of this study was to develop legume management strategies for switchgrass production systems. Four cool- and two warm-season legume treatments were compared to 67 and 135 kg of inorganic N ha^{-1} during 2009 and 2010 at East Tennessee, Plateau, and Milan Research and Education Centers. The cool-season legumes selected were alfalfa (*Medicago sativa* cv “Evermore”), red clover (*Trifolium pretense* cv “Cinnamon Plus”), crimson clover (*Trifolium incarnatum*); and hairy vetch (*Vicia villosa*). The warm-season legumes included were Illinois bundle flower (*Desmanthus illinoensis*), and partridge pea (*Chamaechrista fasciculata*). The legumes were monitored for establishment, persistence, and their effects on yield and forage quality of switchgrass under two harvest systems: single post-dormancy and two-cut, early (boot-stage) plus post-dormancy. In the one-cut system, switchgrass yields for the 67 kg N ha^{-1} treatment (16.6 Mg ha^{-1}) exceeded ($P \leq 0.05$) those for legume-only treatments (12.6 to 14.3 Mg ha^{-1}). In the two-cut harvest system, switchgrass yields from alfalfa (8.7 Mg ha^{-1}) and Illinois bundle flower (8.4 Mg ha^{-1}) were different from the 67 kg N ha^{-1} treatment (12.5 Mg ha^{-1}) in 2010. Red clover showed the most persistence in both harvest systems. Forage crude protein levels were higher ($P \leq 0.05$) with 135 kg inorganic N ha^{-1} versus other treatments, with the exception of red clover, in the first cut of the two-cut harvest system. Soil nutrient levels were not adversely affected by either harvest systems for the N-levels we examined. Clustering of NIR spectral data indicated

differences in switchgrass chemical signatures among locations and nitrogen treatments, but not among the legume treatments.

CHAPTER I

Introduction

Switchgrass, a C4 perennial grass has excellent potential for producing biomass in the warm, dry summer months (Cherney et al., 1991) and is currently the subject of intense research and development as a biofuel crop. Nitrogen (N) increases productivity of established switchgrass and is considered a limiting nutrient. Annual switchgrass biomass yield averages in the upper Southeast are 15.9 Mg ha^{-1} (Lemus et al., 2009) and yields can increase with the use of nitrogen fertilization. Nitrogen fertilization is recommended in switchgrass production systems at a rate of 67 kg ha^{-1} (Garland et al., 2008) or approximately one half the recommended rate for corn production (Sanderson et al. 1996). Nitrogen removal has been shown to be twice as high in two- versus one-cut harvest systems (Fike et al., 2006; Lemus et al., 2009; Reynolds et al., 2000; Yang et al., 2009).

Nitrogen-fixing legumes could supply a portion of the N required for switchgrass production. Experiments have shown that alfalfa and birdsfoot trefoil (*Lotus corniculatus* L.) may transfer a high proportion of N to a companion grass stand (Brophy et al., 1987). Alfalfa can fix 82 to 254kg N ha^{-1} when grown with a companion grass (Heichel and Henjum, 1991). Fall seeded stands of hairy vetch, red clover, alfalfa, common vetch (*Vicia sativa*), arrowleaf clover (*Trifolium vesiculosum*), and crimson clover have been found to supply significant amounts of N to subsequent corn crops (Holderbaum et al., 1990). Corn yields when planted into chemically killed vetch were similar to corn fertilized with 84 kg N ha^{-1} (Tyler et al., 1987).

Legume-switchgrass mixtures produced more upper-canopy yield than monoculture switchgrass treated with 240 kg N ha^{-1} (George et al., 1995). Mallarino et al. (1990) reported average N derived from legumes in tall fescue (*Lolium arundinacea* Schreb) increased from 20% in the first year after seeding to 45-60% in the following year. Experiments with legume-switchgrass stands including white and yellow sweet clovers (*Melilotus alba* Medik and *Melilotus officinalis* L., respectively), birdsfoot trefoil, red clover, alfalfa, and hairy vetch documented yields that exceeded those of N-only stands, even at N levels of 240 kg ha^{-1} (George et al., 1995; Gettle et al., 1996). Hairy vetch and Persian clover (*Trifolium resupinatum*) grown simultaneously in grass swards, are also very effective in providing additional N to the grass crop (Opitz von Boberfeld et al., 2005).

Establishment and persistence of legumes interseeded into switchgrass depends on the growth habit and cycles of the individual legume species and their management. A productive grass crop is maintained by legume persistence, compatible growth habits, and rate of legume maturity that may substantially reduce switchgrass growth early in the growing cycle (Blanchet et al., 1995; Posler et al., 1993). Alfalfa and hairy vetch persistence were both over 60% when seeded into "Cave-In-Rock" switchgrass (Blanchet et al., 1995), an upland cultivar that is shorter and less robust than Alamo. Establishment of legumes, in this study, was based on stand densities in switchgrass. Published recommendations for pasture systems report that legumes should cover 30% of the ground area to fix sufficient amounts of nitrogen to eliminate the need for inorganic N fertilization in the spring (Bates, 1995).

Legume stand densities depended on seeding rates, date of seeding, weather after seeding, legume growing cycle, soil nutrient levels, and switchgrass competition. Legume seeding rates were adjusted from pure stand rates for forage to reduce the potential for competition with switchgrass early in the season. Seeding dates for cool-season legumes are typically in early fall and mid-February through the end of March. Seeding dates for warm-season legumes range from late winter to the beginning of May depending on species. Legumes were seeded in late fall to allow switchgrass harvests to be completed, because planting legumes into uncut, mature switchgrass stands was not possible. Legume seeding rates may need to be adjusted from pure stand rates to avoid legume stands that are so dense that they suppress switchgrass growth during the spring, an outcome more likely for early-seeded cool-season legumes.

Legumes have the potential to increase the crude-protein content in grass forage (Barnett and Posler, 1983; Posler et al., 1993), improve nitrogen availability, and switchgrass yields. The addition of legumes to cool-season pasture consistently improved animal performance by 25% to 50% (Allen et al., 1992) and can improve the overall forage quality. In vitro digestible dry matter (IVDDM) concentrations were shown to improve in switchgrass forage when interseeded with legumes. However, legume reseeding was necessary for continued improvement of IVDDM concentrations after consecutive years of legume growth (Posler et al., 1993). Legumes can enhance switchgrass forage quality early in the season (George et al., 1995; Posler et al., 1993) when a forage harvest would normally be taken. Legumes are added into grass systems to increase plant available N and enhance forage quality while maintaining switchgrass yields and sustainable conservation practices.

The objectives of this research were to: (1) develop efficient legume management strategies for switchgrass biomass and forage production systems, (2) evaluate selected cool- and warm-season legumes for their compatibly with switchgrass, and (3) determine whether switchgrass yields are increased by compatible legumes. A compatible, persistent, warm-season grass and legume combination could maintain switchgrass yields while replacing inorganic N. However, companion species must be able to grow together simultaneously, not eliminate one another from the stand, and take advantage of their companion's growth patterns (Cherney et al., 1991). Legumes are able to fix nitrogen, increase soil quality, and increase species diversity. This increase in plant species diversity helps to maintain stable year to year production, break disease cycles, and increase diversity in arthropod communities that can decrease pest populations harm ful to monoculture crops (DeHaan et al., 2010; Tillman, 2000).

Chapter II

Materials and Methods

Switchgrass Stands

‘Alamo’ variety switchgrass was planted at 9 kg ha^{-1} pure live seed at three Research and Education Centers in Tennessee: in spring 2007 at East Tennessee (ETREC) in Knoxville (35.53^o N -83.57^o W) and Plateau (PREC) in Crossville (36.1^o N -85.8^o W) and in spring 2004 at Milan (RECM) in Milan (35.55^o N -88.44^o W). Soil type at ETREC was Huntington silt loam (fine-silty, mixed, active, mesic Fluventic Hapludolls), at PREC Lily silt loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludults), and at RECM Collins (coarse-silty, mixed, active, acid, thermic Aquic Udifluvents). Previous management practices, mean annual temperature and precipitation differed at each of these locations (Table 1.1). Weeds were controlled at ETREC by using hand cultivation and chemically sprayed with nicosulfuron {2-[[(4,6-dimethoxypyrimidin-2-yl)aminocarbonyl]aminosulfonyl]-N,N-dimethyl-3-pyridinecarboxamide} at a rate of 0.67 oz ac^{-1} in 2009.

Legume and Harvest Treatments

Two experiments were installed, both using a randomized complete block design, with three replications per location. Both experiments used the same six legume species. The cool-season legumes selected were alfalfa (ALF; *Medicago sativa* cv “Evermore”), red clover (RC; *Trifolium pretense* cv “Cinnamon Plus”), hairy vetch (HV; *Vicia villosa*), and crimson clover (CC; *Trifolium incarnatum*). The warm-season legumes were Illinois bundle flower (IBF; *Desmanthus illinoensis*), and partridge pea (PP; *Chamaechrista fasciculata*). Legumes were chosen based on growth habits and reseeding ability that were potentially compatible with switchgrass and for their ability to fix nitrogen in significant enough quantities to positively affect switchgrass yields.

Experiment #1:

Experiment #1 evaluated legumes/switchgrass in a single, post-dormancy harvest and was conducted at ETREC, PREC, and RECM. This experiment included four cool-season legumes (ALF, CC, HV, & RC), two warm-season legumes (IBF and PP), two inorganic N rates (0 and 67 kg N ha^{-1}), and two combinations of legumes (alfalfa and red clover) plus 67 kg N ha^{-1} for a total of ten treatments. The RECM location also included a 135 kg N ha^{-1} treatment and all six legume treatments plus 67 kg N ha^{-1} in addition to the other treatments above for a total of 15 treatments. For the legumes with the N treatments, application occurred once switchgrass broke dormancy and was approximately 30.5 cm tall, typically in late April.

Experiment #2:

Experiment #2 had two harvest treatments, one at boot stage (late May-mid June) and the second, post-dormancy. This experiment was conducted at ETREC and PREC and included the same four cool-season legumes (ALF, CC, HV, & RC), the same two warm-season legumes (IBF and PP), in addition to three inorganic-N treatments (split applications of 0-0, 0-67, & 67-67 kg N ha^{-1}) for a total of nine treatments. For the 0-67 kg N ha^{-1} treatment, N was applied approximately two weeks following the first harvest. For the 67-67 (135 total) kg N ha^{-1} treatment, N was applied at the same time as the April application (in Experiment #1) and after renewed green-up approximately two weeks following the first cut. The control was represented by the 0 kg N ha^{-1} treatment.

Legume Establishment

The legumes were no-till drilled into one-year-old switchgrass stubble at ETREC and PREC using a Hege™ plot drill (Colwich, KS). At RECM, legumes were drilled into three-year-old switchgrass stubble with an 8-row ALMACO plot drill (Nevada, IA). Planting depth ranged from 0.6 to 1.3 cm. The plot sizes at ETREC and PREC were 7.6 x 1.5 m and 7.6 x 1.8 m, respectively with 18 cm row-spacing. The plot size at RECM was 7.6 x 3.8 m with 25.4 cm row-spacing. Legume seeding rates were 13.5, 6.7, 6.7, 9, 13.5, and 9 kg ha^{-1} for ALF, CC, HV, IBF, PP, and RC, respectively (Table 1.2). Seeding rates were adjusted to account for germination rates and hard seed. ALF, CC, and RC were inoculated. HV, IBF, and PP were not inoculated.

At ETREC, RC, CC, and HV were planted on 20 October 2008 and 29 October 2009, and ALF, CC, HV, IBF, PP, and RC on 24 March 2009. At PREC, RC, CC, and HV were planted on 4 November 2008 and 22 October 2009, and ALF, CC, HV, IBF, PP, and RC on 31 March 2009. At RECM, ALF, CC, HV, IBF, PP, and RC were planted on 9 April 2009, and RC, CC, and HV were planted on 17 December 2009. The synthetic nitrogen source, ammonium nitrate, was broadcast by mechanical spreader.

Legume stand densities were estimated annually following green-up in the spring using a 1-m^2 frequency grid (Vogel and Masters, 2001). Four density counts were taken on each legume treatment plot. Legume densities were averaged from all three replications at each location to determine average plant densities and validate forage quality analysis for each legume treatment. Switchgrass heights were taken at each density count (n=4) and averaged for each plot.

Sample Collection

Switchgrass dry matter yields were taken for all plots at harvest. Harvests were implemented in 2009 and 2010. Plots were harvested at ETREC & PREC using a Carter™ harvester (Brookston, IN**)** with a 91 cm cutting width at 20.3 cm height and at RECM with a New Holland ‘Crop Cruiser 850’ forage chopper with a 2.1 m cutting width at 20.3 cm height.

Grab samples of switchgrass (1-2 kg) were collected from all plots at harvest, weighed, dried at 49^{o}C in a batch oven (Wisconsin Oven Corporation, East Troy, WI), and weighed again to determine moisture content. Samples were then ground through a 2 mm sieve on a Wiley mill (Thomas Scientific, Swedesboro, NJ). Soil core samples were taken to a depth of 15.2 cm from all plots. Soil sample were combined by treatment and analyzed for phosphorus, potassium, calcium, and magnesium levels.

Harvests for the first-cut of the two-cut biomass system were taken on10 June 2009 and 26 May 2010 at ETREC and 17 June 2009 and 9 June 2010 at PREC. Harvest dates for the biomass cuts of the one and two-cut harvest systems were taken on 22 October 2009 and 8 November 2010 at ETREC and 21 October 2009 and 21 October 2010 at PREC. At RECM, harvest dates for the biomass cut of the one-cut harvest system were 3 December 2009 and 23 November 2010.

Data Analysis

Forage quality was only analyzed on the forage (first) cut of the two-cut harvest system. The analysis included moisture at harvest, dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), total digestible nutrients (TDN), and net energy lactation (NEL) (Robertson and Van Soest, 1981). NEL is the net energy value of feed used for milk production and is derived from ADF (Martin et al., 2006). Forage samples were analyzed at Cumberland Valley Analytical Services, Inc (Hagerstown, MD).

Near-Infrared Spectroscopy (NIR) was used to develop near-infrared spectral data on ground switchgrass biomass samples. NIR absorption bands are produced when NIR radiation at a specific frequency vibrates at the same frequency as molecular bonds from molecules such as O-H, C-H, and N-H in a sample (Shenk et al.; 2001).

The near-infrared spectra were collected from 2 mm ground switchgrass biomass samples using a LabSpec® Pro Spectrometer (Analytical Spectral Devices, Boulder, Colorado). The scan range was 1003-2500 nm. Five scans were taken from three ground switchgrass subsamples for every treatment in a replication. The NIR data set was transferred to Unscrambler software v. 10.1 (CAMO Software Inc., Woodbridge, NJ). The reflectance spectra were converted to absorbance spectra, reduced by averaging the spectra to a spectral data set of 4nm intervals, mean-normalized, and a multiplicative scatter correction (MSC) was applied to compensate for multiplicative and/or scatter effects in the data (Labbe et al., 2008). The spectra were averaged to reduce size of the spectral matrix and computation time of multivariate models (Labbe et al., 2008).

A principal component analysis (PCA) was used to determine if there were any trends or clusters due to treatments and/or locations. The PCA is an indirect analysis of spectral data. PC's (principle components) are not the true underlying factors causing the data variation, but orthogonal linear combinations of them. The PC's are abstract solutions. The PC's are computed interactively and PC1 explains the most variation in the data followed by PC2 and on down the line. A principle component analysis (PCA) from NIR spectral data was run individually on all treatments at each location, all treatments combined at each location, and all treatments across all locations. The PCA helped to evaluate the variation in molecular bonds (e.g., OH, CH, and NH) across all treatments and then locations. The variations were visually represented as groupings or clusters of spectral data from the chemical signatures of molecules from switchgrass samples.

Switchgrass yields and forage quality were analyzed using PROC Mixed with SAS v. 9.1.3 (SAS Institute, Cary, NC). Fixed effects were legume and nitrogen treatments, and locations and replications were assigned as random effects. Tukey’s mean separation analysis was used to control test treatment means for differences in switchgrass yield and forage quality with alpha = 0.05.

CHAPTER III

Results and Discussion

Experiment #1: Biomass System (One Harvest)

Legume Establishment

Individual locations:

Legume plant densities at ETREC ranged from 8 to 31 and 0 to 23 plants m^{-2} for 2009 and 2010, respectively (Table 1.4). Legume density (plants m^{-2}) was greatest for CC (31), RC (25), and RC+67 kg N ha^{-1} (25) in 2009. These legume densities were also highest in 2010 with CC (23), RC (16), and RC+67 kg N ha^{-1} (17).Densities were lowest for ALF+67 kg N ha^{-1} (9), IBF (8), and PP (7) in 2009 and ALF (0), ALF+67 kg N ha^{-1} (0), IBF (0), and PP (2)in 2010. Legume densities were taken on 12 May 2009 and 24 May 2010. May switchgrass heights increased from 67 cm to 109 cm from 2009 to 2010.

Legume plant densities at PREC ranged from 5 to 37 and 0 to 6 plants m^{-2} for 2009 and 2010, respectively (Table 1.4). Legume density (plants m^{-2}) was greatest for ALF (30), CC (32), RC (36) and RC+67 kg N ha^{-1} (37) in 2009. Legume densities were lowest for IBF (5) in 2009. In 2010, legume densities at PREC did not exceed 6 plants m^{-2} for any species. Legume densities were taken on 14 May 2009 and 25 May 2010. May switchgrass heights increased from 52 cm to 106 cm from 2009 to 2010.

Legume plant densities at RECM ranged from 9 to 34 and 0 to 26 plants m^{-2} for 2009 and 2010, respectively (Table 1.4). Legume density (plants m^{-2}) was greatest for RC (34) and least with IBF+67 kg N ha^{-1} (9) in 2009. In 2010, legume densities were highest for RC (26) and RC+67 kg N ha^{-1} (17). Densities of 5 plants m^{-2} or less were observed for all other treatments.

Legume densities were taken on 19 May 2009 and 19 May 2010. May switchgrass heights increased from 74 cm to 84 cm from 2009 to 2010 (Table 1.4).

<u>Across Locations and Years</u>:

Legume stand densities declined (Table 1.4) and switchgrass height increased (Table 1.4) from 2009 to 2010 at all locations. In 2009, ALF, CC, RC, and RC+67 kg N ha^{-1} legume treatments had the highest densities. In 2010, RC and RC+67 kg N ha^{-1} had the greatest densities across all locations. ALF, ALF+67 kg N ha^{-1}, and IBF had little or no establishment at any location in 2010 (Table 1.4).

Small and non-existent legume stands at PREC could have been caused by poor quality soils. Soil nutrient values were considerably lower at PREC, where phosphorus and potassium levels were deficient, compared to the other two locations (Table 4.3, pp.125). Switchgrass plots had no additional soil amendments added during this study other than the indicated nitrogen treatments.

Precipitation increased at ETREC and PREC, while RECM precipitation was approximately the same in both years (Table 1.1). Heavy rains and flooding at RECM, nutrient deficiency at PREC, and cold temperatures that occurred after fall seeding at all locations could have impacted legume establishment and persistence during 2010. Typically fewer legumes were established, with the exception of RC, with the addition of 67 kg N ha^{-1} than for the legume-only treatments.

Increased legume densities may be achieved by increasing seeding rates, amending soil nutrients, and inoculation of seed with species-specific rhizobia. HV, IBF, and PP were not inoculated, which may have affected successful establishment. Timing of legume stand densities estiamtes may have had an effect on reported stand rates given the growth cycles of warm-season legumes. Accurate legume densities are difficult to take once switchgrass begins rapid growth in early summer. Switchgrass height shades legumes that are slow growing and/or not yet mature by the time switchgrass reaches early elongation stage (Moore et al., 1991). Warm-season legume growth coincides with switchgrass elongation and reduces the legume's access to light and other resources. For example, ALF and IBF did not get above 5 cm in 2009 and were not able to penetrate switchgrass canopy.

Biomass Yields

Individual locations:

Switchgrass yields at ETREC did not differ among treatments (Table 1.5) for 2009 and 2010 ($P \leq 0.05$). Yields ranged from 11.3 Mg ha^{-1} for RC to 16.1 for 67 kg N ha^{-1} in 2009. In 2010, yields ranged from 10.2 Mg ha^{-1} for ALF and PP to 19.4 for ALF. The control treatment reached yields of 15.2 and 11.6 Mg ha^{-1} in 2009 and 2010, respectively. Switchgrass plots with added N fertilizer did not have increased yields.

At PREC in 2009, switchgrass yields with HV (16.9 Mg ha^{-1}) were greater ($P \leq 0.05$) than PP (13.0 Mg ha^{-1}). Switchgrass yields for all treatments were not different from the control in 2010 and did not increase with N fertilizer in 2009 or 2010. In 2010, yields ranged from 13.2 Mg ha^{-1} for ALF to 16.4 for IBF. Switchgrass plots with added N fertilizer did not have increased yields in 2010.

At RECM in 2009, switchgrass yields for legume treatments without inorganic N ranging from 12.6 Mg ha^{-1} for IBF to 14.1 Mg ha^{-1} for HV were lower ($P \leq 0.05$) than for those with 67 kg N ha^{-1} ranging from 18.7 Mg ha^{-1} for HV+67 kg N ha^{-1} and 20.9 for CC+67 kg N ha^{-1}. In 2010, legume treatments with +67 kg N ha^{-1} yields ranging from 18.5 Mg ha^{-1} for ALF+67 kg N ha^{-1} to 20.8 Mg ha^{-1} for RC +67 kg N ha^{-1}were greater than legume only treatment yields ranging from 10.5 Mg ha^{-1} for CC and 12.3 Mg ha^{-1} for HV. ALF (15.9 Mg ha^{-1}did not differ from any of the treatment yields. In 2009 and 2010, switchgrass plots fertilized with additional N were higher yielding than most plots without additional N.

Across locations and years:

Switchgrass yields for legume treatments ranging from 12.6 Mg ha^{-1} for PP to 14.3 Mg ha^{-1} for IBF were less ($P \leq 0.05$) than for those treatments with additional N ranging from 16.6 Mg ha^{-1} for 67 kg N ha^{-1} and RC+67 kg N ha^{-1} to 16.7 Mg ha^{-1} for ALF+67 kg N ha^{-1} when analyzed for both years. Additional N supplied to the switchgrass increased yields across locations and years in this study.

NIR Analysis

Absorbance spectral data were taken from 2009 samples only. Spectral bands were developed from the NIR data using Unscrambler software. The spectral bands indicated if there was a change in frequency or intensity of an overtone or combination vibration from the C-H, O-H, or N-H absorptions bands in the switchgrass molecules. Normal spectral data appears like a random scattering of data. Treatments that have elevated or decreased frequencies or combination vibrations from the overall average of spectral data will appear to cluster or group together on a scores plot. A PCA was run on all treatments to determine if distinct clustering or trends could be observed from the frequencies or intensities of the NIR spectral bands in the switchgrass. Clustering of the spectra for individual treatments shows that the frequencies or their intensities are different from the overall spectral data across the experiment site(s).

Individual locations:

At ETREC, NIR data of CC, RC, and RC+67 kg N ha^{-1}switchgrass treatments were examined together due their high legume densities from both years of the study (Figure 1.1). Crimson clover spectra showed no distinct pattern. Red clover spectra were grouped mainly above the x-axis and can be compared to a 0 N treatment. Red clover+67 kg N ha^{-1} spectra were clustered near the y-axis and were comparable to the clustering 67 kg N ha^{-1} treatments. The patterns of the red clover and red clover+67 kg N ha^{-1} spectra indicate the possibility of different switchgrass compositions due to N treatments.

At PREC, legume densities were limited after two years. A PCA was run on all treatments and replications. No trends or clustering of NIR spectral data was detected among any treatments.

At RECM, NIR data of the 0, 67, and 135 kg N ha^{-1}treatments were compared (Figure 1.2). Grouping occurred among the nitrogen treatments. The 0 N spectra gathered vertically on both sides of the y-axis and the 135 kg N ha^{-1} spectra clustered mainly to the right of the y-axis. The 67 kg N ha^{-1} spectra were grouped on the y-axis between the 0 and 135 kg N ha^{-1} spectra. These patterns of N treatments indicate the possibility of different switchgrass compositions due to the added N treatments. Similar clustering was seen when RC and RC+67 kg N ha^{-1} treatments were analyzed with the 135 kg N ha^{-1} treatment (Figure 1.3). Red clover spectra were grouped mainly to the right of the y-axis and could be compared to a 0 N treatment. Red clover+67 kg N ha^{-1} spectra were grouped near and to the left of the y-axis and were comparable to the clustering 67 kg N ha^{-1} treatments. There was no discernible pattern of the 135 kg N ha^{-1} spectra. The red clover and red clover+67 kg N ha^{-1} spectra indicate the possibility of different switchgrass compositions due to N treatments.

Across locations:

Differences in frequencies or intensities of the spectral data were observed when PCA was used to analyze differences among the three locations for the 0 and 67 kg N ha^{-1} treatments (Figure 1.4). The 0 and 67 kg N ha^{-1}treatments were analyzed individually across the three locations and showed similar spectral groupings. The differences could be the result of different switchgrass compositions from each location or the timing of harvests.

The RC and RC+67 kg N ha^{-1} treatments at ETREC and RECM were compared using PCA and spectra showed similar groupings to that of the 0 and 135 kg N ha^{-1} treatments (Figure 1.5). The differences could be the result of different switchgrass stand compositions from each location.

The legume treatments, when analyzed with NIR, did not show differences in the spectral data of the different switchgrass treatments. The lack of distinct clustering among the legume treatments indicates switchgrass chemistry varied widely among the stands and the legume treatments did not have a direct effect on switchgrass chemistry. Switchgrass chemistry appears to have been affected by both location and nitrogen treatments.

Experiment #2: Forage/Biomass System

Legume Establishment

Individual locations:

Legume establishment in the two-cut harvest system were evaluated for two years at two locations. At ETREC, legume plants densities ranged from 11 to 30 and 1 to 31 plants m^{-2} were observed in 2009 and 2010, respectively (Table 1.6). Legume density was greatest with ALF (30), CC (24), and RC (30) in 2009 and CC (15) and RC (31) in 2010. Densities were least with ALF (1), IBF (1), and PP (3) in 2010. Legume densities were taken on 13 May 2009 and 27 May 2010. Switchgrass heights at the time of legume counts increased from 65 cm to 94 cm from 2009 to 2010.

Legume plants densities ranged from 3 to 35 and 0 to 33 plants m^{-2} were observed at PREC in 2009 and 2010, respectively (Table 1.6). Legume density was greatest with ALF (31), CC (32), and RC (35) in 2009 and RC (33) in 2010. Densities were least with IBF (3) in 2009 and very low or no plant densities were observed for ALF (1), CC (1), HV (0), IBF (0), or PP (2) in 2010.Legume densities were taken on 14 May 2009 and 25 May 2010. Switchgrass heights at the time of legume counts increased from 52 cm to 80 cm from 2009 to 2010.

Only RC had consistently high densities across both locations and years. IBF, when grown with warm-season grasses has been found to die after the first year (Townsend et al., 1975). Alfalfa and IBF, maturing later than some of the other legumes in the study with heights ranging between 3 and 6 cm, may have had trouble with establishment when interseeded into a lowland switchgrass variety. This could have been due to date of seeding, weather after seeding, or switchgrass competition. As was the case with experiment one, low plant densities at PREC could have been caused by poor quality soils (Table 4.4, pp.126). Switchgrass plots had no additional soil amendments added during this study other than the listed nitrogen treatments. Proper management strategies would include a soil test before the incorporation of legumes into switchgrass stands.

Maturity of the legumes may be of importance in the two-cut harvest. Earlier maturing legumes, such as CC and RC, can take advantage of the open canopy that is left after the fall switchgrass harvest and set seed before the forage cut. If legumes have not reached their reproductive state before the forage cut, then the chances of their producing viable seed decline dramatically. Rapid switchgrass growth in late May and early June quickly closes the canopy reducing light and other resources available to legume seedlings such as ALF. The forage cut may reduce the switchgrass canopy enough to allow legumes to accelerate vegetative growth reaching their reproductive stage.

Forage Yields (first cut)

Individual locations:

At ETREC in 2009, switchgrass yields of all treatments, with the exception of ALF (2.1 Mg ha^{-1}), were not different ($P \leq 0.05$) from the 67 kg N ha^{-1} treatment (5.4 Mg ha^{-1}) and ranged from 3.8 Mg ha^{-1} for HV and 5.5 Mg ha^{-1} for PP and 0N (Table 1.7). In 2010, switchgrass yields ranged from 3.9 Mg ha^{-1} for IBF and 6.7 Mg ha^{-1} for RC, 0N, 67 kg N ha^{-1} and no differences were found among treatments.

At PREC in 2009, forage switchgrass yields were not different among treatments ($P \leq 0.05$) and ranged from4.4 Mg ha^{-1} for PP and 5.8 Mg ha^{-1} for 6.7 Mg ha^{-1} (Table 1.7). In 2010, switchgrass yields of the 67 kg N ha^{-1} treatment (4.4 Mg ha^{-1}) were not different from the other treatments and ranged to 3.0 Mg ha^{-1} for ALF and IBF.

Across Locations and Years:

In 2009 (Table 1.7), the 135 kg N ha^{-1} treatment (6.7 Mg ha^{-1}) had greater forage yields ($P \leq 0.05$) than ALF, HV, CC, and IBF treatments, which ranged from 3.3 to 4.9 Mg ha^{-1}. PP, RC, 0 N, and 67 kg N ha^{-1} treatment yields ranging from 5 to 5.6 Mg ha^{-1} were not different from those for 135 kg N ha^{-1}. In 2010, 135 kg N ha^{-1} treatment yields across both locations (6.9 Mg ha^{-1}) exceeded ($P \leq 0.05$) those ALF, HV, IBF, and PP treatments ranging from 3.5 to 4.5 Mg ha^{-1}. Yields for CC, RC, 0 and 67 kg N ha^{-1} treatments ranging from 5.0 to 5.5 Mg ha^{-1} did not differ from yields of the 135 kg N ha^{-1} treatment.

Forage Quality

Forage nutrient concentrations of the forage cut (first) of the two-cut harvest systems were evaluated at both locations for two years (2009-2010) of the study (Table 1.8). ALF and IBF legume treatments were not included in the analysis due to the short height of the plants at harvest in 2009 and inadequate plant densities in 2010. No differences were detected across treatments and years for ADF and NDF ($P \leq 0.05$). Treatment effects were observed in NEL and TDN at ETREC in 2010, but did not appear to reflect meaningful trends given the small relative differences in the values for these parameters. In general, neither the legumes nor the N treatments had any significant ($P \leq 0.05$) effects on forage quality. The exception occurred with the 135 kg N ha^{-1} rate at PREC in 2009 and ETREC in 2010 on crude protein. In those locations and years, the crude protein values of the switchgrass forage from the 135 kg N ha^{-1} treatment (13.3 and 10.4 % respectively) were significantly higher than all the other treatments (Table 1.8).

Biomass Yields (second cut)

Individual locations:

At ETREC in 2009, switchgrass biomass yields were not affected by legumes nor N treatments (Table 1.7). In 2010, the only treatment that had a significant effect on biomass yield was 135 kg N ha^{-1}. However, only yields from RC (5.1 Mg ha^{-1}) and IBF (5 Mg ha^{-}1) were significantly ($P \leq 0.5$) below 135 kg N ha^{-}1 (9.4 Mg ha^{-1}) (Table 1.7). There were no differences among the legume treatments ranging from 5 to 6.3 Mg ha^{-1}, the control (5.3 Mg ha^{-1}), and 67 kg N ha^{-1} (7.7 Mg ha^{-1}) in 2010.

At PREC in 2009, switchgrass biomass yields differed between the 135 kg N ha^{-1} treatment (7.3 Mg ha^{-1}) and the legume treatments of ALF, HV, IBF, PP, and RC ranging from 3.8 to 4.7 Mg ha^{-1} (Table 1.7). There were no differences among legume treatments, control (4.8 Mg ha^{-1}), and the 67 kg N ha^{-1} treatments (5.9 Mg ha^{-1}). In 2010, switchgrass biomass yields between the 135 kg N ha^{-1} treatment (6.8 Mg ha^{-1}) and the ALF treatment (4.5 Mg ha^{-1}) were different. There were no differences among the legume treatments ranging from 4.5 to 5.7 Mg ha^{-1} and the 67 kg N ha^{-1} (6.3 Mg ha^{-1}, Table 1.7) .

Across Locations:

In 2009, yields for 67 (8 Mg ha^{-1}) and 135 kg N ha^{-1}(8.5 Mg ha^{-1}) treatments were higher ($P \leq 0.05$) than those for ALF, HV, IBF, and PP treatments ranging from 4.7 to 5.1 Mg ha^{-1} but did not differ from those for CC, RC, and the 0 N treatments (5.8 to 6.3 Mg ha^{-1}) (Table 1.7). CC (6.1 Mg ha^{-1}) had higher yields ($P \leq 0.05$) than all other legume treatments except RC (5.8 Mg ha^{-1}).In 2010, yields for the 135 kg N ha^{-1} treatment (8.1 Mg ha^{-1}) were greater ($P \leq 0.05$) across both locations than all legume treatments (4.9 to 5.7 Mg ha^{-1}), but not different from the 67 kg N ha^{-1} treatment yield (7.0 Mg ha^{-1}). Yields for the 67 kg N ha^{-1} treatment did not differ from any of the legume treatments (Table 1.7).

Forage + Biomass Yields (total yield)

Individual locations:

At ETREC, comparison of 2009 switchgrass yields between the 135 kg N ha^{-1} treatment (16.5 Mg ha^{-1}) and the legume treatments of ALF, HV, and IBF were different ($P \leq 0.05$) ranging from 7.7 to 10 Mg ha^{-1} (Table 1.7). However, yields from 67 kg N ha^{-1} treatment (15.4 Mg ha^{-1}) did not differ from the legume treatments, except ALF (7.7 Mg ha^{-1}), ranging from 9.4 to 12.4 Mg ha^{-1} nor the control (13.3 Mg ha^{-1}). In 2010, the 135 kg N ha^{-1} treatment (17.5 Mg ha^{-1}) was higher than ALF (9.8 Mg ha^{-1}) and IBF (9 Mg ha^{-1}) ($P \leq 0.05$) but not higher than the other legumes (10.7 to 11.8 Mg ha^{-1}), control (12 Mg ha^{-1}), and 67 kg N ha^{-1} (14.3 Mg ha^{-1}, Table 1.7).

At PREC in 2009, the switchgrass yields (Mg ha^{-1}) from the 135 kg N ha^{-1} treatment (14.2) was significantly higher ($P \leq 0.05$) than the control (9.5) and all of the legumes (8.3 to 10), but not the 67 kg N ha^{-1} treatment (11.7, Table 1.7).The 67 kg N ha^{-1} treatment did not differ from the legume treatments. In 2010, the F+B yields from CC, HV, and RC ranging from 8.6 to 9.6 were equivalent to the 67 kg N ha^{-1} treatment (10.7) and 135 kg N ha^{-1} treatment (12.6). The F+B yields from ALF (7.5), IBF (7.7), and PP (8.5) were ($P \leq 0.05$) less than the high N rate at PREC in 2010.

Across Locations and Years:

Total switchgrass yields (F+B) of the two cut harvest system (Table 1.7) across both locations in 2009 for all legume treatments (8 to 11.2 Mg ha^{-1}) were less than ($P \leq 0.05$) than for the 135 kg N ha^{-1} (15.4 Mg ha^{-1}) treatment. The 67 kg N ha^{-1} treatment yield (13.6 Mg ha^{-1}) was higher ($P \leq 0.05$) than ALF, HV, and IBF treatments (8 to 9.5 Mg ha^{-1}), but not CC, RC, PP, and the 0 N treatments (10.1 to 11.4 Mg ha^{-1}). In 2010, all legume treatment yields, across both locations (8.4 to 10.7 Mg ha^{-1}), were lower ($P \leq 0.05$) than yields of the 135 kg N ha^{-1} treatment (15.0 Mg ha^{-1}). Yields for the 67 kg N ha^{-1} treatment (12.5 Mg ha^{-1}) were similar to those of CC, HV, RC, PP, and the 0 N treatments (10.1 to 10.7 Mg ha^{-1}). Across both harvest systems (one and two-cut), the one-cut system had greater total yields than the two-cut harvest system.

Switchgrass may take up three years to deplete existing soil nitrogen pools and for nitrogen-fixing legumes to have effects on yield (Lemus et al., 2008). Removal of N as a result of one and two-cut harvest systems can exceed N application, which suggests that switchgrass is removing additional N supplied from the soil by mineralization or other processes (Lemus et al., 2009). After three years of legume management, legume rhizobia communities may have increased sufficiently to affect N-fixation. Greater legume densities and associated N-fixation may be achieved by increased seeding rates, inoculation of seed with correct rhizobia, and adjustment of planting times. Soil nutrient levels from initial soil samples and control plots in 2010 did not appear to change over the course of the study (Appendix D 4.3 & 4.4, pp.125-126). As legume seeding rates are increased and stand densities are greater, N-fixation of legumes should enhance switchgrass yield. Care must be taken though, to avoid legume stands that are so dense that they suppress switchgrass growth during the spring, an outcome more likely for early-seeded cool-season legumes.

CHAPTER IV

Conclusions

Results of this study indicate that red clover was the most promising of the legumes tested when seeded into ‘Alamo’ switchgrass stands at all locations and in both harvest systems. Alfalfa and Illinois bundle flower had little or no establishment in the second year of the study. Legume establishment typically declined when nitrogen was applied to plots interseeded with legumes, with the exception of red clover. Establishment of legumes may be increased by seeding rates, legume inoculation, and timely planting of each species. Crimson clover, hairy vetch, and partridge pea need further evaluation to develop appropriate establishment protocols in lowland switchgrass types. Proper legume management needs to be developed that address legume varieties compatible with switchgrass, seed inoculation, seeding dates and rates, and rate of competition with switchgrass. Switchgrass yields in the one-cut harvest systems showed differences among treatments when 67 kg N ha^{-1} was added with or without a legume treatment across all locations and years. In the forage/biomass system, effects of legumes on switchgrass yields were the same as adding 67 kg N ha^{-1}, with the exception of alfalfa and Illinois bundle flower in both years and hairy vetch in 2009, which were lower. Presence of legumes did not significantly alter forage quality among legumes treatments; however, the 135 kg N ha^{-1} treatment increased crude protein content in dry matter compared to the legumes as well as the 67 kg N ha^{-1}. Harvest systems did not appear to affect nutrient levels in the soil. The legume treatments had minimal influence on switchgrass chemistry when analyzed by NIR. It appears that location and nitrogen treatments had an effect on spectral patterns of switchgrass. This means that the relative composition of switchgrass will likely vary from different farmers' fields and with different levels of N fertilization.

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APPENDIX A

Part 1

Table 1.1. Average annual (2009-2010) precipitation, temperatures, and previous experimental site management for the East TN (ETREC), Plateau (PREC), and Milan (RECM) Research and Education Centers utilized in these experiments.

Location	Annual Precipitation		Annual Temperature		Previous Experimental Site Management
	2009	2010	2009	2010	
	-----------cm-----------		-----------°C-----------		
ETREC	173	124	14.3	14.4	Orchardgrass hay (4 yrs)
PREC	192	140	12.7	12.6	Tall fescue pasture
RECM	140	145	14.9	15.2	Row crops

Table 1.2. Legume species variety, seeding rates, and seed inoculation utilized in the one and two–cut experiments for 2009 and 2010.

Legume Species	Variety	Seeding rate	Inoculated Seed	Recommended Rhizobia Species
		----kg ha^{-1}----		
Alfalfa	Evermore	13.5	Yes	*R. meliloti*
Crimson Clover	VNS†	6.7	Yes	*R. trifolii*
Hairy Vetch	VNS	6.7	No	*R. leguminosarum*
IL Bundle Flower	VNS	9.0	No	*R. Desmanthus illinoensis*
Partridge Pea	VNS	13.5	No	*R. leguminosarum*
Red Clover	Cinnamon Plus	9.0	Yes	*R. trifolii*

†VNS= Variety not stated

Table 1.3. Summary of legumes seeding and switchgrass harvest dates at the East TN (ETREC), Plateau (PREC), and Milan (RECM) Research and Education Centers for both single and two-cut harvest system for 2009 and 2010.

	Seeding					
	ETREC		PREC		RECM	
Growing Season	Cool[†]	Warm	Cool	Warm	Cool	Warm
2009	20-Oct-2008 24-Mar-2009[§]	24-Mar-2009	4-Nov-2008 31-Mar-2009[§]	31-Mar-2009	9-Apr-2009	
2010	29-Oct-2009		22-Oct-2009		17-Dec-2009	

	Harvest				
Growing Season	ETREC		PREC		RECM
	Forage	Biomass	Forage	Biomass	Biomass
2009	10-Jun-2009	22-Oct-2009	17-Jun-2009	21-Oct-2009	3-Dec-2009
2010	26-May-2010	8-Nov-2010	9-Jun-2010	21-Oct-2010	23-Nov-2010

†Cool-season legume plantings included: alfalfa, crimson clover, hairy vetch, and red clover.

‡Warm-season legume plantings included: Illinois bundle flower and partridge pea.

§ In 2009, cool-season legumes were replanted in March because of poor emergence in 2008 at ETREC and PREC due to a combination of wet and cold weather damage over winter months.

Table 1.4. Average† legume (LG) plant density and height and switchgrass (SG) height at the East TN (ETREC), Plateau (PREC), and Milan (RECM) Research and Education Centers in the one-cut biomass harvest experiments taken in May 2009 and 2010.

2009

	ETREC			PREC			RECM		
	Plant	Height		Plant	Height		Plant	Height	
Treatment	Density	LG	SG	Density	LG	SG	Density	LG	SG
	No. m^{-2}	--------cm--------		No. m^{-2}	--------cm--------		No. m^{-2}	--------cm--------	
Alfalfa	16	5	67	30	3	48	23	4	64
Alfalfa+67N†	9	3	69	27	3	54	20	4	80
Crimson Clover	31	10	69	32	9	54	23	7	63
Crimson Clover+67 N	-	-	-	-	-	-	22	9	86
Hairy Vetch	12	22	66	15	10	51	17	30	68
Hairy Vetch+67N	-	-	-	-	-	-	13	29	79
IL Bundle Flower	8	3	72	5	3	52	12	5	62
IL Bundle Flower+67N	-	-	-	-	-	-	9	5	83
Partridge Pea	7	8	64	12	7	53	11	9	62
Partridge Pea+67 N	-	-	-	-	-	-	13	13	82
Red Clover	25	6	69	36	10	54	34	9	65
Red Clover+67 N	25	9	65	37	10	53	24	8	84
0 Nitrogen Control	-	-	65	-	-	53	-	-	73
67 Nitrogen	-	-	70	-	-	53	-	-	85
135 Nitrogen	-	-	-	-	-	-	-	-	81

2010

	ETREC			PREC			RECM		
	Plant	Height		Plant	Height		Plant	Height	
Treatment	Density‡	LG	SG	Density	LG	SG	Density	LG	SG
	No. m^{-2}	--------cm--------		No. m^{-2}	--------cm--------		No. m^{-2}	--------cm--------	
Alfalfa	0	-	111	0	-	103	0	-	79
Alfalfa+67N	0	-	115	0	-	105	0	-	81
Crimson Clover	23	49	112	1	7	105	1	40	68
Crimson Clover+67N	-	-	-	-	-	-	1	36	92
Hairy Vetch	7	54	84	0	0	109	5	51	69
Hairy Vetch+67N	-	-	-	-	-	-	4	45	86
IL Bundle Flower	0	-	117	0	-	110	2	4	75
IL Bundle Flower+67N	-	-	-	-	-	-	2	6	99
Partridge Pea	2	14	106	4	16	100	3	16	71
Partridge Pea+67 N	-	-	-	-	-	-	4	18	96
Red Clover	16	42	112	6	19	105	26	20	71
Red Clover+67N	17	51	112	5	18	106	17	13	94
0 Nitrogen§ Control	-	-	110	-	-	108	-	-	78
67 Nitrogen	-	-	114	-	-	108	-	-	99
135 Nitrogen	-	-	-	-	-	-	-	-	108

†Means across treatments and replications

‡Plant density = (frequency of occurrence * 0.4) x 100(Vogel and Masters, 2001)

§Nitrogen applications are in kg ha^{-1}.

Table 1.5. Average[†] dry matter yields (Mg ha^{-1}) of switchgrass per legume or legume+nitrogen treatment from the one-cut biomass harvest at East TN (ETREC), Plateau (PREC), and Milan (RECM) Research and Education Centers from 2009-10.

	2009			2010			2009-10
Treatment	**ETREC**	**PREC**	**RECM**	**ETREC**	**PREC**	**RECM**	**ALL LOC**
	-----	-----	-----	Mg ha^{-1}	-----	-----	-----
Alfalfa	15.0a	14.6ab	15.6bcd	10.2a	13.2a	15.9abcd	14.1b
Alfalfa +67 N[‡]	13.9a	15.3ab	19.6ab	19.4a	15.1a	18.5ab	16.7a
Crimson Clover	11.4a	14.4ab	12.9d	12.7a	15.4a	10.5d	12.9b
Crimson Clover +67 N	-	-	20.9a	-	-	20.4a	-
Hairy Vetch	14.4a	16.9a	14.1cd	12.7a	13.8a	12.3cd	14.0b
Hairy Vetch +67N	-	-	18.7abc	-	-	17.6abc	-
IL Bundle Flower	15.5a	15.9ab	12.6d	12.8a	16.4a	12.5bcd	14.3b
IL Bundle Flower +67 N	-	-	21.7a	-	-	21.5a	-
Partridge Pea	13.4a	13.0b	12.8d	10.2a	14.4a	11.7cd	12.6b
Partridge Pea +67N	-	-	20.8a	-	-	19.5a	-
Red Clover	11.3a	14.1ab	12.9d	14.6a	14.0a	11.8cd	13.0b
Red Clover +67 N	13.4a	15.0ab	20.5a	13.7a	15.9a	20.8a	16.6a
0 Nitrogen Control	15.2a	14.3ab	13.4d	11.6a	13.7a	13.1bcd	13.6b
67 Nitrogen	16.1a	15.5ab	19.0ab	14.2a	15.6a	19.3a	16.6a
135 Nitrogen	-	-	22.3a	-	-	21.8a	-

†Mean separations based on Tukey's at $P \leq 0.05$ apply to columns across treatments within locations and year.

‡Nitrogen applications are in kg ha^{-1}.

Table 1.6. Average[†] legume (LG) stand densities and heights and switchgrass (SG) heights at the East TN (ETREC), Plateau (PREC) Research and Education Centers in the two-cut forage/biomass harvest experiments in 2009 and 2010.

	ETREC						PREC					
	2009			2010			2009			2010		
Treatment	Plant Density[‡]	Height LG	Height SG	Plant Density	Height LG	Height SG	Plant Density	Height LG	Height SG	Plant Density	Height LG	Height SG
	No. m^{-2}	----cm----		No. m^{-2}	----cm----		No. m^{-2}	----cm----		No. m^{-2}	----cm----	
Alfalfa	30	3	54	1	-	85	31	3	51	1	-	83
Crimson Clover	24	10	67	15	46	94	32	10	50	1	4	88
Hairy Vetch	11	14	62	8	67	91	15	13	52	0	-	11
IL Bundle Flower	17	3	62	1	2	88	3	6	53	0	-	82
Partridge Pea	16	7	65	3	14	93	12	6	53	2	15	85
Red Clover	30	9	72	31	54	93	35	10	52	33	47	89
0 Nitrogen Control	-	-	69	-	-	97	-	-	52	-	-	87
67 Nitrogen§	-	-	63	-	-	100	-	-	51	-	-	90
135 Nitrogen	-	-	70	-	-	108	-	-	53	-	-	100

†Means across treatments and replications

‡ Plant density = (frequency of occurrence * 0.4) x 100(Vogel and Masters, 2001)

§ Nitrogen applications are in kg ha^{-1}.

Table 1.7. Average[†] dry matter yields (Mg ha^{-1}) of switchgrass per legume or legume+nitrogen treatments of forage, biomass, and total forage+biomass cuts at East TN (ETREC) and Plateau (PREC) Research and Education Centers for the two-cut forage/biomass experiments from 2009-2010.

2009

	ETREC			**PREC**			**Two Location Average**		
Treatment	**Forage**	**Biomass**	**F+B**	**Forage**	**Biomass**	**F+B**	**Forage**	**Biomass**	**F+B**
	---Mg ha^{-1}---								
Alfalfa	2.1b	5.7a	7.7c	4.5a	3.8b	8.3b	3.3c	4.7c	8.0c
Crimson Clover	5.0ab	7.4a	12.4abc	4.7a	4.9ab	9.6b	4.9bc	6.1ab	11.0bc
Hairy Vetch	3.8ab	6.2a	10.0bc	4.6a	4.0b	8.6b	4.2bc	5.1c	9.3c
IL Bundle Flower	4.0ab	5.4a	9.4bc	4.9a	4.7b	9.5b	4.5bc	5.0c	9.5c
Partridge Pea	5.5a	5.9a	11.4abc	4.4a	4.4b	8.8b	5.0abc	5.1c	10.1bc
Red Clover	5.4a	6.9a	12.3abc	5.4a	4.6b	10.0b	5.4ab	5.8bc	11.2bc
0 Nitrogen Control	5.5a	7.8a	13.3abc	4.6a	4.8ab	9.5b	5.1abc	6.3abc	11.4bc
67 Nitrogen	5.4a	10.0a	15.4ab	5.8a	5.9ab	11.7ab	5.6ab	8.0ab	13.6ab
135 Nitrogen	6.8a	9.7a	16.5a	6.9a	7.3a	14.2a	6.7a	8.5ab	15.4a

2010

	ETREC			**PREC**			**Two Location Average**		
Treatment	**Forage**	**Biomass**	**F+B**	**Forage**	**Biomass**	**F+B**	**Forage**	**Biomass**	**F+B**
	---Mg ha^{-1}---								
Alfalfa	4.3a	5.5ab	9.8b	3.0b	4.5b	7.5b	3.7b	5.0b	8.7c
Crimson Clover	6.0a	5.6ab	11.6ab	3.9ab	5.7ab	9.6ab	5.0ab	5.7b	10.6bc
Hairy Vetch	5.3a	5.4ab	10.7ab	3.5b	5.1ab	8.6ab	4.4b	5.3b	9.7bc
IL Bundle Flower	3.9a	5.0b	9.0b	3.0b	4.7ab	7.7b	3.5b	4.9b	8.4c
Partridge Pea	5.4a	6.3ab	11.7ab	3.5b	5.0ab	8.5b	4.5b	5.6b	10.1bc
Red Clover	6.7a	5.1b	11.8ab	4.3ab	5.3ab	9.6ab	5.5ab	5.2b	10.7bc
0 Nitrogen Control	6.7a	5.3ab	12.0ab	3.3b	5.4ab	8.7ab	5.0ab	5.3b	10.4bc
67 Nitrogen	6.7a	7.7ab	14.3ab	4.4ab	6.3ab	10.7ab	5.5ab	7.0ab	12.5ab
135 Nitrogen	8.1a	9.4a	17.5a	5.7a	6.8a	12.6a	6.9a	8.1a	15.0a

† Mean separations based on Tukeys at $P \leq 0.05$ apply to columns across treatments.

‡Nitrogen applications are in kg ha^{-1}.

Table 1.8. Forage nutrient concentrations of acid detergent fiber (ADF), crude protein (CP), neutral detergent fiber (NDF), net energy lactation (NEL), and total digestible nutrients (TDN) in the first cut of the two-cut forage/biomass harvest at the East TN (ETREC) and Plateau (PREC) Research and Education Centers in 2009 and 2010.

	Acid Detergent Fiber						
Location	**CC**	**HV**	**PP**	**RC**	**0N**	**67N[‡]**	**135N**
	% Dry Matter						
ETREC 2009	38.1a	37.5a	37.4a	39.2a	38.9a	38.2a	38.0a
ETREC 2010	36.9a	36.2a	35.3a	35.6a	35.7a	36.7a	36.7a
PREC 2009	40.5a	38.9a	39.5a	39.7a	39.8a	40.7a	40.3a
PREC2010	35.9a	36.5a	35.6a	34.3a	35.7a	35.9a	36.5a
Across Loc & Years	37.9a	37.3a	36.9a	37.2a	37.6a	37.9a	37.9a

	Neutral Detergent Fiber						
Location	**CC**	**HV**	**PP**	**RC**	**0N**	**67N**	**135N**
	% Dry Matter						
ETREC 2009	69.0a	66.9a	69a	69.8a	69.4a	68.3a	68.8a
ETREC 2010	66.7a	66.8a	66.9a	65.8a	68.6a	68.6a	67.7a
PREC 2009	73.5a	71.9a	72.5a	73.7a	73.7a	73.0a	73.7a
PREC 2010	67.1a	66.7a	66.8a	63.3a	65.1a	66.2a	68.2a
Across Loc & Years	69.1a	68.1a	68.8a	68.1a	69.2a	69.1a	69.6a

	Crude Protein						
Location	**CC**	**HV**	**PP**	**RC**	**0N**	**67N**	**135N**
	% Dry Matter						
ETREC 2009	7.3a	8.1a	7.3a	7.2a	6.9a	7.8a	7.7a
ETREC 2010	8.0b	7.7b	8.0b	8.6b	7.3b	7.0b	10.4a
PREC 2009	8.8b	9.2b	8.8b	8.7b	8.6b	9.5b	13.3a
PREC 2010	10.5a	10.2a	10.6a	12.3a	11.2a	11.7a	10.8a
Across Loc & Years	8.6b	8.8b	8.7b	9.2ab	8.5b	9.0b	10.5a

	Net Energy Lactation						
Location	**CC**	**HV**	**PP**	**RC**	**0N**	**67N**	**135N**
	Mcal/lb						
ETREC 2009	0.60a	0.61a	0.61a	0.60a	0.60a	0.61a	0.61a
ETREC 2010	0.60b	0.61ab	0.62a	0.61ab	0.62a	0.61ab	0.62ab
PREC 2009	0.58a	0.59a	0.59a	0.59a	0.59a	0.59a	0.58a
PREC 2010	0.62a	0.62a	0.62a	0.62a	0.63a	0.62a	0.62a
Across Loc & Years	0.60a	0.61a	0.60a	0.61a	0.61a	0.61a	0.61a

	Total Digestible Nutrients						
Location	**CC**	**HV**	**PP**	**RC**	**0N**	**67N**	**135N**
	% Dry Matter						
ETREC 2009	58.7a	59.0a	59.3a	58.9a	58.9a	59.1a	59.1a
ETREC 2010	58.6b	59.3ab	60.0a	59.6ab	59.9a	59.2ab	59.7ab
PREC 2009	56.8a	57.5a	57.4a	57.2a	57.6a	57.3a	56.9a
PREC 2010	60.3a	59.8a	60.4a	60.1a	60.8a	60.5a	60.6a
Across Loc & Years	58.6a	59.0a	59.3a	59.0a	59.3a	59.0a	59.1a

†Mean separations based on Tukey's at $P \leq 0.05$ apply to rows within locations and years.

‡Nitrogen applications are in kg ha^{-1}.

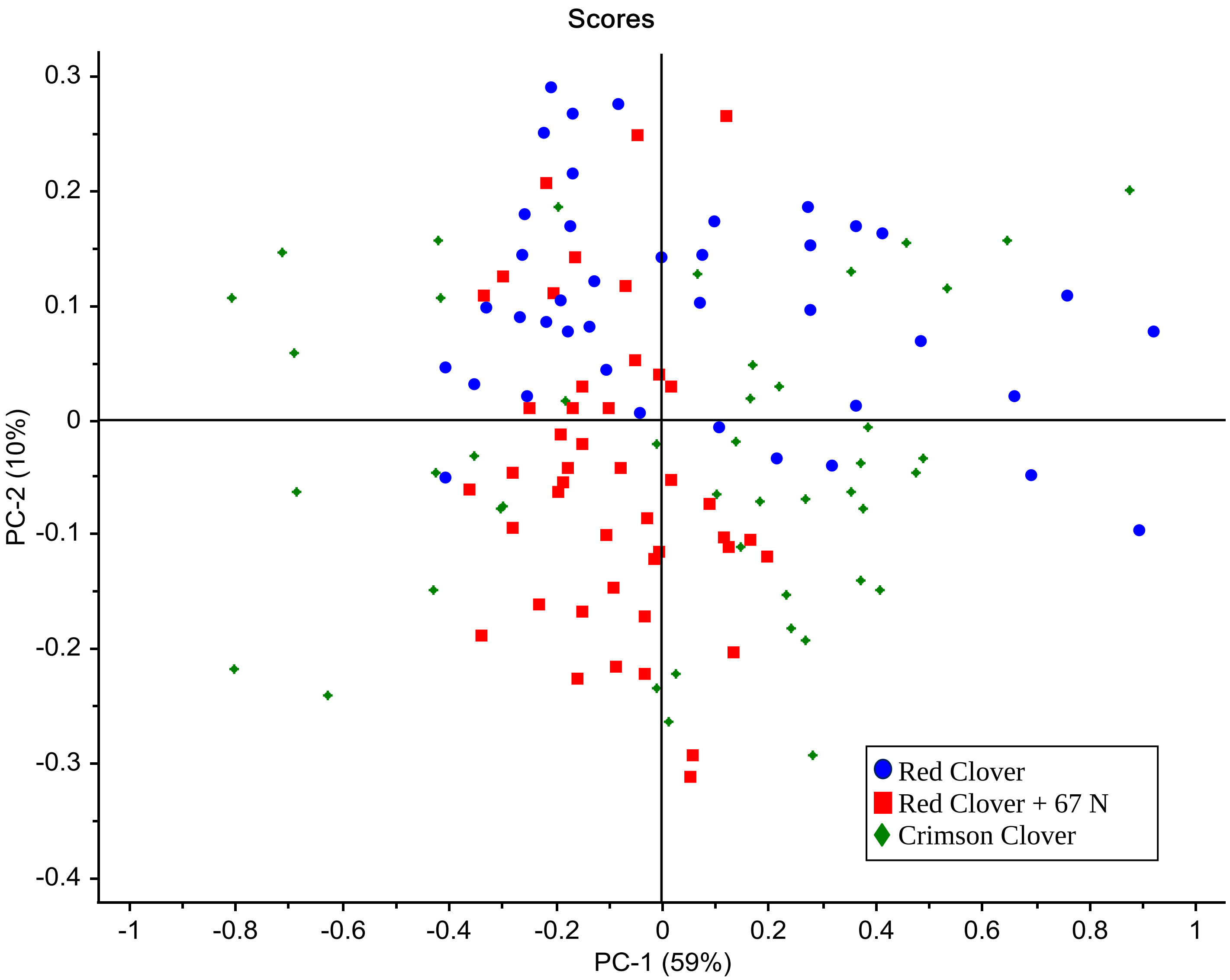


Figure 1.1 Scores plot of the first two principle components derived from NIR absorbance spectra of dried switchgrass samples from red clover, red clover + 67 kg N ha^{-1}, and crimson clover at East TN (ETREC) Research and Education Center in 2009. PC1 explains the most variation in the data followed by PC2.

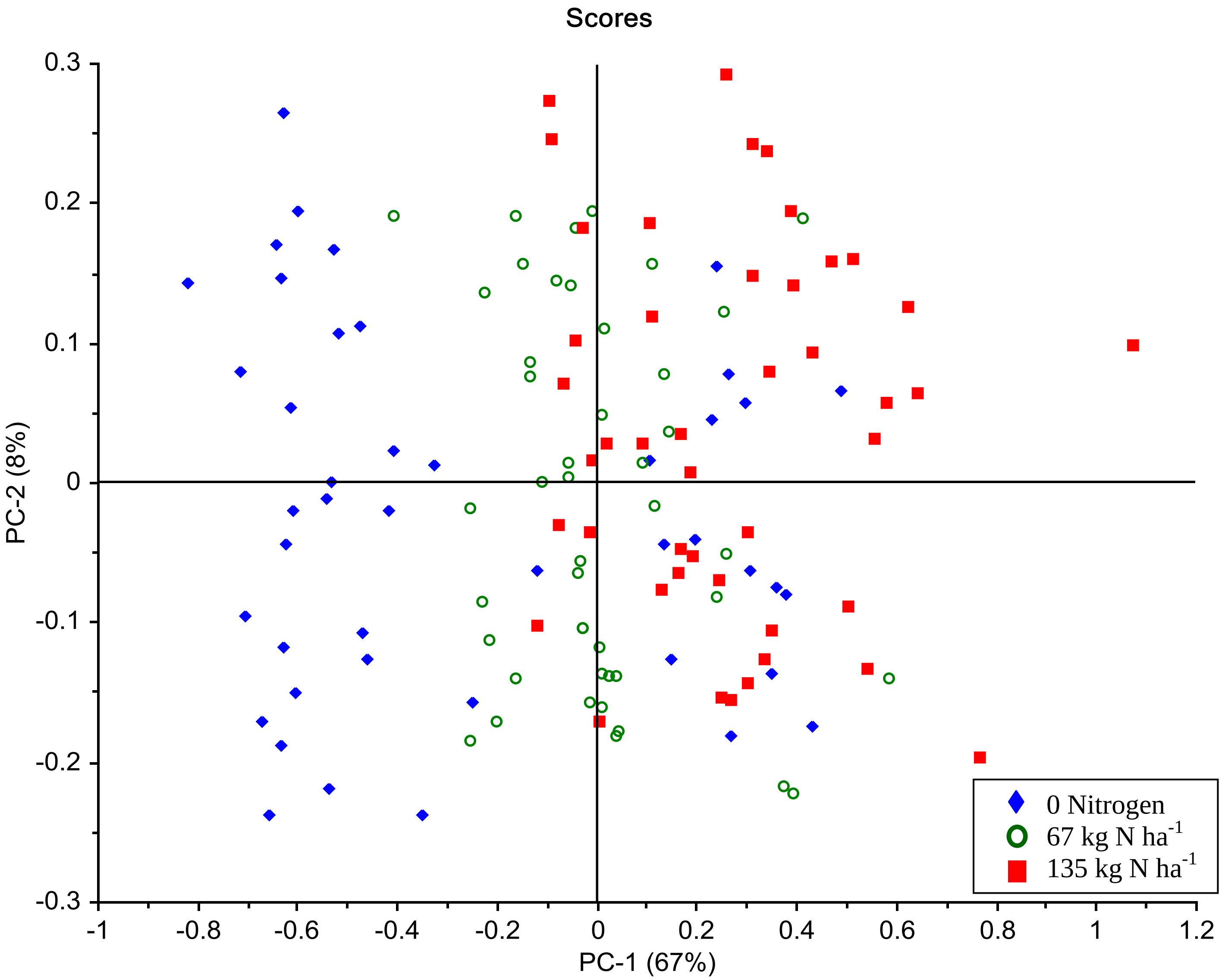


Figure 1.2 Scores plot of the first two principle components derived from NIR absorbance spectra of dried switchgrass samples from 0 Nitrogen, 67 kg N ha^{-1}, and 135 kg N ha^{-1} at Milan (RECM) Research and Education Center in 2009. PC1 explains the most variation in the data followed by PC2.

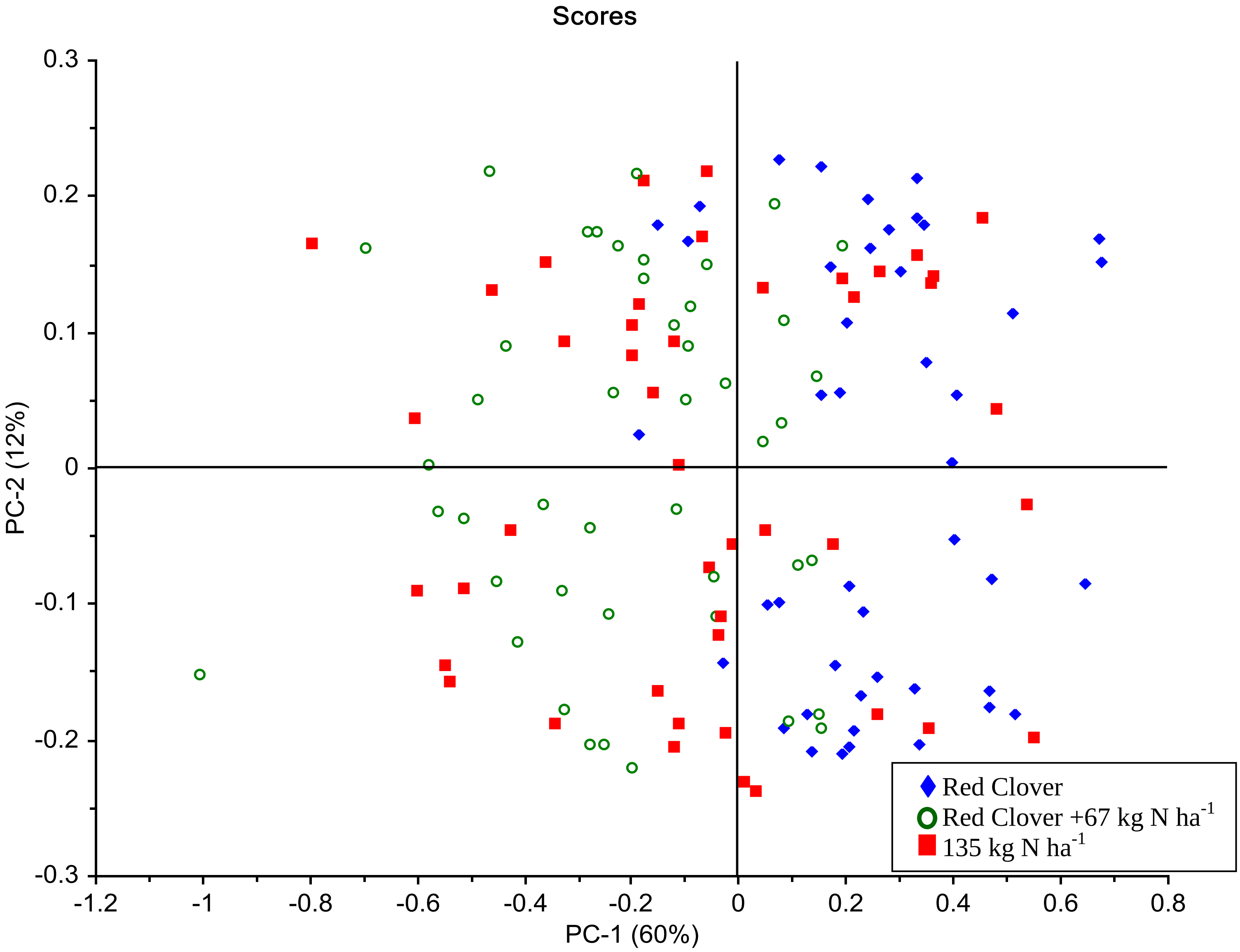


Figure 1.3 Scores plot of the first two principle components derived from NIR absorbance spectra of dried switchgrass samples from red clover, red clover + 67 kg N ha^{-1}, and 135 kg N ha^{-1} at Milan (RECM) Research and Education Center in 2009. PC1 explains the most variation in the data followed by PC2.

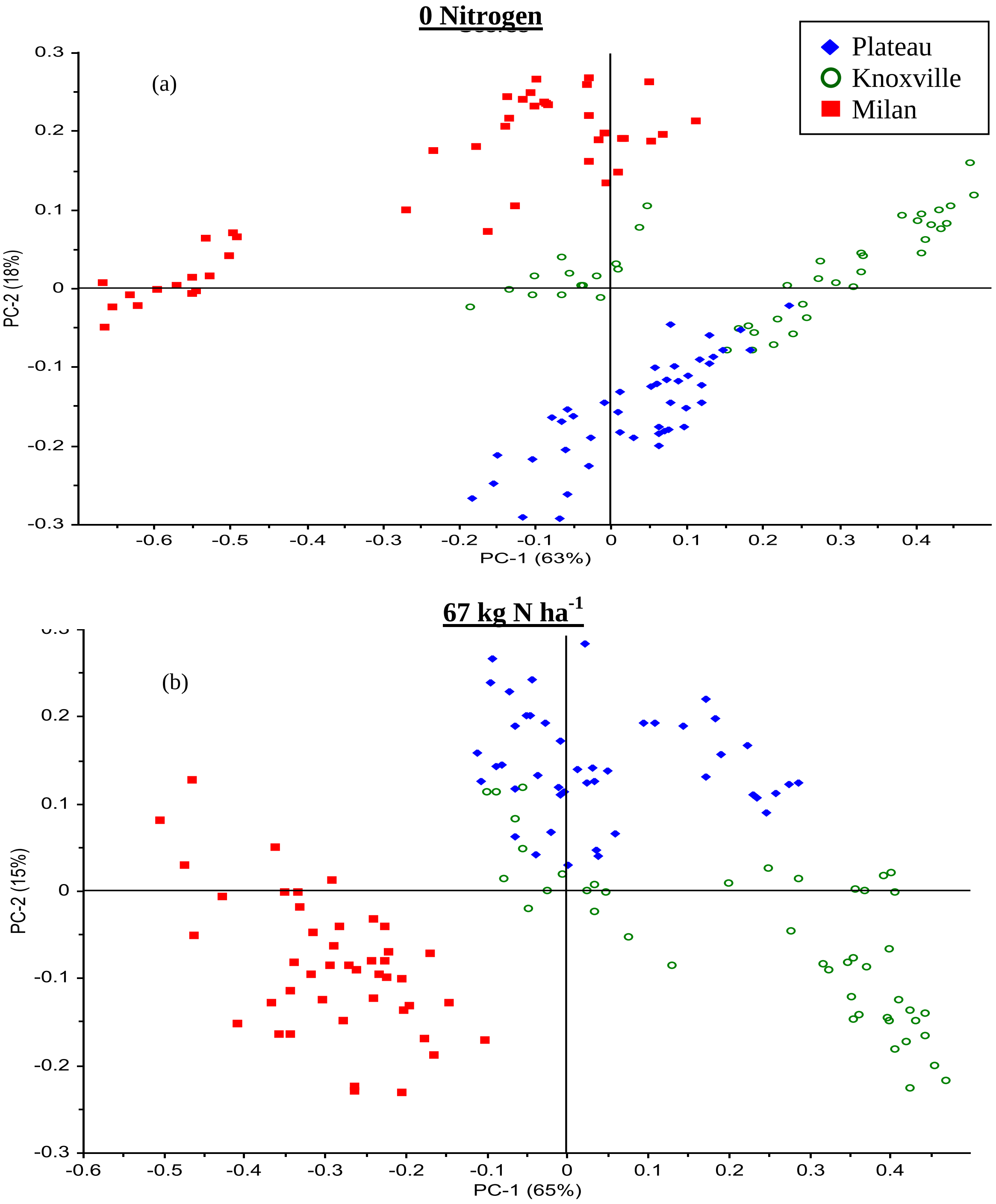
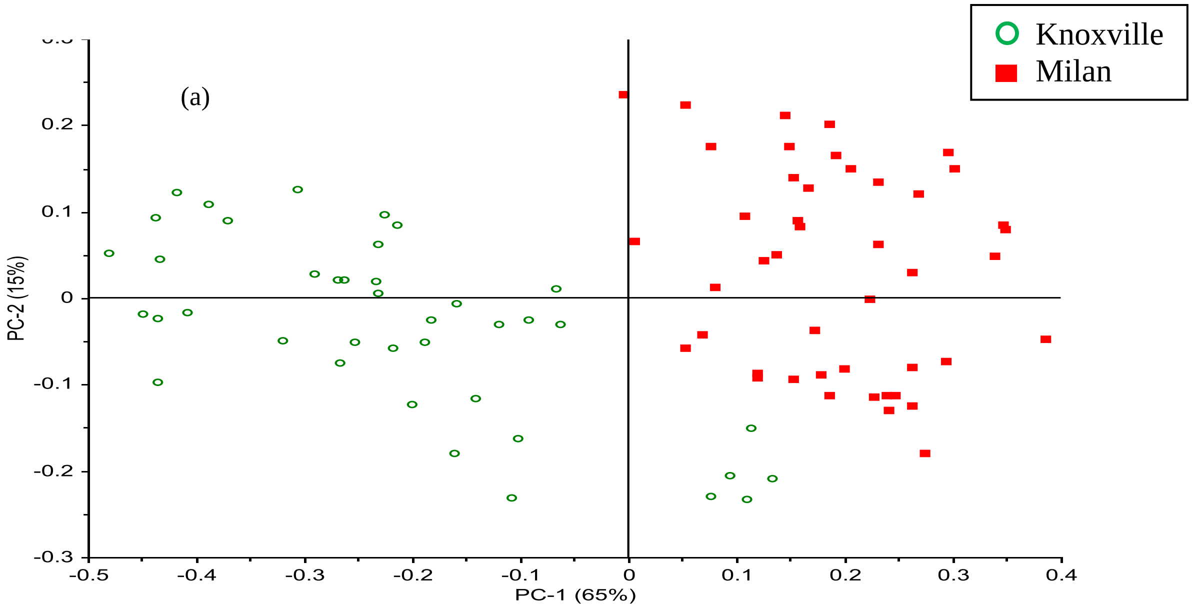
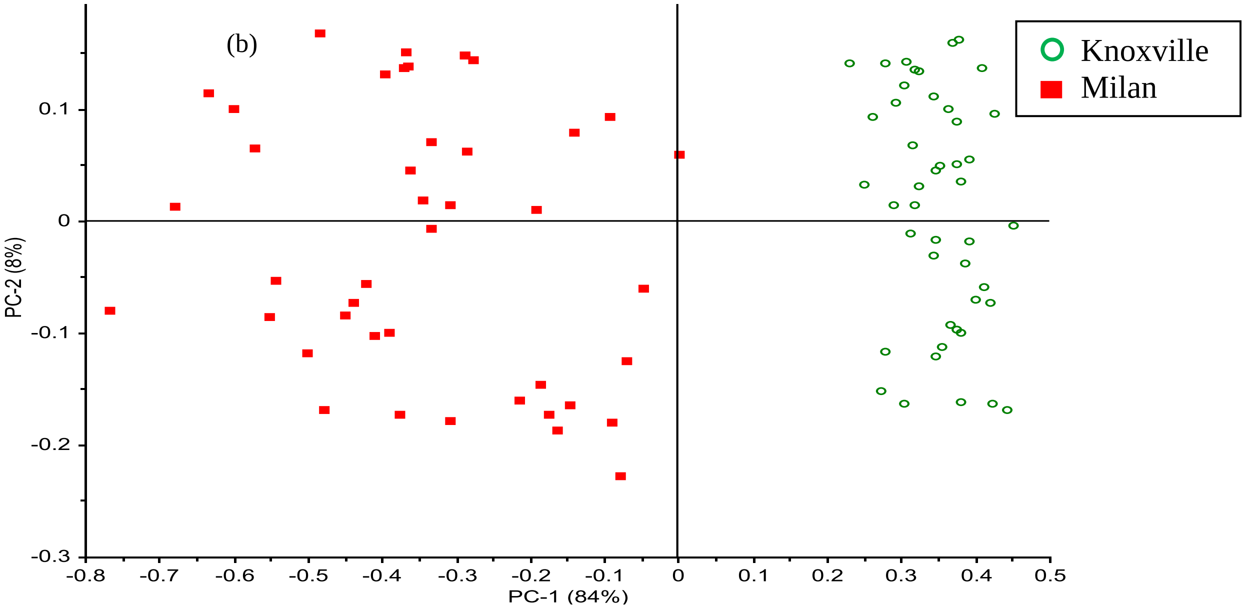


Figure 1.4 Scores plot of the first two principle components derived from NIR absorbance spectra of dried switchgrass samples of (a) 0 Nitrogen treatments and (b) 67 kg N ha^{-1} from East TN (ETREC), Plateau (PREC), and Milan (RECM) Research and Education Centers in 2009. PC1 explains the most variation in the data followed by PC2.



Red Clover treatments



Red Clover + 67 kg N ha^{-1}

Figure 1.5 Scores plot of the first two principle components derived from NIR absorbance spectra of dried switchgrass samples of (a) red clover and (b) red clover + 67 kg N ha^{-1} treatments from at East TN (ETREC) and Milan (RECM) Research and Education Centers in 2009. PC1 explains the most variation in the data followed by PC2.

Part II

Establishment and reseeding of cool and warm-season legumes into established stands of switchgrass.

Abstract

Interest in using legumes as a companion crop to switchgrass (*Panicum virgatum*) to provide an alternative to synthetic nitrogen fertilization is increasing. The objectives of this study were to: (1) develop efficient legume management strategies for switchgrass production systems, (2) evaluate and identify cool and warm-season legumes that can be grown compatibly with switchgrass, and (3) determine whether switchgrass yields are increased by legume N-fixation. Legumes can take advantage of annual switchgrass growth patterns and contribute to switchgrass biomass yields by fixing atmospheric nitrogen. Nine cool- and warm-season legume species were included. Cool-season legumes were alfalfa (ALF; *Medicago sativa*), arrowleaf clover (AC; *Trifolium vesiculosum*), red clover (RC; *Trifolium pretense*), hairy vetch (HV; *Vicia villosa)*, crimson clover (CC; *Trifolium incarnatum*), and crown vetch (CV; *Securigera varia*). Warm-season legumes were Illinois bundle flower (IBF; *Desmanthus illinoensis*), partridge pea (PP; *Chamaechrista fasciculata*), and trailing wild bean (TWB; *Strophostyles helvula*). Legume combinations included four legume species: common vetch (CmnV; *Vicia sativa*), CC, PP, and TWB, as follows: CmnV + PP, CC + PP, CmnV + TWB, and CC + TWB. RC, CC, and AC had the highest densities for both years with annual reseeding. Cool-season legumes, CC and CmnV, in combination with PP, had the best initial establishment after one year. Annual reseeding of RC (17.6 Mg ha^{-1}) and TWB (16.3 Mg ha^{-1}) increased ($P \leq 0.05$) switchgrass yields versus the control (13.4 Mg ha^{-1}) at in 2010. No differences in switchgrass yield were detected between years for the annual reseeding experiment or from the legume combinations after one year.

CHAPTER I

Introduction

Switchgrass (*Panicum virgatum*) is a warm-season perennial grass with excellent potential for producing biomass in the warm, dry summer months due to its C4 photosynthetic pathway (Cherney et al. 1991). Modest inputs are required for production once it has been established. One input for increased productivity is the application of nitrogen after the establishment year. N-fixing legumes may aid in fulfilling, at least in part, the 67 kg N ha^{-1} required for switchgrass (Garland et al, 2008). Grass-legume mixtures benefit switchgrass by taking advantage of the different seasonal growth patterns of the legumes that can improve seasonal distribution of grass forages. Many studies have been done on interseeding legumes into warm-season grasses to increase forage yields, but few included lowland switchgrass.

Legumes fix nitrogen, increase soil quality, and increase species diversity in an otherwise monoculture environment. This increase in plant species diversity helps to maintain stable year-to-year production, break disease cycles, and increase diversity in arthropod communities that may decrease pest populations that can harm monoculture crops (DeHaan et al., 2010; Tillman, 2000).

Legumes have been used extensively as cover crops and have ultimately increased the yield of subsequent crops (Ranells and Wagger, 1996; Tyler et al, 1987). In southeastern USA crop production, release of nitrogen from chemically desiccated legumes to a subsequent crop of corn improved N availability (Ranells and Wagger, 1997). An increase in nitrogen mineralization may be caused by the low C:N ratios found in soil after legume desiccation (Clark et al., 1994; Hargrove, 1986; Wagger, 1989; Ranells and Wagger, 1997). Production costs of legume establishment in no-till systems have been shown to influence the farmer's decision to plant legumes and reduce use of synthetic N (Larson et al., 2001). When vetch was used as a winter cover crop and an additional application of synthetic nitrogen was applied, corn yield under no-tillage production was maximized (Larson et al., 1998).

Annual switchgrass biomass yield in the upper southeastern USA average 15.9 Mg ha^{-1} (Lemus et al., 2009) and increase with the use of nitrogen fertilization. Nitrogen fertilization is recommended in switchgrass production systems at a rate of 67 kg ha^{-1} (Garland et al., 2008) or approximately one half the recommended rate for corn production (Sanderson et al. 1996). Crimson clover and hairy vetch can supply N to a successive crop (Holderbaum at al., 1990); while alfalfa may fix 82 to 254kg N ha^{-1} when grown with a companion grass (Heichel and Henjum, 1991). Corn yields when planted into chemically-killed vetch were similar to corn fertilized with 84 kg N ha^{-1} (Tyler et al., 1987).

Experiments have shown that alfalfa and birdsfoot trefoil may transfer a high proportion of N to a companion grass stand (Brophy et al., 1987). Legume-switchgrass mixtures were shown to produce more total-season, upper-canopy yield than a monoculture switchgrass field fertilized with 240 kg N ha^{-1} (George et al., 1995). According to Mallarino et al. (1990), average N derived from legumes in tall fescue (*Lolium arundinacea* Schreb) increased from 20% in the first year after seeding to 45-60% N in the following year. Experiments with legume-switchgrass stands including white and yellow sweet clovers (*Melilotus alba* Medik and *Melilotus officinalis* L., respectively), birdsfoot trefoil, RC, ALF, and HV found yields that exceeded those of N-only stands, even at N levels of 240 kg ha^{-1} (George et al., 1995; Gettle et al., 1996). HV and Persian clover (*Trifolium resupinatum*) grown simultaneously in grass swards, are also very effective in providing additional N to the grass crop (Opitz von Boberfeld et al., 2005). Legumes that are established and properly maintained may enhance switchgrass yields by contributing N to the soil environment after their desiccation.

Productive grass stands are dependent on seasonal growth habits, management, persistence, and level of maturity of legumes and how those factors may obstruct switchgrass early in its growth cycle (Blanchet et al., 1995; Posler et al., 1993). Conversely, a compatible, persistent warm-season grass and legume combination could significantly increase switchgrass yields. Companion species must be able to grow together simultaneously, not eliminate one another from the stand, and take advantage of their companion's growth patterns (Cherney et al., 1991). ALF, CV, HV, and RC legume establishment in 'Cave-In-Rock' switchgrass has been successful (Blanchet et al., 1995; George et al., 1995; Gettle et al., 1996). Successful establishment of legumes is dependent on grass and legume species competition. The 'Cave-In-Rock' cultivar of switchgrass is an upland variety that is typically less robust and shorter than lowland varieties of switchgrass such as 'Alamo'. Legumes cannot be sown into switchgrass during establishment year due to their competition with switchgrass (Berdahl et al., 2001).

Legume stand densities depended on seeding rates, date of seeding, weather after seeding, legume growing cycle, and switchgrass competition. In these studies, legume seeding rates were adjusted from pure stand rates for forage to reduce competition with switchgrass early in the season. Seeding dates for cool-season legumes are typically in early fall and the last two weeks in February through the end of March. Seeding dates for warm-season legumes range from late winter to the beginning of May depending on legume species. For these studies, legumes were seeded in late fall to allow for switchgrass harvest, due to inability to plant legumes into uncut, mature switchgrass stands. Legume seeding rates may need to be adjusted from pure stand rates to avoid legume stands that are so dense that they suppress switchgrass growth during the spring, an outcome more likely for early-seeded cool-season legumes.

Establishment of legumes, in this study, was based on stands densities in switchgrass. Published recommendations for pasture systems report that legumes should cover 30% of the ground area to fix sufficient amounts of nitrogen to eliminate the need for inorganic N fertilization in the spring (Bates, 1995). Blanchet et al., 1995, reported that alfalfa and hairy vetch had the highest percent establishment at 64% and red clover had 37% establishment in upland switchgrass.

The objectives of this study were to: (1) develop efficient legume management strategies for switchgrass production systems, (2) evaluate and identify cool and warm-season legumes that can be grown compatibly with switchgrass, and (3) determine whether switchgrass yields are increased by legume N-fixation. Annual seeding of nine cool- and warm-season legume species and combination using one cool- and one warm-season legume interseeded into switchgrass to take advantage of winter and early spring dormancy in switchgrass were evaluated for their ability to grow in established switchgrass stands.

Chapter II

Materials and Methods

Switchgrass Stands

‘Alamo’ variety switchgrass was planted in spring of 2007 at 9 kg ha^{-1} pure live seed at the East TN Research and Education Center (ETREC) in Knoxville (35.53° N -83.57° W). Field research was conducted at ETREC (Holston unit) on Huntington silt loam soil (fine-silty, mixed, active, mesic Fluventic Hapludolls). This site had an annual temperature and precipitation of 14.5°C and 142 cm, respectively, and was previously under hay management for the three years prior to the initiation of this experiment. Weeds were controlled by using hand cultivation and chemically sprayed with nicosulfuron {2-[[(4,6-dimethoxypyrimidin-2-yl)aminocarbonyl]aminosulfonyl]-N,N-dimethyl-3-pyridinecarboxamide} at a rate of 0.67oz ac^{-1} in 2009.

Legume Treatments

This study included two experiments. Legume species were selected based on their size, growth habits and cycles, persistence, and productivity.

Experiment #1:

Nine legumes were evaluated in a one-cut switchgrass biomass system that was reseeded every year and assessed legumes for survivability in switchgrass. The cool-season legumes were ALF, AC, RC, HV, CC, and CV. The warm-season legumes were IBF, PP, and TWB. Legume seeding rates were adjusted from pure stand rates for forage to reduce competition with switchgrass early in the season. These nine legumes provided a wide range of plant sizes, growth habits, and growth cycles.

Experiment #2:

A combination of one cool-season plus one warm-season species was sown together into switchgrass stands in a one-cut switchgrass biomass system. Cool and warm-season legumes were combined and planted simultaneously into switchgrass plots. Experiment #2 included four legume species: CmnV, CC, PP, and TWB. The legumes were in four different combinations of CmnV + PP, CC + PP, CmnV + TWB, and CC + TWB. The legumes were evaluated for establishment by determining plant densities and their effects on switchgrass yield for one growing cycle in 2010.

A randomized complete block design with a factorial arrangement of treatments was used in both experiments. In both experiments, treatments were replicated three times. Controls for both experiments were represented by a 0 kg N ha^{-1} treatment. A frequency grid was used to take legume stand densities on switchgrass plots (Vogel and Masters, 2001). The densities were averaged from the three replications of each legume treatment for each experiment to determine establishment.

Legume Establishment

The legumes were no-till drilled into switchgrass stubble at ETREC with a Hege™ plot drill during the fall (cool-season) and spring (warm-season) shown in Table 2.2 for two years in the same plots at a planting depth ranging from 0.6 to 1.3 cm. The plot size was 7.6 x 1.5 m with 18 cm row spacing. Legume seeding rates were 13.5, 11.2, 6.7, 6.7, 14, 6.7, 9, 13.5, 9, and 20.2 kg ha^{-1} for ALF, AC, CmnV, CC, CV, HV, IBF, PP, RC, and TWB (Table 2.1). Seed was adjusted to account for germination rates and hard seed. ALF, AC, CV, CC, and RC were inoculated. CmnV, HV, IBF, PP, and TWB were not inoculated. AC, CC, CV, HV, RC, and

TWB were planted on 20 October 2008. ALF, AC, CC, CmnV, CV, HV, IBF, PP, RC, and TWB were planted on 24 March 2009 and 29 October 2009.

Legume stand densities were taken annually following green-up in the spring with a 1 m^2 frequency grid (Vogel and Masters, 2001). Four density counts were taken on each legume treatment plot. Legume densities were then averaged from the three replications to determine average plant density and establishment for each legume treatment. Switchgrass heights were taken at each density count (n=4) and averaged for each plot.

Data Analysis

Harvests were implemented in 2009 and continued through 2010. Legume plots were harvested using a Carter™ harvester (Brookston, IN**)** with a 91 cm cutting width at 20.3 cm height. In both experiments, a single fall harvest was conducted after the switchgrass had gone dormant on 22 October 2009 and 8 November 2010 (Table 2.2). The harvest plot size was 7.6 x 6.9 m. Grab samples (1-2 kg) of switchgrass were collected from all plots at harvest, weighed, dried at 49^{o}C in a batch oven (Wisconsin Oven Corporation, East Troy, WI), and reweighed to determine moisture content.

Total dry matter switchgrass yields and legume establishment were analyzed for each plot. Switchgrass yield measurements were taken for all plots at time of harvest, and switchgrass dry matter yields were evaluated for significant differences across treatments. Switchgrass yields were analyzed using PROC Mixed with SAS v. 9.1.3 (SAS Institute, Cary, NC). Fixed effects were legume treatments, and replications were assigned as random effects. Tukey's mean separation analysis was used to control test treatment means for differences in switchgrass yield with alpha = 0.05. Soil core samples (15.2 cm depth) were taken from each plot. Soil samples were analyzed for phosphorus, potassium, calcium, and magnesium levels in the soil in 2010.

CHAPTER III

Results and Discussion

Legume Establishment

Experiment #1:

Legume plant densities ranged from 0 to 34 and 0 to 36 plants m^{-2} for annual reseeding of legumes in 2009 and 2010, respectively (Table 2.3). Legume density was greatest with AC (30), ALF (25), CC (32), and RC (34) in 2009 and RC (36), CC (26), AC (17), and PP (15) in 2010. Densities were least with HV (3), IBF (6), PP (9), and TWB (7) in 2009 and ALF (4), IBF (4), and TWB (5) in 2010. Legume densities of HV and PP increased over the two-year period. ALF densities declined, while HV and PP plant densities increased over the length of the study. IBF and TWB stands did not improve after two years. No CV plants germinated or were observed over the course of the study. Legume densities were taken on 13 May 2009 and 27-28 May 2010. May switchgrass heights increased from 73 cm in 2009 to 116 cm in 2010.

Experiment #2:

The combination of cool-season legumes, CC and CmnV with warm-season legume PP, had the highest plant densities (Table 2.4). The CC (8 plants m^{-2}, 36 cm) and PP (10 plants m^{-2}, 26 cm) combination had a switchgrass height average of 110 cm. The CmnV (6 plants m^{-2}, 40 cm) and PP (9 plants m^{-2}, 2 cm) combination had a switchgrass height average of 86 cm. The CC (9 plants m^{-2}, 36 cm) and TWB (2 plant m^{-2}, and 16 cm) combination and the CmnV (9 plants m^{-2}, 52 cm) and TWB (0 plants m^{-2}) combination had very low densities and switchgrass heights of 87 and 86 cm, respectively. Legume densities were taken on 27-28 May 2010. Warm-season legumes may have had decreased germination due to seeding in late fall with cool-season legumes.

Increased legume density, growth, and nitrogen fixation rates may be achieved by increasing seeding rates and inoculation of seed with species-specific rhizobia. HV, IBF, PP, and TWB were not inoculated and may have affected legume densities (Table 2.1). Legume densities were difficult to take after switchgrass began rapid growth in early summer. Time when legume densities were taken may have an effect on reported stand densities when looking at the growth cycles of some of the warm-season legumes. Increase in switchgrass height can rapidly overtake and shade out legume species that are slow growing and/or not yet mature by the time switchgrass reaches early elongation stage (Moore et al., 1991).

Biomass Yields

Experiment #1:

Switchgrass yields for legume treatments in 2009 ranged from 14.4 to 18 Mg ha^{-1} and were not different. Switchgrass yields showed differences between the control (11.6 Mg ha^{-1}) and legume treatments of RC (17.2 Mg ha^{-1}) and TWB (16.3 Mg ha^{-1}) in 2010 (Table 2.5). No differences were found on switchgrass yields among the other legume treatments in 2010. Between years 2009-10, switchgrass legume yields ranged from 14.3 to 17.6 Mg ha^{-1} and were not different from the control treatment (13.4 Mg ha^{-1}).

Bow et al. (2008) found that switchgrass interseeded with AC and CmnV outyielded grass-only plots in the second year of growth. Arrowleaf clover was found to have a fertilizer N equivalence of 50 kg N ha^{-1} to rye (S*ecale cereale L.*) (Lynd et al., 1984), while CC and HV contributed an average of 39 and 102 kg N ha^{-1} to rye in a year (Ranells and Wagger, 1996). RC transferred an average of 28 kg N ha^{-1} per year to tall fescue (Mallarino et al., 1990). In this study, switchgrass yields were not different from control plot yields. Increasing legume seeding rates in switchgrass stands should supply more available nitrogen to the grass and enhance yields.

Experiment #2:

Switchgrass yields did not show differences between any of the legume combination treatments after one year, with a range of 9.7 to 12.4 Mg ha^{-1}in switchgrass yield (Table 2.4). No comparative literature of switchgrass yields was found on legume combinations interseeded into switchgrass stands.

CHAPTER IV

Conclusions

Nitrogen fixing legumes may be a good alternative to inorganic nitrogen fertilization of switchgrass. Arrowleaf clover, crimson clover, partridge pea, and red clover had the highest plant densities after annual reseeding. Cool-season legumes crimson clover and common vetch in combination with warm-season partridge pea had the highest plant densities after one year. Annual reseeding of red clover (17.2 Mg ha^{-1}) and trailing wild bean (16.3 Mg ha^{-1}) had effects on yield of switchgrass when compared to the control treatment (11.6 Mg ha^{-1}) in 2010 ($P \leq 0.05$). No other differences were seen among annual reseeding treatments from 2009 to 2010. Legume combinations showed no differences on switchgrass yields after one year, but combinations of cool-season legumes, common vetch and crimson clover, and warm-season legume partridge are good prospects for additional research. Proper legume management needs to be developed that address legume varieties compatible with switchgrass, seed inoculation, seeding dates and rates, and rate of competition with switchgrass.

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APPENDIX B

Table 2.1. Legume species variety, seeding rates, and seed inoculation used in Experiments #1 and #2 for 2009 and 2010.

Legume Species	Variety	Experiment	Seeding Rate	Inoculated Seed	Recommended Rhizobia Species
			---kg ha^{-1}---		
Alfalfa	Evermore	1	13.5	Yes	*R. meliloti*
Arrowleaf Clover	Apache	1	11.2	Yes	*R. trifolii*‡
Common Vetch	VNS†	2	6.7	No	*R. leguminosarum*
Crimson Clover	VNS	1 & 2	6.7	Yes	*R. trifolii*
Crown Vetch	Penngift	1	14.0	Yes	*Rhizobium spp.*
Hairy Vetch	VNS	1	6.7	No	*R. leguminosarum*
Illinois Bundle Flower	VNS	1	9.0	No	*R. Desmanthus illinoensis*
Partridge Pea	VNS	1 & 2	13.5	No	*R. leguminosarum*
Red Clover	Cinnamon Plus	1	9.0	Yes	*R. trifolii*
Trailing Wild Bean	Tamu-H	1 & 2	20.2	No	no data found

†VNS= Variety not stated

‡Arrowleaf clover requires specific inoculum and will not cross inoculate with other clover species (Jennings, 2005; Lacefield et al., 2002).

Table 2.2. Summary of seeding and harvest dates at the East TN (ETREC) Research and Education Center for Experiments #1and #2.

	Experiment One			**Experiment Two**		
	Seeding		**Harvest**	**Seeding**		**Harvest**
	Cool†	Warm‡	Biomass	Cool	Warm	Biomass
2008	20-Oct	-	-	-	-	-
2009	24-Mar§ 29-Oct	24-Mar 29-Oct	22-Oct	29-Oct	29-Oct	-
2010	-	-	8-Nov	-	-	8-Nov

†Cool-season legume plantings included: alfalfa, crimson clover, hairy vetch, and red clover.

‡Warm-season legume plantings included: Illinois bundle flower and partridge pea.

§ In 2009, cool-season legumes were replanted in March because of poor emergence from fall seeding in 2008

Table 2.3. Average[†] legume (LG) plant densities[‡] and heights and switchgrass (SG) heights at the East TN (ETREC) Research and Education Center in Experiment #1 (annual reseeding) taken on 13 May 2009 and 27-28 May 2010.

	2009			2010		
	Plant	Height		Plant	Height	
Treatment	Density	LG	SG	Density	LG	SG
	No. m^{-2}	---------cm---------		No. m^{-2}	---------cm---------	
Arrowleaf Clover	30	3	74	17	50	114
Alfalfa	25	3	75	4	26	118
Crimson Clover	32	11	81	26	54	119
Crown Vetch	0	0	74	0	0	114
Hairy Vetch	3	10	68	12	76	120
IL Bundle Flower	6	3	73	4	4	121
Partridge Pea	9	8	76	15	12	118
Red Clover	34	9	71	36	61	116
Trailing Wild Bean	7	8	73	5	33	113
0 Nitrogen Control	-	-	65	-	-	110

†Means across treatments and replications

‡Plant density = (frequency of occurrence * 0.4) x 100(Vogel and Masters, 2001)

Table 2.4. Average[†] legume (LG) plant densities[‡] and heights and switchgrass (SG) heights and yields[‡] at the East TN (ETREC) Research and Education Center in Experiment #2 (legume combination) in 2010.

	2010								
	Crimson Clover (CC) & Partridge Pea (PP)		**Crimson Clover (CC) & Trailing Wild Bean (TWB)**		**Common Vetch (CmnV) & Partridge Pea (PP)**		**Common Vetch (CmnV) & Trailing Wild Bean (TWB)**		**0 Nitrogen**
	Plant Density	Height	Plant Density	Height	Plant Density	Height	Plant Density	Height	Height
	No. m^{-2}	----cm----	No. m^{-2}	----cm----	No. m^{-2}	----cm----	No. m^{-2}	----cm----	----cm----
CC	8	36	9	36	-	-	-	-	-
Cmn V	-	-	-	-	6	40	9	52	-
PP	10	26	-	-	9	2	-	-	-
TWB	-	-	2	16	-	-	0	0	-
Total LG	18	-	11	-	15	-	9		
SG ht		110		87		86		100	110
SG yield Mg ha^{-1}		12.0a		9.7a		12.4a		14.2a	11.6a

†Means across treatments and replications

‡ Plant density = (frequency of occurrence * 0.4) x 100(Vogel and Masters, 2001). Density were taken on 27-28 May 2010.

§ Mean separations based on Tukey’s at P≤0.05 apply to rows across treatments.

Table 2.5. Average[†] dry matter yields (Mg ha^{-1}) of switchgrass per legume treatment with annual reseeding in a one-cut biomass system at East TN (ETREC) Research and Education Center from 2009-10.

	Annual Reseeding		
Treatment	**2009**	**2010**	**2009-10**
	--------------------Mg ha^{-1}--------------------		
Alfalfa	16.2a	14.2ab	15.2a
Arrowleaf Clover	17.5a	15.6ab	16.1a
Crimson Clover	16.8a	14.1ab	15.5a
Crown Vetch	14.4a	14.1ab	14.3a
Hairy Vetch	15.2a	15.6ab	15.4a
IL Bundle Flower	16.1a	13.9ab	15.0a
Partridge Pea	18.0a	15.8ab	16.9a
Red Clover	18.0a	17.2a	17.6a
Trailing Wild Bean	16.4a	16.3a	16.3a
Control	15.2a	11.6b	13.4a

†Mean separations apply to columns within a given year followed by a common letter are not statistically significant based on Tukey’s at P≤0.05.

‡nd=no data taken

Part III

Estimation of nitrogen fixation rates of common versus hairy vetch and seeding rates needed for interseeding into established switchgrass stands

Abstract

Interest in alternative sources to synthetic N fertilizer in switchgrass (*Panicum virgatum*) production continues to grow. Nitrogen fixing legumes interseeded into switchgrass may be one alternative. The objectives of this study were to: (1) develop efficient legume management strategies for switchgrass production systems, (2) determine whether switchgrass yields are increased by legume N-fixation, and (3) determine N-fixation of common (*Vicia sativa*) and hairy vetch (*Vicia villosa*). Vetch stand densities were estimated to supply 67 kg ha^{-1}, the recommended rate of N fertilizer for switchgrass biomass production (Garland et al., 2008). Common vetch is a N-fixing legume that occurs naturally throughout the Southeast, as well as other parts of the U.S. It has less hard seed than hairy vetch, making it less of a weed problem, and may fix N at similar rates to hairy vetch. In this study, N-fixation rates via N-difference method were determined to be 59.3 and 43.3 kg N ha^{-1} for aboveground plant matter, assuming a bioavailable rate of 50%, at a seeding rate of 6.7 kg PLS ha^{-1}for common and hairy vetch, respectively based on reported plant densities. A seeding rate of 6.7 kg ha^{-1} of common and hairy vetch did not affect 'Alamo' switchgrass yields after one year. Based on the N fixation rates and vetch plant densities determined in this study, we estimate that seeding rates of 7.6 and 10.4 kg PLS ha^{-1} for common and hairy vetch will be required to obtain plant densities needed to fix the current recommended rate of 67 kg N ha^{-1} for switchgrass biomass production.

CHAPTER I

Introduction

Legumes are agronomically beneficial because they fix nitrogen through a symbiotic relationship with rhizobia. Rhizobia form nodules on the plant root. Inside the nodules, the bacteria convert dinitrogen (N_2) from the atmosphere or soil pore space into ammonium (NH_4^+), a form that plants can uptake through the roots (Graham, 2005). The plant supplies the bacteria with nutrients needed to carry out growth and N-fixation. This beneficial plant-bacteria relationship enhances the soil quality by increasing soil N by plant matter decomposition and reduces the amount of synthetic nitrogen fertilizer applied to switchgrass crops.

Biological nitrogen fixation (BNF) is directly affected by the amount of available nitrogen in the soil. In the long term, a cycle occurs. BNF by legumes leads to accumulation of soil N, companion grass dominance, and then a reduction of nitrogen fixation as soil nitrogen increases (Ledgard and Steele, 1992). Decay of legumes may not be in sync with the peak demand of N by the main crop (Larson et al., 2001). N-fixation of legumes can be affected by weather, available nutrients, and inorganic nitrogen in the soil from fertilizer during dry conditions (Larson et al., 2001; Ledgard and Steele, 1992).

Annual switchgrass biomass yield averages in the upper Southeast are 15.9 Mg ha^{-1} (Lemus et al., 2009), and yields can increase with the use of nitrogen fertilization. Nitrogen fertilization is recommended in switchgrass production systems at a rate of 67 kg ha^{-1} (Garland et al., 2008) or approximately one half the recommended rate for corn production (Sanderson et al. 1996).

Legumes interseeded into switchgrass may be able to supply the N required for biomass production. Experiments have shown that alfalfa (*Medicago sativa*) and birdsfoot trefoil (*Lotus corniculatus* L.) may transfer a high proportion of N to a companion grass stand (Brophy et al., 1987) and alfalfa may fix 82 to 254kg N ha^{-1} when grown with a companion grass (Heichel and Henjum, 1991). Legume-switchgrass mixtures were shown to produce more total-season upper canopy yield than a monoculture switchgrass field fertilized with 240 kg N ha^{-1} (George et al., 1995) and have produced yields equivalent to rye (*Secale cereale* L.) fertilized with 50 to 121 kg N ha^{-1} (Lynd et al., 1984).Experiments with legume-switchgrass mixtures including white and yellow sweet clovers (*Melilotus alba* Medik and *Melilotus officinalis* L.), birdsfoot trefoil, red clover (*Trifolium pretense* L.), alfalfa, and hairy vetch found yields that exceeded those of N-only stands, even at N levels of 240 kg ha^{-1} (George et al., 1995; Gettle et al., 1996). Tyler et al. (1987) reported that corn yields when planted into chemically killed vetch were similar to corn fertilized with 84 kg N ha^{-1}. Hairy vetch and Persian clover (*Trifolium resupinatum*) grown simultaneously in grass swards, are also very effective in providing additional N to the grass crop (Opitz von Boberfeld et al., 2005). According to Mallarino et al. (1990), average N derived from legumes in tall fescue (*Lolium arundinacea* Schreb) increased from 20% in the first year after seeding to 45-60% N in the following year.

Common and hairy vetch can fix nitrogen required for a single-cut biomass system by increasing N in the soil for a subsequent crop (Ranells and Wagger, 1997). Hairy and common vetch cover crops have been reported to contain between 50 to 350 kg N ha^{-1} and 25 to 190 kg N ha^{-1}, respectively in above ground growth (Holderbaum et al., 1990; Ranells and Wagger; 1997). Availability of N supplied by hairy vetch to succeeding crops has been shown to be dependent on climatic conditions such as heavy precipitation, and can be difficult to predict (Cook et al., 2010).

Many methods are used to determine biological nitrogen fixation including N balance, N difference, acetylene reduction, hydrogen evolution, and ^{15}N isotope techniques. Each technique has advantages and disadvantages. Acetylene reduction and hydrogen evolution should be performed in a controlled environment with a sealable container or an incubation vessel for optimal results and are unsuitable for the assessment of field grown legumes (Myrold et al, 1999; Minchin et al., 1994; Peoples et al., 2009). Acetylene reduction uses gas chromatography to detect very low levels of nitrogenase activity in an assay (Peoples et al., 2009; Vessey, 1994; Zuberer, 2005) and may best be used in conjunction with other techniques (Unkovich and Pate, 2000). Hydrogen evolution indirectly measures nitrogenase activity by measuring H_2 concentration from a nodulated root system in a contained environment, which makes it unsuitable for quantifying N^2 fixation of field-grown plants (Peoples et. al, 2009). Errors in calculating N-fixation can occur when soils are high in plant available N, soil moisture content is less than optimal, or by applying fertilizers (Ledgard and Steele, 1992).

The ^{15}N isotope dilution (ID), ^{15}N natural abundance, nitrogen balance, and nitrogen difference are examples of methods suitable for experiments in the field. ID is an effective field measurement technique that requires adding ^{15}N labeled fertilizer. This method may be difficult to track due to different legume species and control plant ^{15}N uptakes levels (Danso et al., 1993; Unkovich and Pate, 2000). The addition of ^{15}N into the soil N environment may have a negative impact on legume rhizobia decreasing the rate of N fixation (Unkovich and Pate, 2000). The isotope ^{15}N natural abundance method utilizes the small, natural enrichment of ^{15}N present in most soils and is similar to the ID method. The difference is that no additional ^{15}N material is used and comparison species do not need similar uptake characteristics (Ledgard and Steele, 1992; Unkovich and Pate, 2000; Zuberer, 2005). The nitrogen balance method works only if all possible inputs and outflows can be accounted for. A problem encountered with these methods is that losses of N through NH_3 volatilization, denitrification, leaching, runoff, and erosion can be difficult to measure (Peoples et. al, 2009).

The nitrogen difference method estimates the amount of N taken up by the legume from soil by comparing the N-fixing legume to neighboring non-fixing control plants. The N-difference method is a simple and inexpensive method that works best under low soil N conditions and when legume fixation levels are high (Danso, 1995; Peoples et al., 2009; Zuberer, 2005). Some disadvantages of the N-difference method include indistinguishable N transfer due to other factors that may increase dry matter yield. Another potential limitation with the N-difference method is the assumption that both legume and control plants absorb equal amounts of soil N for growth (Rennie and Rennie, 1983; Segundo and Boddey, 1987; Ledgard and Steele, 1992) and that plant sizes and/or root morphologies do not differ (Danso et al., 1992; Boddey et al., 1984) resulting in different capacities to exploit soil N (Chalk, 1998; Peoples et al., 2009). Extreme variations of fixation can be seen among different legume species and their physical environment such as rainfall, pH, and inoculation (Graham, 2005; Schultz et al, 1999; Unkovich and Pate, 2000). Significant statistical differences in fixation may only be seen when levels reach a rate of 20 kg N ha^{-1} (Weaver and Danso, 1994; Weaver, 1986; Zuberer, 2005). It has been shown that estimates obtained by the N-difference method are comparable to estimates obtained from more expensive and sophisticated techniques (Phillips et al, 1983; Bell et al, 1994).

Seeding rates for interseeding of legumes into lowland, production switchgrass stands are not well defined. Rates used for previous studies into upland switchgrass have been for frost-seeding into grass pastures, and a reduction of those rates was recommended (Gettle et al., 1996). Legume seeding rates need to be developed to establish persistent legume stands that increase nitrogen availability without competing for space and other resources of switchgrass.

Common and hairy vetches are cool-season legumes that have their growing cycle during switchgrass fall-winter dormancy through spring green-up. It was been reported that hairy vetch can fix between 96 to 149 kg N ha^{-1} per Mg of dry matter (Clark, 1995; Mueller and Thorup-Kristensen, 2001). The legume symbiotic relationship and its interaction with the soil environment will be measured via comparison of switchgrass dry matter yields to help determine the effectiveness of nitrogen fixation by the legume (Unkovich and Pate, 2000).

Several potential advantages of using common vetch in place of hairy vetch can be identified. Common vetch is frequently found growing throughout the Southeast and U.S. on roadsides and in fields and pastures as an escape from cultivation (UC SAREP, 2006). Common vetch typically has less hard seed than most varieties of hairy vetch (Matic et al., 2009; Sattell et al., 1998; Hanaway and Larson, 2004). Hairy vetch seed can range between 5 and 30% hard seed, last 5+ years in the soil, and become a serious weed pest in annual crop rotations (Hanaway and Larson, 2004; Myers and Underwood, 1990; Sattell et al., 1998). Common and hairy vetch N-fixation rates are similar at 134 to 142 kg N ha^{-1} and 174 to 238 kg N ha^{-1}, respectively

(Peoples and Baldock, 2001; Peoples et al., 2001; Rochester et al., 1998; Rochester and Peoples, 2005).

Legume plant densities can be affected by date of legume seeding, weather after seeding, soil nutrient levels, and switchgrass competition. Seeding dates for cool-season legumes are typically in early fall and the last two weeks in February through the end of March. Seeding dates for warm-season legumes range from late winter to the beginning of May depending on legume species. In this study, legumes could not be planted into uncut, mature switchgrass stands and thus were not seeded until late fall to allow for switchgrass biomass harvest. Late seeding, combined with harsh weather conditions, may have influenced survival rates of immature vetch seedlings.

The objectives of this study were to: (1) develop efficient legume management strategies for switchgrass production systems, (2) determine whether switchgrass yields are increased by legume N-fixation, and (3) determine N-fixation of common (*Vicia sativa*) and hairy vetch (*Vicia villosa*).

CHAPTER II

Materials and methods

Switchgrass Stands

'Alamo' variety switchgrass was planted in spring 2007 at 9 kg ha^{-1} pure live seed at two Research and Education Centers in Tennessee: East Tennessee (ETREC) in Knoxville, TN (35.53^{o} N -83.57^{o} W) and Plateau (PREC) in Crossville, TN (36.1^{o} N -85.8^{o} W). ETREC (Holston unit) had soil classified as Huntington silt loam soil (fine-silty, mixed, active, mesic Fluventic Hapludolls), annual precipitation average of 124 cm, and an annual temperature of 14.4^{o}C. PREC (Grasslands Unit) had soil classified as Lily silt loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludults), annual precipitation average of 140 cm, and an annual temperature of 12.6^{o}C.

Legume Establishment

Common and hairy vetch were seeded in fall 2009 into established 3 yr old switchgrass stands of 'Alamo' at two locations (ETREC and PREC). At ETREC and PREC, the legumes were seeded into approximately 20 cm switchgrass stubble on 22 & 29 October 2009 at PREC and ETREC, respectively, with a Hege™ plot drill (Colwich, KS) at a planting depth ranging from 0.6 to 1.3 cm. At ETREC and PREC, the plot sizes were 7.6 x 1.5 m and 7.6 and 1.8 m, respectively, with 18 cm spacing between rows. The seeding rate for both common and hairy vetch was 6.7 kg ha^{-1} and the control was represented by a 0 kg N ha^{-1} treatment.

The seed used for common vetch were collected from volunteer populations at ETREC (Holston and Plant Science Units) in early summer 2009 and treated to break dormancy by stratification and scarification. Seed collected from the Plant Sciences Unit was divided into two lots. Lot one was dried at room temperature (approximately 25^{o}C), and lot 2 was dried at 49^{o}C in a batch oven (Wisconsin Oven Corporation, East Troy, WI). Seed collected from the Holston Unit were dried at room temperature (lot 3). The three seed lots were treated for dormancy by dry cold stratification in a cooler at an average of 8^{o}C for 1-6 weeks plus a control treatment resulting in 21 treatments (7 treatments x 3 lots) including controls. The stratified vetch was then seeded into sand trays in a greenhouse for germination testing (Table 3.1).

Common vetch seed from the Holston Unit was treated for dormancy by physical and chemical scarification with 10 different treatments (Table 3.2). The treatments included a control, physical scarification with 100 grit sandpaper (0.5 kg for 30 sec, 0.5 kg for 1 min, 0.7 kg for 30 sec, 0.7 kg for 1 min, 0.9 kg for 30sec, 0.9 kg for 1min), 3% bleach (sodium hypochlorite) for 10 minutes, 98% sulfuric acid (H_2SO_4) for one minute, and 1% hydrogen peroxide (H_2O_2) treatment for 24 hours. Physical scarification with sandpaper was applied with sandpaper attached to wood boards while using a weigh scale to indicate the required pressure.

The scarified common vetch was then seeded into sand trays in a greenhouse for germination testing. The sulfuric acid seed treatment showed the best rate of germination (Table 3.2). As a result, the remaining bulk of common vetch seed was treated with sulfuric acid (98% for 1 min), rinsed for 15 minutes, force air-dried for 10 minutes, and was direct seeded into switchgrass plots.

A frequency grid (Vogel and Masters, 2001) was used to take legume stand densities on switchgrass plots interseeded with legumes in early summer to allow time for legume growth. Four density counts were taken on each legume treatment plot. Plant densities were averaged from three replications at each location to determine plant densities m^{-2}. Switchgrass heights were taken at each density count (n=4) and averaged for each plot.

Switchgrass Yields

Two harvest systems were used in the experiments. A single, post-dormancy harvest was used at ETREC on 08 November 2010. A two-cut harvest system was harvested with cuts at early-boot stage on 09 June 2010 and post-dormancy of switchgrass at PREC on 21 October 2010. The switchgrass plots were harvested at ETREC & PREC using a Carter™ plot harvester (Brookston, IN). Harvested plot size was 0.9 x 7.6 m area at a 20 cm height. Grab samples (1-2 kg) of switchgrass were collected from all plots at harvest, weighed, dried in a batch oven at 49^{o}C, and weighed again to determine moisture content.

Nitrogen Fixation

Nitrogen content of common and vetch plants at ETREC, Plant Sciences Unit, were compared to wheat and wild barley (monocots) and broadleaf weeds: wild geranium, mare’s tail, and Venus looking glass (dicots). At the Holston Unit, common and hairy vetch plants were compared to switchgrass, wild barley, daisy fleabane, wild geranium, mare’s tail, and Venus looking glass.

Nitrogen fixation of common and hairy vetches was determined by using the N-difference method because it is a simple and inexpensive method that works well in field conditions with low soil N levels (Danso, 1995; Peoples et al., 2009). Shoot samples of common vetch, hairy vetch, and non-N-fixing control plants including wheat (*Triticum spp*.), switchgrass, wild geranium (*Geranium spp*.), barley (*Hordeum murinum* ssp. Leporium), mare’s tail (*Conyza canadensis*), Venus looking glass (*Tridanis perfoliata*), and daisy fleabane (*Erigeron strigosus*) were gathered by cutting the plants flush with the soil with pruning shears in late spring, 2010. All species listed above were in their reproductive stages, with the exception of switchgrass and mare’s tail in their vegetative state. Control plant sizes were all larger than vetch plants with the exception of Venus looking glass plants. Samples were weighed, oven dried, reweighed, and ground with a 1 mm sieve through a Wiley mill (Thomas Scientific, Swedesboro, N.J.). Samples were then analyzed for N content by UT Soil, Plant, & Pest Center (Nashville, TN).

Plant aboveground N was determined by multiplying plant dry matter by its percent N content. Control plant N (wheat and/or other non-nitrogen fixing plants) was then subtracted from legume plant N (vetch) and divided by 100 to obtain legume “fixed” N {(Legume N-Control Plant N)/100} (Danso, 1995; Peoples et al., 2009) (Tables 3.3 & 3.4). The N-difference of the vetch from the control plants was then multiplied by the average plant weights of common and hairy vetch plants found in May 2010 to achieve the aboveground N per vetch plant. Wheat and vetch were both fall-planted crops. Vetch plants were compared to wheat and other control plants that were harvested in late May 2010. Previous reports have stated that below ground plant matter may provide as much as 50% of plant N after decomposition (Peoples and Baldock, 2001; Jorgensen and Ledgard, 1997; McNeill et al., 1997).

Estimated seeding rates for common and hairy vetch to fix the recommended rate of 67 kg ha^{-1} N fertilizer for switchgrass were obtained from N-fixation rates determined by the N-difference method in this study (Table 3.5). Total vetch aboveground plant N m^{-2} was calculated by multiplying by the average plant density, found in 2010 when vetch was planted at a seeding rate of 6.7 kg PLS ha^{-1}, times the aboveground vetch plant N. The total aboveground plant N m^{-2} was then converted to kg ha^{-1} and divided by 2 to account for 50% bioavailability. To achieve the seeding rate required for vetch to supply 67 kg N ha^{-1} to the grass crop, the target N level was divided by the bioavailable vetch N developing a ratio to multiply by the current seeding rate (6.7 kg PLS ha^{-1}) that would give the suggested seeding rates of common and hairy vetch.

Data Analysis

Switchgrass yields and common vetch seed germination following chemical and physical scarification treatments were analyzed using PROC Mixed with SAS v. 9.1.3 (SAS Institute, Cary, NC). Tukey's analysis was used to test treatment means for differences in switchgrass yields and seed germination rates with alpha = 0.05. Fixed effects were legume and seed treatments, and locations and replications were assigned as random effects.

CHAPTER III

Results and Discussion

Seed Treatment

Cold storage seed stratification and seed scarification were used to determine an effective way to break dormancy for seed germination of common vetch. Stratification by seed chilling for 1 to 6 weeks did not increase seed germination in this study (Table 3.1). Germination rates for oven dried seed averaged only 2%, while air dried seed averaged 7%. This could indicate negative effects from high temperatures or decrease in seed moisture content from oven drying. A chilling temperature of 8^oC at 41% relative humidity did not show increased responses in common vetch germination in this study. Other studies involving seed chilling at constant temperatures have not shown accelerated responses in legume seed germination, but increased germination in spring has been achieved by moderate winter temperatures (Van Assche et al, 2003). Hairy vetch germination has been shown to improve by a small percentage when subjected to warmer temperatures (Van Assche et al, 2003).

Mechanical and chemical seed scarification treatments did result in differences in germination (Table 3.2). The sulfuric acid treatment resulted in 40% germination and was higher than all other treatments except the sandpaper treatment of 0.7 kg of pressure for one minute at 31% germination ($P \leq 0.05$). Ortega-Olivencia and Devesa (1997) found that sulfuric acid treatments of 15 and 30 minutes produce 100 % germination rates of vetch seed. Hydrogen peroxide, sodium hypochlorite (bleach), 0.5 kg of pressure for 1 minute, and 0.7 kg of pressure for 30 seconds treatments were not different from the control treatment (Table 3.2). Stronger solutions or longer soaking times may have been needed for the hydrogen peroxide and bleach treatments to become effective. Hydrogen peroxide, bleach, and physical scarification with sandpaper are safer alternatives to sulfuric acid and should be considered further.

Legume Establishment

Legume densities were calculated to determine the level of vetch establishment at each location. Establishment of common and hairy vetch for seeding rates of 6.7 kg ha^{-1} at ETREC were 10 and 7 plants m^{-2}, respectively on 12 & 13 May. At PREC, legume densities were 7 and 0 plants m^{-2}, respectively on 25 May 2010 (Table 3.6). Switchgrass heights were taken on the same dates as legume densities.

Small and none-existent vetch densities at PREC could have been caused by poor quality soil nutrient levels. Soil nutrient values of phosphorus and potassium levels were considerably lower at PREC compared to the ETREC location (Table 4.10, pp. 128). Switchgrass plots had no additional soil amendments added during this study. Proper management strategies would include a soil test before the incorporation of legumes into switchgrass stands.

Seeding rates of common and hairy vetch were lowered from the pure stand rates of 33.6 kg ha^{-1} used for forage (Bates et al., 2008) to 6.7 kg ha^{-1}, reducing competition with switchgrass early in the season. Initial seeding of hairy vetch at pure stand rates outcompeted and substantially reduced switchgrass growth early in the season (data not shown).

Based on the nitrogen fixation rates determined in this study, estimated seeding rates of 7.6 and 10.4 kg PLS ha^{-1} for common and hairy vetch, respectively, would provide sufficient plant densities to positively enhance N-fixation and increase switchgrass yields. Blanchet et al., 1995, reported that alfalfa and hairy vetch had the highest percent establishment (64%) in ‘Cave-in-Rock’ switchgrass.

Nitrogen Fixation

Nitrogen fixation rates of common and hairy vetches above-ground plant matter, using wheat as the control plant, were similar at 59.3 and 43.3 kg N ha^{-1} based on seeding rates of 6.7 kg ha^{-1} and plant densities of 8.5 and 7 plants m^{-2} (Table 3.6). Hairy vetch has been shown to supply 90 to 150 kg N ha^{-1} to subsequent crops (Hargrove, 1986; Ebelhar et al, 1984; Peoples et al., 1995; Ranells and Wagger, 1996). Common vetch has the potential to supply 106 to 146 kg N ha^{-1} to subsequent crops (Hargrove, 1986; Peoples et al., 1995). Given the preceding N-fixation rates and plant densities, estimated seeding rates of common and hairy vetch would need to be 7.6 & 10.4 kg PLS ha^{-1}, respectively, to achieve 67 kg N ha^{-1} contribution. If achieved, these rates should supply the recommended rate of N for switchgrass.

Control plants and nitrogen fixing plants must be similar in size to absorb the same amount of N for plant growth (Danso et al., 1992; Boddey et al., 1984). Vetch plants sown in early fall were considerably larger in size than those sown in late fall (data not shown). The use of both dicot and monocot non-N-fixing control plants revealed little difference in the N-fixation rates of common and hairy vetch at either location (Tables 3.3 & 3.4). The use of switchgrass and other weeds to determine n-fixation of common and hairy vetch showed similar results. Mare’s tail revealed to smallest amount of N-fixation for the vetch plants. This could have been a result of plant maturity levels where Mare’s tail was in its vegetative stage not a reproductive stage like the other weed species.

Switchgrass Yields

Switchgrass yields showed no differences among common vetch (13 Mg ha^{-1}), hairy vetch (12.4 Mg ha^{-1}) or the 0 nitrogen control treatment (10.7 Mg ha-1) across both harvests and locations after only one year (Table 3.7). Hairy vetch cover crops have been shown to increase corn yields by 2.5 Mg ha^{-1} (Ebelhar et al., 1984). In this study, common vetch (13 Mg ha^{-1}), hairy vetch (12.4 Mg ha^{-1}), and grass-only yields averaged of 10.7 Mg ha^{-1}.

Common vetch was seeded into the switchgrass plots for only one year. Neither common nor hairy vetch seed was inoculated prior to planting. The presence of nodules on plants following establishment indicated that rhizobia were present in the soil at the experiment sites, but concentrations of soil rhizobia prior to seeding were not determined. When seeding common or hairy vetches into established stands of switchgrass, it is advisable to inoculate seed with the appropriate species of rhizobia. Inoculation should occur prior to planting to ensure nodulation and effective plant stands for nitrogen fixation rates sufficient to produce a yield response in switchgrass. In cases of low rhizobia levels in the soil and/or no inoculation when seeding, it may take two to three years to naturally develop enough soil rhizobia for vetch to fix the nitrogen needed to elevate switchgrass yield. Based on the results obtained in experiments reported herein, switchgrass yields are expected to show a significant response when seeded with inoculated common or hairy vetch seed at seeding rates of 7.6 and 10.4 kg PLS ha^{-1}. These estimated seeding rates are determined to be necessary to achieve 67 kg N ha^{-1}, the recommended rate of nitrogen for switchgrass.

CHAPTER IV

Conclusions

Common, as well as hairy, vetch have the potential to be viable alternatives to offset part of the nitrogen inputs suggested for switchgrass. Common vetch seed germination can be increased through a sulfuric acid pretreatment before seeding, but such pretreatment can be unsafe and cost prohibitive. A cost effective alternative to break seed dormancy and increase vetch seed germination is the use of 100 grit sandpaper at 0.7 kg of pressure for one minute. Establishment of legumes may be increased by effective seeding rates for companion planting into switchgrass and accurate planting times for each legume species. Similar above-ground N-fixation rates of common and hairy vetch plants (59.3 and 43.3 kg N ha^{-1}), respectively were reported. Legumes need to be inoculated with species-specific rhizobia when first seeded in order to enhance nitrogen fixation. Common vetch, as well as, hairy vetch can supply nitrogen 67 kg N ha^{-1}, the recommended rate of N fertilizer for switchgrass if sufficient plant densities are achieved. Based on the results reported herein, it is estimated that switchgrass yield will increase with common or hairy vetch seeding rates of approximately 7.6 or 10.4 kg PLS ha^{-1}. Proper legume management needs to be developed that address legume varieties compatible with switchgrass, seed inoculation, seeding dates and rates, and rate of competition with switchgrass.

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APPENDIX C

Table 3.1. Average[†] seed germination rates of dry, cold stratification treatments of common vetch seed.

	Holston Unit		Plant Science Unit			
	Air Dried		Air Dried		Oven Dried (49°C)	
Treatments[‡]	No. of Seed Germed ‡	% Germination	No. of Seed Germed	% Germination	No. of Seed Germed	% Germination
1 Week	1.5	6.	2.5	10	0	0
2 Weeks	3.5	14	1.5	6	1	4
3 Weeks	2.5	10	2.0	8	1	4
4 Weeks	2.0	8.	1.0	4	.5	2
5 Weeks	1.0	4.	1.0	4	0	0
6 Weeks	0.5	2.	1.5	6	.5	2
Control§	2.5	10	1.5	6	1	4

† Means across treatments and replications.

‡Treatments used 25 seeds for each of two replications from three different seed collections (Holston Unit, Plant Sci. Unit (oven and air dried)) and were chilled at 8°C with 41% humidity.

§Air dried and control seed was stored at ambient temperature and averaged 24°C with 58% humidity

Table 3.2. Physical and chemical seed scarification methods, treatments, and average[†] germination rates of common vetch seed.

Scarification Method	Treatment[‡]	No. of Seed Germinated[†]		% Germination
Control	Air dried seed (Holston)	8	cde	16
Sandpaper§	0.5 kg for 30sec	10	bcd	20
Sandpaper	0.5 kg for 1min	9	cde	17
Sandpaper	0.7 kg for 30sec	7	cde	14
Sandpaper	0.7 kg for 1min	16	ab	31
Sandpaper	0.9 kg for 30sec	12	bc	23
Sandpaper	0.9 kg for 1min	10	bcd	20
Chlorine Bleach	3% sodium hypochlorite/10 min	4	de	7
Sulfuric Acid	98%H_2SO_4/1 min	20	a	40
Hydrogen Peroxide	1% H_2O_2/ 24hrs	2	e	4

† Mean separations based on Tukey's followed by same letter are not significantly different at $P \leq 0.05$ level.

‡ Treatments had three replications consisting of 17 air dried seeds each from Holston Unit seed collection

§100 grit sandpaper.

Table 3.3. Nitrogen fixation rates for common vetch using the N-Difference method at Knoxville Plant Science Unit and Holston Unit of the East TN (ETREC) Research and Education Center.

	Common Vetch				
	KPSF				
Control Plants	N from Vetch	N from control plant	% N-Difference of vetch	Average[†] vetch plant weight	Vetch Aboveground N plant^{-1}
	g kg^{-1}			g	
Wheat	24.7	10.1	14.6	9.6	1.4
Wild Barley	24.7	13.1	11.6	9.6	1.1
Geranium spp.	24.7	13.4	11.3	9.6	1.1
Mare’s Tail	24.7	14.4	10.3	9.6	1.0
Venus Looking Glass	24.7	11.6	13.1	9.6	1.3
	Holston Unit				
	g kg^{-1}			g	
Switchgrass	25.8	12.2	13.6	9.6	1.3
Wild Barley	25.8	8.5	17.3	9.6	1.7
Daisy Fleabane	25.8	9.0	16.8	9.6	1.6
Geranium spp.	25.8	1.2	13.7	9.6	1.3
Mare's Tail	25.8	1.7	8.7	9.6	0.8
Venus Looking Glass	25.8	1.0	16.1	9.6	1.5

[†] Means of vetch plant weights across all samples

Table 3.4. Nitrogen fixation rates for hairy vetch using the N-Difference method at Holston Unit of the East TN (ETREC) Research and Education Center.

	Hairy Vetch				
Control Plants	N from Vetch	N from control plant	% N-Difference of vetch	Average[†] vetch plant weight	Vetch Aboveground N plant^{-1}
	g kg^{-1}			g	
Wheat[‡]	26.0	10.1	15.9	7.8	1.2
Switchgrass	26.0	12.2	13.8	7.8	1.1
Wild Barley	26.0	8.5	17.5	7.8	1.4
Daisy Fleabane	26.0	9.0	17.0	7.8	1.3
Geranium spp.	26.0	12.1	13.9	7.8	1.1
Mare's Tail	26.0	17.1	8.9	7.8	0.7
Venus Looking Glass	26.0	9.7	16.3	7.8	1.3

[†] Means of vetch plant weights across all samples

[‡] N-difference values using wheat were taken from wheat samples from the Knoxville Plant Science Unit.

Table 3.5. Estimated seeding rates for common and hairy vetch to obtain one-half the recommended rate of nitrogen fertilizer for switchgrass using the N-difference method to calculate N-fixation rates of common and hairy vetch from East TN Research and Education Center.

Control Plant	Vetch Aboveground N plant^{-1}	Observed Average Vetch Density†	Total Aboveground Vetch N	Bioavailable Vetch N	Target N	Vetch seeding rate to supply 67 kg ha^{-1} N to Switchgrass
Common Vetch						
	----------g-----------	-----m^{-2}-----	---g m^{-2}---	-------------kg ha^{-1}	-------------	---kg PLS ha^{-1}---
Wheat	1.4	8.5	11.9	59.3	67	7.6
Switchgrass	1.3	8.5	11.0	55.2	67	8.2
Hairy Vetch						
	----------g-----------	-----m^{-2}-----	---g m^{-2}---	-------------kg ha^{-1}	-------------	----kg PLS ha^{-1}---
Wheat	1.2	7	8.7	43.3	67	10.4
Switchgrass	1.1	7	7.5	37.6	67	12.0

†Common vetch plant density was averaged from both ETREC and PREC locations in 2010. Hairy vetch plant density was taken from ETREC due to no seedling emergence at PREC. Both common and hairy vetch densities were obtained with seeding rates of 6.7 kg ha-1.

Table 3.6. Average† common and hairy vetch legume (LG) plant densities‡ and heights and switchgrass (SG) plant heights at the East TN (ETREC) and Plateau (PREC) Research and Education Centers in 2010.

	Common Vetch			Hairy Vetch			Control
	Plant	Height		Plant	Height		Height
Location	Density	LG	SG	Density	LG	SG	SG
	No. m^{-2}	--------cm--------		No. m^{-2}	---------cm--------		------cm------
ETREC	10	59	119	7	54	84	110
PREC	7	43	86	0	4	109	87

†Means across treatments and replications

‡Plant density = (frequency of occurrence * 0.4) x 100(Vogel and Masters, 2001)

Table 3.7. Average† dry matter yields (Mg ha^{-1})of switchgrass per common vetch or hairy vetch treatment from a one-cut biomass and two-cut forage/biomass harvest systems at East TN (ETREC) and Plateau (PREC) Research and Education Centers in 2010.

	KPSF	PREC			All Locations
Harvest System‡	Biomass	Forage	Biomass	F+B	Both Harvest Systems
Treatment	--Mg ha^{-1}--				
Common Vetch	15.9a	3.8a	6.2a	10.0a	13.0a
Hairy Vetch	12.7a	3.5a	5.1a	8.6a	12.4a
Control	11.6a	3.3a	5.4a	8.7a	10.7a

†Mean separations based on Tukey's at P≤0.05 apply to columns across treatments.

‡ Switchgrass harvests were shown in two different systems: One-cut biomass harvest (ETREC) and two-cut forage/biomass harvest (PREC).

Summary

Nitrogen fixing legumes are a good alternative to inorganic nitrogen fertilization of switchgrass. Red clover was the most promising of the legumes when seeded into ‘Alamo’ switchgrass at all locations in both one and two-cut harvest systems. Legumes alfalfa and Illinois bundle flower germinated but did not persist in competition with switchgrass in either harvest systems. Legume density typically declined when nitrogen was applied to plots interseeded with legumes, with the exception of red clover. There was insufficient time to determine reseeding and persistence of crimson clover, hairy vetch, and partridge pea in lowland switchgrass types.

Switchgrass yields in the one-cut biomass harvest systems showed differences among treatments when 67 kg N ha^{-1} was added to the plot with or without a legume treatment across all locations and years. The legume treatments in this system did not have a considerable influence on the chemical signatures from switchgrass samples when using NIR. It appears that location and nitrogen treatments may have had an effect on spectral patterns of switchgrass, but the NIR data was not precise enough to determine accurate chemical compositions.

In the two-cut forage/biomass system, effects of legumes on switchgrass yields were the same as adding 67 kg N ha^{-1}, with the exception of alfalfa and Illinois bundle flower in both years and hairy vetch in 2009. Presence of legumes did not significantly alter forage quality among legumes treatments with the exception of the 135 kg N ha^{-1} treatment that increased crude protein content in forage dry matter. Harvest systems did not appear to affect nutrient levels in the soil. Inadequate soil nutrient levels may have affected legume densities at the PREC location.

In the reseeding experiment, arrowleaf clover, crimson clover, and red clover had the highest densities after annual reseeding. Annual reseeding of red clover and trailing wild bean legumes had effects on yields of switchgrass when compared to the control treatment in 2010. No other differences were seen among annual reseeding treatments from 2009 to 2010.

A combination of cool and warm-season legumes were planted and grown concurrently in established switchgrass stands in 2010. Cool-season legumes crimson clover and common vetch in combination with warm-season partridge pea achieved better plant densities than when combined with warm-season trailing wild bean after one year. Legume combinations showed no differences on switchgrass yields after one year.

Effective establishment of legumes may be increased by seeding rates, legume inoculation, and timely planting times of each species. Legumes need to be inoculated with specific rhizobia to provide adequate soil bacteria communities for nitrogen fixation. Time when legume stand counts are taken may have an effect on reported stand rates when looking at the growth cycles of some of the warm-season legumes. Accurate legume counts are difficult to take once switchgrass begins rapid growth in early summer.

Nitrogen-fixing common vetch has the potential to be a viable alternative to offset part of the nitrogen inputs suggested for switchgrass. Common vetch seed germination can be increased through a sulfuric acid pretreatment before seeding, but the pretreatment can be toxic and cost prohibiting. A cost effective alternative to break seed dormancy and increase vetch seed germination may be the use of 100 grit sandpaper at 0.7 kg of pressure for one minute with no chemical disposal issues. Common vetch, as well as, hairy vetch can supply nitrogen equal to recommended N rate of 67 kg N ha^{-1} for switchgrass. It is estimated that switchgrass yield will increase with common vetch and hairy vetch seeding rates of 7.6 and 10.4 kg PLS ha^{-1}, respectively.

Proper legume management needs to be developed that addresses legume varieties compatible with switchgrass, seed inoculation, soil nutrient quality, seeding dates and rates, and rate of competition with switchgrass. Species-specific legume seed inoculation is required when legumes are planted into a field that has not recently or previously contained the legume. Soil testing is recommended to determine if adequate nutrients are available for your companion legume. Legume seeding rates can be developed that can supply adequate nitrogen fixation for switchgrass biomass production. Switchgrass must be able to penetrate the legume stands when it starts to grow in the spring. Maturity of the legumes is a key part in determining legume compatibility with switchgrass. Early maturing legumes can reseed themselves before a forage-cut or before switchgrass growth shades out the legumes. Later maturing varieties may benefit from a forage-cut reducing the switchgrass canopy and allowing legumes to accelerate vegetative growth reaching their reproductive stage.

APPENDIX D

Table 4.1. Summary of treatment and plot randomization for single-cut harvests at the East TN (ETREC), Plateau (PREC), and Milan (RECM) Research and Education Centers.

	ETREC			PREC			RECM		
Treatment	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
Red Clover	101	204	306	101	204	306	114	204	313
Partridge Pea	102	208	303	102	208	303	107	201	310
67 Nitrogen† (N)	103	209	307	103	209	307	111	206	314
Alfalfa	104	203	304	104	203	304	105	211	311
Alfalfa + 67 N	105	201	305	105	201	305	102	203	306
Hairy Vetch	106	206	309	106	206	309	112	214	301
IL Bundle Flower	107	210	308	107	210	308	103	210	304
Crimson Clover	108	207	301	108	207	301	104	213	302
0 Nitrogen	109	205	310	109	205	310	115	208	305
Red Clover + 67 N	110	202	302	110	202	302	109	207	308
Hairy Vetch + 67 N							101	209	303
Partridge Pea + 67 N							106	202	307
IL Bundle Flower + 67 N							108	205	315
135 Nitrogen							110	212	309
Crimson Clover + 67 N							113	215	312

† Nitrogen applications are in kg ha^{-1}.

Table 4.2. Summary of treatment and plot randomization for two-cut harvest systems at the East TN (ETREC) and Plateau (PREC) Research and Education Centers.

	ETREC			PREC		
Treatment	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
Alfalfa	111	219	318	111	219	318
Hairy Vetch	212†	212†/215	319	112	215	319
Red Clover	113	213	312	113	213	312
135 Nitrogen‡	114	216	314	114	216	314
Partridge Pea	115	211	316	115	211	316
IL Bundle Flower	116	218	317	116	218	317
0 Nitrogen	117	214	313	117	214	313
Crimson Clover	118	217	311	118	217	311
67 Nitrogen	112†/119	112†	315	119	212	315

† Plots inadvertently switched during planting.

‡Nitrogen applications are in kg ha^{-1}.

Table 4.3. Soil nutrients levels of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in the one-cut harvest systems at the East TN (ETREC), Plateau (PREC), and Milan (RECM) Research and Education at Centers in kg ha^{-1}.

	ETREC				PREC				RECM			
Treatment	**P**	**K**	**Ca**	**Mg**	**P**	**K**	**Ca**	**Mg**	**P**	**K**	**Ca**	**Mg**
	-------kg ha^{-1}-------											
Initial (control)	56	194	2691	381	9	127	1276	72	45	110	-	-
Alfalfa	114	137	3366	489	7	109	2098	158	58	198	2657	147
Alfalfa+67N†	113	94	3465	450	6	95	2525	156	76	191	2889	146
Crimson Clover	124	102	3301	475	7	113	2412	208	59	168	2849	127
Hairy Vetch	117	102	3408	482	7	81	2374	162	55	164	2615	124
IL Bundle Flower	123	109	3519	514	8	110	2175	164	78	194	2847	168
Partridge Pea	101	135	3099	441	6	70	2328	111	59	197	2760	132
Red Clover	93	110	3003	426	6	99	2516	177	68	234	3039	169
Red Clover+67N	132	107	3383	453	8	105	2354	161	73	219	2509	151
0 Nitrogen	131	131	3708	526	7	124	2390	229	64	185	2524	136
67 Nitrogen	93	113	3268	446	6	81	2262	143	65	206	2686	140
Crimson Clover+67N									44	155	2495	128
Hairy Vetch+67N									52	148	2691	147
IL Bundle Flower+67N									63	179	3027	132
Partridge Pea+67N									77	209	2900	146
135 Nitrogen									80	201	2321	129

†Nitrogen applications are in kg ha^{-1}.

Table 4.4. Soil nutrients levels of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in the two-cut harvest systems at the East TN (ETREC) and Plateau (PREC) Research and Education Centers in kg ha^{-1}.

	ETREC				PREC			
Treatments	**P**	**K**	**Ca**	**Mg**	**P**	**K**	**Ca**	**Mg**
	---------------------------------kg ha^{-1}---------------------------------							
Initial (control)	56	194	2691	381	9	127	1276	72
Alfalfa	191	158	4214	613	7	111	2136	183
Crimson Clover	145	128	3698	434	8	81	2797	244
Hairy Vetch	181	122	4181	571	9	104	2059	183
IL Bundle Flower	242	182	5519	598	9	89	2505	214
Partridge Pea	168	164	4412	517	6	100	2115	211
Red Clover	117	113	3499	437	7	118	2192	211
0 Nitrogen	156	104	3740	412	8	85	2445	182
67 Nitrogen	140	105	3840	442	7	121	2461	220
135 Nitrogen	196	111	4503	425	6	99	2171	198

Table 4.5. Summary of treatment and plot description for annual reseeding experiment at the East TN (ETREC) Research and Education Center.

	Annual Reseeding Experiment		
Treatment	**Rep 1**	**Rep 2**	**Rep 3**
Crown Vetch	R111	R212	R312
Arrowleaf Clover	R112	R213	R311
Trailing Wild Bean	R113	R211	R313
Crimson Clover	R101	R203	R304
Red Clover	R102	R201	R303
Alfalfa	R103	R205	R306
IL Bundle Flower	R104	R204	R305
Partridge Pea	R105	R202	R301
Hairy Vetch	R106	R206	R302
Control (0 Nitrogen)	109	205	310

Table 4.6. Summary of treatment and plot description for cool and warm-season legume combination experiment at the East TN (ETREC) Research and Education Center.

	Legume Combination Experiment		
Treatment	**Rep 1**	**Rep 2**	**Rep 3**
Crimson Clover & Partridge Pea	111C	311C	R307C
Crimson Clover & Trailing Wild Bean	120C	211C	R207C
Common Vetch & Partridge Pea	121C	220C	321C
Common Vetch & Trailing Wild Bean	R107C	221C	320C
0 Nitrogen	109	205	310

Table 4.7. Soil nutrients levels of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in the one-cut harvest system for the annual reseeding experiment at East TN Research and Education Center in kg ha^{-1}.

	Alfalfa	Arrowleaf Clover	Crimson Clover	Crown Vetch	Hairy Vetch	IL Bundle Flower	Partridge Pea	Red Clover	Trailing Wild Bean	Initial Control
	kg ha^{-1}									
P	90	67	63	66	175	150	138	66	61	63
K	124	137	138	126	150	137	164	146	131	218
Ca	3619	3259	3708	3101	4488	4437	4470	3697	3224	3019
Mg	470	440	488	417	503	509	567	455	410	427

Table 4.8. Soil nutrients levels of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in the one-cut harvest system for the legume combination experiment at East TN Research and Education Center in kg ha^{-1}.

	Crimson Clover Partridge Pea	Crimson Clover Trailing Wild Bean	Common Vetch Partridge Pea	Common Vetch Trailing Wild Bean	Initial Control
	kg ha^{-1}				
P	216	165	228	195	63
K	149	147	166	154	218
Ca	4907	4300	4686	4755	3019
Mg	570	512	616	673	427

Table 4.9. Summary of treatment and plot randomization for common vetch, hairy vetch, and control treatments at East TN (ETREC) and Plateau (PREC) Research and Education Centers.

	ETREC			**PREC**		
Treatment	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 1**	**Rep 2**	**Rep 3**
Common Vetch	R114	R214	R314	120	220	320
Hairy Vetch	106	206	309	112	215	319
Control (0 Nitrogen)	109	205	310	117	214	313

Table 4.10. Soil nutrients levels of phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in common and hairy vetch plots at East TN (ETREC) and Plateau (PREC) Research and Education Centers in kg ha^{-1}.

	ETREC			PREC		
	Common Vetch	Hairy Vetch	Control	Common Vetch	Hairy Vetch	Control
	-- kg ha^{-1}			--		
P	86	117	63	6	7	9
K	170	102	218	86	81	127
Ca	3375	3408	3019	2439	2374	1276
Mg	437	482	427	205	182	72

VITA

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