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ESTABLISHMENT, FERTILITY AND HARVEST MANAGEMENT FOR OPTIMIZING SWITCHGRASS YIELD AND QUALITY AS A CELLULOSIC ETHANOL FEEDSTOCK IN THE GREAT LAKES REGION

presented by

Katherine Kelly Withers

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Kurt D. Thelen
Major Professor's Signature

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By

Katherine Kelly Withers

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ABSTRACT

ESTABLISHMENT, FERTILITY AND HARVEST MANAGEMENT FOR OPTIMIZING SWITCHGRASS YIELD AND QUALITY AS A CELLULOSIC ETHANOL FEEDSTOCK IN THE GREAT LAKES REGION

By

Katherine Kelly Withers

Agronomic management specific to the Great Lakes Region is needed for producing switchgrass (*Panicum virgatum* L.), in an economically and environmentally sustainable manner for the bioconversion platform. Two field studies were conducted to determine: the best seeding date and planting methodology for stand establishment; and agronomic N rate and harvest management system for optimizing switchgrass yield and quality as a bioenergy feedstock. Three seeding dates (early, mid, and late- spring), and three planting methodologies (no-till, and conventionally tilled and planted with either a grain drill or double- roller seeder) were assessed. The harvest and fertility study consisted of three harvest management systems (one- cut fall, one- cut overwintered, and a two- cut system), and four N rates (0, 39, 78, and 157 kg N ha^{-1}). Both were randomized complete block designs with five replications, the former arranged as a split-plot. A mid- spring seeding date, with or without tillage resulted in acceptable stand frequencies and subsequent yields. A single harvest made in the fall after a killing frost was found to be the best harvest management system with a mean yield of 12.3 Mg ha^{-1}. Biomass yields did not differ between 78 and 157 kg N ha^{-1}, indicative of a yield plateau. The yield response to N was best modeled with a linear response plateau.

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INTRODUCTION

The quest for a renewable liquid transportation fuel has generated criteria applicable to the biofuel feedstocks under consideration as well as the resultant fuel. Scientists, policy makers and society insist that the production of the fuel be sustainable and that the needs of national security, climate security, and the environment are met. These important issues have led to extensive research and the preliminary development of a cellulosic ethanol industry. Cellulosic ethanol is a viable fossil fuel alternative because it is renewable, can lead to a reduced dependence on foreign oil, and is compatible with existing automobile standards (Mitchell et al., 2008). Switchgrass (*Panicum virgatum L.*), a native, warm-season, perennial grass, has been selected as a model herbaceous perennial crop for cellulosic ethanol production by the U.S. Department of Energy (DOE) (McLaughlin and Wullschleger, 1999).

The Energy Independence and Security Act of 2007 calls for the Renewable Fuels Standard (RFS) to be increased to 36 billion gallons of renewable fuels by 2022, of which 21 billion gallons must be derived from cellulosic feedstocks. Additionally, the new farm bill, the Food, Conservation and Energy Act of 2008, contains provisions that will provide payments to producers in an effort to support and expand production of cellulosic ethanol. Michigan is in a great position to benefit from these policy implementations as it has approximately 238,120 acres of ideal conservation reserve program (CRP) land (FSA 2008), and over 4.5 million acres of land that is being neither actively cropped nor forested according to National Land Cover Data. The three components of sustainable development: profitability, socio-economic factors, and the environment, provide a practical framework for assessing why growing switchgrass as a cellulosic ethanol feedstock is viable option for Michigan.

Profitability

Profitability can be viewed from the perspectives of both the switchgrass grower and future biorefineries. It is imperative that growers can produce consistently high-yielding switchgrass in a cost-effective manner and obtain a reasonable price for their crop (Sokhansanj et al., 2009). It is also reasonable to assume that biofuel refineries will have constraints on what they can afford to pay for ethanol feedstocks if they are to be profitable (Sokhansanj et al., 2009).

The ability to use existing farming equipment for stand establishment and biomass harvest is a great advantage to farmers, as it makes the purchase of new equipment or capital expenditure unnecessary (Parrish, 1999). Additionally, methods of adding value to the harvested crop before farm-gate would be advantageous, rather than simply producing a raw commodity that will likely command a low price.

Cellulosic ethanol biorefineries need to be profitable over the long-term, which can only be achieved by purchasing high quality, competitively priced feedstocks with the option of co- or by-product generation (Sokhansanj et al., 2009). For a high-quality energy crop, growers should seek to maximize the concentration of lignocellulose in the feedstock, minimize N and mineral concentrations, and limit water concentration (Lewandowski and Kicherer, 1997). It is understood that the profit margins for fuel production are relatively low and thus the ability to co-produce animal feed or biomaterials, biotextiles, or biochemical is an important consideration (Laser et al., 2009).The use of waste streams to power the refineries, such co-firing or combusting the residual lignin, is also an important aspect because it would lower production costs and improve production efficiencies (Lynd et al., 2009).

Socio-Economic Factors

Many socio-economic factors on both a national and global scale govern the transition to second-generation biofuels. Population growth rates and the need for increased food production in the face of climate change, land degradation, and desertification, require that arable land be reserved for food and feed production (Robertson et al., 2008). Increased global demands for energy at a time when oil reserves are thought to have peaked or at least reaching peak oil, support the case for acquiring renewable fuels (Farrell et al., 2006).

A domestically produced, liquid transportation fuel that can replace those derived from nonrenewable energy sourced from increasingly politically volatile foreign nations is being sought in an attempt to enhance national security (Farrell et al., 2006). Stimulating rural economic development is an important domestic issue (Kim and Dale, 2005). Biorefineries situated in appropriate fuelsheds are likely to significantly improve rural economies and strengthen a rediversification of agriculture (Sarath et al., 2008b). Localized production and use of biofuels in Michigan can aid in job creation and the generation and retention of money in rural communities. Michigan's urban centers have experienced economic woes from the decline of the once prominent manufacturing sector and stand to benefit from the emerging bioeconomy.

Environmental attributes of cellulosic ethanol

The exploration, production, and combustion of petroleum fuels result in atmospheric pollution that reduces air quality, and greenhouse gas (GHG) emissions that contribute to climate change (Robertson et al., 2008). Using a carbon neutral bioenergy feedstock such as switchgrass to produce cellulosic ethanol is a superior alternative because the combustion of cellulosic ethanol results in 94 % less GHGs relative to gasoline (Schmer et al., 2008), and the cultivation of switchgrass can reduce atmospheric carbon through $C0_2$ sequestration (Ma et al., 2000). Making ethanol from switchgrass also has environmental advantages over corn-grain ethanol because switchgrass requires less energy and agronomic inputs during cultivation (Mitchell et al., 2008). Switchgrass production requires less diesel fuel usage because it is perennial. Perennials do not have to be established each year and thus have an economic benefit and require fewer net energy inputs relative to annual crops. These include a lower need for herbicides, fertilizer and other chemical inputs (Mitchell et al., 2008). Reduced need for annual cultural inputs, minimizes fossil fuel use in production, and thereby improves the overall energy balance of the fuel (Hill, 2007). Additional benefits of the perennial nature of switchgrass include a large and deep rooting mass (Ma et al. 2000b), which can reduce soil erosion, enhance carbon sequestration rate, and contribute to soil organic matter

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matter levels and quality overall (Robertson and Swinton 2005). Switchgrass demonstrates high nitrogen use efficiency (NUE) and remobilizes nutrients to the root system while undergoing senescence, which means reduced nutrient export from the field and a lower nitrogen fertilizer requirement (Muir et al., 2001). Nitrogen fertilizer is energy intensive to manufacture and has adverse environmental impacts when used inefficiently. Nitrogen that is not taken up can lead to contaminated groundwater or eutrophication of surface bodies of water when leached to groundwater or lost through lateral runoff as nitrate ($N0_3$-) (Carpenter et al., 1998). Alternatively it can be released to the atmosphere as nitrous oxide (N_20), a greenhouse gas with a high global warming potential (Mosier et al., 1998). Nitrous oxide has a global warming potential (GWP) of 296 g eq. CO_2 (g), based on its capacity to trap heat and its atmospheric residency time (Mosier et al. 1998).

The need for agro-ecological specific agronomic information

Agro-ecological region-specific information is needed to inform producers on how to maximize yield and quality of switchgrass grown as cellulosic ethanol feedstock (McLaughlin et al., 1999). The information needed can be classified into two categories: stand establishment and post-establishment management. Successful stand establishment in the first year is critical as switchgrass takes three-four years to establish (Parrish et al. 1999), and plant populations obtained in the first year largely determines consequent yield potential and plays a large role in reducing production costs (Schmer et al., 2006).

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There is limited information on the influence of tillage and seeding equipment on plant population and stand yield (Parrish et al. 1999). No-till establishment is desirable due to reduced soil erosion potential and soil carbon oxidation, especially if establishment is being considered for fallow land such as that enrolled in the Conservation Reserve Program (CRP) or land abandoned due to marginal soil quality (Mitchell et al., 2008). If growers opt to use tillage they will need to know which type of seeding equipment they should use to enhance stand establishment. Producers will also need to be informed of the best seeding date for their particular region and of viable weed control options. Ways of overcoming the inherent seed dormancy of switchgrass also need to be identified.

Once switchgrass has been established questions arise as to how much nitrogen fertilizer should be applied as the nitrogen requirement depends on the yield potential of the site, productivity of the cultivar, and the management practices under which it is grown (Vogel et al., 2002). The effects of harvest frequency on switchgrass productivity and quality is still not clearly understood (Monti et al., 2008). Discovering the optimal harvest timing and frequency is critical, as this will govern stand longevity, yield, and cell wall composition. The proportions of cellulose, hemicellulose and lignin are affected by both nitrogen fertility and the time at which it is harvested (Vogel et al. 2002, Reynolds et al. 2000, Adler et al. 2006).

Cellulosic ethanol derived from warm-season grasses such as switchgrass will not replace our entire fossil fuel need but it is a method of producing a liquid transportation fuel while reducing GHG emissions, improving air and soil quality, and stimulating rural economic development. Much agronomic research has been done on switchgrass but more information is needed on improving stand longevity after multiple years of harvest, optimizing biomass quality and net energy yield from the feedstock, and achieving cost effective feedstock production.

Objectives

The major objective of the overall study is to develop agronomic and economic data and information that will lead to commercially viable and sustainable systems for establishing and producing switchgrass as a high-quality cellulosic ethanol feedstock in Michigan and the Great Lakes Region. Agronomic Information on seeding date, planting methodology, nitrogen fertility, and harvest system management is much needed in the wake of the emerging cellulosic ethanol industry. Additionally, we need to understand how harvest regimes and nitrogen fertility rates influence cell wall composition, which in turn determines feedstock quality.

Two studies were conducted to assess establishment methods and post-establishment nitrogen-fertility and harvest management in central Michigan. The first experiment evaluated the influence of seeding methodology and planting date on plant populations and biomass yields at two sites between 2007 and 2010. The second study conducted during the same period on a different site evaluated the effect on N-fertilization rate and harvest management on biomass yield and feedstock quality.

The objective of the harvest management and response to nitrogen study was to determine the nitrogen fertilizer rate and harvest timing for optimizing yield and quality of switchgrass grown as a cellulosic ethanol feedstock while minimizing environmental externalities. The second study conducted at two sites, assessed three methods of planting switchgrass across three seeding dates. The objective of the establishment study was to determine the best time for switchgrass to be planted and whether or not tillage and seeding machinery play a role in plant populations and first-year biomass yields.

CHAPTER 1

EARLY-, MID-, AND LATE-SPRING ESTABLISHMENT OF SWITCHGRASS UNDER THREE DIFFERENT PLANTING METHODOLOGIES

Abstract: Economically viable production of switchgrass (*Panicum virgatum* L.) as a bioenergy feedstock over the long-term requires successful stand establishment in the first year. Establishment can be hampered by grassy weed pressure and poor germination from unsuitable environmental conditions. A field experiment was conducted with the 'Cave-in-Rock' switchgrass cultivar at two sites at the East Lansing Field Research Facilities (42.75°N -84.47°W) in Michigan. The objective of this study was to determine the most suitable seeding date (early-, mid-, or late-spring) and planting methodology for the Great Lakes Region, to ensure successful stand establishment and high yields in subsequent production years. Planting methodologies examined were: no-till drilled (NT); conventionally tilled and planted with a conventional grain drill (CGD); and conventionally tilled and planted with a double-roller seedbed conditioning system (DRS). Planting methodology and seeding date combinations resulted in stand frequencies that exceeded the desired 40% establishment threshold. Yields for the 2009 seeding-year were highest when tilled and planted in the mid-spring, with either the CGD or DRS. Second-year yields at the 2008 seeding-year were highest when tilled and planted in mid- or late-spring with a CGD. Data suggest that a variety of planting methodologies can be employed for successful establishment, but a seeding date that is combined with frequent precipitation and warm air and soil temperatures, as well as proper weed control, is imperative for obtaining satisfactory first- and second-year yields.

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) was identified as a biomass feedstock for energy production based on economic and environmental assessments by the Oak Ridge National Laboratory's Biofuels Feedstock Development Program. Reasons for its selection include: adaptation to a wide geographic region; its perennial life cycle and associated positive environmental attributes; high-yield potential on marginal lands; and it can be established via direct seeding (Wright and Turhollow, 2010). However, like other perennial warm-season grasses, switchgrass has a long establishment period. Reports on the time it takes switchgrass to reach full yield potential vary from three years (Heaton et al., 2004), (McLaughlin and Kszos, 2005), to between three and four years (Sharma et al., 2003) or more (Christian et al., 2002). A lengthy establishment period means potential stand encroachment from weeds and delayed profitability for farmers facing operating costs without a harvestable crop. It is understood that switchgrass is not generally harvested in the first year because its energy allocation to below-ground development in the establishment year results in above-ground biomass yields that are not economical to harvest (McLaughlin et al. 1999). With proper agronomic practices and normal climatic conditions, however, year-one biomass yields of 50% of full yield potential can be obtained (Schmer et al., 2006)

A major research priority is to identify reliable establishment tools and methods that will ensure productive yields in establishment and post-establishment years, eliminate the need for re-seeding, and thereby reduce establishment costs (Sanderson et al., 2006). McLaughlin et al. (1999) stated that this research priority needs to be addressed before farmer adoption and the scale-up of switchgrass production can be realized. There is limited information on the influence of tillage and seeding equipment on switchgrass plant population and stand yield (Parrish et al. 1999). Few studies compare conventional and no-till methods of establishment without the added factors of unequal skill or management and weed control methods (Sanderson et al. 2004). Poor stand establishment can delay biomass production by one or more years (Schmer et al., 2006). The inability to obtain rapid and consistent establishment of productive switchgrass stands is a major economic constraint for bioenergy production (Perrin et al., 2008; Sarath et al., 2008b; Schmer et al., 2008; Schmer et al., 2006).

Establishing an acceptable stand in the seeding year and obtaining harvestable biomass early on has significant economic effects on the production of switchgrass for bioenergy. Growing switchgrass in an economically viable manner requires establishing a good stand with a stand frequency of 40% or greater in the establishment year (Schmer et al., 2006). Stand frequency can be defined as the number of times a species occurs within a given repeated sample area (Greig-Smith, 1983). Low second-year yields from stands with initial stand frequencies of 40% or lower can be directly attributed to the initial low-stand frequency. Low second-year yields from stands that met the establishment threshold are more likely a result of poor environmental conditions.

The ability to use existing farm equipment for stand establishment and biomass harvest is a great advantage to farmers, as it means the purchase of new equipment or capital expenditure is unnecessary (Parrish et al. 1999). In a willingness-to-grow study conducted by Jensen et al. (2007), farmers indicated that they would be more likely to grow switchgrass if they owned hay-making equipment -- as it is readily adapted to harvesting switchgrass -- which suggests that data in support of existing forage crop establishment equipment could be a motivating factor in farmer willingness-to-grow. Furthermore, farmers indicated a strong willingness to try environmentally friendly technologies and thus the authors concluded that no-till is positively associated with switchgrass adoption. The Biomass Crop Assistance Program (BCAP) recently amended by Title IX of the Food, Conservation, and Energy of 2008 Act (known as the 2008 Farm Bill) supports farmers in establishing and producing eligible crops for conversion to bioenergy. The recently proposed changes to BCAP will likely provide incentive for adopting establishment and management practices that enhance delivery of ecosystem services such as sustaining biodiversity and soil conservation.

It is hypothesized by economists and fuel vs. food observers that bioenergy feedstocks will be grown primarily on lands that are classified as marginal due to poor soil quality or slope. No-till establishment would reduce potential soil erosion events on converted land (Monti et al., 2001; Wolf, 1989). Additionally, the lack of a tillage event would maintain existing soil aggregate-size distribution (Grandy and Robertson, 2006) and minimize soil organic carbon (SOC) mineralization relative to establishment with tillage, thereby providing a potential to maintain or even improve existing SOM levels. Another ecological benefit of no-till establishment is keeping Arbuscular Mycorrhizal hyphal networks intact, with positive implications for AM colonization of roots and future P uptake potential (Goss and de Varennes, 2002). From an agronomic standpoint, the soil moisture conservation that a no-till system provides could be helpful in preventing stand failure when spring rains are not frequent after planting (Wolf et al. 1989). Switchgrass should not be planted into soils with heavy crop or weed residues (Parrish et al 1999). No-till establishment could be enhanced with a fall burndown with a non-selective herbicide, a spring burn-down and pre- and post-emergence herbicides (Masters and Nissen, 1998; Masters et al., 1996).

An economic benefit of no-till establishment is fewer trips across the field, thereby reducing diesel fuel expenses and other costs involved with establishment, such as depreciation of tillage machinery. Tillage costs constitute a large portion of establishment costs. Diesel fuel usage accounted for 29% of establishment costs in a large multi-location field-scale study by Schmer et al. (2008), compared to 18% in post-establishment years. Limiting tillage passes across the field could reduce establishment costs despite the need for additional herbicides. The concept of no-till switchgrass establishment will be further developed in this study by verifying its ability to produce stands that meet the threshold for bioenergy feedstock production determined by Schmer et al. (2006), and by determining which planting dates will result in the best stands and post-establishment yields. No-till establishment has many potential ecological, agronomic, and economic benefits, provided that satisfactory yields are achieved.

There are many ecological challenges governing successful switchgrass establishment. Soil moisture has implications for seed imbibition, seedling survival, and root development. The planting date is an important consideration regarding soil moisture and weed competition (Smart, 1997) Studies have found that the soil surface must be moist for three to five days before adventitious roots will form (Newman and Moser, 1988; Wilson and Briske, 1979). Research conducted in Texas by Ocumpaugh et al. (2002) indicates that rainfall as frequent as at least once in a seven to 10 day period is critical for seedling survival in that climate, or in areas with low soil moisture, high evaporation or non-ideal pH. The need for regional agro-ecological-specific agronomic information means that many of the planting dates stated in the literature will not be directly applicable to all locations. Researchers in eastern Nebraska found mid-March to be a superior seeding date to late-April or May. The proposed seeding date of within three weeks of the recommended corn seeding date (Panciera and Jung, 1984) has become a general rule of thumb (Parrish et al. 1999, (Mitchell et al., 2008) but has not been widely tested (Moser et al. 2004 as discussed in Parrish and Fike, 2005). Switchgrass is photoperiod sensitive (Moser, 1995) with biomass accumulation peaking with the onset of anthesis and seed development. This implies that seedlings that are slower to emerge will reach peak biomass at the same time as those seeded earlier, even though they underwent less vegetative growth. A premature shift to reproductive growth because of a late seeding date could possibly have adverse consequences for root development. Parrish and Fike (2005) recommend planting non-dormant seeds after soils have warmed to at least 15 or 20°C and while rainfall patterns are favorable.

The Great Lakes Region experiences a shorter growing season, and cooler-wetter springs than the study sites of previous planting date research. Our major objective, therefore, was to develop agro-ecological, region-specific information needed to inform producers on how to establish switchgrass to obtain high-plant populations and maximize yield in production years. A field study involving a side-by-side comparison of conventional and no-till (NT) switchgrass establishment was conducted to determine which seeding date and planting methodology result in the highest second-year yields and that present the best likelihood for establishment success. The following hypotheses were formulated based on previous research: (i) switchgrass can be successfully established as a bioenergy feedstock without tillage; (ii) both a conventional grain drill (CGD) and a dual-roller seeder (DRS) can be used (with tillage) to successfully establish switchgrass for energy purposes; and (iii) a mid-spring seeding would result in the highest first-year stand frequency and, subsequently, second-year yields in the central Great Lakes Region.

MATERIALS AND METHODS

Site Description and Experimental Design

Field experiments were conducted on two sites at the Michigan State University Agronomy Farm in East Lansing (42°25′48′′ N, 84°16′48′′ W), over a three-year period beginning in the spring of 2008. Soils were a mixture of a Capac loam (fine-loamy, mixed, mesic Aeric Ochraqualfs) and a Colwood-Brookston loam (fine-loamy, mixed, active, mesic Typic Endoaquolls and Typic Haplaquolls). Mean pH levels at the two sites ranged from 6.9-7.9, and K levels were similar; however the P levels at the 2009 seeding-year location were twice that of the 2008 seeding-year (Table. 1). The previous crops at the 2008 seeding-year location were corn (*Zea mays* L.) and soybean (*Glycine max* L.) in 2006 and 2007, respectively, using a conventional chisel plow tillage system. The previous crop for the second seeding-year was canola (*Brassica napus*).

A randomized complete block design was arranged as a split-plot with five replications at two sites, with three planting methodologies (whole plots) and three seeding dates (subplots). Planting methodologies were: (NT) drilled; conventionally tilled and conventionally drilled (CGD); and thirdly, conventionally tilled and planted with a Brillion Sure Stand grass seeder (DRS) (Brillion Iron Works, Brillion WI). The Brillion consists of a double-roller seedbed conditioning system which firms the seedbed with the first roller and presses the seed in place with the second.

Conventional tillage refers to using a chisel-plow in the fall and a soil finisher in the spring. A John Deere conventional grain drill (CGD) (Model No. 450, Westminster,.MD) on a 19cm row spacing was used for both the no-till and conventional seeding, and a cultipacker (Brillion Iron Works, Brillion WI) followed the grain drill used in the NT treatment to ensure good seed-to soil contact. Seeding dates (Table 2) were later in 2009 due to excess precipitation resulting in soils that were too wet for earlier seeding operations. Switchgrass cultivar 'Cave-in-Rock' was seeded into plots that were 12m X 3m at the 2008 seeding-year and 9m X 3m at the 2009 seeding-year, at a seeding rate of 9 kg ha^{-1}. No soil amendments were applied in the establishment year. Nitrogen fertilizer was applied to the 2008 seeding-year the following year on May 8, 2009, as urea (46-0-0 N-P-K) at a rate of 79 kg N ha^{-1}.

Table 1. Soil properties obtained from soil samples taken prior to fertilization in 2009 growing year.

Soil Property	**Seeding- Year**	
	2008	**2009**
pH	6.9	7.2
P	23.4	48.8
K	111.8	125.6
Ca	1049.4	1617.8
Mg	215.4	280.8
Nitrate	2	3.8
Ammonium	2.9	2.9

Table 2. Agronomic operations for both seeding- years over course of field experiment 2008- 2010

Agronomic Event	Spring Seeding Date					
	2008			2009		
	Early	Mid	Late	Early	Mid	Late
Tillage & Seeding Event	22-April	15-May	6-June	12-May	26-May	15-June
Herbicide Control Program						
Pre-plant burndown (no- till):						
glyphosate (4.68 L ha^{-1} + AMS (1-2% v/v)	n/a			18-May		
Pre-emergence:						
atrazine (0.6 kg a.i. ha^{-1}) + quinclorac (0.6kg a.i. ha^{-1})	n/a			18-May	31-May	22-June
Post-emergence:						
atrazine (1.12 kg a.i. ha^{-1})	19-May			n/a		
bromoxynil (1.16 L ha^{-1})	25-June + 22-July			n/a		
Second- year N fertilizer Application						
(79 kg N ha^{-1} Urea 0-0-46)	8-May 2009			30-April 2010		

Weed Control

In the 2008 seeding-year no pre-emergence herbicides were used. Atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] and bromoxynil [3,5-Dibromo-4-hydroxybenzonitrile] were used post-emergence to obtain broadleaf control. Heavy weed pressure from three C_4 grasses; fall panicum (*Panicum dichotomiflorum*), giant foxtail (*Setaria faberi),* and large crabgrass (*Digitaria sanguinalis*) was observed at the site. In 2009, a more rigorous chemical weed-control program was employed. This included a pre-plant glyphosate [N-(phosphonomethyl) glycine] burndown for the NT treatments, and a pre-emergence application of a quinclorac [3,7-dichloro-8-quinolinecarboxylic acid] and atrazine tank-mix. A post-emergence application of the salt of bromoxynil [3,6-dichloro-2-methoxybenzoic acid] was made to the early-seeded treatments to control an escape of redroot pigweed (*Amaranthus retroflexus).*

Biomass Measurements

Stand frequency was measured using a frequency grid as a means of quantifying establishment success. The grid frequency method was originally developed by Vogel and Masters (2001), and then used to determine stand thresholds for growing switchgrass as a commercial biomass energy crop by Schmer et al. (2006). The grid is comprised of 25 (15- X 150cm) cells, and was placed randomly within the plot and then turned over four times for a total of 100 cells and each cell is marked for the presence or absence of switchgrass. A conservative estimate of plant population (plants m^{-2}) can be obtained by multiplying the stand frequency by 0.4 (Vogel and Masters, 2001).

First-year yields for the 2009 seeding-year were harvested with a flail-type forage harvester (Carter Mfg Co., Brookston, IN) equipped with an automated weigh system to determine biomass yield, set at an 18cm cutting height on October 26, 2009. Second-year cuttings of the 2008 seedings were made on October 20, 2009 with a commercial forage harvester (Model No. 7650 Hesston Co., Hesston, Kansas), set at an 18cm cutting height that was modified for plot research with the addition of a weigh bin for yield determination from individual plots. Biomass content for each plot was recorded and biomass moisture content for each plot was determined via subsamples that were placed in a forced-air oven at 100°C for 72 hours or until a consistent dry weight was achieved.

Statistical Analysis

Data were analyzed using PROC MIXED in SAS v9.1 (2003 SAS Institute Inc., Cary, NV.) Analysis of variance (ANOVA) was conducted to measure the treatment effects on stand frequency and yield. The analysis was performed as a RCBD arranged as a split-plot design. Seeding date and planting methodology were treated as fixed effects while block, seeding-year, and the interaction between the whole-plot factor and block were considered to be random effects. Normality of the residuals and homogeneity of variances were evaluated by examining normal probability plots and box plots. When variances were found to be heterogeneous, the REPEATED/GROUP option of PROC MIXED was implemented. Fisher's protected least significant difference (LSD) multiple comparison procedure was used for mean separation when ANOVA was significant. Results were reported as statistically significant at $\alpha = 0.05$. Linear contrast statements were constructed in addition to the multiple comparison procedure to test the significance of prior hypothesized effects of the main factors.

RESULTS AND DISCUSSION

Climatological Summary

The 2008 growing season tended to be drier than both the 30-year average and 2009 (Table 3). April and May were 41% and 46% drier than the 30-year mean and 53% and 75% drier than 2009, respectively. August was also a very dry month, receiving approximately 82% less precipitation than 2009 and the 30-year average. Precipitation from April through the month of June was wetter than average in 2009, and in August precipitation was similar to the 30-year mean. Mean air temperatures did not vary considerably between years or within the 30-year average, although April of 2008 was slightly warmer than either 2009 or the climatic norm, indicating that April of 2008 was unusually warm and dry which had implications for the early-seeded treatment in that year (Table 3).

Stand Frequency and Yield

Although site -year was considered to be a random effect and found to be non-significant in the full model, it is useful to consider each year separately in relation to the environmental conditions and relative weed pressure for further insight into the risk of potential stand failure. A discussion on significant findings and trends for data from both years will follow, with a discussion of the results of hypothesis testing.

Table 3. Monthly rainfall totals and average mean monthly air temperatures at the Michigan State Agronomy Farm, East Lansing, MI, in the 2008 and 2009 growing seasons and the 30-year average (1971-2000).

	Mean Monthly Rainfall			Mean Monthly Temperature		
Month	2008	2009	30-yr average	2008	2009	30-yr average
	mm			°C		
April	43.4	147.3	83.1	10.3	8.35	7.6
May	29.5	109.0	69.1	12.8	14.0	14.0
June	112.5	126.3	81.0	20.2	18.9	19.2
July	96.25	60.7	75.7	21.4	18.8	21.4
August	16.51	104.7	87.5	20.5	20.1	20.3
September	206.8	24.1	89.2	17.2	16.8	16.0
April-September	84.2	95.3	80.9	17.0	16.2	16.4

Results of the 2008 Seeding-year

For the 2008 seeding-year, soil and air temperature did not start to rise until the end of May (Fig. 1). There were two significant dips in air temperature with corresponding decreases in soil temperature within 10 days of the seeding event, which could potentially have induced seed dormancy. There were no precipitation events immediately preceding the early-spring (ES) seeding date, and few precipitation events after the mid-spring (MS) seeding date, which is necessary for seed imbibition. Soil temperatures were not conducive to rapid germination for the early-, and mid- dates, however conditions improved greatly for the late-spring (LS) seeding event.

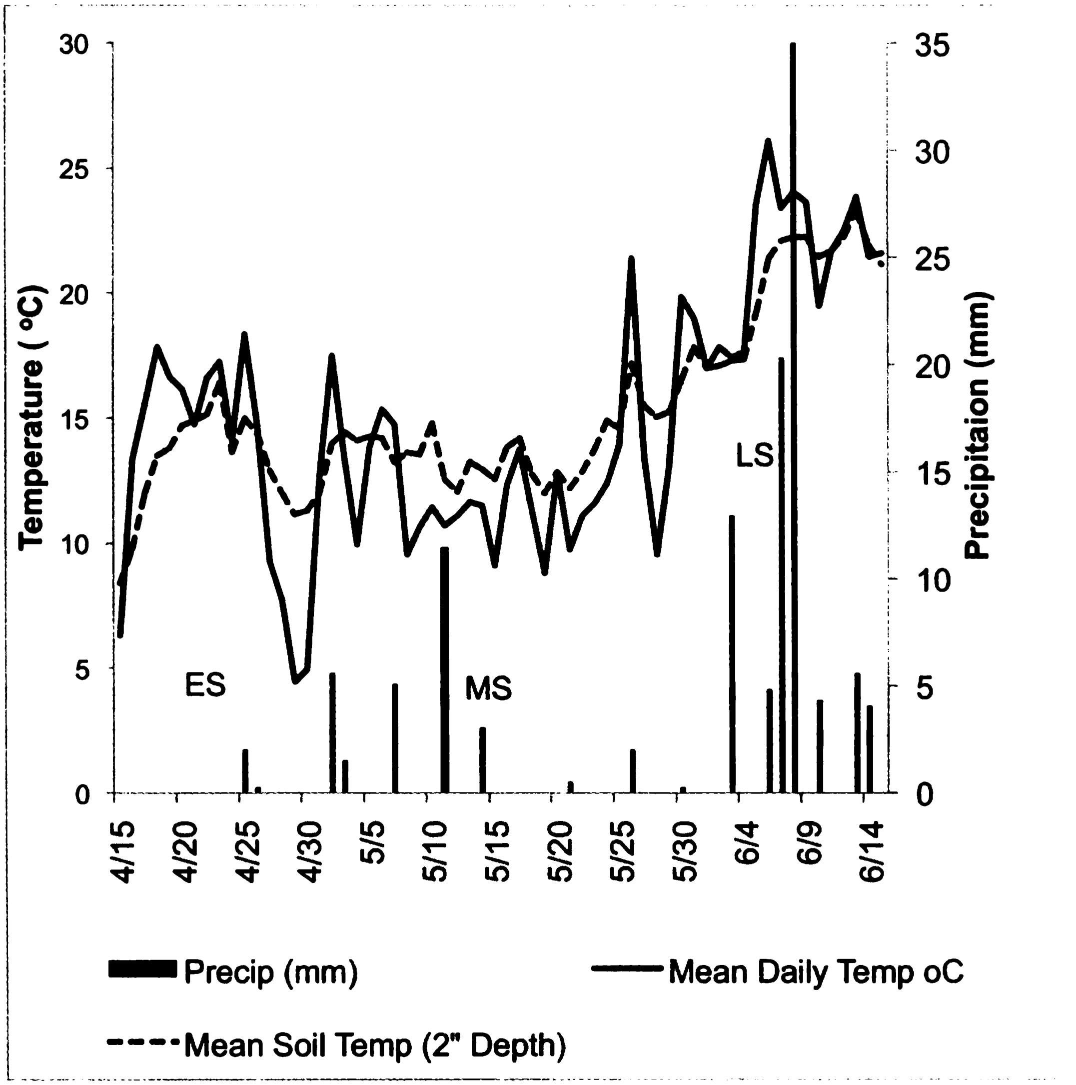

Figure 1. Environmental Conditions during planting in the 2008 Seeding- year. Weather information was obtained from the Michigan Automated Weather Network.

Stand Frequency

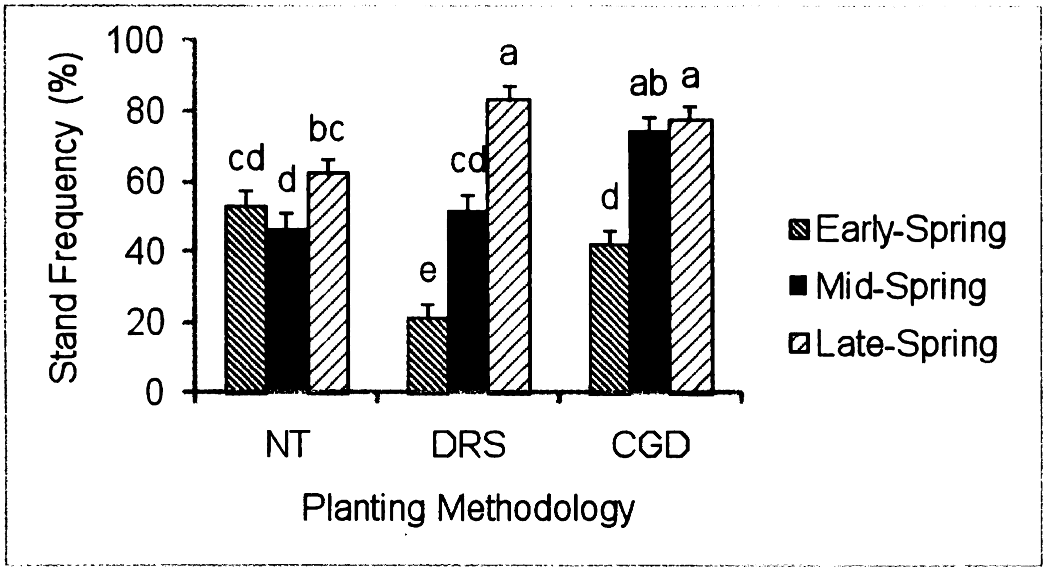

Figure 2. Stand frequencies obtained at the 2008 seeding-year. Means with the same letters are not significantly different at a=0.05. Abbrev: No-till (NT), Dual-roller Seeder (DRS), Conventional Grain Drill (CGD).

The highest stand frequencies were obtained when tillage was used at either the mid-, or late-spring seeding date when the CGD was used, and at the LS date when seeded with the DRS (Fig. 2). The ES seeded DRS treatment failed to reach the desired 40% establishment threshold determined by Schmer et al. (2006), and the ES conventional treatment came very close to failing, and may have, had this been on a larger scale with increased spatial variation. Contrast statement estimates indicted that the CGD performed significantly better than the DRS at the ES and MS seeding dates but did not differ at the LS date when moisture was less limiting. The current, un-improved varieties of switchgrass are known to have issues with the pre-determined length of the subcoleoptile internode, and the cessation in growth of the coleoptile when it encounters light (Newman and Moser 1988). Adventitious roots form at the crown node, which can often be very close to the soil surface, especially with shallow plantings like that afforded by the DRS. If the soil surface is dry, or if there is a lack of rain, the highly necessary, adventitious roots will abort or simply cease to grow. Breeding efforts are underway to select for lower crown node placement (McLaughlin and Kszos, 2005), but in the meantime, drilling switchgrass to a depth of 1cm is advantageous in dry years (Evers and Butler 2000) and rolling the seed-bed after a shallow seeding event can also be advantageous (Monti et al., 2008), as discussed in Parrish and Fike (2005).

The results of the linear contrast statements used to test the hypothesis indicated that NT treatments performed better than tilled treatments at the ES seeding date, however at the late-seeding date, NT resulted in stand frequencies that were significantly lower than either of the tilled treatments. Stand frequencies in NT plots exceeded the desired establishment threshold, and did not vary as much as the tilled plots, indicating that they were not affected by planting date or environmental conditions to the same extent. Although the soil temperatures under NT were likely cooler (Cox et al., 1990; Kravchenko and Thelen, 2007), it was not to the detriment of establishment success. The plots that underwent tillage were observed to have significantly higher weed pressure from C_4 annual grasses than the NT plots at the ES and MS seeding dates, which reduced the potential establishment success under tillage that was evident at the later seeding date. The tilled treatments likely had a competitive advantage at later dates with increased soil temperatures, decreased weed pressure (flushes of annual summer grasses were controlled with a delayed tillage event), and from improved seed-to-soil contact over the NT system. Strong competition from three C4 grassy weeds was observed in plots that were tilled and seeded at the ES and MS seeding date, particularly from giant foxtail, large crabgrass and fall panicum and by early July these plots had a closed canopy comprised of weedy biomass, implying competition for light and water. Surviving seedlings had a spindly appearance. Giant foxtail was particularly problematic as it was listed as a possible weed species present on the seed tag, has a shallow rooting system, and was present in the seed bank. Crabgrass has been previously reported as a concern for switchgrass establishment due to its similar germination timing and morphological development (Nelson, 1986). Fall panicum, although an annual, is closely related to switchgrass, which could have implications for selecting a herbicide based on physiological differences.

Although winter-annual, biennial and perennial weed species were noted in the NT plots, the canopy did not close due to the presence of weeds as observed in the tilled treatments. Interference from crop residue, living weeds and remnants of annual bluegrass (*Poa annua*) appear to have reduced seed-to-soil contact. Species that were present, such as marestail (*Conyza canadensis*) and dandelion, could be treated with a fall burndown of a systemic herbicide in the future. No-till drills could be fitted with row cleaners to prevent potential hair-pinning of residue, and wave or bubble coulters to improve seed-to-soil contact, particularly in excessively wet or dry soils.

Yield

First-year yields for the 2008 seeding-year were not calculated due to the presence of weedy biomass and an inability to differentiate the cut switchgrass from grassy weeds in the post-senescent state. There was a significant interaction between seeding date and planting method (p = 0.0026) for second-year yields of the 2008 seeding-year. Both seeding date (p=0.0004) and planting method (p=0.0179) were found to be significant (Fig. 3). The highest second-year yields were achieved when tilled and planted with the CGD at either the mid-, or late-spring seeding date, similar to the results of stand frequency in the establishment year (Fig 3). The late-seeded DRS had comparable yields to the late seeded conventional treatment. According to contrast statements the DRS and CGD performed similarly, and only differed for the MS seeding date (P=0.0004). The MS date was only advantageous in second-year yield gains for the conventional treatment. The early seeded DRS treatment, which failed in the establishment year, had the lowest second-year yields, as did the early seeded conventional treatment, which just met the 40% stand frequency establishment threshold. The NT treatments did not differ significantly from either of the low-yielding aforementioned treatment combinations despite having met the establishment threshold. They were also comparable to the yields obtained under the DRS treatment. The MS and LS conventional treatments yielded significantly greater than the other two methodologies and it does not appear to be related to stand frequency, and is possibly due to being planted at an optimal depth with frequent rains that supported adventitious root proliferation allowing for improved over-wintering capabilities.

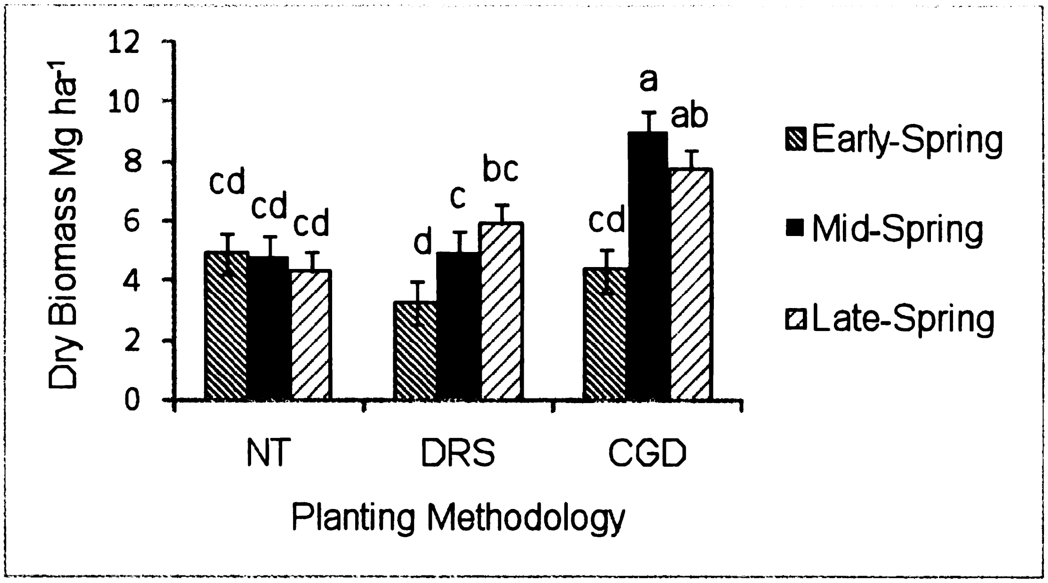

Figure 3. Second-year yields for the 2008 seeding-year. Means with the same letters are not significantly different at α=0.05 level of significance. Standard error was 0.70. Abbrev: No-till (NT), Dual-roller Seeder (DRS),Conventional Grain Drill (CGD).

Results of the 2009 Seeding-year

The 2009 seeding-year was marked by frequent and plentiful rainfall prior to, and after, each seeding event (Fig. 4). There were 19, evenly spaced precipitation events between mid-May and the end of June that likely facilitated seed imbibition, high emergence rates and a low incidence of seedling death. Both primary and adventitious root development would also have benefited from the frequent rains. Ambient air and soil temperatures rose shortly after the ES seeding event and soil temperatures remained above 15°C for all seeding events, climbing to over 20°C at the LS seeding date (Fig. 4). Air temperatures were favorable for seedling growth; the two dips in air temperature that occurred after both the springs and MS seeding events occurred during their respective germination and emergence periods and therefore did not affect seedling growth.

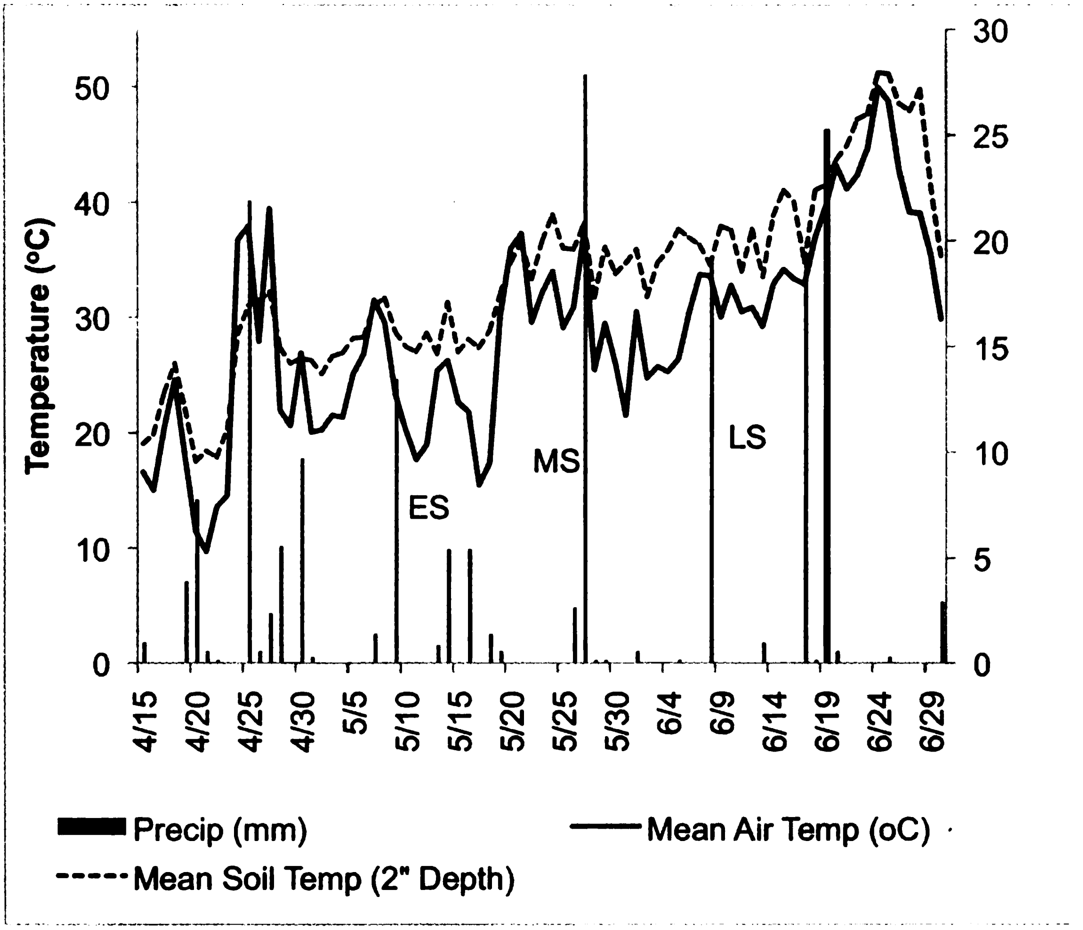

Figure 4. Environmental conditions at planting time for the 2009 seeding year. Weather information was obtained from the Michigan Automated Weather Network.

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Stand Frequency

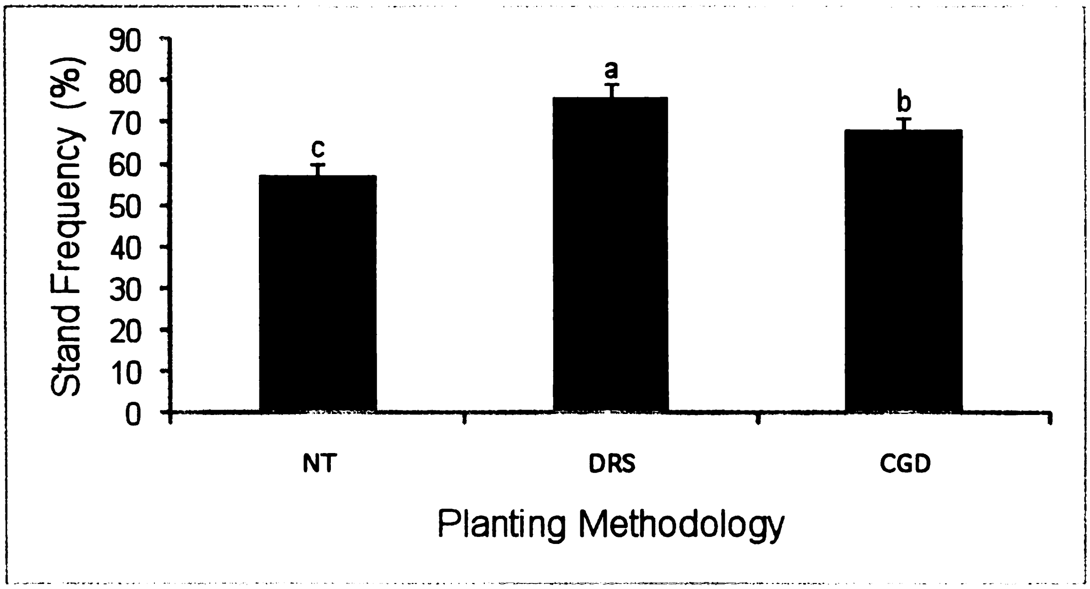

Figure 5. Stand frequencies obtained at the 2009 seeding-year, Means with the same letters are not statistically different at the α=0.05 level of significance. Stand error was 2.96 Abbrev: No-till (NT), Dual-roller Seeder (DRS), Conventional Grain Drill (CGD).

In 2009, all treatment combinations resulted in stand frequencies above 40%. Although there was an interaction (p=<0.0001) between planting methodology and seeding date, seeding date itself was not found to be significant, but planting methodology had a significant effect (p=<0.0001) (Fig.5). The highest stand frequency was obtained with the DRS, and the lowest under NT. The Brillion double-roller seedbed conditioning system appears to have fared well in this seeding-year, which had adequate precipitation, warm ambient air and soil temperatures, and minimal weed competition.

Yield

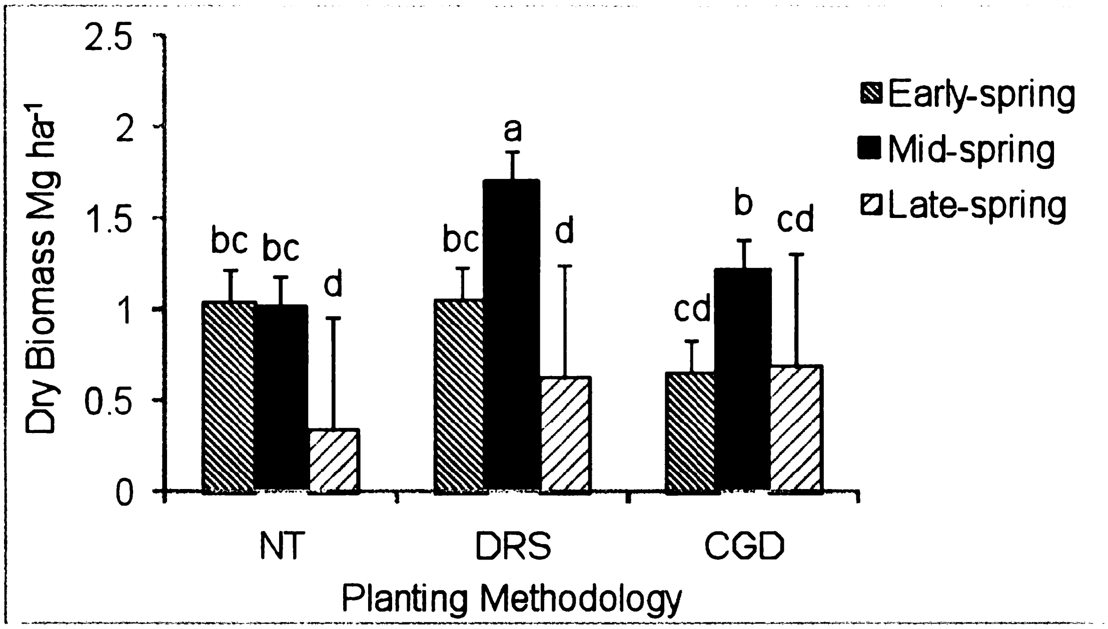

Figure 6. First-year yields in the 2009 seeding-year. Means with the same letters are not significantly different at the α=0.05 level. The standard error was 0.176 Abbrev: No-till (NT), Dual-roller Seeder (DRS), Conventional Grain Drill (CGD).

There was a significant interaction present in first-year yields in the 2009 seeding-year between planting method and seeding date (p=0.0179), and although both main effects, planting method (p=0.048), and seeding date (p=<0.0001) were significant, it is apparent that seeding date was a bigger driver of yield than planting method (Fig. 6). The highest yields were obtained with the DRS treatment at the MS seeding date, and the lowest yields were obtained at the LS seeding date for all planting methods, and when tilled and seeded early with the conventional CGD (Fig. 6).

No-till yield estimates derived from linear contrasts were only significantly lower than other planting methodologies at the LS date (p=<0.0001). These results appear to agree with Rehm (1990), who found no yield differences between conventional and NT establishment in Nebraska. Delayed emergence and subsequently a shorter vegetative growth period meant that the LS NT seeding shifted into reproductive growth at approximately the same time as the other treatment combinations and did not accumulate as much biomass. A MS seeding date was beneficial when planting with the DRS and CGD, into tilled soil (Fig. 6). DRS and CGD seedings were comparable except for the MS DRS, which out-yielded the MS conventional treatment (p=0.0004). Under optimal establishment conditions like that of the 2009 seeding-year, the DRS appears to have had rapid emergence and vigorous growth, allowing for ample shoot-growth. The consequences for second-year yield will be investigated at the conclusion of the upcoming growing season (2010).

Summary of Establishment Results Across Planting Sites

Pooling stand frequency data across seeding-years was possible because seeding-year was considered as a random effect, and found to be non-significant in the full model based on its Pr> f Value from the covariance parameter table. When stand frequency data was combined across both seeding-years there was also an interaction between planting method and seeding date (p=0.008) and both planting method (p=0.0315) and seeding date (p=0.0091) were significant (Fig. 7).

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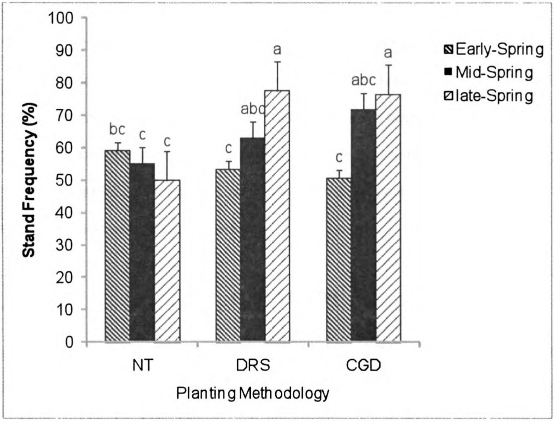

Figure 7. Stand frequencies across both seeding- years. Means with the same letters are not statistically different at the α=0.05 level of significant.

When stand frequency data were combined across years it was still apparent that tillage and either a mid- or late-spring seeding date resulted in the highest stand frequencies (Fig. 7). Contrast statements used for hypothesis testing indicate that there were no differences in final stand frequencies between MS seeding date and all other dates, and that the results of the CGD and DRS treatment did not differ. All NT treatments achieved successful stand frequencies in both years of establishment, and although there was less variation, LS NT stand frequencies were significantly lower (p= 0.0001) with a trend noted for the MS date (p=0.06) (Fig 7). This is in agreement with an establishment study of grass mixtures in Nebraska by Oldfather *et al.* (1989) that found emergence to be higher when tillage was used rather than direct seeding into undisturbed soil. It is not in accordance with King et al. (1989), who found a markedly increased number of seedlings with NT vs. conventional establishment. Although the planting methodologies that employed tillage achieved higher stand frequencies, the levels obtained are not necessary for cost-effective establishment, and might carry a higher inherent risk than NT, where more consistent and less variable stand frequencies were observed.

CONCLUSION

The data suggest that tillage is not critical for obtaining a stand frequency that exceeds the 40% establishment threshold, although first- and second-year yields lagged behind tilled treatments in some instances. However, the use of one or two pre-plant herbicide burndown treatments, proper residue management, optimization of NT drills, and seeding into warmed-up soils could potentially enhance NT stands and yields. Further research is needed to verify this. Additional growing seasons are needed to determine if the establishment gap closes among planting methodologies. The data also support the hypothesis that with tillage, both a CGD and a double-roller seeder can be used to successfully establish switchgrass for energy purposes. These results suggest existing equipment can be used to prepare seedbeds, which would mean less capital expenditure and an increased likelihood of farmer adoption.

The hypothesis that a mid-spring seeding offers the best possibility of establishment success in the central Great Lakes Region was not fully su

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supported by the results as there were interactions between planting methodology and seeding date in some cases, and performance varied between planting methodologies across seeding dates. The data do not indicate a benefit from early planting like that found by Smart and Moser (2007) in Nebraska and Vassey et al. (1985) in central Iowa. However, the wet springs and frequent precipitation events in Michigan may allow for better establishment success with a mid-spring seeding than in Nebraska. Planting up to three weeks before the recommended planting date for corn (Panciera and Jung, 1984) would likely be on the early side for our region.

However, all planting method and seeding date combinations that ranked highest in yield for both seeding-years included the mid-spring treatment. A mid-spring seeding date could potentially offer the best possibility for increasing yield and profitability in early production years. The seeding date results are in agreement with Parrish and Fike (2005) and Hsu and Nelson (1986b) who have conducted similar research in Virginia and Missouri, respectively, and found that planting non-dormant seed into warmed soils while precipitation is still frequent is best for optimizing establishment.

Further research on optimizing no-till establishment systems would be beneficial for enhancing eco-system services delivery from perennial cropping systems with the possibility of reducing establishment costs. Research on chemical control programs for switchgrass establishment and early stand management would also be of benefit as it would lead to registration of effective herbicides that could further enhance establishment and cost-effectiveness.

CHAPTER 2

SWITCHGRASS BIOMASS YIELD RESPONSE TO VARYING RATES OF NITROGEN FERTILIZER AND HARVEST TIMING IN THE CENTRAL GREAT LAKES REGION

Abstract: Switchgrass (*Panicum virgatum* L.) is a C_4, perennial grass that has been identified as a suitable bioenergy feedstock for marginal land in the United States. A field experiment was conducted at the MSU Field Research Facilities in East Lansing (42.75°N- 84.47°W). The objective of this study was to determine switchgrass biomass yield response to nitrogen fertilizer in the Great Lakes Region. The cultivar 'Cave-in-Rock' was established in a RCBD and uniformly harvested in 2007. Four different nitrogen rates (0, 38, 79, and 157 kg ha^{-1}) were applied to the plots that were then harvested either under a two-cut system (anthesis and post-senescence), late fall or left to overwinter and then harvested in the spring. Agronomic recommendations on rate of N application and harvest timing for this agroecoregion will be derived from these findings. Yields across all N rates and harvest systems ranged from 4.0-10.25 Mg ha^{-1} in 2008, and 9.0-12.25 Mg ha^{-1} in 2009.

INTRODUCTION

The Energy Independence and Security Act of 2007 (EISA) mandates the production of 36 billion gallons of renewable fuel by 2022, of which 21 billion gallons must be from advanced fuels such as cellulosic ethanol or biobutanol. A number of possible feedstock sources are under consideration for cellulosic ethanol production, including residues from annual crops, herbaceous perennial grasses and woody perennials. Financial assistance to establish and produce this material is available to producers through a provision of the Farm Services Bill 2008, the Biomass Crop Assistance Program (BCAP). The government will match each dollar paid out by a Biomass Conversion Facility (BCF), up to a maximum of \$45 ton^{-1} over two years of production. It is expected that the emerging cellulosic ethanol industry will consist of regional BCFs that will acquire biomass feedstock from the land surrounding the facility in a 48-mile radius, known as a fuelshed (Sarath et al., 2008b). The boundaries of the fuelshed are determined by the distance biomass feedstock can be transported without the costs of hauling the material becoming uneconomical (Sarath et al., 2008b). Three years of field trial data conducted by the ORNL Bioenergy Feedstock Development Program (BFDP) between 1993 and 1996 emphasized the need for regional specificity of optimum harvest strategies (McLaughlin et al. 1999).

Switchgrass populations can be divided into two ecotypes: lowland and upland (Porter 1966). This was confirmed by Hultquist et al. (1996), who found that upland ecotypes can be either hexaploids (2n=6x=54) or octaploids

(2n=8x=72), and that lowland ecotypes are tetraploids (2n=4x=36). Lowland ecotypes were developed in lower-lying, 'hydric' sites, and tend to be more sensitive to moisture stress and have recently been regarded as a facultative wetland species (Barney et al., 2009). Upland ecotypes were developed on higher, 'mesic' sites and are known to be more drought resistant. Switchgrass also demonstrates adaptation to geographic areas and performs better within its latitude of origin (Casler et al., 2004). From a morphological standpoint, the upland ecotypes tend to be shorter, and have thinner stems than lowland ecotypes. Northern upland populations, for instance, would be more productive in the northeast. Upland cultivars have a greater potential for yield response with two-cut management due to phonological differences – uplands have a faster rate of development, maintain higher rates of photosynthesis, and a greater capacity to recover from stress (e.g. higher drought tolerance) (Wullschleger et al., 1996).

Obtaining high biomass yields, *inter alia,* is a top priority for switchgrass bioenergy production, regardless of the conversion platform or the desired end use. The quantity of dry matter produced by a biomass species per unit area of production determines the potential energy production capacity or yield of the available land area (McKendry, 2002). High yields equates to better land- and water- use efficiency, and higher profit per acre, the latter depending on the ratio of input costs to feedstock value. Management factors, such as fertility programs and harvest frequency, determine stand survival, productivity, feedstock quality, and economic returns (Fike et al., 2006). N fertilizer application rate and harvest management systems must be optimized to minimize dry matter loss and maximize biofuel quality (Lemus et al., 2008). Nitrogen fertilizer is a costly input from both a monetary and energy standpoint, and is a significant source of global warming potential (GWP) in cropping systems fertilized with synthetic nitrogen (Robertson, 2004). Furthermore, the profitability of switchgrass as a biomass crop would be enhanced if acceptable yields were produced with a minimum amount of applied N (Madakadze et al., 1999).

Harvest Management Systems

Seasonal time of harvest affects switchgrass yield (Adler et al. 2006, Madakadze et al., 1999; Sanderson et al., 1999; Vogel et al., 2002; Casler and Boe, 2003). The effect of harvest frequency on switchgrass productivity and quality as a bioenergy crop is still not clearly understood (Vogel et al. 2002, Monti et al. 2008). Producing switchgrass as a biofuel feedstock has different management objectives than that of a forage crop, or production as a feedstock for energy production via combustion. Vogel et al. (2002) report that the optimal time to harvest switchgrass for maximizing biomass yields in the Midwest is at the R3 to R5 stages of maturity, which means that the cut would be made between panicle emergence and postanthesis (Moore et al., 1991). They also state that with the right growing conditions, sufficient regrowth could be obtained in time for a killing frost. However, long-term yield depression is not precipitation related, below ground root reserves are depleted from not being replenished by mineral nutrients during natural crop senescence (Sanderson et al., 1996). Decreased productivity over the long-term has been reported with multiple harvests (Casler and Boe, 2003; Cuomo and Anderson, 1996; Mulkey et al., 2008). Optimal harvest timing is not based on maximizing yield in the short-term, but on obtaining high yields while maintaining stand longevity, and economic viability in terms of reducing N-P-K inputs.

One-cut harvest system Described

A harvest management system consisting of a single cut made after switchgrass has naturally senesced and at least two weeks after a killing frost is generally believed to be the most suitable from a biogeochemical standpoint. This system facilitates the mobilization of carbohydrates and nutrients into the below-ground, which means less nutrient mining of the soil with harvest, and, consequently, lower fertilizer requirements.

Adler et al. (2006) found that yield decreased when harvest was delayed until spring by 20-24% and 32-43% at the plot and field scale respectively. These yield losses were attributed to biomass not picked up by the baler, and a decrease in standing tiller weight, which accounted for <10% of the loss.

Two-cut Harvest System Described

The feasibility of a two-cut harvest system is contingent on quality (N and ash content), seasonal yield, long-term regrowth capacity, economic lifespan of the stand, and cost of multiple harvests (Monti et al. 2008). Cutting switchgrass twice per year may increase biomass production but the impact on fuel quality and economic return needs to be investigated to determine if the additional yields from the two-cut system are warranted (Fike et al., 2006). 'Cave-in-Rock', a northern upland ecotype could potentially provide higher yields with a two-cut system; however, this yield increment could be countered by the need for additional N fertilization; harvest operations and associated variable costs; and reduced fuel quality due to high N concentrations. It is well understood that a two-cut harvest system takes up and exports more nitrogen than a single-cut system does (Reynolds et al., 2000). In northern regions that have shorter growing seasons, the timing of the first-cut in a two-cut system is critical as it affects regrowth potential. The earlier the first harvest occurs the higher the regrowth will be; provided that the switchgrass is harvested above the growing points and that there is adequate precipitation.

Decreased productivity over the long-term has been reported with multiple harvests (Cuomo et al.1996, Casler and Boe 2003, Mulkey et al. 2007). The two-cut system leads to increased seasonal biomass yield in the two years following establishment (+15% and +38%, respectively) but third-year yields were significantly lower (28% compared to the one-cut system) (Monti et al., 2008). Reynolds et al. (2002) found that in Tennessee, the two-cut system only out-yielded the single-cut system when summer precipitation was not limiting. The two-cut system performed poorly at the Texas research site. This is believed by the authors to have been caused by the effect of the cutting on root persistence, and the consequent impediment on late-season water uptake under dry growing conditions (McLaughlin et al. 1999). Stand losses can occur if there is not a six-week span between the last harvest and a killing frost (Moser and Vogel 1995).

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Yield Response to Applied Nitrogen Fertilizer

Achieving high Nitrogen Use Efficiency (NUE) in switchgrass is important if this biofuel feedstock is to be grown in an environmentally sustainable manner and with producer profitability in mind. Important considerations in selecting a nitrogen rate in, addition to increasing yield, include minimizing nitrous oxide emissions to the atmosphere, and the loss of nitrate-N to groundwater through leaching. Minimizing excessive N fertilizer in a cropping system translates into lower nitrous oxide (N_2O) emissions, which are 310 more potent than CO_2 on an equivalent basis as a GHG (Fargione et al., 2008).

Switchgrass production is optimized and quality stands are maintained with good fertility management (Sarath et al., 2008b). While the calculation of the most economic rate of nitrogen is important for cost-effective production, a more pressing research need is estimating the optimal nitrogen rate for maximizing yield: yield potential data are lacking in the region; no commercial cellulosic ethanol production currently exists; the expected feedstock value is not known; and the price of nitrogen will fluctuate over time and from region to region.

The main fertilizer requirement of switchgrass is nitrogen (N) (Vogel et al. 2002). Economically viable production of perennial grass monocultures coupled with substantial quantities of annual biomass removal would be expected to require inputs of N fertilizer (Heaton et al., 2004). The N requirement of switchgrass is a function of a number of factors ,including: harvest timing and frequency, location, rainfall and length of growing season, yield potential of the site, productivity of the cultivar, and other agronomic management practices (McLaughlin and Kszos, 2005; Vogel et al., 2002). Nitrogen recommendations should be site-specific and take into account soil type, and the crop's use of N and system NUE (Brejda et al., 1998).

Soil moisture and water-holding capacity can be important factors in yield (Fike et al., 2006). These factors interact with the above stated environmental considerations and thus information specific to the particular agro-ecological region, coupled with local trials, are needed to determine how these management factors affect yield and to fine-tune N-fertilization levels to local conditions (McLaughlin and Kszos 2005). A major objective of the fertility regime, in addition to economic and environmental concerns, should be to avoid application of N-fertilizer that could cause stand lodging, which has implications for harvesting and yield.

Previous research on the estimation of switchgrass biomass yield response to N has been summarized by McLaughlin and Kszos (2005) and more recently in Haque et al. (2009). Considerable differences were found across location, switchgrass varieties, and harvest timings; further, genotype environment interactions play a large role in switchgrass production (Fike et al., 2006). Therefore this review will focus on studies that are applicable to our agroecological region, and that used upland varieties (e.g. Cave-in-Rock) under similar harvest regimes.

In a study conducted at Ames, Iowa, and Mead, Nebraska, that examined switchgrass yield response to nitrogen fertilizer and harvest frequency, Vogel et al. (2002) found that the response to increased nitrogen was linear for both first-cut (late-boot to early anthesis), and total biomass yields at Mead, and curvilinear at Ames. They reported a yield range of 10.5-12.6 Mg ha^{-1} and an N level of 120 kg ha^{-1} associated with maximum yield. A yield increase of ≤ 0.6 Mg ha^{-1} was gained with N rates above 60 kg ha^{-1} for the first-cut harvests made at Mead, and that yield plateau was present at Ames, with no increase in yield with N rates above 180 kg ha^{-1}. Further, their regrowth yields made from successive harvests of the plots that were cut at late-boot at two-week intervals, increased linearly with increasing N at Mead but did not respond to nitrogen at the Ames site.

In southern Iowa, Lemus et al. (2008) obtained a yield of 8.5 Mg ha^{-1} when switchgrass was harvested after a killing frost (single-harvest) and found the N level associated with maximizing yield was 112 kg N ha^{-1}. The authors noted a non-linear response to nitrogen, with diminishing yields occurring at higher N levels and calculated the most efficient rate of fertilizer to be 56 kg N ha^{-1} from a fertilizer use efficiency (FUE) standpoint.

Haque et al. (2009) compared two harvest systems in Stillwater, Oklahoma: one harvest per year in October, and a two-harvest system (July and October) in which the nitrogen rates were split and applied at the beginning of the season and again after the first harvest. They found that the one-cut system out-yielded the two-cut system, and that there was no response to nitrogen above their applied rate of 67 kg N ha^{-1}, indicative of a yield plateau.

Much of the previous work used quadratic polynomials or quadratic response models to model the yield response of switchgrass to nitrogen. A recent paper by Haque et al. (2009) and the associated work by (Aravindhakshan, 2008) examined the nitrogen response of four perennial grass species that are bioenergy candidates. The researchers found that the linear response plateau (LRP) model was the most appropriate model for switchgrass in their case, based on the likelihood dominance criterion of Pollack and Whales (1999), being 12 times more likely than the linear model and five times more likely than the quadratic model (Aravindhakshan et al. 2008).

Information is needed on growing and harvesting switchgrass (*Panicum virgatum* L.) as a bioenergy feedstock in the Central Great Lakes region. In order for rapid scale-up of commercialized cellulosic ethanol production, large quantities of biomass feedstock will be required. Farmers require agro-ecological specific information for economic production of switchgrass. A combination of low N inputs and a single harvest taken at the end of the season may be an optimal and sustainable strategy for managing switchgrass for biomass for bioenergy production.

Objectives

The objectives of this study were to determine the optimum nitrogen rate and harvest timing and frequency to maximize switchgrass yield on a dry matter, per hectare basis in the Great Lakes Region. Three harvest management systems will be compared: one-cut fall, one-cut overwintered, and a two-cut system in which a cut is made in July (two-cut summer) and then re-harvested after a killing frost in the fall (two-cut fall). Further analysis was conducted on the components of the two-cut system alone over the two growing seasons. It was

hypothesized that yield would increase with increasing N until a plateau was reached due to moisture, fertility or other limitations and that N-fertilizer rate would not significantly impact crop quality. Harvest timing is hypothesized to significantly impact yield. Regrowth in the two-cut system was not anticipated to contribute enough yield to justify the two-cut system due to our short growing season.

MATERIALS AND METHODS

Site Description and Experimental Design

Field experiments were conducted on the Michigan State University Agronomy Farm in East Lansing (42°25′48′′ N, 84°16′48′′ W), MI, over a three-year period beginning in the fall of 2007. Soils at East Lansing are classified as a mixture of Aubbeenaubbee-Capac sandy loams (fine-loamy, mixed, active, mesic Aeric Epiaqualfs) and Colwood-Brookston loams (fine-loamy, mixed, active, mesic Typic Endoaquolls and Typic Haplaquolls). The upland switchgrass cultivar 'Cave-in-Rock' was seeded at a rate of 9 kg ha^{-1} on May 24, 2007, into 4.6m X 12.2 m plots. The previous crop was soybean (*Glycine max* (L.) Merr.) Switchgrass was seeded into relatively weed-free plots that were chisel-plowed in the fall of 2006 and cultivated again in the spring of 2007. The switchgrass was seeded using a dual roller seeder to a depth of 0.5cm. A double roller seeder (Brillion, Brillion Iron Works, WI) was used to plant the seeds to a ~1.25 cm depth, while simultaneously acting as a cultipacker, thus firming the seedbed. Plots were fertilized in the spring at green-up in the years after establishment year with (0, 38, 79, 157 kg N ha^{-1}) applied as granular urea (46-0-0 N-P-K).

Weeds were controlled with a tank-mix of S-metalochlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl] acetamide) and atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) at rates of 1.3 and 1.8 kg a.i. ha^{-1}, respectively.

The experimental design was a randomized complete block design with five replications. Soil testing was done in April 2007 to measure pH, P, and K levels and re-tested again in April of 2008 and 2009 more intensively to measure soil nitrate levels. The field has two different soil types and consequently two distinct pH ranges; the eastern two thirds of the field has a mean pH of 6.4 while the sandier, western third of the field had a mean pH of 5.6. The entire field has a lime index of 68-71. Initial P and K levels were within a close range but lower than desirable for initial nutrient levels of these two important macronutrients.

Biomass Measurements

The various harvest management systems were imposed in 2008 (Table 4), the first year of production after establishment. Cuttings were made with a commercial forage harvester (Model No. 7650 Hesston Co., Hesston, Kansas), set at an 18cm cutting height that was modified for plot research with the addition of a weigh bin for yield determination from individual plots. Total fresh weight and biomass content for each plot was recorded and biomass moisture content for each plot was determined via subsamples that were placed in a forced air oven at 100°C for 72 hr or until a consistent dry weight was achieved.

The total dry matter (DM) per plot was then converted to Mg DM ha^{-1}.

Table 4. Harvest Management Systems employed during the study (2008-2010) with associated harvest dates and plant developmental stages from Moore et al. (1991).

Harvest Treatment	Harvest Date		Plant Developmental Stage (Moore et al. 1991)	
	2008-2009	2009-2010	2008-2009	2009-2010
	One-Cut Harvest Management Systems			
One-Cut Fall	Nov. 5	Oct. 26	Post Senescent	Post Senescent
One-Cut Overwintered	March 22	March 19	Post Senescent	Post Senescent
	Two-Cut Harvest management Systems			
Two-Cut Summer	July 21	July 17	3.7 (Anther emergence/and or anthesis)	3.0 (Boot stage) -3.1 (Inflorescence emergence)
Two-Cut Fall	Nov. 5	Oct. 26	Post Senescent	Post Senescent

Statistical Analysis

Data were analyzed using PROC MIXED in SAS v9.1 (2003 SAS Institute Inc., Cary, NV.) Analysis of variance (ANOVA) was conducted to measure the treatment effects on biomass yield. Year, nitrogen rate and harvest timing were treated as fixed effects while blocks were considered to be random effects. Repeated measures analysis was not employed at this time because there have not been enough yields taken over time to warrant its use. Fisher's protected least significant difference (LSD) multiple comparison procedure was used for mean separation when ANOVA was significant (Saxton, 1998). Single degree of freedom contrast statements were used in PROC MIXED to assess the effects of harvest timing on yield, how the harvest timings responded in each year, and specifically where yield plateaus occurred within a given harvest timing.

Yield response to nitrogen was modeled using the NLMIXED procedure to fit three functional forms: quadratic polynomial (QP), linear (L), and linear response plateau (LRP). N fertilizer was listed as a continuous variable, whereas harvest timing was considered a categorical variable and listed in the class statement. Model appropriateness was based on comparisons of the −2 log likelihood value and Akaike's information criterion (AIC). The log likelihood value was used to compute coefficients of determination (R^2) values using the method from (Nagelkerke, 1991). In the LRP model, the plateau of the regression estimates the maximum yield, and the join point of the regression estimates the N fertilizer rate at which the maximum biomass yield was achieved. In the quadratic model, the level of N that optimizes yield was calculated by taking the derivative of the slope and setting it to zero and solving for the independent variable (N) (Bowley, 2008). Response fit was visually inspected against observed yield to further ensure the best model was chosen.

Table 5. Three functional forms: linear (L), Quadratic Polynomial (QP), and Linear Response Model (LRP), used to model yield response to N.

Linear Model	$Y = mX + b$
Quadratic Model	$Y = a + bX - cX^2$
Linear Response Model	$Y = a + bX \quad \text{if } X < C$ $Y = P \quad \text{if } X \geq C$

Where Y is the yield of biomass (Mg ha^{-1}) and X is the rate of N application (kg ha^{-1}), b is the intercept, m, is the linear coefficient and C is the critical rate of N which occurs at the intersection of the linear response and the occurrence of the plateau (Cerrato and Blackmer 1990). In the LRP model, P is the join point or the N fertilizer rate at which the plateau begins, and a, b, c, and plateau are model coefficients. Coefficients of determination (R^2) values were calculated as follows:

$$R^2 = 1 - [L(0) \div L(\theta)]^{2/n}$$

Where *0* and θ represent the log likelihood values for the full and the null (intercept only) model, respectively.

Normality of the residuals and homogeneity of variances were evaluated by examining normal probability plots and box plots. When variances were found to be heterogeneous, the REPEATED/GROUP option of PROC MIXED was implemented. Results were reported as statistically significant at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Climatological Summary

Mean air temperatures did not vary considerably between years or against the 30-yr average (Table 6). The median date for first frost (28°) at East Lansing was 18 October with temperatures falling to (28°) by November 4, 90% of the time over the last 30 years.

Table 6. Mean monthly rainfall (mm) and temperatures (°C) during the experiment in comparison to the 30-year mean (1971–2000)

	Mean Monthly Rainfall			Mean Monthly Temperature		
Month	2008	2009	30 yr-avg.	2008	2009	30 yr-avg.
April	4.8	10.4	8.3	10.3	8.4	7.6
May	3.7	15.0	6.9	12.8	14.0	14.1
June	9.2	10.7	8.1	20.0	18.9	19.2
July	9.5	5.9	7.6	21.4	18.8	21.4
August	1.5	8.7	8.7	20.5	20.1	20.3
September	15.7	3.5	9.2	17.2	16.8	16.0
April-September	7.4	9.0	8.1	17.0	16.2	16.4

The 2008 growing season tended to be drier than both the 30-yr average and 2009 April and May were 41% and 46% drier than the 30-year mean and 53% and 75% drier than 2009, respectively (Table 6). August 2008 was also a very dry month, receiving approximately 82% less precipitation than 2009 and the 30-year average, which had negative consequences for regrowth in the two-cut system (Table 6). Precipitation from April through the month of June in 2009 was wetter than average, and in August 2009 precipitation was similar to the 30-year mean and hence more conducive for regrowth of switchgrass under the two-cut system.

Three Harvest Management Scenarios Compared

A three-way interaction was not present between year, N and harvest timing, nor was there a two-way interaction between year and nitrogen in the ANOVA results. Harvest timing interacted with nitrogen ($p=0.0161$) and year ($p=0.0001$). The fixed effects: harvest timing ($p=<0.0001$), N ($p=0.0003$), and year ($p=<0.001$) were significant. Overall yields in 2009 were significantly higher than in 2008 ($p=<0.0001$). Yields across all N rates and harvest systems ranged from 4.0-10.25 Mg ha^{-1} in 2008, and 9.0-12.25 Mg ha^{-1} in 2009. The stand was at two-thirds and possibly full yield potential in 2008 and 2009, respectively, which mirrors the concept put forth by McLaughlin and Kszos, 2005) in their summary of the 10-year assessment of switchgrass in the ORNL BFDP program, and (Madakadze et al., 1998) in the short growing season of eastern Ontario.

Year By Harvest Interaction

Yields in the second production year (2009) were higher due to either precipitation or the fact that the stand in 2008 was not at full-yield potential. In assessing the effect of harvest timing on yield in 2008, we found that the two-cut system out-performed the one-cut system ($p=0.001$), however the combined system yielded less than the one-cut fall harvest system in 2009 (Fig. 2). In both years the one-cut fall yields were significantly higher than one-cut overwintered yields ($p=0.001$). This is in agreement with Adler et al. (2006), who found that yields generally decreased by more than 20% when harvest was delayed from fall to spring. The researchers estimated that leaf and panicle weights and tiller weights (-7%) decreased, while stem weights increased. Mechanical harvest losses were also incurred during their research, with harvest equipment not picking up 21% and 45% of the harvested biomass in the fall and following spring, respectively. Our use of a commercial forage harvester with a direct -cut header was efficient at collecting harvestable biomass. Yield losses on a plot scale varied between (≤1 - ≤ 9%) in the spring of 2009 and 2010 (data not shown).

Nitrogen By Harvest Interaction

There were no significant differences among N rates in either the one-cut fall or one-cut overwintered system (Table 7). This was not due to a lack of response to nitrogen, but rather a loss of biomass over time due to translocation of carbohydrates and nutrients, leaf and seed drop, and further physical losses due to environmental conditions (e.g. peduncles being lost to wind). The results of the combined yield from the two-cut system demonstrated different responses to N when tested with linear contrast statements. A significant difference was found between the 39 and 78 kg N ha^{-1} rate (p=0.0351), but not between 78 and 157 kg N ha^{-1}, indicative of a yield plateau.

Table 7. Switchgrass dry biomass yields (Mg ha^{-1}) in response to three different harvest management systems, and four nitrogen rates in 2008-2010

Harvest Management System	Nitrogen Fertilizer Application Rate kg N ha^{-1} 0	39	78	157
	2008 Mg ha^{-1}			
One-Cut Fall	6.31	6.77	7.18	6.99
One-Cut Overwintered	4.06	4.34	3.88	3.64
Two-Cut Combined (Summer + Fall)	8.34**	10.52	10.91	11.24
	2009 Mg ha^{-1}			
One-Cut Fall	11.82	12.02	12.36	12.80**
One-Cut Overwintered	8.56	8.95	9.31	9.34**
Two-Cut Combined (Summer + Fall)	9.54**	10.30	12.20	12.28

** Yields differed from other means within the same row at α =0.05
LSD values for N*Harvest is 0.91

In 2008, one-cut overwintered yields did not respond positively to applied nitrogen fertilizer (Fig. 9). The one-cut fall harvest management system was best modeled with a quadratic polynomial that reaches a plateau at 7.07 Mg ha^{-1} with a corresponding N rate of 60 kg N ha^{-1}. The log likelihood values for the two-cut combined system were 83.2 and 83.5 for the LRP and QP functions, respectively, and corresponding AIC values were similar (Table 13). The outcomes for critical agronomic N values and plateaus varied. The LRP model predicts a plateau in yield at 11.07 Mg ha^{-1}, while the QP predicts a plateau at 11.56 Mg ha^{-1}. The former occurs at 111 kg N ha^{-1}, and the QP model predicts the plateau to occur at 106.9 kg N ha^{-1}.

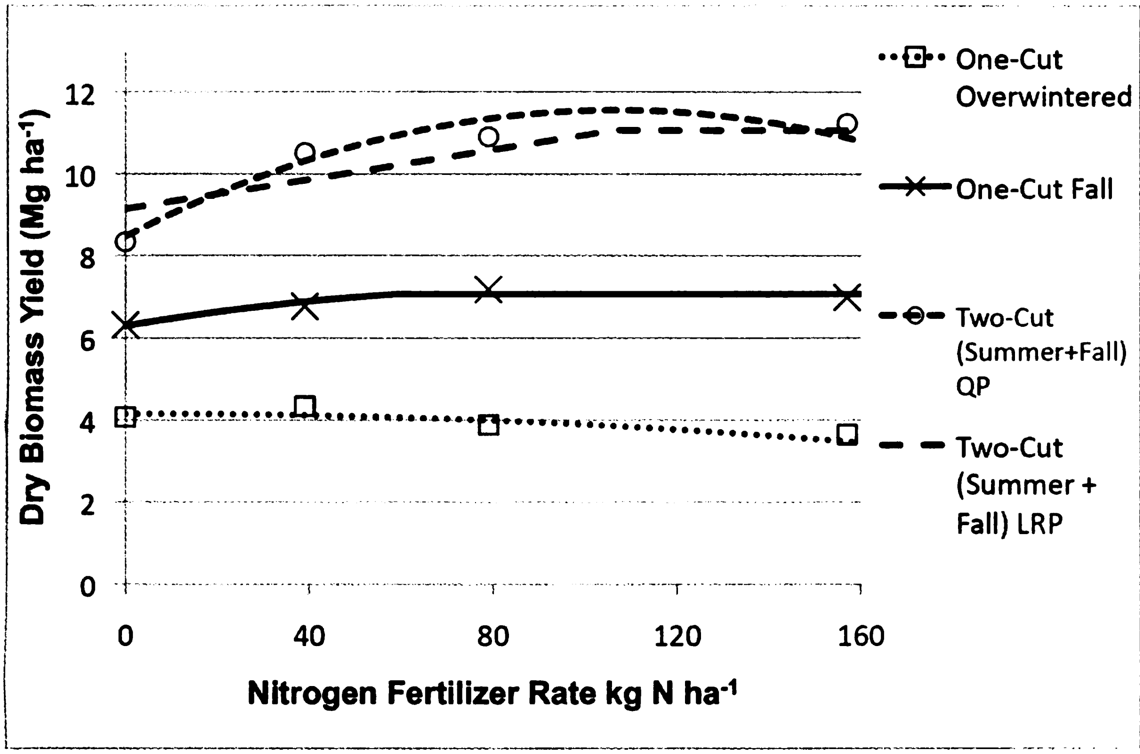

Figure 8. Switchgrass dry biomass yield (Mg ha^{-1}) response to nitrogen fertilizer 2008-2009. Corresponding regression equations and parameters can be found in Table 8.

Table 8. Switchgrass biomass yields in response to three different harvest management systems, and four nitrogen rates in 2008

Harvest Management System	Model	Functional Form	Estimated Requirement, kg N ha^{-1}	AIC	-2 log Likelihood Fit Statistic	R^2
One-Cut Fall	$Y = 6.29 + (0 .019 X N) - 0.0001 X N^2)$	QP	58.5	37.4	27.4	0.38
Two-Cut (Summer + Fall)	$Y = 9.15 + (0.018 X N^2)$	LRP	106.9	83.5	93.5	0.31
	$Y = 8.48 + (0.058 X N) + (-0.00027 X N^2)$	QP	111	83.2	93.2	0.30
One-Cut Overwintered	$Y = 4.15 + (0.00044 X N) + (-0.00003 X N^2)$	QP	n/a	40	50	0.08

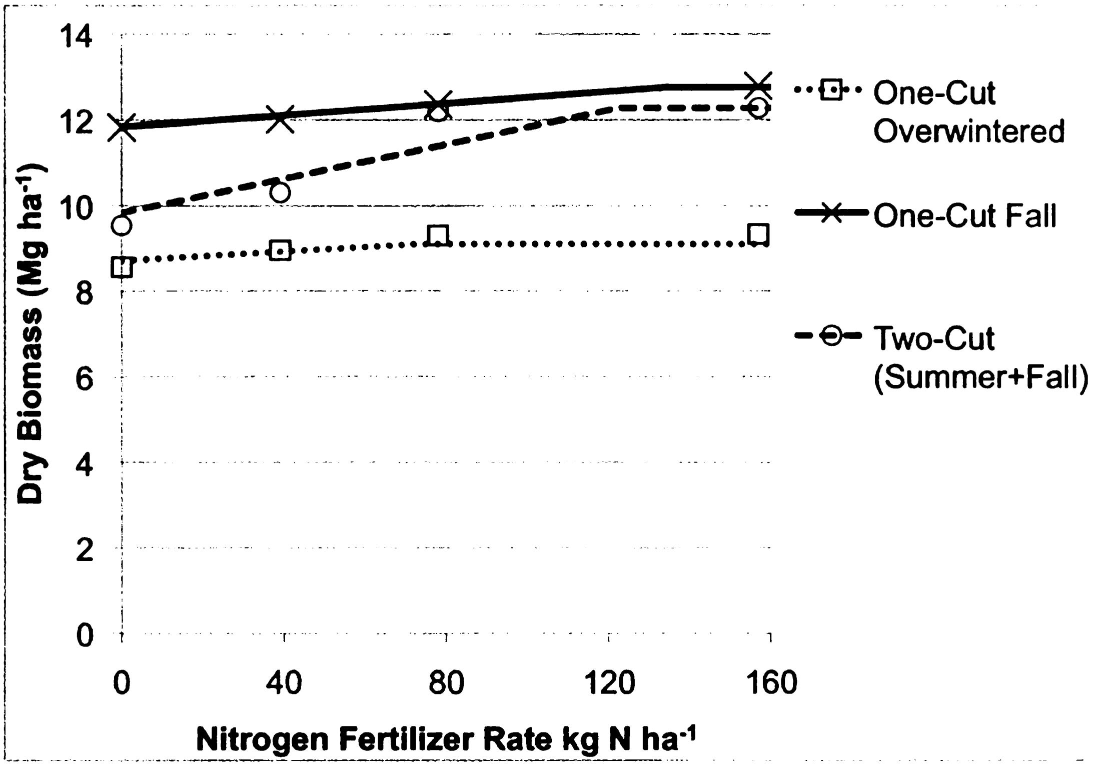

Figure 9. Switchgrass dry biomass yield (Mg ha^{-1}) response to Nitrogen in 2009. Regression equations and parameters can be found in Table 9.

In 2009, one-cut overwintered switchgrass yields did not vary with applied nitrogen, yield reached a plateau at 9.1 Mg ha^{-1}. Both the one-cut fall system and the combined two-cut system were best modeled with the LRP functional form with a plateau occurring at 12.80 Mg ha^{-1} and 12.28 Mg ha^{-1}, respectively. These yields would be obtained with a nitrogen rate of 134 kg N ha^{-1} for the fall-one cut system, and 123 kg N ha^{-1} for the latter system.

Table 9. Switchgrass biomass yields (Mg ha^{-1}) in response to three different harvest management systems, and four nitrogen rates in 2009.

Harvest Management System	Model	Estimated N Requirement, kg N ha^{-1}	AIC	-2 log Likelihood Fit Statistic	R^2
One-Cut Fall	Y = 11.84 + (0.0069 X N)	134	58.4	48.4	0.16
Two-Cut (Summer + Fall)	Y= 9.83 + (0.020 X N)	123	79.0	69.0	0.51
One-Cut Overwintered	Y = 8.71 + (0.0053 X N)	72.5		54.7	0.22

Break Down of the Two-Cut Harvest Management System

The statistical analysis of the two-cut system did not result in a three-way interaction between N, yield and harvest, nor were there two-way interactions between year and harvest, or year and nitrogen. There was, however, a significant interaction between harvest timing and nitrogen ($p=0.0024$). Fixed effects, nitrogen rate and harvest timing were significant ($p=<0.0001$), and yields were higher in 2009 than in 2008 ($p=0.0352$). Despite the lack of Year X H and Year X N interactions, data are reported and discussed for each year individually to improve our understanding of what occurred. This was done because 2008 was the first production year and thus the stand was not at full yield potential and there may have been a decreased response to applied N fertilizer due to the residual N in the system from the previous soybean crop in 2006.

Table 10. Dry switchgrass biomass yields (Mg ha^{-1}) responses within the two-cut system at four different rates of nitrogen fertilizer in 2008 and 2009.

Harvest Management System	Nitrogen Fertilizer Application Rate kg N ha^{-1} 0	39	78	157
	2008 Mg ha^{-1}			
Two-Cut Summer	7.6**	9.5	9.9	10.2
Two-Cut Fall	0.7	1.0	1.0	1.0
Two-Cut Combined (Summer + Fall)	8.3**	10.5	10.9	11.2
	2009 Mg ha^{-1}			
Two-Cut Summer	8.2**	9.0	10.4	10.4
Two-Cut Fall	1.3	1.3	1.5	1.9**
Two-Cut Combined (Summer + Fall)	9.5**	10.3	12.2	12.3
** Yields differed from other means within the same row at α=0.05 LSD values for N*H is 1.14				

Harvest

Two-cut summer and the combined yields (summer and fall) from the two-cut system did not differ between 2008 and 2009. The regrowth in the two-cut system was higher in 2009 than in 2008 ($p=<0.0001$) because there was less precipitation during the latter half of the 2008 growing season (Table 1.) and due to the timing of the first-cut. The first cut made in the two-cut system varied with calendar date and plant growth stage and so yield differences cannot be directly attributed to year effect. In 2008 switchgrass was harvested during anthesis (Moore et al. 1991 staging 3.0-3.3), which meant there was less of a growing season (shorter photoperiod) to accumulate yield prior to senescence that was further confounded by low, late-summer precipitation (Table 1). In 2009, switchgrass was harvested at the late-boot stage, which meant the crop likely had higher carbohydrate and nutrient reserves available and there was also ample precipitation to facilitate the regrowth (Table 1).

Harvesting the switchgrass earlier in the season in 2009 and obtaining more regrowth resulted in the combined and one-cut system having smaller yield gaps than in 2008. The one-cut fall system resulted in higher yields than the two-cut combined system at the 39 kg ha^{-1} rate, and had comparable yields at 79 and 157 kg N ha^{-1}. The stand was in its second production year, with more than adequate precipitation for optimal growth, and the two-cut system accumulated a large amount of biomass prior to the first-cut and had appreciable regrowth yields (1.57 Mg ha^{-1}), 39.02% more than regrowth yields in 2008.

In 2009, the two-cut system did not perform better than the one-cut system, this is in agreement with findings by Casler and Boe (2003), Monti et al. (2008) and elsewhere reported in with Sanderson et al. (1999), and in the review by McLaughlin and Kszos (2005). Haque et al. (2009) found that switchgrass did not respond well to a harvest in July followed by a harvest in October. Our two-cut system is not directly comparable to (Haque et al. 2009) because we applied the full rate of N at the beginning of the season, with no additional input after the first harvest. Hence our yield for the second cut was a function of residual soil nitrate and not from an application of additional fertilizer.

Nitrogen

In 2008 there was no difference in regrowth yields of the two-cut system between the 39, 78 and 157 kg N ha^{-1} rates, and in 2009, there was no significant yield response to N fertilizer applied at the beginning of the season.

Single-degree of freedom contrasts applied to the pooled data from 2008 and 2009 show that yields from the two-cut summer treatment did increase with increased nitrogen, but there was no difference in yield between the 39 and 78 kg N ha^{-1} rates, indicative of a yield plateau. For yields from the second cut made in the two-cut system, a response was noted between the 0 and 157 kg N ha^{-1} rates, with a trend ($p=0.0580$) noted for the difference in yield between the 0 and 78 kg N ha^{-1} rate. Total yields from the two-cut system were higher with applications of 78 and 157 kg N ha^{-1}, but not 39 kg N ha^{-1}, and there were no differences in yield between either the 39 and 78, or 78 and 157 kg N ha^{-1} rates.

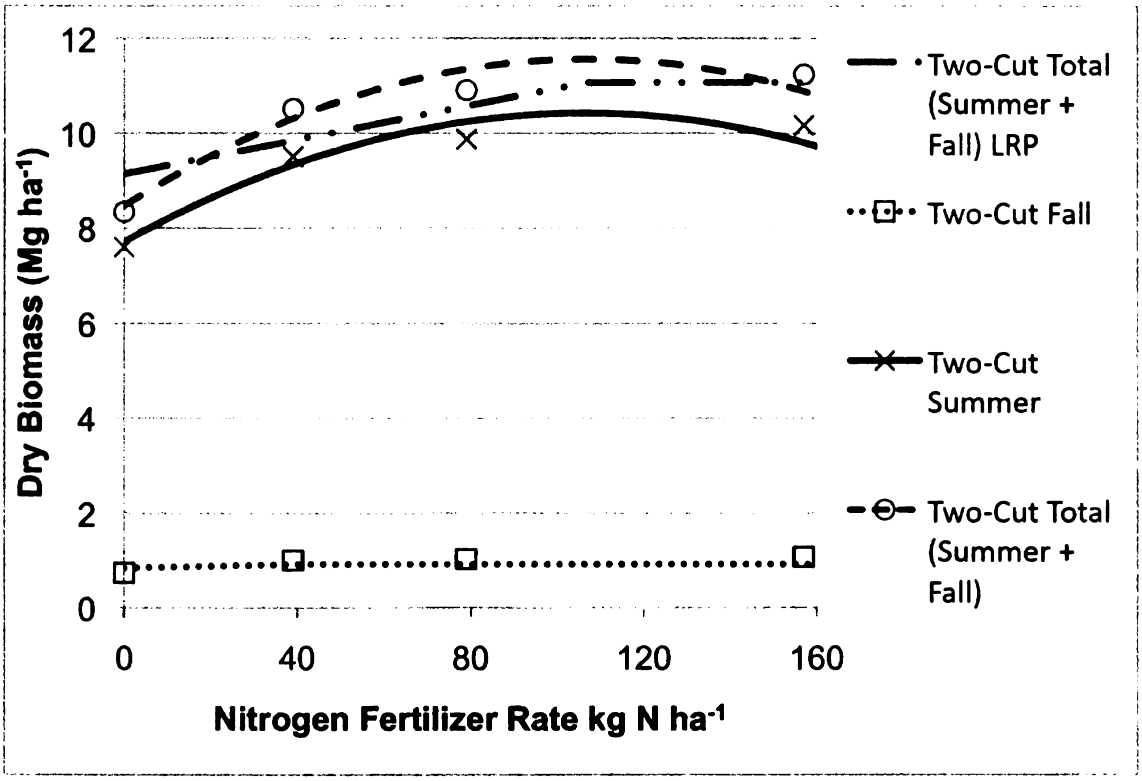

Figure 10. Switchgrass biomass yield response to nitrogen under the two-cut harvest management system in 2008. Corresponding regression equations can be found in Table 11.

Table 11. Regression equations and parameters for the two-cut harvest system in 2008.

Harvest System	Model	Estimated N Required kg N ha^{-1}	AIC	-2 log Likelihood Fit Statistic	R^2
Two-Cut Summer	Y= 7.72+ (0.051 X N)+ (-0.00024 X N^2)	148	69.7	59.7	0.28
Two-Cut Fall (Re-growth)	Y= 0.84 + 0.002 X N) Plateau = 11.07 Mg ha^{-1}	41	17.6	7.9	0.23
Two-Cut (Summer + Fall)	Y = 8.47+ (0.057 X N)+ (-0.000027 X N^2)	112	83.5	93.5	0.30

In 2008, the two-cut summer treatment was best modeled by a quadratic polynomial, and the regrowth yields from that system was best modeled by a LRP function, although the regression parameters for the second-cut were very small because there were no significant differences between N rates (Fig. 11). The summer cut of the two-cut system reached a plateau at 10.03 Mg ha^{-1}, obtained with an N rate of 148 kg N ha^{-1}. The log likelihood values from the combined yields from the two-cut system were very similar for both the QP and LRP functional forms as previously discussed. The QP model predicted the N rate that maximizes biomass to be 112 kg N ha^{-1} at a yield of 11.09 Mg ha^{-1}, whereas the LRP model predicts an agronomic optimal N rate of 109 kg N ha^{-1} at a join point yield of 11.07 Mg ha^{-1} (Fig 11).

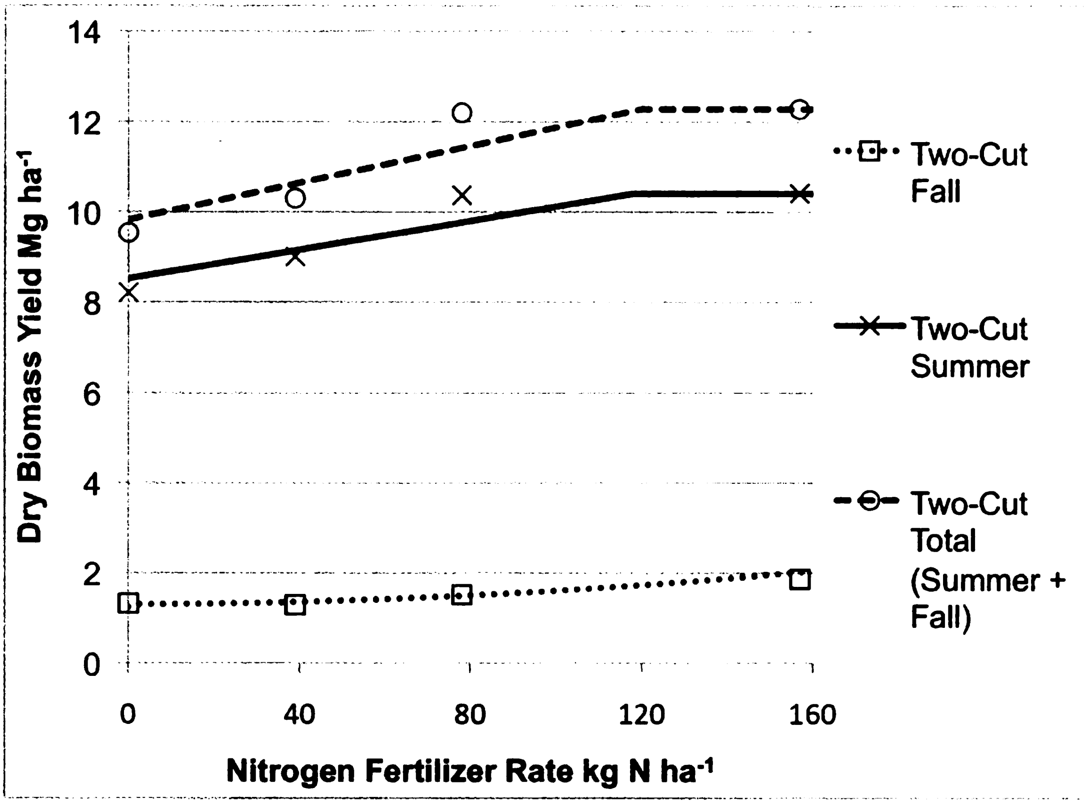

Figure 11. Switchgrass biomass yield response to nitrogen under the two-cut harvest management system in 2009. Corresponding regression equations are found in Table 12.

In 2009, yield responses to nitrogen were best modeled with the LRP functional form. The critical value at which the linear portion joins the plateau region of the non-linear response was 120 kg N ha^{-1} for the combined system, where yield was maximized at 12.28 Mg ha^{-1}. The yield from the summer cut reached a plateau at 10.41 Mg ha^{-1}, and required 118 kg N ha^{-1}. The response and consequent N requirement for regrowth was negligible for the two-cut fall harvest.

Table 12. Switchgrass biomass yields in response to three different harvest management systems, and four nitrogen rates in 2009.

Harvest Management System	**Model**	**Estimated N Requirement, kg N ha^{-1}**	**AIC**	**-2 log Likelihood Fit Statistic**	**R^2**
Two-Cut Summer	Y= 8.53 + (0.016*N)	118 kg N ha^{-1}	68.9	58.9	0.51
Two-Cut Fall (Re-growth)	Y= 1.24+ (0.0042*N)	n/a	n/a	n/a	0.37
Two-Cut (Summer + Fall)	Y = 9.83 + (0.020*N)	121 kg N ha^{-1}	83.2	93.2	0.51

Table 13. Maximum likelihood estimates and coefficients of determination (R^2) of regression parameters for quadratic, linear, and linear response plateau (LRP) equations for the various switchgrass harvest management systems in 2008 and 2009.

	2008			2009		
Equation	**Two-Cut Total**	**One-Cut Fall**	**One-Cut Spring**	**Two-Cut Total**	**One-Cut Fall**	**One-Cut Spring**
Linear (L)	Y=9.89 + 0.015N -	Y=10.0082+0 .0068N	Y=8.35 + 0.0071N	Y=9.69+ 0.020N	Y=11.35 + 0.011N	Y=8.71+ 0.0054N
	R^2 = 0.21	R^2 = 0.20	R^2 = 0.07	R^2 = 0.41	R^2 = 0.16	R^2 = 0.16
2log likelihood	69.0	50.8	66.8	62.9	64.9	46.0
Linear Response Plateau (LRP)	Y=11.380 + (- 0.076N	Y=10.77- 0.030(N)	Y=7.34	Y=12.28 – 0.03N	Y=12.80 - 0.019N	Y= 9.33 - 0.011(N)
	R^2= 0.31	R^2= 0.37	R^2= 0.072	R^2= 0.51	R^2= 0.16	R^2= 0.22
2log likelihood	63.7	45.2	n/a	61.7	64.1	44.7
Quadratic Polynomial (QP)	Y= 9.17 +0.053N - 0.00026 N^2	Y= 9.60 +0.031N- 0.00016 N^2	Y= 8.23 +0.00012N - 0.00005 N^2	Y= 9.44 +0.035N - 0.00011N^2	Y= 11.11 +0.026N - 0.0001 N^2	Y= 8.55 +0.015N - 0.00007 N^2
	R^2= 0.30	R^2= 0.38	R^2= 0.08	R^2= 0.49	R^2= 0.16	R^2= 0.21
-2log likelihood	66.7	45.3	66.6	62.0	63.9	44.8

CONCLUSION

Data from the formative years of this switchgrass stand indicate that yield response to nitrogen is best modeled with a linear response plateau. Quadratic and LRP functional forms were very similar in some instances and had minor implications for the predicted optimal N within the range tested. Yields across all N rates and harvest systems ranged from 4.0-10.25 Mg ha^{-1} in 2008, and 9.0-12.25 Mg ha^{-1} in 2009, which is similar to reported yields in the mid- west, and north- east regions of the U.S. The efficacy of deriving agronomic and optimal N rates for switchgrass biomass production from either an LRP or QP model needs to be ascertained beyond the formative years and across all years of a mature stand. In 2009, the fall one-cut system outperformed the two-cut system. However, data representative of a mature stand is required before we can conclude that a single cut-system is superior to the two-cut system in the Great Lakes Region. Delaying harvest until two weeks after a killing frost or the following spring appears to make any gains in yield from nitrogen fertilizer application negligible during the early years of the stand. Further research is needed on how nitrogen contributes to increased biomass yield and why those gains diminish with delaying harvest. In both years there were sizeable yield losses when switchgrass was overwintered, (41.5% and 26.3% in 2008 and 2009, respectively). It does not appear to be an ideal harvest management system for the bioconversion platform.

CHAPTER 3

CRYSTALLINE CELLULOSE, LIGNIN CONTENT AND MATRIX HEMICELLULOSE POLYSACCHARIDE COMPOSITION OF SWITCHGRASS UNDER VARYING NITROGEN RATES AND HARVEST MANAGEMENT SYSTEMS

Abstract: Understanding the chemical composition of switchgrass is an important issue for future utilization of switchgrass for biofuel production by- and co- product generation. The objective of this research was to determine the effects of nitrogen fertilizer and harvest timing on switchgrass biomass composition. Four different nitrogen rates (0, 38, 79, and 157 kg $ha^{-1)}$ were applied to the plots that were then harvested either under a two-cut system (anthesis and post-senescence), late fall or left to overwinter and then harvested in the spring. Crystalline cellulose content increased with maturity but did not increase with increased nitrogen fertilizer. Acetyl bromide soluble lignin content did not differ between the fall and spring, but was higher with 79 kg and 157 kg N ha^{-1} compared to the two lower application rates of N. Hemicellulose monosaccharide composition varied with harvest timing, and mannose, rhamnose and galactose concentrations were significantly reduced at the 79 and 157 kg N ha^{-1} rate of fertilizer.

Key Words: lignocellulosic, ethanol, cellulose, hemicellulose, lignin, xylose, harvest, nitrogen

INTRODUCTION

Cellulosic ethanol is being pursued as a domestically produced, renewable liquid transportation fuel in the United States as part of a mandate stating that 21 billion gallons of renewable, advanced fuels must be produced annually by 2022. More specifically, the 2005 Energy Act mandates that 250 million gallons of ethanol must be produced from cellulose materials by 2012. The positive attributes of domestic production of cellulosic ethanol from perennial grasses has been discussed in previous chapters.

Understanding the chemical compositions of switchgrass is an important issue for future utilization of switchgrass for biofuel production (Hu et al., 2010). With a thorough understanding of the composition of feedstocks, we are better equipped to maximize cellulosic ethanol production from various feedstocks, as well as derive co- and by-products. Utilizing all major components in the plant improves the cost effectiveness of the production system (Cherney et al., 1988; Pauly and Keegstra, 2008). Feedstock quality is the primary determining factor in conversion efficiency. Increasing cell wall carbohydrate (principally as cellulose) content in biomass can be expected to increase the conversion efficiency and yield of liquid fuel (Weimer et al., 2005; Dien et al., 2006, (Sarath et al., 2008b).

The processing steps for depolymerization by both chemical or enzymatic processes and the subsequent fermentation of the various sugars will need to be optimized to accommodate different feedstocks. Within a given species, information on the ease of digestibility due to lignin content and composition, crystalline cellulose content, and the composition of the hemicelluloses and other matrix polysaccharides will also be important for feedstock processing optimization (Foster et al, 2010) It follows that information regarding specific feedstock characteristics form feedstock grown under varying levels of nitrogen fertilizer and harvest frequencies/timing will be needed for planning harvest and delivery logistics.

If Bioconversion Facilities (BCF) desire specific quality parameters for improved conversion efficiency, growers could be compensated accordingly (e.g. increased cellulose, low lignin content). Economic incentives will have to be provided for producers who choose to grow cultivars with quality characteristics that have been improved through genetic manipulation (Vogel and Jung, 2001). Vogel and Jung (2000) speculated on a scenario in which future bioconversion facilities would probe and collect feedstock samples at delivery and be able to evaluate feedstock quality value in ≤ 30 min. In the absence of improved cultivars for conversion to cellulosic ethanol, agronomic management serves as the primary method of increasing yield and optimizing quality traits.

Biomass Cell Wall Constituents

Lignocellulosic biomass is a mixture of carbohydrate polymers (cellulose, hemicellulose and pectin in varying ratios) and the non-carbohydrate polymer lignin (Doran-Peterson et al., 2008). The breakdown of crystalline cellulose yields glucose, while hemicellulose yields mostly pentoses (xylose) and some hexoses (Sarath et al., 2008a). Grass cell walls consist of a network of cellulose fibers surrounded by a matrix of non-cellulosic polysaccharides (Vogel 2008).

Switchgrass, being a monocot and a member of the Poales family, has a Type II primary cell wall. It is comprised of cellulose fibers encased in glucuronoarabinoxylans (GAX), high levels of hydroxycinnamates, quantities of mixed linkage glucans to varying degrees, and levels of pectin and structural proteins (Harris and Smith, 2006). Once the cell walls have ceased elongation, secondary cell wall deposition initiates and cell walls lignify, replacing water with lignin for improved protection from pathogens, viruses and mechanical strength. Secondary cell walls are largely composed of cellulose, GAX, and lignin (~20%) and are prominent features of xylem, fibers and sclerenchyma (Vogel, 2008). They account for the dominant fractions of lignin, hemicellulose and cellulose in the biomass of mature plants. Tables 14-17 provide information on switchgrass chemical composition from the literature.

Table 14. Selected lignocellulosic biomass compositions (% dry weight) from Wyman (1996) and Li et al. (2001)

Cell Wall Component	Wyman (1996)	Li et al., (2001)
	% D.M.	
Cellulose	32.0	37.3
Hemicellulose	25.2	28.5
Xylan 5C	21.1	22.8
Arabinan 5C	2.8	3.1
Mannan 6C	0.3	0.3
Galactan 6C	1.0	1.4
Lignin	18.1	19.1

Table 15. Range of Compositions of whole plant 'Cave-in-Rock' and leaves and stems across numerous varieties as a percentage of dry weight from Wyman (1996)

Feed-stock	Glucan	Xylan	Galactan	Arabinan	Lignin	Mannan
Whole-plant	31.4-35.0	20.2-23.8	0.9-1.6	2.7-2.9	11.4-16.5	0.2-0.5
Stems	33.5-37.5	21.8-25.0	0.6-1.2	2.2-2.7	13.6-17.2	0.2-0.4
Leaves	26.8-30.9	17.6-21.8	1.2-2.6	3.2-3.7	5.5-15.4	0.2-0.6

Table 16. Chemical compositions of four populations of switchgrass based on (%) of oven dry weight from Hu et al. 2010 (%) of oven dry weight from Hu et al. 2010

Variety/Populations (Whole plant)	Arabinose	Galactose	Glucose	Xylose	Lignin
Alamo	3.8	1.2	38.8	23.1	21.2
Kanlow	3.4	1.3	37.4	23.1	22.6
GA993	3.7	1.3	37.8	23.1	22.4

In a study by Sarath et al. (2007) that looked at lignification of successive internodes in switchgrass, significant secondary cell wall deposition was noted in the cortical fibers, the fiber sheaths surrounding the vascular bundles, and in the cortical parenchyma. Grabber et al. (1991) also showed increased amounts of lignin in older parenchyma cells of switchgrass.

Table 17. Comparison of average chemical compositions between three morphological portions of switchgrass and other published results from Hu et al. (2010)

	Ara	Gal	Gluc	Xyl	Lignin	Reference
Whole-plant Switchgrass	3.7	1.3	38.0	22.8	22.1	Hu et al. 2010
	3.2	1.1	34.3	20.9	17.5 *	Agblevor et al. 1994
	3.6	2.1	34.8	23.4	21.4*	Thammasouk et al. 1997

*Klason lignin content

Cellulose

Cellulose is the most abundant organic polymer on earth, and an important trait for biofuels being used to produce ethanol via fermentation (Lemus et al., 2002). Cellulose is made up of long microfibrils containing repeating units of beta-linked (β 1,4) dimmers of glucose molecules known as cellobiose (Vogel and Jung 200, Doran-Peterson et al. 2008). Cellulose from 1 kg of dry straw yields 0.111 kg of ethanol, it has an enzymatic conversion efficiency of 0.76 and a fermentation efficiency of 0.75 (Badger, 2002). The susceptibility of cellulose to hydrolysis is restricted due to lignin matrix and hemicellulose protection surrounding these cellulose microfibrils (Alizadeh et al., 2005).

Hemicellulose

Hemicellulose is the second most renewable biomass polymer next to cellulose (Saha, 2003). Hemicellulose is a branched polysaccharide composed of both 6-carbon and 5-carbon sugars. Hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids (Saha, 2003). It can be used in the production of xylitol, 2,3-

butanediol, and other value-added fermentation products in addition to fuel ethanol (Saha, 2003). Although more susceptible to enzymatic hydrolysis than lignin, it is not as readily fermented by the industrial yeast Saccharomyces cerevisiae. Further research is needed to identify microorganisms that can ferment sugars derived from hemicellulose in an economic and efficient manner (Saha, 2003). Improvements in hemicellulosic conversion will improve the economic efficiency of the conversion process (Pauly and Keegstra, 2008).

Lignin

Lignin is a biologically resistant, non-carbohydrate polymer that surrounds cellulose and hemicelluloses. Lignin improves water conductance through the xylem, provides mechanical strength to the plant and prevents the infiltration and spread of pathogens in cell walls (Iiyama et al., 1994). As plants mature, the efficiency of enzymatic degradability of cell walls is decreased due to the accumulation and progressive lignification of vascular and sclerenchyma tissue cell walls (Grabber, 2005). The primary cell walls of parenchyma and epidermal tissues undergo lignification in stem, and to a lesser extent, leaf cell walls (Wilson and Hatfield, 1997). Lignin content negatively affects hydrolysis of structural carbohydrates because it restricts access to enzymes, which can limit the conversion of plant material into liquid fuels (Cherney et al. 1988).

Bioconversion of biomass to ethanol is hindered by the structure and content of lignin in the cell wall. Many researchers have proposed modifying lignin biosynthetic pathways to either reduce the recalcitrance of the lignin or reduce its quantity. Hu et al. (1999) found that repressing lignin promotes cellulose. However, this has implications for plant defense mechanisms, vascular integrity, plant fitness, and lodging (Anterola and Lewis, 2002).

The remaining lignaceous biomass after saccharification and fermentation can be used as a combustion fuel source for the distillation process and to power the cellulosic ethanol biorefinery with the possibility of selling surpluses to the electrical grid (Farrell et al., 2006; Lynd et al., 2009).

The Bioconversion Platform and the Bio-refinery Concept

Most conversion technology research efforts in the United States are focused on the sugar or bioconversion approach due to the strong interest in the development of alternative liquid transportation fuel (Sanderson et al., 2006). In the biological conversion of feedstock carbohydrate fractions cellulose, hemicellulose and pectins are chemically separated from lignin and split into hexose and pentose monomers that are then fermented to produce ethanol (Patzek 2008). The bioconversion platform is made up of an array of pretreatment, hydrolysis or saccharification, fermentation and distillation processes. In some forms of the platform, processes can be combined, including simultaneous saccharification and fermentation (SSF), a process in which cellulose hydrolysis and glucose fermentation as well as C6 hemicellulose sugars (galactose and mannose) occur in the same vessel (Chang et al., 2001). SSF is thought to reduce inhibition caused by glucose fermentation and thereby increase the rate of saccharification (Chang et al., 2001). Consolidated bioprocessing (CBP) is a single step, mature technology (not yet developed) process, that combines cellulase production, cellulose hydrolysis, and hexose and pentose fermentation, thereby eliminating the need for separate enzyme production (Lynd et al., 2005).

Pretreatment

The susceptibility of cellulose to hydrolysis is restricted due to lignin matrix and hemicellulose protection surrounding the cellulose microfibrils (Bals et al., 2010a; Wyman, 1996). Effective pretreatment is required to increase the surface accessibility for hydrolysis, promote cellulose decrystallization, partially depolymerize hemicelluloses, and reduce the lignin recalcitrance in the treated biomass (Alizadeh et al., 2005). An ideal pretreatment must reduce lignocellulosic recalcitrance while minimizing the formation of degradation products that inhibit subsequent hydrolysis and fermentation. Pretreatment processes can be physical or chemical. Examples of available pretreatment techniques include acid hydrolysis, steam explosion, ammonia fiber expansion, alkaline wet oxidation, and hot water pretreatment. AFEX is an important pretreatment technology that utilizes both physical (high temperature and pressure) and chemical (ammonia) processes to achieve effective pretreatment (Alizadeh et al., 2005; Bals et al., 2010b). AFEX offers nearly complete recovery of the pretreatment chemical (ammonia) and does not require a washing step, which facilitates high solid load hydrolysis. The residual ammonia on the pretreated biomass serves as a nutrient addition for microbial growth.

Hydrolysis

The disrupted biomass then undergoes hydrolysis (saccharification), which liberates glucose, xylose and other monomer sugars which are then fermented and distilled to produce bioethanol (Yuan et al., 2008). There are four major structural characteristics of lignocellulosic biomass that hinder enzymatic hydrolysis: cellulose fibre crystallinity, hemicelluloses acetylation, inaccessible surface area and lignin content. Glucose can be fermented by industrial yeast strains (*Saccharomyces*). Hemicellulosic pentoses, however, are difficult to ferment with currently available yeast strains (Saha, 2003).

Effect of nitrogen fertilization and harvest timing on cell wall composition

It is generally understood that the proportions of cellulose, hemicellulose, and lignin components vary from plant to plant, but less is known about the extent to which these proportions differ within in a given species, or how these proportions can be manipulated with harvest timing and frequency, or nitrogen fertilizer.

Nitrogen fertilization and its effect on chemical composition of biomass is complex. A survey discussed in Cherney et al. (1998) in which Wilson (1982) looked at studies that examined the effects of nitrogen on composition and degradability found that a third of them stated there was no effect, another third said there was a positive effect and the remainder stated that there was a negative effect. A previous study by Lemus et al. (2008) found that cellulose and lignin increased with increased N rate.

The Effect of Nitrogen Fertilizer and Harvest Timing on Feedstock Quality

The different responses to harvest date and location/ecotype have large implications for biomass refining (Table 18, Bals et al., 2010a). Harvest management systems must be tailored to local needs of the BCF in order to maximize ethanol production (Bals et al., 2010). The authors found that harvesting in July provided lower costs for pretreatment and higher potential ethanol yields, which may help offset the cost of a second harvest, with the possibility for additional revenue from co-product generation. However, harvesting prior to senescence and a killing frost has implications for stand longevity as discussed in the previous chapter.

Table 18. Composition analysis (as a percentage of total dry weight) for the July and October harvests of Cave-in-Rock (CIR) switchgrass from Bals et al. 2010

Cell-Wall Constituent	CIR: July	CIR: October
	(% of total dry weight)	
Glucan	30.6 ± 0.2	33.6 ± 0.5
Sucrose*	5.1 ± 0.3	2.4 ± 0.2
Xylan	19.4 ± 0.3	25.3 ± 0.2
Arabinan	2.0 ± 0.1	2.0 ± 0.1
Lignin	10.4 ± 0.4	16.7 ± 0.5

*Soluble carbohydrate

Cell wall component differences also exist between switchgrass harvested in the fall and the spring. Adler et al. (2006) found that switchgrass that was over-wintered and harvested in the spring had higher concentrations of cell wall glucose and non-glucose sugars than switchgrass harvested in the fall but that lignin increased over the same period. They attribute the higher cell wall carbohydrates values in spring to the leaching of soluble components such as sugars, protein, and organic acids when overwintered (Adler et al., 2006).

Maturity is the single most important determinate of cell-wall concentration and composition (Vogel and Jung 2000). As plants mature, they undergo many processes that affect the relative concentrations of cellulose, hemicellulose, lignin, pectin, proteins, ash, and other cell wall constituents. Some of the processes include: cell elongation; a shift in leaf:stem ratio; secondary wall deposition; lignification; carbohydrate translocation; and senescence. The stem component increases as switchgrass matures and leaf components decrease as a percent of total biomass, primarily because of stem internode (Table 19, Lee et al., 2007) elongation (Sanderson, 1992). The proportion of leaves to stems is an important determinant in biomass yields and potentially for conversion. On a component basis, leaves are generally lower in lignin, higher in solubles, cellulose and hemicellulose, whereas switchgrass stems will contain greater proportions of lignin and cellulose (Sarath et al., 2008a). Table 20 provides information on changes in cell-all carbohydrates with maturity from Dien et al. (2006).

Table 19. Changes in composition of 'Nebraska 28' switchgrass with crop Maturity from Lee et al. (2007)

	Anthesis	Post Physiological Maturity	Overwintered
	------------------- % of dry matter -------------------		
Cellulose	33.0	35.2	41.8
Hemicellulose	31.0	31.1	33.7
Acid Detergent Lignin	3.8	4.6	6.2

Table 20. Cell wall carbohydrates (g kg^{-1}) from Dien et al. (2006)

Switchgrass	Gluc*	Xyl	Ara	Gal	Man	Rha	Fuc
Pre-boot	273	179	31	13	5	2	1
Anthesis	283	195	27	10	4	1	1
Post-frost	322	223	30	12	5	2	1

*Abbrev: Glucose (Gluc); Xylose (Xyl); Arabinose (Ara); Galactose (Gal); Mannose (Man); Rhamnose (Rha); Fucose (Fuc).

Objectives

Cost effective lignocellulosic ethanol production from switchgrass requires high biomass yields and a high concentration of cellulose. The objective of this research was to quantify the crystalline cellulose and polyphonol lignin content of switchgrass cell walls, and assess the composition of matrix polysaccharide content mainly from hemicelluloses at a whole-plant level under three harvest management systems and four nitrogen fertilizer rates. Research on pretreatment optimization, hydrolysis/saccharification, SSF, BCP, enzyme and catalyst design could benefit from this feedstock quality data.

MATERIALS AND METHODS

Site Description and Experimental Design

Field experiments were conducted at the Michigan State University Agronomy Farm, in East Lansing (42°25′48′′ N, 84°16′48′′ W), MI over a 3-yr period beginning in the spring of 2007. The experimental design was a randomized complete block design with five replications and was conducted in a field with predominantly Capac loam (Fine-loamy, mixed, mesic Aeric Ochraqualfs) soils. Plots were fertilized (0, 38, 79, 157 kg N ha^{-1}) in the spring applied as granular urea (46-0-0 N-P-K). Plots were then harvested under a one, or two- cut system. The plots under the one-cut system were cut either in the fall approximately two weeks after a killing frost (one-cut fall), or left to overwinter, and harvested in the spring (one-cut overwintered). The two cut-system consisted of a harvest during the late-boot to early anthesis stage (two-cut summer), and again in the late fall (two-cut fall) after a killing frost (<28° C for a sustained period of time). Total fresh weight per plot and biomass content for each plot was recorded, and biomass moisture content for each plot was determined via subsamples that were placed in a forced air oven at 100°C for 72 hours or until a consistent dry weight was achieved. The total dry matter (DM) per plot was then converted to Mg DM ha^{-1}. Samples were finely ground with a Christy-Norris mill (Christy and Norris Limited, Chelmsford, England) fitted with a 1-mm screen for further analysis.

Compositional Analysis

Crystalline cellulose content and the analysis of the monosaccharide constituents of cell wall hemicellulose were based on five field replicates and all four nitrogen rates and harvest timings, while lignin content analysis was based on three field replicates, two harvest timings (one-cut fall and one-cut overwintered) and three N rates (0, 79, 157 kg ha^{-1}). Prior to compositional analysis, the dried biomass was ball milled to a fine powder (<1mm). From the powder, the alcohol insoluble residue (AIR) was prepared and thereafter, underwent amylase treatment to remove the residual starch resulting in purified lignocellulosic material (York et al., 1986).

Cellulose and Hemicellulose

For hemicellulose compositional analysis, the destarched AIR was used for the weak acid hydrolysis using [2M] trifluoroacetic acid. The hydrolysate was separated from the insoluble material and the monosaccharides were derivatized into their corresponding alditol acetates and quantitated using a GC-MS system (Albersheim, 1967). The remaining insoluble material from the hydrolysis was stripped further of hemicelluloses and amorphous glucan by washing with the Updegraff reagent, an acetic acid and nitric acid mixture (Updegraff 1969). The remaining material, primarily crystalline cellulose, was fully hydrolyzed with 72% sulphuric acid and the glucose in the resulting hydrolysate was quantitated using the colorimetric Anthrone assay (Seaman hydrolysis; (Selvendran and Oneill, 1987).

Lignin

Samples collected from three replications, and not including the 38 kg N ha^{-1} rate, or the two- cut system treatment, were used for lignin content analysis. Starting from the purified lignocellulosic material collected from the sugar compositional analysis, the lignin was quantified with the acetyl bromide soluble lignin assay. The material was treated with a 1:4 acetyl bromide/acetic acid (v/v) solution to render the lignin acetic acid soluble. After a volumetric dilution with glacial acetic acid, the solublized lignin was quantitated using a UV spectrophotometer set at 280 nm wavelength. The lignin content data was expressed as percent acetyl bromide soluble lignin from the dry weight of the isolated cell wall material (Fukushima et al., 1991).

Statistical Analysis

All compositional analyses were done in triplicate, and data were corrected to a 100% dry matter (DM) basis. Data was analyzed using PROC MIXED is SAS v9.1 (2003 SAS Institute Inc., Cary, NV.) Nitrogen fertilization level, harvest timing, and year were considered as fixed effects, and replication was considered to be a random effect. Unequal variances were analyzed using the REPEATED/Group option. Comparisons between individual factor level or treatments were conducted using multiple t-tests with the post-hoc scheffe adjustment to generate post-specified contrasts and estimate statements. When nitrogen fertilizer was found to be significant in the ANOVA linear contrast statements were used to examine the nitrogen effect. Contrast statements were also used to determine if concentrations of the various hemicellulose sugars varied with harvest timing. Matrix polysaccharide composition data were transformed with the arcsine square-root transformation prior to analysis and year was treated as a random effect for this analysis. Results were reported as statistically significant at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Crystalline Cellulose Content

There was no interaction between nitrogen fertilizer and harvest timing, and nitrogen was not significant at $\alpha=0.05$, but a trend is evident ($P=0.0667$) (Fig. 12). Harvest timing was a significant factor ($p=<0.0001$), plots that were harvested under the two-cut harvest system contained ~12.8% less crystalline cellulose ($\mu g\ mg^{-1}$) than those that reached full maturity and underwent natural senescence (Fig. 12).

Studies of lowland ecotypes in Alabama found that cellulose contents decreased by 12% between the first and second harvests within a two-cut system (Sladden et al., 1991). Although the second-cut of the two-cut system was made at the same time as the one-cut fall, plants experiencing regrowth may have had an accelerated vegetative growth and therefore a reduced period of secondary cell wall deposition; and may have ceased growth as a result of the killing-frost as opposed to reaching full maturity, and undergoing natural senescence. The resulting tissue may have higher proportions of soluble carbohydrates at harvest time.

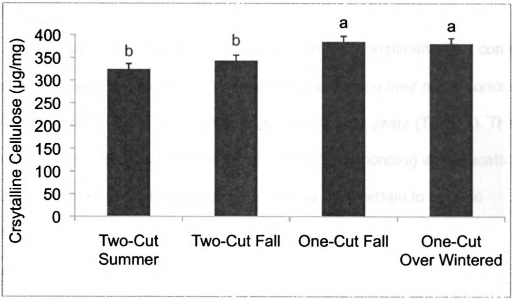

Figure 12. Crystalline cellulose under varying harvest timings. Means with the same letter are not statistically different at $\alpha=0.05$. Standard error was 12.1

The lack of a change in cellulose content with increased fertilizer present in our data is not in agreement with previous research. Bilal and Sindhu (2001) found that cellulose content increased with nitrogen fertilizer level and advancing growth stage with the maximum concentrations occurring in the stem portion. Lemus et al. (2008) conducted a study that looked at quality as affected by nitrogen rate, but had a single harvest treatment in which switchgrass was harvested between September 27 and November 27 over a five-year period. They found that cell wall constituents differed between years, with strong linear and quadratic responses for all traits and nitrogen content of earlier cut switchgrass to differential leaching severity among their harvest dates. They do not mention the possible change in stem:leaf ratio or potential for enhanced lignification of the secondary cell wall with the delayed onset of frost in some years.

Matrix polysaccharides composition

The molar concentrations of seven cell wall constituents that comprise or are associated with hemicellulose were quantified and their molar concentrations were compared among harvest timings, across both years (Table 8). The monosaccharides were derivatized into their corresponding alditol acetates and thus we are not able to discuss the results as they pertain to specific hemicellulose types, or other measures of quantification such as NDF and ADF. A measure of total change of hemicellulose over time is not reported. All monosaccharide's molar concentrations were significantly affected by harvest timing ($p = < 0.0001$) and nitrogen was found to be a significant effect in the derivation of three of the sugars, namely arabinose ($p = 0.0155$) (Fig 14), rhamnose ($p = 0.0002$) (Fig 15) and galactose ($p = 0.0002$) (Fig 16) although there was no interaction between the main effects.

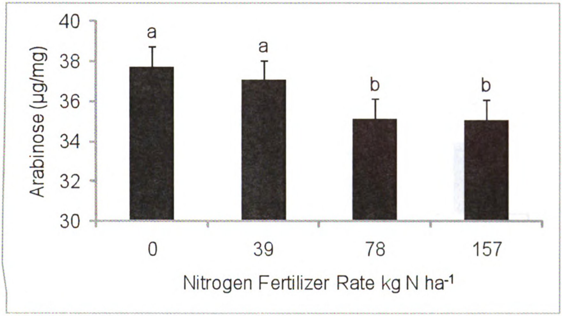

Figure 13. Figure 14. Arabinose molar concentration (%), as a proportion of the matrix polysaccharides at four different N rates across harvest timings. Means with the same letters are not statistically different at a=0.05. Standard error was 1.02

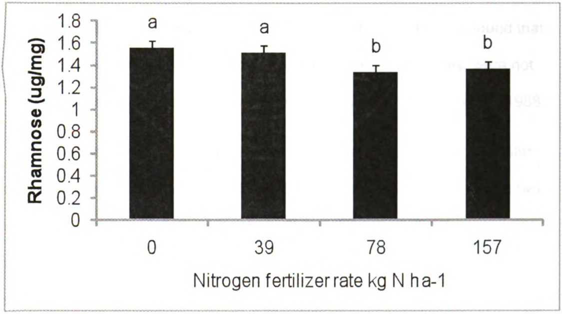

Figure 14. Rhamnose molar concentration (%) , as a proportion of the matrix polysaccharides at four different nitrogen rates, across harvest timings. Means with the same letters are not significantly different at a=0.05. Standard error was 0.06.

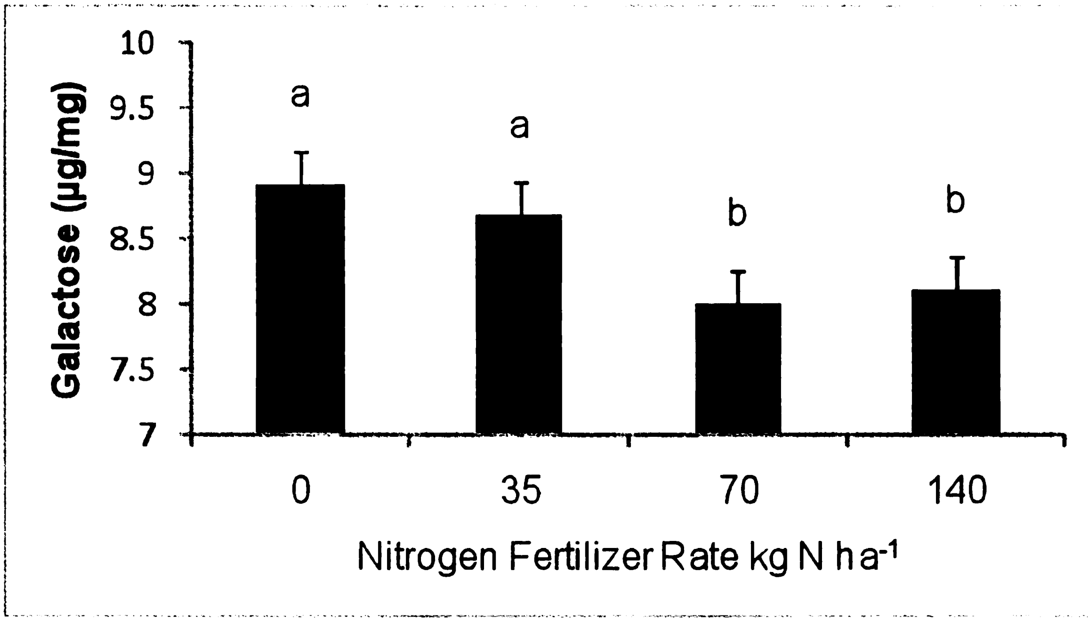

Figure 15. Galactose molar concentration (%), as a proportion of the matrix polysaccharides at four different N rates across harvest timings. Means with the same letters are not statistically different at a=0.05. Standard error was 0.22

Our data is not in agreement with Cherney et al. (1998) who found that arabinose concentrations, among other cell wall monosaccharides, were not influenced by environmental conditions or N fertilization (Cherney et al., 1988).

The two-cut summer biomass had high levels of xylose (80.1% molar concentration) and the lowest levels of all other monosaccharides. Of the two harvests made in the fall, there were significant differences in all monosaccharides between harvest systems except for fucose and glucose. The regrowth from the two-cut system had the highest proportions of all monosaccharides except in the case of xylose, which was relatively higher in the one-cut fall harvested biomass. The one-cut system had similar levels of xylose, rhamnose and galactose across harvest systems. However, glucose, mannose and arabinose were all higher in proportion in the biomass that was over-wintered. The highest glucose concentration was present in the one-cut overwintered biomass.

Table 21. Matrix polysaccharide composition across varying harvest timings expressed as molar concentration

Monosaccharide Constituents	Two-Cut Summer	Two-Cut Fall	One-Cut Fall	One-Cut Overwintered	Standard Error
	Molar Concentration (%)				
Xylose	80.10[a*]	68.93[c]	74.70[b]	69.65[c]	0.002
Arabinose	9.81[d]	13.48[a]	11.20[c]	12.25[b]	0.0031
Mannose+	0.17[d]	1.35[a]	0.39[c]	0.84[b]	0.0050
Fucose	0.09[b]	0.12[a]	0.14[a]	0.09[b]	0.0023
Rhamnose+	0.29c	0.39a	0.72b	0.45b	0.0022
Galactose+	1.94[c]	3.59[a]	2.32[b]	2.45[b]	0.0037
Glucose	7.58[c]	11.82[b]	10.88[b]	14.28[a]	

*Means with the same letters within the same row are not different at a=0.05
[+] N was a significant factor but no interaction was present

Lignin Content

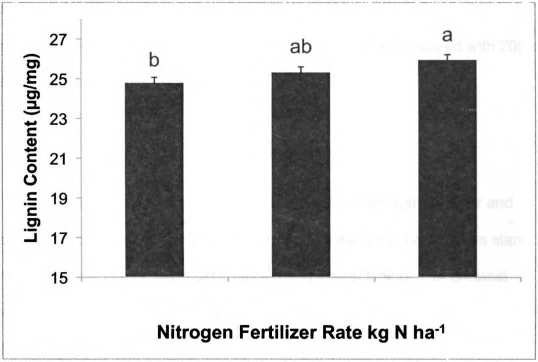

Figure 16. Lignin content expressed as a weight percentage measurement of the acetyl bromide soluble lignin as a function of applied nitrogen fertilizer. Means with the same letters are not statistically significant (a=0.05; scheffe adjusted means). Standard error was 0.273.

The lignin content was determined as a weight percentage measurement of the acetyl bromide soluble lignin based off the dry weight of the isolated cell walls. Lignin content of the biomass did not differ when harvested in the fall or delayed until the spring and there were no significant interactions between any of the fixed effects. Analyses on the summer one- and two-cut samples were not performed. Both year ($p=<0.001$) and nitrogen ($p=0.0099$) were affected although caution is exercised in the interpretation of the year results as samples were analyzed at separate times. Switchgrass that was fertilized with 157 kg N ha^{-1} had a lignin content that was 4.5% greater than unfertilized switchgrass, whereas switchgrass fertilized at the 79 kg N ha^{-1} rate was not statistically different from either the unfertilized biomass, or the 157 kg N ha^{-1} rate (Fig. 9). Lignin content was increased by 16% when fertilized with 200 kg N ha^{-1} (Cherney et al., 1988).

CONCLUSION

The data do not show a correlation between nitrogen fertilizer and crystalline cellulose content during the initial years of the switchgrass stand. Hemicellulose composition did change with harvest timing. The greatest amount of xylose was present in the two-cut summer harvest. Nitrogen fertilizer negatively affected the concentrations of arabinose, galactose and rhamnose. Further research is needed to examine why nitrogen might affect these specific hemicelluloses monosaccharides. Lignin content did not differ between the fall and spring but did increase with increased rates of applied nitrogen fertilizer. Increased sampling in terms of harvest timing and nitrogen rates is needed to further validate this finding. Farmers should harvest once annually, once the crop has senesced to enhance feedstock quality.

CONCLUSIONS

The data from three sites over two years demonstrate that switchgrass can be successfully established in the Great Lakes Region. The data suggest that tillage is not critical for obtaining a stand frequency that exceeds the 40% establishment threshold. The data also support the hypothesis that with tillage, both a CGD and a double-roller seeder can be used to successfully establish switchgrass for energy purposes. These results suggest that existing equipment can be used to prepare seedbeds, which would mean less capital expenditure and an increased likelihood of farmer adoption. A mid-spring seeding date (mid-may to early June) could potentially offer the best possibility for increasing yield and profitability in early production years.

A yield of 12.0 Mg ha^{-1} per year is a reasonable estimate of yield potential for the fall one- cut harvest system, Yield losses from delaying harvest until the spring is of concern if switchgrass is being grown for the bioconversion platform. Nitrogen rate was a significant determinant of yield for the two- cut summer, and two- cut combined yields and was best fir with a LRP functional form. Less differentiation by level of nitrogen fertilizer was evident when harvest was delayed until the fall or following spring.

We have shown that lignin content was increased with increasing N and that crystalline cellulose was not. Crystalline cellulose increased with harvests made after maturity had been reached. Hemicellulose composition was affected by harvest timing, which subsequently affects the concentration of hemicellulosic monosaccharides. Arabinose, rhamnose, and galactose concentrations were decreased with higher rates of N,

Further research is needed to optimize the no- till establishment systems, This includes the use of two pre-plant herbicide burndown treatments, proper residue management, optimization of NT drills, and seeding into warmed-up soils could potentially enhance NT yields. Research on chemical control programs for switchgrass establishment and early stand management would also be of benefit as it would lead to registration of effective herbicides that could further enhance establishment and cost-effectiveness, The efficacy of deriving agronomic and optimal N rates for switchgrass biomass production from wither an LRP or QP model needs to be ascertained, Lastly, research is needed on how nitrogen contributes to increased biomass yield and why those gains diminish with delaying harvest.

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