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EVALUATING THE AGRONOMIC PERFORMANCE OF SWITCHGRASS

(*Panicum virgatum* L.) ON MARGINAL vs. PRIME FARMLAND

by

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ABSTRACT OF THE THESIS

Evaluating the Agronomic Performance of Switchgrass (*Panicum virgatum* L.) on
Marginal vs. Prime Farmland

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Dr. Stacy A. Bonos

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial grass native to North America. The difference in biomass production between and within switchgrass ecotypes (upland and lowland) and populations due to genotype x environment interaction (GxE) has been documented. Breeding research for increased biomass production in switchgrass has been conducted on University research farms with prime farmland. This study aims to evaluate the agronomic performance of 14 cultivars and 45 high biomass producing clones of switchgrass in marginal vs. prime farmland. Additionally this study investigates the effects of increasing biodiversity (1 grass species, 3 grass species or 4 species-grass/legume combinations) on biomass production. The cultivars and biodiversity studies were seeded in 2008 and 2009 in six locations (Maryland, New Jersey, New York, Pennsylvania, South Dakota and Wisconsin) and three locations (New Jersey, New York, Pennsylvania), respectively, in paired fields (marginal vs. prime land). Each field had a nitrogen treatment 0 or 100 kg of N·ha⁻¹·year⁻¹. Stand establishment (% coverage), plant height (cm), tiller density (tillers·m⁻²) and dry biomass yield (Mg·ha⁻¹) data was collected to determine agronomic performance. The clonal material was transplanted in 2009 in two locations (New Jersey and South Dakota). In addition to agronomic data collected,

heading date, anthesis date (Julian date) and visual ratings for disease presence were recorded. Cultivars were shorter in marginal soils. For stand establishment and biomass yield, 50% of cultivars showed differences due to soil quality. For tiller density, 40% of the cultivars presented differences due to soil quality; some cultivars had higher tiller density in marginal soils. For the biodiversity study low diversity plots (one grass species) were not significantly different than yields of high diversity plots (four species-grass/legume). For the clone study, soil quality may have influenced a delay in flower initiation and other traits, such as plant height, etc. It was also observed that genotype may have been the most influential factor in tolerance to anthracnose (*Colletotrichum navitas*) and rust (*Puccinia emaculata*).

DEDICATION

Quiero dedicar este documento a mi hijo Samuel Matias Sosa por ser mi inspiración, espero que encuentres tu verdadera pasión así como yo la encontré en las ciencias agrícolas. Te quiero mucho hijo.

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TABLE OF CONTENTS

ABSTRACT OF THE THESIS	ii
DEDICATION	iv
ACKNOWLEDGEMENT	iv
TABLE OF CONTENTS.....	v
LIST OF TABLES	viii
LIST OF FIGURES	xvii
LITERATURE REVIEW	1
Switchgrass a Renewable Source of Fuel	1
Soil Considerations and Variability of Switchgrass Establishment	5
Genetic and Phenotypic Diversity of Switchgrass.....	8
Switchgrass Ecotypes.....	9
Genetic Diversity of Switchgrass.....	17
Genotype by Environment Interactions	20
Influence of Nitrogen Fertilization on Switchgrass Performance.....	23
Improving Yield in Low Productivity Environments	28
The Use of a Multi-Species Approach to Improve Biomass Yield and Reduce Nitrogen Fertilization	37
Literature Cited	42
CHAPTER 1: Agronomic Performance of 14 Switchgrass Cultivars in Marginal vs. Prime Farmland in Six Locations	53
Abstract.....	53
Introduction.....	55

Materials and Methods.....	59
Plant Material and Soil type.....	59
Data Collection	63
Data Analysis	68
Results and Discussion	69
Stand Establishment.....	69
Plant Height	77
Tiller Density	86
Dry Biomass Yield.....	94
Conclusions.....	103
Literature Cited	106
CHAPTER 2: A Comparative Study of the Agronomic Performance of Switchgrass Clones Selected from Different Environments in Marginal vs. Prime Farmland in New Jersey and South Dakota.....	
	111
Abstract.....	111
Introduction.....	113
Materials and Methods.....	118
Plant Material and Soil Type	118
Data Collection	120
Data Analysis	123
Results and Discussion	124
Heading and Anthesis Date.....	124
Plant Height	133

Tiller Density	142
Dry Biomass Yield.....	151
Anthracnose Disease Ratings.....	163
Rust Disease Ratings.....	167
Conclusions.....	170
Literature Cited	172
CHAPTER 3: Bioenergy Characteristics of Native Warm-season Grass Monocultures and Multi-Species Mixtures in Marginal vs. Prime Farmland in New Jersey, New York and Pennsylvania	
	175
Abstract.....	175
Introduction.....	177
Materials and Methods.....	181
Plant Material and Soil type.....	181
Data Collection	184
Data Analysis	185
Results and Discussion	186
Stand Establishment.....	186
Plant Height	195
Dry Biomass Yield.....	201
Conclusions.....	207
Literature Cited	209
CONCLUSIONS OF THE THESIS	215
Curriculum Vitae	218

LIST OF TABLES

Chapter 1

Table 1. List of 14 cultivars and experimental selections of switchgrass evaluated in marginal vs. prime farmland in six locations. Switchgrass ecotypes: southern lowland (SL), northern lowland (NL), central upland (CU) and northern upland (NU).	60
Table 2. Description of soil types of the marginal and prime farmland of the six states.....	62
Table 3. Analysis of variance of stand establishment (% coverage) of 14 switchgrass cultivars evaluated at five locations in marginal vs. prime farmland at each location. Switchgrass plots were established in 2008 in NY, SD and WI and in 2009 in NJ and PA.....	71
Table 4. Stand establishment (% coverage) of 14 switchgrass cultivars evaluated at five locations in marginal vs. prime farmland at each location. Switchgrass stands were established in NY, SD and WI in 2008 and in NJ and PA in 2009.....	72
Table 5. Comparison of stand establishment (% coverage) means by cultivar in marginal vs. prime farmland sites across five locations.	76
Table 6. Analysis of variance of plant height (cm) of 14 switchgrass cultivars evaluated at four locations in marginal vs. prime farmland at each location with 0 or 100 Kg of N Ha ⁻¹ . Switchgrass stands were established in NY and WI in 2008; in NJ and PA in 2009.	78
Table 7. Plant height (cm) of 14 switchgrass cultivars evaluated at four locations in marginal vs. prime farmland with 0 or 100 Kg of N Ha ⁻¹ . Switchgrass stands were established in NY and WI in 2008; in NJ and PA in 2009.	82
Table 8. Means of plant height (cm) separated by location, site and nitrogen treatment. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.....	83

Table 9. Means of plant height (cm) separated by genotype. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without the marginal site at NJ and prime at NY due to missing values in the data set.	84
Table 10. Comparison of plant height (cm) means by cultivar in marginal vs. prime farmland sites across four locations. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.	85
Table 11. Analysis of variance of tiller density (tillers·m ⁻²) of 14 switchgrass cultivars evaluated at three locations in marginal vs. prime farmland at each location with 0 or 100 kg of N·ha ⁻¹ . Switchgrass stands were established in WI in 2008; in NY and PA in 2009 due to missing values in the data set.	87
Table 12. Tiller density (tillers per m ²) of 14 switchgrass cultivars evaluated at three locations in marginal vs. prime farmland at each location with 0 or 100 Kg of N Ha ⁻¹ . Switchgrass stands were established in WI in 2008; in NY and PA in 2009.	90
Table 13. Means of tiller density (tiller per m ²) separated by location, site and nitrogen treatment. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.	91
Table 14. Means of tiller density (tillers per m ²) separated by genotype. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without the marginal site at NJ and prime at NY due to missing values in the data set.	92
Table 15. Comparison of tiller density (tillers per m ²) means by cultivar in marginal vs. prime farmland sites across three locations. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.	93

Table 16. Analysis of variance for dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) of 14 switchgrass cultivars evaluated at six locations in marginal vs. prime farmland at each location with 0 or 100 kg of $\text{N}\cdot\text{ha}^{-1}$. Switchgrass stands were established in NY, SD and WI in 2008; in MD, NY and PA in 2009.	96
Table 17. Dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) of 14 switchgrass cultivars evaluated at six locations in marginal vs. prime farmland at each location with 0 or 100 kg of $\text{N}\cdot\text{ha}^{-1}$. Switchgrass stands were established in NY, SD and WI in 2008; in MD, NJ and PA in 2009.	97
Table 18. Means of dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) separated by location, site and nitrogen treatment. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.	98
Table 19. Pearson correlation coefficients for dry biomass yield (DBY) and secondary traits: stand establishment (SE), plant height (PH) and tiller density (TD), of 14 switchgrass cultivars evaluated at six locations in marginal vs. prime farmland at each location with 0 or 100 kg of $\text{N}\cdot\text{ha}^{-1}$. Switchgrass stands were established in NY, SD and WI in 2008; in MD, NY and PA in 2009.	99
Table 20. Means of dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) separated by genotype. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without the marginal site at NJ and prime at NY due to missing values in the data set.	101
Table 21. Comparison of dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) means by cultivar in marginal vs. prime farmland sites across three locations. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.	102

Chapter 2

Table 1. List of 45 switchgrass clones from breeding programs in New Jersey (NJ), South Dakota (SD), and Wisconsin (WI), evaluated in marginal vs. prime farmland in two U.S. states (NJ and SD). Switchgrass ecotypes evaluated: lowland (L) and upland (U).	119
Table 2. Analysis of variance of heading date of 45 switchgrass clones from three breeding programs (NJ, SD and WI) evaluated in NJ in marginal vs. prime farmland with 0 or 100 kg of N·ha ⁻¹	127
Table 3. Analysis of variance of anthesis date of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated in NJ in marginal vs. prime farmland with 0 or 100 kg of N·ha ⁻¹	128
Table 4. Means of heading date separated by genotype for 45 switchgrass clones evaluated in marginal vs. prime farmland sites in NJ. Means were separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entry WS08-R1.	129
Table 5. Means of anthesis date separated by genotype for 45 switchgrass clones evaluated in marginal vs. prime farmland sites in NJ. Means separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entry WS08-R1.	130
Table 6. Heading date means of 45 switchgrass genotypes in marginal vs. prime farmland sites evaluated in NJ. Statistical analysis was conducted without entry WS08-R1.	131
Table 7. Anthesis date means of 45 switchgrass genotypes in marginal vs. prime farmland sites evaluated in NJ. Statistical analysis was conducted without entry WS08-R1.	132
Table 8. Analysis of variance of plant height (cm) of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated on marginal vs. prime farmland in NJ and SD with 0 or 100 kg of N·ha ⁻¹	137

Table 9. Plant height means of 45 switchgrass clones from SD, NJ and WI evaluated under marginal and prime soil types in NJ and SD. Mean separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.....	138
Table 10. Means of plant height separated by genotype for 45 switchgrass clones evaluated in NJ and SD. Means separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.	140
Table 11. Comparison of plant height means in marginal vs. prime farmland sites by genotype. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11 NJ.	141
Table 12. Analysis of variance of tiller density (tillers·plant ⁻¹) of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated at two locations in marginal vs. prime farmland at each location with 0 or 100 kg of N·ha ⁻¹	146
Table 13. Tiller density (tillers·plant ⁻¹) means of switchgrass clones from SD, WI and NJ. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.	147
Table 14. Comparison of tiller density (tillers·plant ⁻¹) means in NJ vs. SD locations. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.....	149
Table 15. Comparison of tiller density (tillers·plant ⁻¹) means in marginal vs. prime farmland sites. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.	150

Table 16. Analysis of variance of dry biomass yield ($\text{kg}\cdot\text{plant}^{-1}$) of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated at two locations in marginal vs. prime farmland at each location with 0 or 100 kg of N ha^{-1}	156
Table 17. Means of 45 clones dry biomass yield ($\text{kg}\cdot\text{plant}^{-1}$) separated by genotype. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.	157
Table 18. Comparison of dry biomass yield ($\text{kg}\cdot\text{plant}^{-1}$) means by genotype in NJ vs. SD locations. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.	159
Table 19. Comparison of dry biomass yield ($\text{kg}\cdot\text{plant}^{-1}$) means by genotype in marginal vs. prime farmland sites. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.	160
Table 20. Comparison of dry biomass yield ($\text{kg}\cdot\text{plant}^{-1}$) means by genotype in 0 vs. 100 kg of $\text{N}\cdot\text{ha}^{-1}$. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.	161
Table 21. Pearson correlation coefficients for dry biomass yield (DBY) and secondary traits: heading date (HD), anthesis date (AD) plant height (PH) and tiller density (TD), of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated at two locations in marginal vs. prime farmland at each location with 0 or 100 kg of N ha^{-1}	162
Table 22. Analysis of variance of anthracnose (<i>Colletotrichum nativas</i>) disease ratings of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated in NJ on marginal vs. prime farmland with 0 or 100 kg of $\text{N}\cdot\text{ha}^{-1}$	164
Table 23. Anthracnose disease (<i>Colletotrichum nativas</i>) ratings of 45 switchgrass clones. Means were separation with the LSMEANS option and adjusted with Tukey's method for multiple comparisons.....	165

Table 24. Comparison of anthracnose disease (<i>Colletotrichum nativas</i>) ratings means by genotype in marginal vs. prime farmland sites. Statistical analysis was conducted without entries WI08-R1 and WI08-U2.....	166
Table 25. Analysis of variance of rust disease (<i>Puccinia emaculata</i>) ratings of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated in SD on marginal vs. prime farmland with 0 or 100 kg of N·ha ⁻¹	168
Table 26. Means rust disease (<i>Puccinia emaculata</i>) ratings of 45 switchgrass clones evaluated in South Dakota. Means were separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons.	169

Chapter 3

Table 1. Native grass and legume biodiversity treatments evaluated in marginal vs. prime farmland in three U.S. states New Jersey (NJ), New York (NY) and Pennsylvania (PA).....	182
Table 2. Analysis of variance of stand establishment (% coverage) of 13 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location. Research plots were established in 2008 in NY and in 2009 in NJ and PA.....	187
Table 3. Stand establishment (% coverage) of 13 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.	191
Table 4. Calculated means and standard deviations (SE) of stand establishment of 13 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location.	192
Table 5. Stand establishment (% coverage) of 6 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location. Mean	

separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.	193
Table 6. Comparison of stand establishment (% coverage) means sorted by biodiversity treatment in marginal vs. prime farmland sites across three locations.	194
Table 7. Analysis of variance of plant height (cm) of 13 warm-season grass/legume biodiversity treatments evaluated at two locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha ⁻¹	197
Table 8. Means of plant height (cm) of 13 warm-season grass/legume biodiversity treatments evaluated at two locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha ⁻¹ . Means were averaged across locations, soil quality and nitrogen treatments. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.	198
Table 9. Means of plant height (cm) of 13 warm-season grass/legume biodiversity treatments evaluated at two locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha ⁻¹ . Means were averaged across locations and nitrogen treatments and compared by soil quality. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.	199
Table 10. Means of plant height (cm) of 13 warm-season grass/legume biodiversity treatments evaluated at two locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha ⁻¹ . Means were averaged across locations, soil quality and compared by nitrogen treatment. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.	200
Table 16. Analysis of variance of dry biomass yield (Mg·ha ⁻¹) of 13 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha ⁻¹	205

Table 17. Dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) of 13 biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.	206
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LIST OF FIGURES

Chapter 1

Figure 1. Picture of the 0.75 x 0.75 m frequency grid (Vogel and Masters, 2001). For this study the frequency grid was a metal frame with 25 squares (5 x 5) made out of re-bar and painted orange for better visibility	65
Figure 2. Picture of plant height being recorded by placing a measuring stick from the soil surface to the top of the panicles in three random spots within plot.....	65
Figure 3. Picture of tiller density being recorded by counting the number of tillers within a 1 ft ² metal square in three random spots within the plots.....	66
Figure 4. Switchgrass being harvested in a 0.30 x 1.8 m strip from the 1.8 x 1.8 m seeded plot with a two-wheel sickle bar mower (BCS, Hector, NY).....	66
Figure 5. Harvested switchgrass weighted on the field to record fresh weight.	67
Figure 5. Picture of the inside of the tobacco dryer where field samples were placed, at 43°C for 10 days, after fresh weight was recorded.	67

Chapter 2

Figure 1. Ready for harvest large plant, tied with twine and tagged with an identification number.	121
Figure 2. Plants cut at 30 cm from the soil surface with a hedge trimmer (KHT600, Kawasaki Motor Corp., Irvine, CA)	122
Figure 3. Field samples were placed in a tobacco dryer (DeCloet Bulk Curing Systems, Tillonsburg, Canada) for 10 days at 38° C.....	122

LITERATURE REVIEW

Switchgrass a Renewable Source of Fuel

There is a great interest to increase the production of biomass for energy. This revival of bioenergy is based on worldwide concerns about the security of the national energy supply and the changes in global climate (Farrell et al., 2006). Governments in Europe and the United States have issued mandates for the usage of renewable sources of fuel and energy, for example, the U.S. Congress passed a bill named the Energy Policy Act of 2005 (EPACT 2005) which was signed into law on August 8th, 2005 and mandates the use of up to 7.5 billion gallons of renewable fuel in gasoline by 2012 (Farrell et al., 2006) intensifying the pressure to develop agricultural systems dedicated to produce crops that can supply the biomass necessary for the growing demand for fuel and energy (Groom et al., 2007; Johnson and Runge, 2007). This resolution to support the production and commercialization of renewable sources of energy has two main objectives: a) to curb the production and usage of fossil fuels avoiding the depletion of the petroleum reserves and b) to reduce the impact of fossil fuels on the environment (Parrish and Fike, 2005).

In order to fulfill these objectives, the development of agricultural systems for energy should follow certain guidelines that guarantee a sustainable and environmentally safe production of biomass (McLaughlin and Walsh, 1998). Crops destined for bioenergy or biofuel should be grown with agricultural practices that have a minimal negative impact on the ecosystem (Tilman et al., 2006). Bioenergy crops should avoid competing with food crops and native species for land use (Groom et al., 2007; Johnson and Runge, 2007). Ultimately these crops should have a negative

or at least neutral carbon footprint in their production and conversion cycle, as well as, in their storage and transport phase (Tilman et al., 2006).

Currently in the U.S. the production of biofuel is dominated by corn (*Zea Mays* L.) based ethanol (Groom et al., 2007; Johnson and Runge, 2007). This ethanol fuel is produced by fermentation of non-fiber or non-structural carbohydrates inside plant cells because these types of carbohydrates are easily broken down and converted into ethanol (Lynd et al., 1991). However, the current energy policies that favor corn-based ethanol have overlooked important issues, for instance, the increase in the price of staple foods in the U.S. and around the world, and the detrimental impact that intensive corn production has on the environment (Johnson and Runge, 2007). Although the production of corn-based ethanol utilizes far less petroleum-based products than gasoline, it has greenhouse gas emissions similar to those of gasoline (Farrell et al., 2006). Corn production utilizes the greatest quantities of fertilizer and pesticides in comparison to any other major U.S. crops and has the lowest energy conversion efficiency ratio (energy output/fossil energy input) when compared to other energy crops (Groom et al., 2007; Hill et al., 2006).

The current U.S. energy policies regarding ethanol production and commercialization are not supportive for developing alternative bioenergy sources (Groom et al., 2007; Johnson and Runge, 2007). Cellulosic biomass from native perennial grasses or wood fibers is being researched as an alternative (McLaughlin and Walsh, 1998). Cellulosic biomass consists of the structural carbohydrates that make up the cell wall in plant cells (Carpita and McCann, 2000). These carbohydrates are harder to breakdown and convert into ethanol than the non-structural ones; nevertheless there are efficient processes to accomplish this task (Sanderson et al.,

1996). Moreover native perennial crops may be produced more sustainably and with environmentally safe cultural practices compared to row-crop agriculture (Tilman et al., 2006).

Fortunately, The U.S. Department of Energy (DOE) has shifted its research focus towards non-edible crops, such as, native perennial prairie grasses for the production of biomass for fuel and energy (Parrish and Fike, 2005). In 1985, the DOE funded several research studies focused on finding alternative sources of energy. In this early cycle of studies several perennial grasses and legumes species were tested and switchgrass (*Panicum virgatum* L.) showed a great potential for high biomass production (McLaughlin et al., 1999; Parrish and Fike, 2005; Sanderson et al., 1996). Consequently, in 1992 the DOE initiated a five-year research program with the main purpose of developing switchgrass as a sustainable herbaceous energy crop from which a renewable source of fuel and/or biomass-generated electricity could be produced (Sanderson et al., 1996). This program concentrated on several areas, such as breeding for improved biomass yields, regional field trials, cultural practices, physiology of switchgrass and tissue culture (McLaughlin et al., 1999; Sanderson et al., 1996). The long-term improvement of switchgrass through breeding, performing regional trials, optimizing harvest frequency and date, and reducing levels and timing of nitrogen fertilization has generated biomass yield increases of approximately 50%. In turn, this has reduced the estimated production cost of this herbaceous energy crop by about 25% (McLaughlin and Kszos, 2005).

Switchgrass is a warm-season perennial grass native to North America. The natural geographic distribution of switchgrass populations ranges from Southern Canada to Central America (Hitchcock, 1935). Switchgrass has several attributes that

make it appealing for biomass production, it has the potential for high biomass yield with low agrochemical input compare to other herbaceous crops; it has broad adaptability and tolerance to unfavorable edaphic factors; it has a positive influence on soil and water stabilization, and it has high carbon sequestering characteristics (McLaughlin et al., 1999) These characteristics make switchgrass a major candidate for sustainable herbaceous energy crop production on less costly land or marginal land (Sanderson et al., 1996;).

There are several benefits of using a perennial crop as a bioenergy source instead of an annual crop. For instance the energy flux and the carbon footprint of bioenergy production systems are important measurements of success and sustainability and switchgrass-based bioenergy production systems have a favorable flux of energy (Parrish and Fike, 2005). Calculations show that the energy inputs used to produce, store and deliver biomass from switchgrass are about 3.5% of the energy outputs thus, demonstrating that these systems are energetically efficient (Metcalf and Bullard, 2001). Switchgrass-based energy systems may be considered carbon-neutral (C-neutral) because the C released by their consumption is cycled to and from the atmosphere rather than being extracted from a fossil source (Parrish and Fike, 2005). Additionally, as a long term advantage of biofuel systems, part of the carbon removed from the atmosphere will be utilized by switchgrass plants to develop underground biomass which in turn will create soil organic matter (Ma et al., 2000).

Another advantage of this species is that switchgrass plants have a dense canopy and an extensive and deep root system that may reduce the adverse effects of heavy raindrop, runoff and erosion (Self-Davis et al., 2003). The federal Conservation Reserve Program of the US Department of Agriculture has utilized switchgrass to

minimize soil erosion (Moser and Vogel, 1995). There are several studies suggesting that switchgrass may facilitate the breakdown or removal of soil contaminants, such as, herbicides, trinitrotoluene, chromium, radionuclides, etc. (Belden and Coats, 2004; Dzantor et al., 2000; Entry and Watrud, 1998; Shahandeh and Hossner, 2000).

Additionally the biomass obtained from switchgrass can be combusted by itself or co-fired with coal to produce electricity (Parrish and Fike, 2005). Many studies have suggested that the biomass of switchgrass plants harvested later in the season have generally lower ash content which makes it more acceptable for combustion (Parrish and Fike, 2005). High ash content biomass reduces the efficiency of the energy producing system because it leaves residues containing potassium, phosphorus and nitrogen in the walls of the boilers which means the system will need more heat to burn the biomass (Boylan et al., 2000). Research suggests that switchgrass plants may be able to retranslocate mobile nutrients, such as, nitrogen, phosphorus, potassium and carbohydrates, to store them in the crowns and root systems as plants approach senescence later in the growing season, thus contributing to the low nutrient requirements of the crop and low ash content of the biomass produced (Boylan et al., 2000; Parrish and Fike, 2005). In Iowa there was an extensive ongoing study where switchgrass production was devoted to electricity production by biomass combustion and according to the results, direct combustion of switchgrass biomass or co-firing the biomass with coal could be ready for commercial utilization (Tillman et al., 2000).

Soil Considerations and Variability of Switchgrass Establishment

It has been suggested that switchgrass plants can tolerate harsh edaphic conditions, such as, acidic soils and nutrient deficient soils (Parrish and Fike, 2005).

However this species has a characteristic shared by many warm-season perennial grasses, a difficult and many times very slow establishment (Aiken and Springer, 1995; Hintz et al., 1998, Moser and Vogel, 1995). Thus, establishing switchgrass plots may be even more difficult in low productivity or marginal soils and there is limited research on this subject. Additionally slow germination and seedling establishment will encourage weed competition and attacks by insects or grass-feeding animals during critical developmental stages (Parrish and Fike 2005). The difficulties with establishing a good stand of switchgrass may stem from factors inherent to the species such as postharvest seed dormancy, seed size and seedling morphology (Aiken and Springer 1995; Elbersen et al., 1999; Knapp, 2000; Smart and Moser, 1999; Mullen et al., 1985) or to environmental factors, e.g., soil texture, soil temperature and soil moisture (Sanderson et al., 1996; Vassey et al., 1985; Zhang and Maun, 1990).

Postharvest seed dormancy in switchgrass is one of the main problems which affect germination rates and seedling development (Burson et al., 2009). The degree of dormancy may vary depending upon the cultivar in use (Mullen et al., 1985). In various switchgrass cultivars postharvest seed dormancy can be as high as 95% for recently harvested seed (Sanderson et al., 1996). Seed exposure to combinations of low temperatures and high humidity treatments, as well as, to plant growth regulator treatments, such as ethylene and gibberellic acid, may improve germination in switchgrass seeds by breaking dormancy (Beckman et al., 1993; Zarnstorff et al., 1994). However in low productivity or marginal environments, where drought may combine with high soil temperatures, stratification treatments may be negatively affected and bring secondary dormancy to switchgrass seeds (Shen et al., 2001).

Postharvest seed dormancy may also decline naturally but it could require up to 2 years of after-ripening for seeds to become ready to germinate (Sanderson et al., 1996; Shen et al., 2001). Thus, progress in breeding programs to improve desirable agronomic traits can be delayed when long periods are required to reduce dormancy (Sanderson et al., 1996). Further research should be focused on finding a more reliable and permanent approach to minimizing postharvest seed dormancy in switchgrass by utilizing the genetic potential of the species and develop cultivars with reduced postharvest seed dormancy (Sanderson et al., 1996; McLaughlin and Kszos, 2005; Burson et al., 2009).

Some research studies suggest that larger switchgrass seeds may have an advantage over smaller seeds in germinating and emerging (Aiken and Springer 1995). Smart and Moser (1999) found that larger seeds produced seedlings which grew adventitious roots faster than seedlings from smaller seeds; however there is evidence that in stable environments these early differences, in seedling development associated with seed size, are no longer noticeable later in the growth cycle (Aiken and Springer, 1995; Zhang and Maun, 1991). In relation to seedling morphology it has been suggested that the positioning of the seedling's growing point or crown during emergence may have an impact on switchgrass establishment (Elbersen et al., 1998). If the crown is located above ground, especially in highly permeable soils or in dry environmental conditions, adventitious root growth may be unsuccessful leading to low seedling survival due to dehydrations (Evers and Parsons, 2003).

There are several studies suggesting different planting dates for switchgrass seed depending on location. Vassey et al. (1985) suggested that in Iowa it was more advantageous to plant in early spring due to cooler soil temperatures and the rainfall

patterns in the area. Hsu and Nelson (1986) observed quicker emergence in late spring in Missouri. In Nebraska it was found that early spring plantings produced larger seedlings compared to plantings in June or July (Smart and Moser, 1999). The difference in planting dates may be related to soil temperatures varying in the spring in different states. In laboratory trials it was found that switchgrass can germinate at temperatures ranging from 10 to 35°C; with an optimum germination rate at 20 to 30°C (Hsu et al., 1985). Besides soil temperature, there is an important soil characteristic that promotes good switchgrass establishment, water-holding capacity (Evers and Parsons, 2003). Fine-textured soils have greater water-holding capacity and support better germination and seedling survival of switchgrass in warmer or drier environments (Stout et al., 1986). Proper seed planting depth may also ameliorate establishment problems in arid conditions (Parrish and Fike, 2005)

Genetic and Phenotypic Diversity of Switchgrass

Switchgrass (*Panicum virgatum*) is a warm-season perennial grass native to North America. In the United States switchgrass swards can be found from the eastern seaboard to as far west as Arizona and Nevada and from the Dakotas in the north to the Texas Coastal plains in the south (Hitchcock, 1935). This species is a typical component of the tall- and mixed-grass prairies of the Great Plains regions of the U.S. and has served for hundreds years as a forage to feed draft animals, especially during the summer months when cool season grasses are less productive (Cornelius and Johnston, 1941; Eberhart and Newell, 1959; Newell and Eberhart, 1961; Nielsen, 1944; Vogel, 1996). Since the 1930s, switchgrass has been used to for soil conservation purposes (Cornelius and Johnston, 1941; Nielsen, 1944). Currently switchgrass is one of the species used in programs such as the Soil Bank and the

Conservation Reserve Program to improve depleted farmland (Moser and Vogel, 1995). Additionally this species is continuously being studied for ecosystem restoration, soil erosion control, vegetative filter strips and phytoremediation projects involving herbicide contamination (Belden et al., 2004; Bilbro and Fryrear, 1997; Blanche et al., 2003).

Switchgrass Ecotypes

Several researchers have conducted botanical descriptions of switchgrass and they have indicated the presence of two distinct types of switchgrass plants. Cornelius and Johnston (1941) tested thirty-four accessions of switchgrass, from different parts of the Great Plains, in Manhattan, Kansas. In this study the researchers observed considerable variation in growth habit and disease resistance among accessions. Furthermore the researchers realized that accessions originated in higher altitude locations were early maturing, short in stature, with lodging stems; they had few leaves and coarse leaves. They acknowledged that accessions from northern locations had similar growth habit to those from high altitude locations, except for the leafiness of the plants. Accessions that originated from lower altitude sites were described as higher in forage and seed yield, taller in stature and later in maturity than the upland types. The results also suggested that accessions from southern latitudes were similar in growth to the lowland types. Additionally southern lowland types were highly resistant to rust (*Uromyces graminicola*) (Cornelius and Johnston, 1941).

Eberhart and Newell (1959) compared endemic strains of switchgrass collected from native grasslands in Nebraska. These researchers reported similar results to Cornelius and Johnston (1941) suggesting that strains from a more southern origin were more vegetatively productive. They also described two distinct growth

types among their collections: (1) the blue-green type originated from sandy soils from northern and western Nebraska with a rhizomatous decumbent plant growth, large seed size, and early maturity, and (2) the green type from southeastern Nebraska with large forage yield, late maturity and resistance to rust.

Benedict (1940) studied the effects of different day length and temperatures on switchgrass plants. The results suggested that switchgrass is a short day plant which may explain the late maturity of southern types in northern latitudes. However the author failed to specified the origin of the plant material used in this study. Additionally the researcher reported that a large day and night temperature differential increased biomass production of plants in the short day treatment but decreased biomass production of plants in long day treatment. Additional it was determined that root to shoot ratio was greater of switchgrass plants in the short day regimen than in the long day.

Porter (1966) evaluated the extent of the variation between the switchgrass types grown in the native grasslands of Oklahoma and determined the basis for the morphological differences between the types. The researchers conducted large field collections and grew plants under various environmental conditions to determine consistent patterns of morphological variability and whether this variability was due to genetic differences, to distinct environmental influences, or an interaction of the two. In general, lowland clones of switchgrass were more robust than those of the upland type in the field trials (Porter, 1966). However lowland clones varied significantly in height, from 2 to 10 ft. This variation was suggested to be the result of age differences and not so much because of localized habitat conditions. It was also reported the lowland plants grew in bunches whereas upland plants grew in scattered

culms. Additionally the researcher found a significant relation between the height of a plant and the length, width or diameter of aerial vegetative organs, but no such relationship was evident between height and the length of the spikelets or the caryopses. The researchers also provided evidence for the genetic differences between upland and lowland types which exhibited essentially the same morphological variation when grown under uniform conditions inside a greenhouse as in the field trials.

In this study, the relative water requirements of upland and lowland types were also tested. Upland types grew better under moderate soil moisture conditions while lowland types grew better under flooded conditions. Lowland plants were much larger than upland types whether grown in higher or lower soil moisture conditions. The researcher suggested that even though morphological differences between lowland and upland types may be genetic, there also may be an underlying physiological difference between types in water requirements. Additionally it was proposed that underperformance of lowland or upland types in adverse environmental conditions may be related to the influence of those conditions on the genotype.

Lastly, the researcher conducted reciprocal transplanting experiments where upland switchgrasses were grown in lowland environment and vice versa. The results showed that upland switchgrass did not grow better in a lowland location with greater availability of water and that plants of the lowland type became reduced in size in the drier upland location. However lowland types grew larger in both environments. These results substantiate the presence of genetic differences between the types and the influence of the environment on the genotype.

Stroup et al. (2003) examined two lowland cultivars Alamo and Kanlow and two upland cultivars Blackwell and Caddo for differences in response to water deficit and nitrogen fertilization. The results suggested that growth potential of the cultivars was determined by nitrogen availability and not by water availability. As previously reported, by other researchers, Stroup et al. (2003) observed that lowland cultivars produced higher biomass yields than upland cultivars, although upland cultivars were less susceptible to drought stress. In this study, Alamo was the cultivar with the greatest biomass yield among the cultivars tested. However the researchers did not specify the location of the trial which may have influenced the response in biomass yield of the cultivars. In our study the location and the quality of the soil is specified to provide a better understanding of the results.

Cytological studies have also attempted to determine the basis for the morphological differences between switchgrass types. Church (1940) was the first to show the existence of multiple ploidy levels within populations of switchgrass. He found accessions originating in Kansas and Oklahoma to have somatic chromosome numbers of 36 and 72. He also reported the somatic complement of an accession of *P. virgatum* var. *spissum* L. from Massachusetts to be 36. Burton (1942) found a switchgrass accession with a chromosome number of 72 which originated in Florida. Nielsen (1944) found 59 chromosomes in a switchgrass collected in an area extending from Wisconsin to Montana and south to Arkansas and Arizona. He found that the species has a polyploidy series of 18, 36, 54, 72, 90 and 108 somatic chromosomes. McMilland and Weiler (1959) studied clones that represented local populations from Manitoba and eastern Montana to Texas. They reported haploid numbers of 18, 27, 36 and 54, with 18 and 36 being predominant chromosome numbers in their collections.

Porter (1963) conducted a comparative analysis of upland and lowland types of switchgrass in the vicinity of Norman, Oklahoma. He reported tetraploid, hexaploid and octoploid plants in this area. Bragg (1964) found switchgrass plants with haploid chromosome numbers of 36 which originated in Texas.

McMillan and Weiler (1959) found no significant relationship between the ploidy level of switchgrass plants and the size of the plants. However Church (1940) and Bragg (1964) reported octoploid plants were shorter in stature than tetraploid plants. Church (1940) also noted that octoploid switchgrass plants and tetraploid plants differed in glume shape and pilosity distribution. Porter (1963) found that large lowland plants were tetraploid and smaller, finer upland plants were hexaploid or octoploid. Similarly, Barnett and Carver (1967) reported tall, coarse and glabrous plants of the lowland type were tetraploid and shorter finer plants of the upland type with varying amount of leaf pubescence were mostly octoploid.

Nielsen (1944) found no regional segregation of switchgrass types based on chromosome number and it was suggested that more than one ploidy level can exist within a switchgrass population. However McMillan and Weiler (1959) reported that plants with $2n = 36$ to be more of northwestern origin than plants with $2n = 72$. Nielsen (1944) and McMillan and Weiler (1959) both indicated that many morphological characteristics differed to a higher degree in plants of the same chromosome number. In a subsequent study, Nielsen (1947) observed that polyploidy was not associated to winter hardiness of switchgrass plants or to the ability of genotypes with higher ploidy level to grow in more rigorous environments compared to diploid counterparts.

Bruken and Estes (1975) surveyed upland and lowland populations of switchgrass in Oklahoma to determine chromosome number and morphology. They found that all the lowland plants from their collections were tetraploid and the upland plants were octoploid and aneuploidy variants formed at the octoploid level. The researchers did not detect any genetic interaction between the types in sympatric populations. Cluster analysis of morphological differences suggested that lowland types are more closely related to southern populations of switchgrass than to upland and/or northern populations.

Until 1982 the reports of chromosome numbers of switchgrass population had been from field collections in native grasslands. Riley and Vogel (1982) reported the chromosome numbers of five released cultivars: $2n = 4x = 36$ for Kanlow and Summer and $2n = 6x = 54$ for Blackwell, Pathfinder and Nebraska 28. Vogel et al. (1991) registered Trailblazer as a new upland type cultivar and reported it to be hexaploid. Hulquist et al. (1996) conducted a study to determine if chloroplast DNA restriction fragment length polymorphisms occurred among switchgrass cultivars that differ in ploidy level or ecotype classification. The researchers detected one polymorphism associated with the lowland and upland classification. They showed that the lowland type cultivars contained a restriction site change that was absent in the upland type cultivars. Additionally they used flow cytometry to show that lowland type cultivars had 3 pg DNA/nuclei while the upland type cultivars had either 3 or 6 pg DNA/nuclei.

Hopkins et al. (1996) determined chromosome number and DNA content of several switchgrass populations by means of light microscopy and laser flow cytometry. They concluded that all lowland types were tetraploid and all upland

types were octoploid except for Summer which was a tetraploid. Their results also showed that switchgrass cultivars Blackwell, Pathfinder, Cave-in-Rock and Trailblazer contained only octoploid plants, contradicting previous reports from Riley and Vogel (1982) and Vogel et al. (1991). The DNA content findings were in accordance with the results from Hultquist et al. (1996). There is strong evidence that supports the premise of variation in genotype and phenotype in switchgrass as a species, McLaughlin et al. (1999) suggested that this variability may be attributed to three main features: the genetic diversity associated with a reproductive method that favors cross pollination; an extensive and well developed underground biomass system, and an efficient physiological metabolism.

Certainly the reproductive scheme in switchgrass has played an important role in the large genetic variability of the species. The main characteristic of the reproductive system is a self-incompatibility mechanism that contributes significantly to cross pollination and promotes genetic variation within the species (Martinez-Reyna and Vogel, 2002; Casler, 2005). Martinez-Reyna and Vogel (2002) carried out research studies to characterize this self-incompatibility system in switchgrass. They reported that self-pollination was very low ranging from 0.35 to 1.39% in tetraploid and octoploid parents, respectively. Pre-fertilization incompatibility mechanism in switchgrass, to avoid self-pollination, is similar to the S-Z system previously reported in the Poaceae family. They also reported the occurrence of a post-fertilization incompatibility system that inhibits intermating among octoploid and tetraploid plants. This self-incompatibility system of switchgrass appears to have worked in concert with evolutionary processes, such as gene migration, random genetic drift, mutation and natural selection at each specific ecosystem where switchgrass can be

found to produce an apparent unique pool of genes for each ecosystem's population and in turn generated distinctive phenotypes for each ecosystem (Parrish and Fike, 2005; Casler et al., 2007).

Zhang et al. (2011) attempted to identify regional diversity, gene pools, and centers-of-diversity of switchgrass. The objectives of this study were to increase the understanding of this species evolution and to identify both the geographic range and potential overlap between functional gene pools. They sampled a total of 384 genotypes from 49 accessions which included the three main groups of switchgrass (lowland 4x, upland 4x, and upland 8x). They were able to identify several major centers of diversity for switchgrass in the eastern and western Gulf Coast regions. As suggested by previous research groups, Zhang et al. (2011) found evidence that migration, drift, and selection may have led to adaptive radiation in switchgrass, forming regional gene pools within each of the main switchgrass groups. In accordance with Huang et al. (2003), this group estimated that both upland-lowland divergence and 4x-to-8x polyploidization within switchgrass began approximately 1.5-1 M ybp and that subsequent ice age cycles may have resulted in gene flow between ecotype lineages and between ploidy levels.

Because there is a great amount of variation in ploidy, morphology, growth pattern, and zone of adaptation between and within the upland and lowland switchgrass ecotypes, it is important to be able to distinguish switchgrass plants belonging to different genetic pools. It is also very important to develop the genetic tools that can rapidly and systematically classify switchgrass ecotypes and use this information to develop switchgrass complementary gene pools that may be capable of

providing a substantial heterotic increase in biomass yield (Zalapa et al., 2010; Zhang et al., 2011).

Genetic Diversity of Switchgrass

Currently, there are several researchers who are focusing their efforts on determining the diversity of the germplasm within and among types and/or populations of switchgrass. Because upland and lowland types have been suggested to be genetically different populations with little gene flow between them (Barnett and Carver, 1967; Brunken and Estes, 1975; Porter, 1966), the accurate assessment of the germplasm diversity within and among cultivars is essential for the success of programs dedicated to improve the agronomic performance of switchgrass. Huang et al. (2003) attempted to establish the evolutionary history and linkage of switchgrass based on nuclear DNA. The researchers used the genetic variation in the nuclear gene that encodes plastid acetyl-CoA carboxylase from six cultivars of switchgrass. They reported that the genomes of tetraploid and octoploid switchgrass are closely related to each other regardless of upland and lowland classification. They also estimated that the most recent polyploidization event, which established the new switchgrass lines, happened approximately less than two million years.

A better understanding of the switchgrass genome is also needed. Gunter et al (1995) tried to characterize and assess the genetic diversity among 14 populations representing upland and lowland switchgrass types by using Random Amplified Polymorphic DNA (RAPD) markers. They made 128 primers of pooled genomic DNA from individual genotypes of each population and 45 primers produced polymorphic markers. The genetic analysis showed an overall similarity of 65% among populations compared to 81% within populations. Switchgrass cultivars

Alamo and Forestbur' presented minimal similarity (53%) compared to the similarity between Caddo and Blackwell (78%).

Missaoui et al. (2005) investigated the genomic organization and chromosomal transmission in switchgrass based on restriction fragment length polymorphism (RFLP) markers. They reported that switchgrass is an autotetraploid species and it has a high degree of preferential pairing. In a later study, Missoaui et al. (2006) presented results suggesting higher genetic variation between upland and lowland types than between genotypes within the types; which may explain higher genetic similarity within populations and low genetic similarity among populations if they belong to different types. Additionally a phylogenetic analysis of chloroplast non-coding region *trnL* (UAA) intron sequences of 34 switchgrass accessions divided the accessions into two major groups. All the accessions grouped in the lowland group had a deletion of 49 nucleotides and this was verified by phenotypic identification of greenhouse grown plants.

Narasimhamoorthy et al. (2008) used simple sequence repeat (SSR) markers to examine the level of genetic diversity in collections obtained from the USDA Germplasm Resource Information Network in order to identify unique genotypes that would be useful in switchgrass breeding programs. This group reported that variability within populations was significantly higher (80%) than among populations (20%). Cluster analysis was successful in differentiating between upland and lowland types and the genotypes were grouped into different adaptation zones based on the geographical locations of the collections.

Cortese et al. (2010) used a combination of morphological and molecular characteristics of 12 switchgrass populations grown in New Jersey to assess genetic

diversity and determine differences within and among these populations. Sixteen of 32 expressed sequence tag-simple sequence repeat (ETS-SSR) primers pairs produced polymorphic markers and were chosen for the genetic analysis. Plant measurements such as plant height, panicle height and flag leaf height, length and width, heading and anthesis date were collected and analyzed to determine morphological differences. Molecular variation within the populations was 64% and among populations was 36%. The combined analysis of molecular and morphological data was the most useful in finding difference among populations base on point of origin of the cultivars. The information gathered through genetic diversity studies is really important to the development of switchgrass breeding program. This information may assist these crop improvement programs to focus in selecting and breeding superior varieties, as well as to establish and maintain these superior varieties.

Zalapa et al. (2011) identified patterns of variation between 18 switchgrass cultivars from the different types, 7 lowlands and 11 uplands and from different geographic locations as well as varying ploidy levels, by using 55 SSR loci and 6 chloroplast DNA sequences. This group provided evidence to the accuracy of their molecular tools to differentiate between the types and the ploidy levels. They were also able to identify patterns of DNA polymorphisms related to geographic origin and ecological region. They also suggested that SSR markers have great potential as a fingerprinting tool for switchgrass cultivars and for identifying switchgrass plants from unknown origin. The importance of this research resides in developing complementary gene pools that can provide the genetic potential to improve biomass production in switchgrass to be tested in multi-environment trials that can aid the search for renewable sources of energy.

Genotype by Environment Interactions

Switchgrass can produce high biomass yield compared to other herbaceous crops across a broad range of growing conditions and it also exhibits tolerance to harsh edaphic conditions (Moser and Vogel, 1995). In 1992 the US Department of Energy launched a research effort to assess the performance of available switchgrass germplasm, test new cultural practices, study switchgrass physiology and tissue culture techniques, and to improve biomass yield (Sanderson et al. 1996). However, the agronomic performance of switchgrass may vary depending on the genotype and the influence of the environment on the genotype (Porter, 1966). Additionally desirable agronomic traits, such as, biomass yield, grain and forage yield, etc. are considered quantitative or complex traits that are controlled by many genes that interact with the environment. For example, the amount of biomass produced by a certain switchgrass cultivar may vary not only due to the cultivar's genetic composition but also due to the environmental conditions of where it is grown (Sanderson et al., 1996; Sleper and Poehlman, 2006).

Variation in genotypic response due to different ecological conditions is a significant concern in plant breeding programs. Das et al. (2004) investigated the genetic variability between southern upland, northern upland and southern lowland populations of switchgrass; and the relationship between biomass yield and yield components, such as, tiller number, tiller length, leaf blade width, leaf blade length, etc. The results suggested that there is adequate genetic variability within populations to improve biomass yield through breeding and that tiller number per plant is a good characteristic to select for in order to improve biomass yield under spaced-plant conditions.

Eberhart and Newell (1959) reported that considerable population x year interaction influenced seed and forage yield of switchgrass germplasm from the Great Plains. Hopkins et al. (1995a and b) conducted two separate studies to determine the degree of environmental influence on switchgrass genotypes. For both studies the plants were evaluated in three different locations (Mead, NE; Ames, IA; and West Lafayette, IN) and data was collected for two years after establishment. In the first study the research group tested twenty elite switchgrass populations and they suggested that the interaction between genotype and environment affected the forage yield and biofuel traits of these populations. For the second study, switchgrass accessions collected from remnant Midwestern prairies were tested. It was reported that significant variation was observed across locations and years for heading date, forage yield at heading and vegetative in vitro dry matter digestibility.

Casler and Boe (2003) studied the effects of genotype by environment (G x E) interactions on agronomic and biofuel traits of six switchgrass cultivars. These cultivars were grown in two locations in the north central USA, Brookings, SD and Arlington, WI. There was a great difference in biomass yield among cultivars; however this response was also affected by environmental factors, such as location, year and date of harvest. They noticed that five of the six cultivars had positive and negative responses to harvest date with a general trend for biomass yield to become more positive with later harvest dates and with advancing years at each location. In this study it was suggested that differential changes in ground cover may have influenced the response of biomass yield to later harvest date, because they observed that plots harvested in August endured more acute loss of ground cover than plots harvested in September and October. The influence of harvest date on the

accumulation of neutral detergent fiber (NFD), which is the measure of hemicellulose, cellulose and lignin, was not very significant across location and years; however the interactions of cultivar with location and year were more important than the main effect of cultivar alone. They showed that NFD concentration among cultivars varied greatly depending on the location and year, coinciding with results obtained by Hopkins et al (1995a) for holocellulose concentrations, which measures hemicellulose and cellulose accumulation.

The genetic variability of switchgrass is also expressed in the considerable variation in adaptation across a geographical area that ranges from 36 to 46° N lat. (Casler et al., 2004). Sanderson et al. (1999) showed that upland cultivars from northern origin performed poorly in Texas and they observed that these upland cultivars would mature earlier compare to lowland cultivars. Casler et al. (2004) suggested that the relation between latitude of origin and the genetic response of a switchgrass genotype may be a function of photoperiodism, heat and cold tolerance. Upland types tend to be adapted to mid- to northern latitudes and tend to have higher relative biomass yield and survival when moved to more northern latitudes while lowland cultivars tend to be adapted to southern latitudes and may perform better when moved to more southern latitudes origin (Barnett and Carver, 1967; Brunken and Estes, 1975; Casler et al., 2004).

Cassida et al. (2005) attempted to characterize the G x E interaction and its influence on the chemical composition and yield of biofuel components among switchgrass genotypes from different origins (northern or southern) and types (upland or lowland). They tested these genotypes in five different locations in Texas, which differ in latitude and rainfall distribution. The results showed there was a difference in

performance across regions; however southern lowland genotypes consistently yielded greater amounts of lignocellulose. Additionally it was shown that latitude was influential mostly on lignocellulose yield of upland genotypes as well as the availability of water at certain developmental stages, especially to lowland types. They also observed that nitrogen and phosphorus concentrations in harvested biomass were very low, suggesting low nutrient removal from soil and low nutrient requirements from the crop.

In general these findings support the argument that suitability of switchgrass as a bioenergy crop and ethanol production can be improved by selection; however breeding research should be conducted in a multi-environment setting to enhance the efficiency of the program. Moreover, several studies have attempted to demonstrate that switchgrass cultivars vary widely in their adaptation zones and that some cultivars cannot survive or perform poorly when taken too far from their latitude-of-origin (Casler, 2005; Casler et al., 2004; Casler and Boe, 2003; Cassida et al., 2005; Fuentes and Taliaferro, 2002). Based on the results reported by several research groups it can be suggested that marginal land will affect the performance and biomass yields of switchgrass or that switchgrass cultivars that are taken off deep and nutrient-rich prairie soils where they are selected and grown on marginal soils may fail to produce the same yields that in the selection environment or the survival rate of this cultivar may also decline.

Influence of Nitrogen Fertilization on Switchgrass Performance

Nitrogen management is an important factor in cropping systems where legumes are not included; for bioenergy cropping systems nitrogen is especially important as it represents not only an economic cost but energetic and potential

ecologic cost because it may contribute to air and stream pollution (McLaughlin et al., 1999). There are many studies reporting a wide range of recommendations for nitrogen fertilization on switchgrass but there is not a consistent trend (Parrish and Fike, 2005).

Balasko et al. (1984) evaluated forage production of the switchgrass cultivar 'Blackwell' grown without adding fertilizer or lime on a site of marginal fertility at different cutting schedules that combined one to three cuts with different cutting dates. The highest forage yields of 8 Mg/Ha per year were observed from the one-cut and two-cut schedules. However for the two-cut schedule less than 20% of the annual yield was in the second harvest. They also observed that switchgrass grown without nitrogen had low crude protein concentration an unwanted characteristic for forage production but desirable for biomass production.

Bredja et al. (1994) studied the effect of a single 88 Kg N/Ha application in the spring or 4-weeks after green-up, or split applications of 44 kg N/Ha in the spring and 44 kg N ha⁻¹ following defoliation. The trial was carried out at the University of Missouri Agronomy Research Center and the switchgrass cultivar utilized was 'Blackwell'. They observed that split applications of nitrogen stimulated stem regrowth and increased total tiller density. They suggested that one spring cut of switchgrass for forage prior to stem elongation followed by a postharvest nitrogen application of 44 Kg N/Ha was the best practice to produce both forage and seed.

Sanderson and Reed (2000) grew 'Alamo' switchgrass under 22 or 112 Kg N/Ha, and under field capacity or water-deficit conditions at the Texas A&M University Agricultural Research and Extension Center at Stephenville, TX. Plant spacing varied from 10 to 70 cm. They observed that dry biomass yield and tiller

number were not influenced by nitrogen in the establishment year. In the post-establishment year they observed that dry biomass yield was positively affected by higher nitrogen treatment when switchgrass was planted in the wider row spacing. This increase in dry biomass at the higher nitrogen rate was driven by an increase in individual tiller weight and not by increase in tiller number. Finally they indicated that at higher plant densities the response of switchgrass in relation to dry biomass yield and plant morphology was controlled by the competition of aboveground resources and not by water and nitrogen.

Muir et al. (2001) grew switchgrass cultivar 'Alamo' in two different sites in Texas for seven years. The objective of this study was to determine the response of this cultivar to nitrogen and phosphorus fertilization as affected by row spacing. They reported that biomass production benefited the most from a single spring application of 168 Kg of N/Ha. The higher rate of 224 Kg N/Ha produced lodging in both sites. On the other hand the plots that did not receive any nitrogen deteriorated over time. They did not find any evidence that switchgrass benefited from phosphorus application and they suggested because this species is native to the area, it may be sufficiently efficient at extracting soil-bound phosphorus. This research group observed that increasing row spacing was not advantageous in the long run because tiller density was not able to compensate for the wider spacing which led to slow canopy closure and greater weed incidence.

Vogel et al. (2002) aimed to determine the optimum harvest period and nitrogen rate for biomass production in the Midwest. The study was carried out at Ames, IA and Mead NE and the switchgrass cultivar 'Cave-in-Rock' was grown under six nitrogen rates ranging from 0, 60, 120, 180, 240 or 300 Kg N/Ha. They

reported that the largest amount of dry biomass was obtained with 120 Kg N/Ha when switchgrass was harvested two times, before anthesis and after a killing frost. Additionally they observed that at this application rate the amount of nitrogen removed from the soil was approximately the same as the amount applied.

Thomason et al. (2004) reported that the highest amount of dry biomass (18 Mg/Ha) was observed when switchgrass was harvested three times after receiving 448 Kg N/Ha in April. However the group also observed that plots receiving no nitrogen and harvested three times yielded 16 Kg N/Ha. The research group concluded that while multiple harvests in a year may maximize dry biomass yield in the short-term but the productivity of the stand may decline after 3-4 years.

Mulkey et al. (2006) investigated the effects of harvest timing and nitrogen fertilization on dry biomass production of switchgrass land enrolled in the Conservation Reserve Program (CRP) as well as the impact of the number of cuts performed in a year. They used switchgrass stands of 9, 12 and 13 years of age. They reported that 56 Kg N/Ha increased total dry biomass and higher rates did not provided additional benefits. They also suggested that in order to ensure the continuance of long-standing switchgrass swards enrolled in CRP they should be harvest only once a year after a killing frost.

Wullschleger et al. (2010) conducted a statistical analysis of a database compiled of 1190 observations of yield from 39 field trials carried out across the United States. Their data analysis suggested that variation in dry biomass yield is influenced by genotype as well as by several environmental and management factors including nitrogen fertilization. They also indicated that variability in yield due to nitrogen fertilization was high; however they observed a different response depending

on the ecotypes. For lowland ecotypes there was a hint of an optimum around 100 Kg N/Ha, but in several cases the unfertilized plantings did as well as fertilized stands. In upland ecotypes dry biomass yields appeared to be positively affected to total rates of nitrogen application up to approximately 100 Kg/Ha and decreased with higher rates.

Switchgrass has been described as a thrifty user of nitrogen because the concentration of nitrogen found in leaf biomass is generally lower than other C₄ species and very low compared to C₃ species (Hargrave and Seastedt, 1994). Additionally switchgrass has been known to have a common strategy of tall prairie grasses for conserving nitrogen, at the end of the season senescent leaves translocate nitrogen to the perennial parts of the plant mainly to the underground biomass (Hargrave and Seastedt, 1994). Madakadze et al. (1999) evaluated the changes in chemical composition of three switchgrass cultivars 'Cave-in-Rock', 'Pathfinder' and 'Sunburst'. They reported that the nitrogen concentration of dry biomass declined in a curvilinear manner from 25 g/kg at the beginning of the season to 5 g/kg at the end of the season. It has been suggested that the amount of nitrogen removed in the biomass of switchgrass harvested two times a year is greater than the nitrogen removed by a one harvest system and the amount of nitrogen removed by a two harvest system may even exceed the amount of nitrogen applied in the soil (Reynolds et al., 2000).

According to the information from these studies biomass production from switchgrass may benefit from moderate nitrogen fertilization applied in the spring; however there is not a consensus on the optimal rates for high dry biomass production. Additionally, these studies suggest that to improve biomass production and to promote stand longevity switchgrass should be harvested once a year at end of the season or after a killing freeze when leaves start to senesce, avoiding the removal

of large amounts of nitrogen from the soil-plant system and letting nitrogen be recycled inside the plant. Another solution to the nitrogen fertilization issue may be to exploit the great genetic potential in the species to develop switchgrass genotypes with the ability to maximize biomass yields under marginal concentration of nitrogen in the soil and/or genotype that are able to respond more efficiently to infrequent nitrogen fertilization.

Improving Yield in Low Productivity Environments

Typically, agricultural systems established in low productivity or marginal environments may primarily encounter poor quality soils with low nitrogen levels. The U.S. Department of Agriculture has a classification system that determines farmland as category I, II, or III. Land classified as category IV may be considered as marginal farmland and has limitations to cultivation, such as, slope and erosion. Higher categories may have progressively steeper slopes, certain percentage of rock outcrop, advance levels of erosion, etc. Other soil conditions such as inadequate drainage, low amounts of organic matter, or disturbed areas (strip mines, etc.) may also deem land marginal (U.S. Department of Agriculture, Natural Resources Conservancy Service).

Additionally abiotic stresses in farmers' fields, mainly low soil fertility and drought, rarely occur individually; they are often a combination of factors that may be exacerbated by competition from weeds and/or biotic stresses such as disease and insect attack, thus becoming a limiting factor in the profitability of a crop because they may increase costs and reduce yields (Bänziger et al., 1999). The majority of the research conducted on the sustainability and productivity of the different switchgrass cultivars has been conducted on prime farmland or high performance environments.

Various research studies in other crops, such as, barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and maize (*Zea mays* L.) have suggested that conducting breeding research programs in environments that differ greatly from the target environments are not the most efficient method to obtain significant genetic improvement (Atlin and Frey, 1990; Bänziger et al., 1997; Bänziger et al., 1999; Ceccarelli and Grando, 2003; Simmonds, 1991). In order for switchgrass to succeed as a bioenergy crop, research has to focus on improving biomass productivity on marginal land or low productivity environments (Sanderson et al., 1996).

Switchgrass has several attributes, as a species, which make it appealing for biomass production in marginal or low productivity environments (Moser and Vogel, 1995). This species has shown to require low agrochemical inputs compared to other herbaceous crops and it has been demonstrated to have broad adaptability and tolerance to unfavorable edaphic factors (McLaughlin et al., 1999; Porter, 1966; Sanderson et al., 1996). However several researchers have given evidence that environmental conditions, such as, temperature and day length may influence positively or negatively the performance of released switchgrass cultivars (Casler and Boe, 2003; Das et al., 2004; Eberhart and Newell, 1959; Porter, 1966). Drought and nutrient deficiency, especially nitrogen deficiency, are major components of marginal or low productivity environments and it may be expected that these factors could also affect the productivity of switchgrass cultivars as well (Porter, 1966; Stroup et al., 2003). Plant breeders focused in producing switchgrass cultivars adapted to low productivity environments or cultivars with higher biomass yields in low productivity environments have to take this in consideration when choosing the selection environment for new cultivars.

There are two basic schools of thought when it comes to the best selection environments in a breeding program (Shabana et al., 1980; Zavala-Garcia et al., 1992): (1) in order to maximize heritability the selection environment should have optimal growing conditions and (2) the selection environment should have similar conditions to the target environment, meaning if the cultivar is to be used in a low productivity environment it should be selected under low productivity conditions. Switchgrass cultivars have been mostly selected in research farms with high productivity soils. Little research has been conducted to define an optimal environment, in which maximum genetic gain could be achieved, for switchgrass breeding programs to improve biomass yield in low productivity environments.

Because choosing the environment for selection is such an important decision there has been several criteria developed to assist in the task. Finlay and Wilkinson (1963) proposed performing a regression of the mean yield of new cultivars on selection site mean yields and checking for deviations from the regression line. Frey (1964) suggested the use of heritability estimates to determine the best selection environment. Allen et al. (1978) proposed that in addition to heritability, the genetic correlation between the mean value of a genotype in the selecting environment and all environments should be used as well. Lagner et al. (1979) found there was a high correlation between regression coefficients and varietal ranges (highest mean yield minus lowest mean yield) and suggested that varieties could be grown in two very different environments to measure stability. Falconer (1989) suggested that yield in low productivity environments and high productivity environments may be considered as separate traits which may not be necessarily maximized by identical sets of alleles. He proposed the use of correlated response to identify the best

environment for selection. Allen et al. (1978) and Falconer (1989) concluded that genetic correlation and heritability between yield in the selection environment and the target environment could be used to identify the best environment that would optimize correlated response.

There are several research studies that have reported positive results when conducting selection in high productivity environments for performance in low productivity environments. Shabana et al. (1980) selected among two groups of random oat (*Avena sativa* L.) lines under low, medium and high productivity conditions. They reported that the lines selected under high and low productivity conditions produced virtually equal yield advances, although it slightly favored the high productivity environment and the yield of lines selected under medium productivity conditions were somewhat inferior to those from the other two conditions. Quisenberry et al. (1980) reported significant genetic advance for lint yield in cotton (*Gossypium hirsutum* L.) at different testing locations when the lines were selected in an environment considered optimal for upland cotton growth in Texas. Pfeiffer (1987) compared random soybean (*Glycine max* L.) genotypes selected for higher yield in early planted environments to high yielding genotypes selected in late planted, heat-stressed environments in Kentucky. He observed that early planted soybean genotypes had a greater response than late, stressed planted genotypes regardless of whether selection response was evaluated in early or late plantings. Johnson and Geadelmann (1989) investigated whether moisture stress would influence the response of maize genotypes from mass selection and full-sib recurrent selection when evaluated in environments with different levels of moisture stress. They reported that on average, genotypes selected for grain yield under

irrigated conditions had superior production than genotypes obtained from selection under dry-land conditions. Additionally they proposed that the effectiveness of selection under irrigated condition was as advantageous as selection under dry-land conditions and it also resulted in superior responsiveness of the selected genotypes to favorable environmental conditions.

There are several other researchers who have conducted experiments wherein the largest yield gains in low productivity environments were observed or predicted from genotypes selected in environments with similar stress conditions rather than in high productivity environments. Arboleda-Rivera and Compton (1974) reported that maize genotypes selected in drought-stressed environments produced higher yields than genotypes selected in the absence of drought when evaluated under drought stress conditions. Muruli and Paulsen (1981) observed that maize populations selected under low nitrogen performed better under similar conditions than the selections from high nitrogen environments.

Atlin and Frey (1990) compared the responses of random populations of oat lines, selected in low (LPE), medium (MPE) and high (HPE) productivity environments, at each of the productivity levels. They observed that replication of the selection unit influenced the relative effectiveness of direct and indirect selection for yield in LPE. At low levels of replication, the selection from LPE was less effective in predicting genotype response at similar environmental conditions. However when replication increased, direct selection was more effective in predicting genotype response than indirect selection. They also suggested that even though heritability may be greater in HPE than LPE if the genetic correlation between the two environments is significantly less than 1, the gains from selection in HPE may not be

observed when testing in LPE. They also proposed if heritability is greater in HPE than LPE there may be a positive correlation between the relative effectiveness of direct selection in LPE and increasing in the number of replications within and across environments.

Several studies have reported the importance of the genetic correlation between selection and target environments. When this correlation is very low it may suggest that alleles controlling high yield in LPE are at least partially different of those controlling high yield responses in HPE and testing in either environment alone may not be the most valuable method for increasing productivity in some crops (Atlin and Frey, 1990; Ceccarelli et al., 1992; Ud-Din et al., 1992).

Generally crop breeding programs are established to select genotypes for target areas with variable environmental conditions (Bänziger et al., 1997; Byrnes et al., 1995). The variation in these target areas is not limited to the different sites but also within the same site over years (Rosielle and Hamblin, 1981). Because of this variability the selection environment should encompass a minimum combination of environmental conditions that will enhance the genetic gain both overall and within each individual environment. Zavala-Garcia et al. (1992) compared the efficiencies of indirect selection criteria, such as, mean productivity, rank summation and selection indices to improve yield in two sorghum (*Sorghum bicolor* L.) populations across four dry-land locations and two years. They suggested that indirect selection criteria calculated from yield combination from stress, intermediate and non-stress environments were higher than those calculated from yield from any single environment. Thus, in order to achieve gain in the selection of genotypes for

unpredictable environments the yield data representing the range of environments in the target areas should be included in the definition of the selection criterion.

Bänziger et al. (1997) evaluated the value of low-nitrogen vs. high-nitrogen selection environments for improving lowland tropical maize for low-nitrogen target environments. They reported that broad-sense heritability for grain yield was 29% smaller under low-nitrogen compared to high nitrogen; however selection under low-nitrogen conditions became statistically significant with relative yield reductions of more than 43% was obtained from indirect selection under high-nitrogen conditions. Results from previous studies suggested that under stress conditions, lower heritability for grain yield is related to lower genotypic variance and less error variances (Atlin and Frey, 1990; Frey, 1964; Quisenberry et al., 1980; Ud-din et al., 1992). Thus direct selection under low-nitrogen became more efficient than indirect selection (Bänziger et al., 1997). Edmeades et al. (1999) evaluated the grain and biomass yield of maize populations selected for drought tolerance. They reported that simultaneous selection in well-watered environments and under carefully managed water stress environments bestowed new maize genotypes with improved drought tolerance.

Bänziger et al. (1999) examined the influence of selection for drought tolerance on performance of tropical maize under different levels of nitrogen because target environments are rarely characterized by only one abiotic stress, and it would be valuable to increase the tolerance of crops to various stresses. The genotypes selected during midseason drought were evaluated in two experiments under severe nitrogen stress, one experiment under intermediate nitrogen stress and two well-fertilized experiments. They reported the average above ground biomass

accumulation of nitrogen for the five studies at: 52, 63, 105, 151 and 163 kg N/Ha and the average grain yield obtained were 3.0, 2.9, 5.2, 6.0 and 6.5 Mg/ Ha. Additionally they reported that genotypes selected for tolerance to midseason drought increased grain yields by an average of 86 Kg/Ha/year. They also observed delayed leaf senescence, higher biomass and nitrogen accumulation at maturity. They concluded that selection for tolerance may have led to constitutional changes in the plants that also allowed adaptive advantages under nitrogen stress, such as more efficient use of nitrogen, increased production of carbohydrates by increased light absorption and a more extensive root system. These results are very important for breeding programs which target crops cultivated under low productivity environments as drought and nitrogen stress are the most widely occurring stresses (Atlin and Frey, 1990; Bänziger et al., 1997; Bänziger et al., 1999).

A preliminary experiment to support the current study was conducted by Dr. Arvid Boe in South Dakota from 2001 to 2004. The experiment was set to compare switchgrass biomass yields on marginal soils (glacial till blackslope [Class IV or higher]) in Kimball, SD vs. deep prairie soils (silty clay loam [Class I]) in Aurora, SD. Both of the locations were tilled cropland, precipitation was similar, and no fertilizer was applied at either location. Thirty half-sib families from two switchgrass cultivar ‘Sunburst’ and ‘Summer’ and 19 half-sib families from ‘OKNU 92-4’ (experimental upland population from C. Taliaferro, Oklahoma State University) were evaluated. Biomass was collected from a single harvest in October from 2001-2004. The marginal soils of the Kimball location resulted in a 60% reduction in yields compared to the high quality soils of the Aurora location. In this study it was observed that the top five families selected from the high yield selection environment

yielded 25% lower dry biomass than the best five families from the low yield selection environment when tested in a low yield target environment.

In fact for ‘Sunburst’, ‘Summer’, and ‘OKNU’, only 2 families, 3 families, and 2 families, respectively, occurred in the top 30% in both environments. The inter-location correlations among families (Pearson correlations) was <0.20 for each population, indicating very little similarity in family ranks between the two environments. These results indicated that performance across environments was not consistent and that the top performing genotypes were not the same on both soil types. Thus, suggesting that family selection in low yield environments would definitely result in different families being chosen when selection is conducted in high yield environments.

Rose et al. (2007) conducted a research study to assess the effects of high and low biomass yield environments on recurrent selection for general combining ability in a lowland population of switchgrass. They reported that mean biomass yield from genotypes originated from the low yielding environment protocol were significantly larger than those genotypes from the high yielding environment protocol when tested for performance in both environments.

Plant breeding programs that use indirect selection may not be beneficial when the target environments are extremely variable (Atlin and Frey, 1990; Bänziger and Cooper, 2001; Ceccarelli and Grando, 2007). This is the case for switchgrass because the objective of using this species as a bioenergy crop is to utilize the least amount of agronomic inputs in its cultivation and to grow it on marginal soil where it will not compete for land with edible crops. It is possible to improve yield under stress conditions, however, the effectiveness of the selection resides on the similarities of

the selection and target environments (Ceccarelli et al., 1998). There is plenty evidence indicating that estimates of heritability alone are not enough to identify a proper selection environment and that selection in high-yielding environment may produce negative or no correlated response of a genotype when tested in low-yielding environment (Atlin and Frey, 1990; Ceccarelli et al., 1992; Falconer, 1990). Ceccarelli et al. (1998) and subsequent studies Ceccarelli et al. (2003) and Ceccarelli and Grando (2007) suggest that the most effective method to improve crop productivity in marginal environments may be to use locally adapted germplasm and to make the selection in the target environment, also advocating for participatory plant breeding programs that would include the farmer's knowledge of the crop to increase effectiveness of the selection. In conclusion, target environments for commercial switchgrass cultivations will necessarily be different than the currently utilized selection environments. Breeding programs focused on increasing biomass yield of switchgrass may greatly benefit from direct selection, as well as from participatory plant breeding type programs.

The Use of a Multi-Species Approach to Improve Biomass Yield and Reduce Nitrogen Fertilization

The ultimate objective of cultivating bioenergy crops is to create multifunction environments that not only provide energy but reach the goals of sustainability by reducing inputs of fertilizer and pesticides; protecting the soil from erosion and contamination, sequestering carbon from the environment; protecting the crop from weeds, insects and disease and providing a habitat for wildlife (Groom et al., 2007; Johnson and Runge, 2007). Greater plant species diversity is required for a multifunction environment (Hector and Bagchi, 2007; Sanderson et al., 2004). An

increase in species diversity in grassland plant communities has been linked to stability in response to environmental disturbance (McNaughton, 1977; Frank and McNaughton, 1991; Tilman and Downing, 1994), reduction in the amount of foreign, unwanted species through the better utilization of available resources by the established species (Naeem et al., 2000; Tilman, 1997; Tracy and Sanderson, 2004a), and enhanced nutrient retention (Tilman et al., 1996). However if improving biomass feedstock production is the primary objective, functional diversity could be achieved by utilizing a select group of plant species that work well together (Tracy and Sanderson 2004b).

The cultivation of legumes along with grasses for forage has several advantages over grass monocultures. Baylor (1974) observed that incorporating legumes generally resulted in increased yield and higher quality forage. Legume-grass mixtures have great influence in reducing weed encroachment and erosion and have led to greater stand longevity than grass or legume monocultures, which may be related to the nitrogen fixation characteristics of legumes (Drosom and Smith, 1976). However, the level of success of legume-grass mixtures depends on the compatibility of the associated species, not only with respect to the aboveground biomass growth habit, but also with respect to the root system interrelationships (Papadakis, 1941; Virtanen and Hausen. 1935). Harmful root interrelationships may be due to various phenomena, such as, competition for nutrients, competition for water, excretion of toxic substances detrimental to other species growth, etc. (Ahlgren and Aamodt, 1939; Loehwing, 1937).

Roberts and Olson (1942) conducted an investigation to compare various legumes and their influence on a particular grass. They used two grass species: red top

(*Agrotis alba*) and Kentucky bluegrass (*Poa pratensis*), and six legumes: alfalfa (*Medicago sativa*), lespedeza (*Lespedeza striata*), white clover (*Trifolium repens*), sweet clover (*Melilotus alba*), red clover (*Trifolium pretense*) and Alsike clover (*Trifolium hybridum*). They reported an increase in forage yield and nitrogen content for both grasses when grown in association with Lespedeza and white clover compared to monocultures. They suggested that the greater yields of grasses may be due less competition exerted by these legumes. Jung et al. (1985) suggested that mixed stands of warm and cool season grasses along with legumes can be used for grazing and hay production with an increase in seasonal yield distribution.

George et al. (1995) investigated the role legumes in supplying nitrogen and improving herbage yield when grown in association with switchgrass. This research group evaluated the use cool-season legume renovation vs. nitrogen fertilization for switchgrass stands. They used 10 forage legumes and a legume mixture compared to 0, 60, 120 and 240 Kg N/Ha. The results from this study showed that legume renovation was not very effective during the first year (establishment year), but produced higher forage yield than grass fertilized with 240 Kg of N in the second year. They also reported that forage yields in year two for the inter-seeded swards was significantly improved compared to the 120 or 240 Kg N/Ha. They suggested that cool season legumes could substitute for N fertilization after the establishment year; however adequate defoliation in early summer is important to lessen legume competition to established switchgrass. Gettle et al. (1996) compared the dry matter yield for legume-renovated switchgrass and nitrogen-fertilized switchgrass. Established swards of 'Cave-in-Rock' switchgrass were frost-seeded with forage legumes and legume mixtures. They concluded that dry matter yields of legume-

renovated switchgrass swards were generally larger compare to the mid to high levels of nitrogen-fertilized swards during the second year. The association of legumes with grass cultivation has also been suggested to improve the seasonal distribution of forage yield as well as in-vitro-dry-matter-digestibility (IVDMD), crude protein (CP) content and neutral detergent fiber (NDF) content of the biomass harvested (Sleugh et al. 2000). Improved IVDMD and NDF are desirable traits for ethanol production and biomass combustion but not higher crude protein content because it leads to higher ash content which may reduce the efficiency of the energy production system (Boylan et al., 2000; Calser and Boe, 2003).

Typically pasture lands in the northeastern USA are species rich; however this richness may be dominated by seasonal weedy plants that contribute little to forage value (Tracy and Sanderson, 2000). The plant species composition of managed pasture lands is dominated by mostly two species a perennial grass and a legume (Tracy and Sanderson, 2000). Recent research suggests that pasture productivity and stability may be enhanced by a more complex, multi-species plant community (Bullock et al., 2001; Hector et al., 1999; Naeem et al., 1994; Tilman et al., 2001). However Tracy and Sanderson (2004) observed that increasing the diversity of grass mixtures and legume-grass mixtures beyond three species did not consistently improved yields. They also reported that inter-annual yield stability was not improved by two species mixture and was not significantly related to increasing species diversity. The suggested that in order to obtain higher productivity and inter-annual yield stability it was best to plant two or three species known to work well together in specific environmental conditions rather than planting a random assemblage of species in a complex mixture. Mulkey et al. (2008) found that a mixture of only two grass

species, switchgrass and big bluestem, was enough to produce greater biomass yield, higher concentration of lignocellulose and lower concentrations of total nitrogen and ash. They also found that this grass mixture had relative low nitrogen requirements, thus reducing production input costs. Research is needed to determine an optimum grass mixture or grass-legume mixture combination to maximize biomass yield of switchgrass, improve yield stability and promote biodiversity on low productivity environments.

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CHAPTER 1: Agronomic Performance of 14 Switchgrass Cultivars in Marginal vs. Prime Farmland in Six Locations

Abstract

Switchgrass (*Panicum virgatum* L.) is a warm-season perennial grass native to North America. The difference in biomass production between and within switchgrass ecotypes (upland and lowland) and populations due to genotype x environment interaction (GxE) has been well documented. The majority of the research for increased biomass production in switchgrass has been conducted on University research farms with prime farmland. This research study aimed to determine the effects of marginal land on the performance of 14 switchgrass cultivars and if direct selection on marginal land should be considered to improve switchgrass biomass production in environments with poor soil quality; to identify cultivars with high biomass potential on marginal land in a specific region and to identify cultivars that may have broader adaptation across several U.S. regions on marginal land. The cultivars were seeded in either 2008 or 2009 in six locations (Maryland, New Jersey, New York, Pennsylvania, South Dakota and Wisconsin) in paired fields (marginal vs. prime land). Each paired location also had a nitrogen treatment of 0 or 100 kg of N·ha⁻¹·year⁻¹. In the year after establishment, stand establishment (% coverage) and agronomic data, such as, plant height (cm) and tiller density (tillers·m⁻²) was collected, in addition to dry biomass yield (Mg·ha⁻¹) to determine the performance of the cultivars. Our results were consistent with previous research suggesting a relationship between stand establishment and dry biomass yield. Cultivars with percent coverage above 60% had good dry biomass yield across locations. The analysis of variance suggested that location and the interaction of location x soil quality accounted for the

largest portion of variation in stand establishment and tiller density while soil quality and nitrogen treatment were the most influential factors in plant height and dry biomass yield, respectively. All cultivars were shorter in marginal soils and did not rank the same in prime vs. marginal soil. For stand establishment and biomass yield approximately 50% of cultivars showed differences due to soil quality. For tiller density only 40% of the cultivars presented differences due to soil quality; however some cultivars had higher tiller density in marginal soils. Overall these results may indicate direct selection in marginal land may be necessary for some cultivars, but they also suggest that for cultivars less affected by growing conditions, selection may be possible in a single environment. Nevertheless, it is important to individually evaluate sources of germplasm in multi-environment trials to determine the extent of the influence of environment as well as to identify specific environmental factors that may be the most important so breeders can maximize selection and growers can maximize productivity on poor quality soil.

Introduction

Native perennial grasses, such as, switchgrass, are expected to be used as bioenergy crops and to perform well on low productivity soils or in marginal environments (McLaughlin et al., 1999; Moser and Vogel, 1995; Porter, 1966; Sanderson et al., 1996). However, as in many other crops there has been little to no research to select and evaluate the productivity of switchgrass genotypes under stressful environmental conditions. Thus, the performance of released switchgrass cultivars under low productivity conditions is unknown. This knowledge is critical to the successful development of bioenergy crops that can produce enough biomass on marginal soils to make it economically feasible for farmers to cultivate them on their farms.

Thus far, most if not all of the research and selection of perennial grasses for biomass production, including the selection of switchgrass cultivars, has been conducted by scientists on prime farmland on their research farms. These cultivars are expected to perform well in all environmental conditions. However, biomass yield is considered a complex trait regulated by many genes and influenced by the environment (Sleper and Poehlman, 2006). Falconer (1989) suggested that yield in low productivity environments and high productivity environments may be considered as separate traits which may not be regulated by the same sets of alleles. The interaction of the genotype with the environment may result in different biomass yields depending on the environmental conditions (Falconer, 1989; Sleper and Poehlman, 2006).

Ceccarelli (1987) conducted a breeding study with barley (*Hordeum vulgare* L.) comparing the efficiency of direct and indirect selection in the Aleppo province in

northern Syria. The selection environments differed in the amount of annual rainfall: the environment with favorable conditions received an average of 375 mm of annual rainfall and the unfavorable environment received an average of 175 mm of annual rainfall. The F_3 families originated from F_2 families selected under the driest conditions were taller, more vigorous; earlier in maturity and had greater grain yields than F_3 families derived from F_2 's selected under wetter conditions. It was determined that the correlation coefficients between the drought susceptibility index and grain yield at the different sites were extremely different, suggesting the existence of desirable traits under one set of conditions that may become undesirable under another set of conditions. The conclusions suggested that to improve barley yields in dry areas it is essential to test early segregating populations in the target environment, to avoid the risk of dropping highly drought tolerant genotypes by selecting only in environments with favorable annual rainfall (Ceccarelli, 1987). Additionally, it may be beneficial for switchgrass improvement programs to evaluate early segregating generations in multi-environment trials, with stress conditions, non-stress and moderately stress conditions to identify genotypes which may occur in low frequency and may be able to respond well under stress as well as with sufficient plasticity to respond well to favorable environmental conditions (Atlin and Frey, 1990; Ceccarelli, 1987; Fischer and Maurer, 1978).

There are several research studies with similar findings to Ceccarelli (1987), suggesting that selection under appropriate types of stress and non-stress conditions similar to the target environment together with selection for increase yield and for secondary traits, such as, plant height, tiller density, etc., are extremely beneficial to improve tolerance to unfavorable environmental conditions and increase yield in

maize (*Zea mays* L.) (Arboleda-Rivera and Compton, 1974; Bänziger et al., 1997; Bänziger et al., 1999; Muruli and Paulsen, 1981); oats (*Avena sativa* L.) (Atlin and Frey, 1990); sorghum (*Sorghum bicolor* L.) (Igartua et al., 1996; Zavala-Garcia et al., 1992) and rice (*Oriza sativa* L.) (Guan et al., 2010). Additionally the presence of large genotype-by-environment (GxE) interaction may complicate selection because the performance of the genotype will vary in different environment, decreasing the accuracy of the prediction (Ceccarelli, 1989)

It has been well documented that switchgrass genotypes are influenced by the environment and will perform differently depending on the conditions of the site (Casler and Boe, 2003; Casler et al., 2004; Cassida et al., 2005a and b; Fuentes and Taliaferro, 2002). Switchgrass breeders have to be concerned with the wide distribution of the species and the large amount of within species variability (Hitchcock, 1935; Cornelius and Johnston, 1941; Eberhart and Newell, 1959; Newell and Eberhart, 1961; Nielsen, 1944; Porter, 1966). Furthermore there are two types of switchgrass, upland and lowland, they are genetically distinct, they have different growth habits and are adapted to different types of environments (Cornelius and Johnston, 1941; Eberhart and Newell, 1959; Porter, 1966). Upland types are typically octaploids, have shorter, finer stems and are better adapted to drier habitats (Cornelius and Johnston, 1941; Eberhart and Newell, 1959; Hopkins et al., 1996; Porter, 1966; Lewandowski et al., 2003). Upland types are also typically earlier maturing than lowland types (Benedict, 1940; Cornelius and Johnston, 1941; Eberhart and Newell, 1959; McMillan, 1965; Porter, 1966). Lowland types are generally tetraploid, taller and more robust than upland types (Hopkins et al., 1996). They have coarser stems, a more bunch type growth habit and are adapted to wetter sites (Cornelius and

Johnston, 1941; Eberhart and Newell, 1959; Porter, 1966; Lewandowski et al., 2003). They typically mature later than upland types and require a longer growing period (Cornelius and Johnston, 1941; McMillan, 1965; Porter, 1966).

Day length partly controls the agronomic performance of individual plants or populations of switchgrass, such that most populations cannot be moved north or south more than one hardiness zone without adversely affecting vigor, survival or flowering (Benedict, 1940; Casler et al., 2004; Porter, 1966). In addition to day length, there are other factors, such as, temperature, rainfall, nutrient availability and soil type that could increase the potential for additional phenotypic variation (Casler, 2005; Casler et al., 2004; Porter, 1966). Drought and nutrient deficiency are major components of low productivity environments and may be expected to affect the biomass production of released switchgrass cultivars (Porter, 1966; Stroup et al., 2003).

The two switchgrass types have also been found to have different biomass yields in different environments (Casler et al., 2004; Cassida et al., 2005; Sanderson et al., 1999). Comparisons of agronomic performance among switchgrass cultivars selected in high yielding environments suggests that the most promising cultivars for biomass production are Alamo for the deep South, Kanlow for mid-latitudes and Cave-in-Rock for the central northern states (Cassida et al., 2005; Lewandowski et al., 2003; Sanderson et al., 1996). It has also been indicated that Cave-in-Rock is more adapted to high humidity areas, but Kanlow is more drought tolerant than Alamo or Cave-in-Rock (Lewandowski et al., 2003). Fike et al. (2006) evaluated switchgrass cultivars in several different environments to determine which cultivars performed better in specific environments and which cultivars performed consistently well

across environments. They reported that in TN, WV, VA and KY cultivars Alamo and Kanlow (both lowland types) had greater yields than Cave-in-Rock and Shelter (two upland types). Improving switchgrass dry biomass yield in marginal soils may be more efficient if selection is conducted in environments with similar conditions to the target environments, the key factor is being able to determine those stress and non-stress conditions that will help identify genotypes with increase tolerance for stress and superior yield (Atlin and Frey, 1990; Ceccareli, 1987; Ceccarelli, 1989; Bänziger et al., 1999; Simmonds, 1991).

Materials and Methods

Plant Material and Soil type

Fourteen switchgrass cultivars and selections, which represent both upland and lowland types (Table 1), were evaluated in this study. In addition to standard switchgrass cultivars, including Cave-in-Rock and Alamo which are known to have better adaptability, the study included new cultivars bred for increased biomass such as BoMaster and Performer from North Carolina State University and several experimental cultivars from the different states' breeding programs (Table 1).

The study was conducted in paired fields (marginal vs. prime farmland) in six states: Maryland (MD), New Jersey (NJ), New York (NY), Pennsylvania (PA), South Dakota (SD) and Wisconsin (WI). The soil types are summarized in Table 2. Each paired location also had a nitrogen treatment of 0 or 100 Kg of nitrogen·Ha⁻¹·year⁻¹. Urea (46-0-0) or ammonium nitrate (33-0-0) was used as a nitrogen source and the fertilizer was applied during the spring of the year after establishment.

Switchgrass seeds were stratified to improve establishment success. Ernst Conservation Seed (Meadville, PA) assembled all the seed lots 60 days prior to planting and stratified the seeds according to Shen et al. (2001). Seeds were treated with Thiram and Poncho (Bayer CropScience, U.S.). Stratified and non-stratified seed lots from each entry were sent to the Ohio Seed Improvement Association (Dublin, OH) for germination tests. Based on those results, seeding rates were calculated and standardized to approximately 11.2 kg of Pure Live Seed·ha⁻¹ (PLS) or (10 lb of PLS·acre⁻¹). The research plots were 1.8 x 1.8 m and were arranged in a randomized complete block design with three replications. The plots were seeded during the spring of 2008 at all the locations. Satisfactory establishment was obtained at the NY, SD and WI prime and marginal sites; however because of poor establishment at the MD, NJ and PA at the marginal sites new plots had to be seeded in 2009 in both paired fields.

Table 1. List of 14 cultivars and experimental selections of switchgrass evaluated in marginal vs. prime farmland in six locations. Switchgrass ecotypes: southern lowland (SL), northern lowland (NL), central upland (CU) and northern upland (NU).

Cultivar	Type	Seed Source – Provided by
Alamo	SL	Ernst Conservation Seeds
Blackwell	CU	Ernst Conservation Seeds
BoMaster	SL	Ernst Conservation Seeds/NC State
Carthage	CU	Cape May Plant Materials Center
Cave-In-Rock	CU	Missouri Plant Materials Center
High Tide	CU	Cape May Plant Materials Center
Kanlow	NL	Ernst Conservation Seeds
KY 1625	CU	USDA-ARS Dairy Forage Research Center
Pathfinder	CU	South Dakota State University
Performer	SL	Ernst Conservation Seeds/NC State
Shawnee	CU	Univ. of Nebraska/Ernst Conservation Seeds
Summer	NU	South Dakota State University
Sunburst	NU	South Dakota State University
Timber	NL	Cape May Plant Materials Center

Table 2. Description of soil types of the marginal and prime farmland of the six states.

State	Site	Annual Average Mean Daily Temp (°C)	Annual Precipitation (cm)	Soil Map Unit Name/ Land Capability Class of Marginal Soil	Soil Map Unit Name/ Land Capability Class of Prime Farmland Soil	Dominant Limiting Factors of Marginal Soil
Maryland	Snow Hill, MD	12.8	104	Hurlock loamy sand Class IV	Sassafrass sandy loam Class I	High water table, subject to erosion
New Jersey	Somerset, NJ (marginal) Freehold, NJ(prime)	11.7	116.8	Kleinsville shale 0-2 % slope Class IV	Freehold sandy loam 2-5% slope Class II	Nutrient deficiency, poor water-holding capacity
New York	Ithaca-New Ketola, NY (marginal) Ithaca-McGowan, NY (prime)	8.6	101.6	Honeoye and Lansing gravelly silt loam 15-25 % slope Class IV	Conesus gravelly silt loam Class I	Slope steepness, subject to erosion
Pennsylvania	Rock Springs, PA	9.3	95.1	Andover channery silt loam 0 to 15 % slope Class IV	Hagerstown silt loam 0 to 8% slope Class I, II	Restrictive layer in B (fragipan) or C horizon, poor water-holding capacity and acidic soil
South Dakota	Pierre, SD (marginal) Brookings, SD(prime)	9.8	50.8	Opal-Lakoma clay 9-15% slope Class VI	Lowry silt loam Class II	Shallow over shale
Wisconsin	Hancock, WI	9.9	83.1	Plainfield loamy sand, 0 to 2% slope Class IV	Plano silt loam, 0 to 2% slope Class I	Deep, sandy soil and poor water-holding capacity

Data Collection

Stand establishment was taken the following spring after seeding using the 0.75 x 0.75 m frequency grid (Vogel and Masters, 2001). For this study the frequency grid was a metal frame with 25 squares (5 x 5) made out of re-bar and painted orange for better visibility (Fig. 1). The frequency grid was systematically placed within the research plot because of the plot's size. After placing the grid on the plot the researcher counted the number of cells containing one or more switchgrass seedlings, flipped the frame end-over-end and counted again. The process was repeated until a total of 100 cells were counted within the seeded plot area. The counts were converted into a percentage of plot coverage by dividing the total number of cells that contained at least one switchgrass seedling by 100. These percentages of coverage were utilized in the statistical analysis. The stand establishment data from MD was not used because it was taken by visual rating and not with the frequency grid.

In the year after establishment additional agronomic traits, such as, plant height (cm) and tiller density (tillers·m⁻²) were measured, in addition to dry biomass yield (Mg·ha⁻¹), to determine the performance of the 14 switchgrass cultivars. Plant height was measured at maturity by placing a measuring stick in three random spots within the seeded plot. The height from the soil surface to the top of the panicles was recorded in each random spot (Fig. 2). The three measurements were averaged per plot. This average was used for the statistical analysis. Plant height data was reported from NJ, NY, PA and WI but not from SD. The plant height data from MD was not utilized because it was not taken at maturity.

Tiller density was measured at maturity by using a 1 ft² metal square (Fig. 3). The number of tillers within the metal square was counted. The process was repeated

three times by placing the square in three random spots within the seeded plot. The counts were converted from $\text{tillers}\cdot\text{ft}^{-2}$ to $\text{tillers}\cdot\text{m}^{-2}$ and averaged per plot. The average was used for the statistical analysis. Tiller density data was reported only from NJ, PA and WI.

Dry biomass yield was determined by harvesting a 0.30 x 1.8 m strip of switchgrass from the 1.8 x 1.8 m research plot with a two-wheel sickle bar mower (BCS, Hector, NY) (Fig. 4). The plots were cut at 8-10 cm from the soil surface. The fresh weight of the field sample was recorded (Fig. 5) subsamples were placed in a paper bags (S-9621) (U-line, U.S.). The fresh weight subsamples were recorded and then they were placed in a tobacco dryer (DeCloet Bulk Curing Systems, Tillonsburg, Canada) at 43°C for 10 days (Fig. 6). The fresh and dry weights of the subsamples were used to calculate moisture content which was then subtracted from the fresh weight of the field samples. The adjusted dry weight of the field samples was converted to $\text{Mg}\cdot\text{ha}^{-1}$ and used for the statistical analysis.



Figure 1. Picture of the 0.75 x 0.75 m frequency grid (Vogel and Masters, 2001). For this study the frequency grid was a metal frame with 25 squares (5 x 5) made out of re-bar and painted orange for better visibility



Figure 2. Picture of plant height being recorded by placing a measuring stick from the soil surface to the top of the panicles in three random spots within plot.



Figure 3. Picture of tiller density being recorded by counting the number of tillers within a 1 ft² metal square in three random spots within the plots.



Figure 4. Switchgrass being harvested in a 0.30 x 1.8 m strip from the 1.8 x 1.8 m seeded plot with a two-wheel sickle bar mower (BCS, Hector, NY).



Figure 5. Harvested switchgrass weighted on the field to record fresh weight.



Figure 5. Picture of the inside of the tobacco dryer where field samples were placed, at 43°C for 10 days, after fresh weight was recorded.

Data Analysis

The experiment was arranged in a split block plot design. The locations, sites and the replications within sites were the blocks while the nitrogen treatment was the main plot and the cultivars were the split plot; however only the replications were considered random effects. The analysis of variance was performed with the MIXED procedure in SAS (SAS Institute, Cary, NC). The means were separated with the LSMEANS option and adjusted with Tukey's method for multiple comparisons. The PDMIX800 macro was utilized to convert mean separation output to letter groupings in the MIXED procedure (Saxton, 2003).

Three southern lowland cultivars 'Alamo', 'BoMaster' and 'Performer' and one central upland cultivar 'High Tide' had the poorest establishment in the marginal site in NJ; in the prime site in NY and, in the prime and marginal sites in SD (Table 3). These four cultivars established so poorly in these locations that they did not survive which resulted in missing values for the rest of the agronomic variables measured in this study including plant height, tiller density and dry biomass yield (Table 6; 11 and 16). Because of the number of variable in the model and the amount of missing values for these previously mentioned cultivars the LSMEAN option was not able to separate the means for plant height, tiller density and dry biomass yield and the model was not as informative.

The data was modified to conduct the statistical analysis and to obtain the most information from the study. The first modification was implemented to separate the means for plant height, tiller density and dry biomass yield. A new variable was created called LSQ (a factorial between location and soil quality) and the sites with the most missing data were deleted. This analysis provided a way to observe the

differences among cultivars across locations (Table 8, 13 and 18). The second modification was added to compare the interaction between environments and genotypes. For this analysis the first model was utilized but previously mentioned cultivars were eliminated from the dataset. This analysis provided information on the variation among locations and sites (Tables 7, 9, 12, 14, 17 and 19).

Results and Discussion

Stand Establishment

Stand establishment was measured before the nitrogen fertilizer was applied to the soil, thus the nitrogen treatment was not included in the statistical model for the analysis of the response variable stand establishment. According to the analysis of variance, location and the interaction of location and soil quality were the most influential factors in the establishment of switchgrass cultivars (Table 3). Soil quality and cultivar were also important for switchgrass establishment but to a lesser extent (Table 3). The interaction effects with two and three factors that included the combination of location, soil quality and cultivar were also significant according to the analysis except for the cultivar by soil quality interaction (Table 3). This species has a characteristic shared by many warm-season perennial grasses which is a difficult and many times very slow establishment rate (Aiken and Springer, 1995; Hintz et al., 1998). Factors that can affect establishment of switchgrass plants can range from seed dormancy, seed size and speed of germination and development (Aiken and Springer 1995; Elbersen et al., 1999; Knapp, 2000; Smart and Moser, 1999; Mullen et al., 1985) to soil conditions, e.g., soil texture, temperature and moisture (Aiken and Springer, 1995; Sanderson et al., 1996; Vassey et al., 1985; Zhang and Maun, 1990).

The switchgrass stand establishment in NJ, SD and WI was better in the sites with prime soil quality (Table 4). In contrast, switchgrass cultivars established better in the marginal sites in NY and PA (Table 3). The most dramatic differences in stand establishment between marginal and prime sites were found in NJ (prime 97% compared to marginal 37%) and NY (marginal 77% compared to prime 28%) (Table 4). The best stand establishment was observed at NJ prime site 97.36% and the poorest at NY prime 28.54% (Table 4). Across locations, switchgrass cultivars established best in WI 81% and worst in South Dakota 51% (Table 4). Successful stand establishment in the seeding year is a major economical concern for the viability of growing switchgrass for bioenergy (Perrin et al., 2008). The negative consequences of poor establishment may be observed in biomass yield for several years until the density of the stand increases by plant tillering (Schmer et al., 2006). Weed pressure combined with slow germination of certain cultivars may have been one of the reasons for poor stand establishment at both the marginal site in NJ and the prime site in NY (Moser, 2000; Lemus et al., 2002). Additionally lack of rainfall during the establishment period, inadequate planting techniques including seedbed preparation and planting depth, as well as, poor seed quality may also cause failure to establish (Mitchell and Vogel, 2012). It has also been suggested that stand losses may also be related to insufficient cold tolerance of some switchgrass germplasm in northern latitudes, in our case, upstate NY and SD (Casler et al., 2004).

Table 3. Analysis of variance of stand establishment (% coverage) of 14 switchgrass cultivars evaluated at five locations in marginal vs. prime farmland at each location. Switchgrass plots were established in 2008 in NY, SD and WI and in 2009 in NJ and PA.

Source	Df	F-value	Pr > F
Location (State)	5	83.43	< 0.0001
SQ (Soil Quality)	1	34.13	< 0.0001
Loc*SQ	5	216.73	< 0.0001
Cv (Cultivar)	13	62.47	< 0.0001
Loc*Cv	65	8.84	< 0.0001
SQ*Cv	13	1.75	0.0516
Loc*SQ*Cv	65	4.12	< 0.0001

Table 4. Stand establishment (% coverage) of 14 switchgrass cultivars evaluated at five locations in marginal vs. prime farmland at each location. Switchgrass stands were established in NY, SD and WI in 2008 and in NJ and PA in 2009.

		Stand Establishment (% coverage)										
		NJ		NY		PA		SD		WI		Mean
		Adelphia Somerset		Syracuse		Rock Springs		Brookings Pierre		Columbia County		Across
Cultivar	Type	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Location
Shawnee	CU	99.17	52.67	43.67	95.00	77.00	75.17	90.67	64.67	100.00	100.00	79.80 a ¹
Blackwell	CU	96.33	49.00	57.50	96.00	75.33	77.00	87.00	56.67	99.67	100.00	79.45 a
Pathfinder	CU	95.33	43.00	46.17	82.17	67.89	74.67	100.00	71.33	100.00	98.67	77.92 a
Sunburst	NU	97.50	47.50	44.17	92.67	69.67	66.33	100.00	50.00	100.00	99.33	76.71 ab
Carthage	CU	98.17	34.50	47.83	93.17	69.67	70.50	98.33	54.67	99.67	99.67	76.61 ab
Summer	NU	97.83	45.50	42.50	93.00	74.67	77.00	72.00	48.00	100.00	100.00	75.05 ab
Cave-In-Rock	CU	99.67	43.83	38.00	93.00	71.67	75.17	84.33	24.67	100.00	99.67	73.00 abc
KY 1625	CU	95.83	40.17	22.67	50.83	70.17	75.67	85.33	36.00	100.00	99.67	67.33 bcd
Timber	NL	97.83	38.33	13.83	80.00	67.33	72.50	41.33	36.00	99.00	95.67	64.18 cd
Kanlow	NL	98.50	36.17	20.83	84.33	74.00	74.33	19.67	52.00	95.33	66.67	62.53 d
High Tide	CU	96.83	29.17	5.67	48.33	65.83	73.50	31.67	16.67	87.33	45.00	53.00 e
BoMaster	SL	97.17	21.17	11.00	80.17	71.50	71.67	20.67	46.00	35.67	21.00	47.60 e
Alamo	SL	96.67	13.00	2.67	41.67	54.83	76.17	22.00	16.67	30.33	13.00	36.65 f
Performer	SL	96.83	24.83	3.17	24.17	36.17	60.17	11.00	16.17	57.67	32.33	36.25 f
Mean		97.36	37.06	28.54	77.71	67.55	72.63	61.71	42.11	86.05	76.47	
		A ²	FG	G	BC	DE	CD	E	F	B	CD	

¹ Cultivar means with the same lower case letter were not statistically different at 0.05 level of probability.

² Location means with the same upper case letter were not statistically different at 0.05 level of probability.

In general, upland switchgrass cultivars had better establishment than lowland cultivars across locations (Table 4). More specifically, central upland types established better in relation to cultivars from other types across locations except for High Tide which had the poorest establishment (53%) among central upland cultivars (Table 3). Cultivars, Shawnee and Blackwell had the best establishment among upland cultivars, with over 79% plot coverage, across locations (Table 4). In general lowland type cultivars had the poorest establishment across locations, especially the southern lowland cultivars Alamo, BoMaster and Performer, with under 50% plot coverage across locations (Table 4). These results are not surprising because all locations included in this study were in the northern regions of the U.S. where upland cultivars exhibit better survival, growth and performance (Casler and Boe, 2003; Casler et al., 2004). Casler et al. (2007) suggested that ground coverage of switchgrass ecotypes may be highly related to the latitude-of-origin, meaning that coverage may increase or decrease significantly depending on the distance a cultivar is planted from its place of origin. This may explain the poor performance of southern lowland types such as ‘Alamo’ from Texas and ‘BoMaster’ and ‘Performer’ both from North Carolina. Additionally it may be expected that these results would be reversed if this study would have been conducted in southern regions of the U.S. (Cassida et al., 2005a and b). Lowland types originated in northern latitudes, such as, Kanlow and Timber had good establishment in some locations and a better overall coverage than southern lowland types (Casler et al., 2004).

According to the analysis of variance the interaction of location x soil quality was the most influential factor on stand establishment while soil quality alone was only slightly influential on stand establishment (Table 3). This may indicate that

establishment of switchgrass is affected genotype x environment interactions and factors such as temperature or soil quality may be as influential as genetic factors in the establishment of switchgrass stands. For six cultivars significant differences in stand establishment were observed due to the quality of the soil (Table 5). The upland cultivars Blackwell, Carthage, Sunburst and KY 1625 and the lowland cultivars Alamo and Performer established better in prime soil sites across locations compared to marginal sites suggesting a strong genotype x environment influence for these cultivars (Table 5). Establishment in marginal vs. prime soil were not statistically significant for cultivars Shawnee, Pathfinder, Cave-in-Rock, Summer, Timber, Kanlow, High Tide and BoMaster indicating that these cultivars were less influenced by the environment than Blackwell, Carthage, Sunburst and KY 1625 (Table 5). These data are supported by previous research that found Cave-in-Rock had consistent performance across numerous locations (Casler and Boe, 2003; Lee and Boe, 2005; Lemus et al., 2002). This data also suggests that for some cultivars, direct selection on marginal soil may be needed to improve establishment while for other cultivars that were not affected by genotype x environment interactions indirect selection on prime quality soil may be an option. However variation in establishment is specific to cultivar and not ecotype (upland vs. lowland) initial multi-environment trials need to be conducted on the germplasm of interest to determine whether it is sensitive to environmental conditions during germination.

It is important to determine the effects of stand establishment on switchgrass biomass production. Farmers cultivating switchgrass as a biomass feedstock need this information to make the necessary adjustments to produce economically viable switchgrass fields in post-establishment years (Schmer et al., 2006). Vogel and

Masters and Vogel (2001) conducted switchgrass establishment studies in the central Great Plains and reported that a stand frequency of 50% or higher produced successful stands; a stand frequency of 25 to 50% indicated marginal to adequate establishment, and a frequency less than 25% was considered unsuccessful establishment. Research studies on other grass species such as crested wheatgrass (*Agropyron cristatum*) have found a strong correlation between biomass production and measurements of stand establishment (Hyder and Sneva, 1954). Establishment studies on switchgrass and big bluestem (*Andropogon gerardii*) have suggested that when stand frequencies were 40% or higher, establishment was not a limiting factor in biomass production (Masters, 1997; Vogel, 1987). Schmer et al. (2006) conducted a study to determine the relation between establishment year, second year establishment and biomass yield in the northern Great Plains states of Nebraska, South Dakota and North Dakota. They found a linear relationship between low initial stand establishment, second year establishment and biomass yield. They reported that initial stand frequencies of less than 40% negatively affected establishment and biomass production in the second year. Additionally they observed that when initial stand frequencies were over 40% switchgrass stands and biomass production were more likely to be affected by variation in growing conditions than by initial stand establishment. Ground coverage levels of 25% in the establishment year may be adequate for switchgrass conservation plantings in which harvest is not performed for several years after planting (Schmer et al., 2006). In our study southern lowland cultivars with less than 25% did not recover to adequate levels to obtain other agronomic data in the post-establishment year (Table 4, 7, 12 and 17). As mentioned previously the difficulties with establishing good stands of switchgrass may not only

be a function of the genotype but also of growing conditions, such as, soil texture, temperature, moisture, etc. (Aiken and Springer, 1995; Casler et al., 2004; Sanderson et al., 1996; Vassey et al., 1985; Zhang and Maun, 1990). The seeding rates for this experiment were calculated on a pure live seed basis (PLS) [seedlot purity (%)·germination rate (%)·100⁻¹]; Mitchell and Vogel (2012) have observed poor quality seedlots failed to become well established even though they had germination rates similar to seedlots that produced acceptable stands during the establishment. They recommend the quality of switchgrass seedlots to be tested when using switchgrass for biomass production to reduce the risk of stand establishment failure.

Table 5. Comparison of stand establishment (% coverage) means by cultivar in marginal vs. prime farmland sites across five locations.

Cultivars	Type	Mean Stand Establishment (% coverage)		
		Prime	Marginal	
Blackwell	CU	83.17	75.73	*
Carthage	CU	82.73	70.50	*
Sunburst	NU	82.27	71.17	*
Shawnee	CU	82.10	77.50	NS
Pathfinder	CU	81.88	73.97	NS
Cave-In-Rock	CU	78.73	67.27	NS
Summer	NU	77.40	72.70	NS
KY 1625	CU	74.80	59.87	*
Timber	NL	63.87	64.50	NS
Kanlow	NL	61.67	63.40	NS
High Tide	CU	57.47	48.53	NS
BoMaster	SL	47.20	48.00	NS
Alamo	SL	41.20	32.10	*
Performer	SL	40.97	31.53	*

Cultivar means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

Plant Height

Soil quality had the largest effect on plant height followed by location and the interaction of location x soil quality (Table 6). Casler et al. (2004) suggested that there is a strong relationship between plant height and latitude. Switchgrass is considered a short day species (Benedict, 1940). Lowland types have been known to mature late and grow taller in northern latitude due to the longer summer days (Casler et al 2004; Das et al., 2004; Madakadze et al., 1998)., Our study suggests that soil quality may also be influential on the variation of plant height of switchgrass cultivars.

Nitrogen and the interaction effects of nitrogen with location and soil quality also significantly affected variation in plant height (Table 6). As expected, genotype (cultivar) also had a significant influence on plant height, but most of the interaction effects that contained the cultivar term were not statistically significant ($P < 0.05$) except for the location x cultivar interaction (Table 6). This indicates that the genotype x environment interaction was more influential than genotype on plant height. This may also suggest that soil management and other inputs to improve soil quality may dramatically increase plant height which in turn could improve biomass yield because these two traits have been found to be correlated (Lemus et al; 2002; Schmer et al., 2010; Staley et al., 1991; Stout and Jung, 1995). There are studies suggesting that moderate amounts of nitrogen may optimize switchgrass biomass production in northern latitudes (Mulkey et al., 2006 and 2008). However there is still a wide range of recommendations for nitrogen fertilization rates because of the multitude of environmental and management factors that may affect the nitrogen

utilization efficiency in warm-season grasses (Brejda, 2000; Parrish and Fike, 2005). It may be concluded that genotype x environment interactions in this study may have played a strong role in the variation of plant height among the switchgrass cultivars tested; suggesting that selecting individual genotypes based on plant height in one environment may not result in taller plants in marginal soils or locations with different environmental conditions than the selecting environment. The results for plant height also support the notion that switchgrass ecotypes may need to be selected in the target environments on which they will be grown.

Table 6. Analysis of variance of plant height (cm) of 14 switchgrass cultivars evaluated at four locations in marginal vs. prime farmland at each location with 0 or 100 Kg of N Ha⁻¹. Switchgrass stands were established in NY and WI in 2008; in NJ and PA in 2009.

Source	Df	F-value	Pr > F
Location (State)	3	203.55	< 0.0001
SQ (Soil Quality)	1	395.36	< 0.0001
Loc*SQ	3	185.63	< 0.0001
N (Nitrogen)	1	62.86	< 0.0001
Loc*N	3	62.68	< 0.0001
SQ*N	1	12.46	0.0028
Loc*SQ*N	3	6.17	0.0055
Cv (Cultivar)	13	14.40	< 0.0001
Loc*Cv	39	5.64	< 0.0001
SQ*Cv	13	0.83	0.6237
Loc*SQ*Cv	36	1.32	0.1179
N*Cv	13	1.60	0.0807
Loc*N*Cv	39	0.98	0.5056
SQ*N*Cv	13	1.41	0.1562
Loc*SQ*N*Cv	33	1.29	0.1488

Due to poor stand establishment and subsequent lack of survival of four lowland switchgrass cultivars Alamo, BoMaster, Performer and High Tide missing values were present for these cultivars (Table 7). The quality of the soil affected the height of the plants in two of the four locations, NJ and NY (Table 8). The largest difference in plant height between prime and marginal quality soil was observed in NJ, switchgrass plants were 66% taller in the prime site compared to the plants in the marginal site (Table 8). Switchgrass plants were tallest in NY and WI prime sites fertilized with 100 kg of N ha⁻¹, at 174 and 170 cm, respectively (Table 8). The shortest plants were observed in the NJ marginal site with and without nitrogen fertilizer, 47 and 54 cm, respectively (Table 8). Even though the analysis of variance suggested that nitrogen treatment was a statistically significant ($P < 0.05$) source of variation in plant height, according to the letter grouping significant ($P < 0.05$) differences between fertilized and unfertilized plots were only observed in NY and WI (Table 8). It was observed that in NY fertilized plots in the prime site were approximately 18 cm taller than unfertilized plots, on average, while in WI, fertilized plots were 57 and 39 cm taller in the prime and marginal sites compared to unfertilized plots in the prime and marginal sites, a 33% and 23% decrease in height, respectively (Table 8). Although switchgrass has been described as a thrifty user of nitrogen, moderate fertilization positively influences plant height and dry biomass production (Brejda, 2000; Mulkey et al., 2006 and 2008; Parrish and Fike, 2005). Jung et al. (1990) observed that switchgrass cultivars Caddo, Kentucky 1625 and Carthage produced plants with taller tillers when fertilized with 75 kg·ha⁻¹ of nitrogen compared to unfertilized plants. Schmer et al. (2010) showed that cultivars Cave-in-Rock and Trailblazer grown in fertilized plots with 60 and 120 kg·ha⁻¹ of nitrogen

also produced taller plants compared to unfertilized plots. However nitrogen availability in the soil is subject to variation due to environmental and soil conditions such as temperature (Craswell and Godwin, 1984); annual rainfall (Sala et al., 1988); soil pH (Bohn et al., 1979), and water-holding capacity (Staley et al., 1991). The results concerning the nitrogen treatment indicates that in some locations there may be issues with timing of nitrogen applications, availability of nitrogen in the soil or a delay in the response to nitrogen fertilization by switchgrass plants (Bredja, 2000; Jung et al., 1990). The results also suggest that addition of nitrogen in NY and WI could improve plant heights on marginal soils and may improve biomass yields.

Kanlow, Timber and BoMaster produced the tallest plants across all locations, 150, 146 and 144 cm, respectively (Table 9). In general, in this study lowland cultivars produced taller plants than upland cultivars (Table 7, 9 and 10). Alamo had the shortest plants among the lowland types across locations, 138 cm (Table 9). Cave-in-Rock exhibited the tallest plants among the central upland types and Summer was the tallest of the northern upland type, 144 and 135 cm, respectively (Table 9). Many researchers have shown evidence that the longer days in northern latitudes may delay maturation in lowland types producing taller plants (Casler et al., 2004; Lemus et al., 2002; Madakadze et al., 1998; McMillan, 1965). These results are consistent with previous research suggesting that after establishment lowland types may grow taller even in conditions that favor upland types (Casler et al., 2004; Cornelius and Johnston, 1944; Porter, 1966). This indicates that genotype x environment interactions were more influential in plant height than genotype in the differences observed. The northern lowland cultivars Kanlow and Timber were well adapted to the locations evaluated in this study and exhibited tall plant heights. These two lowland cultivars

would be good lowland cultivar choices for northern regions of the country. Casler et al. (2004) concluded that moving lowland populations slightly north of the latitude-of-origin may potential increase agronomic performance.

Previous research studies have not shown the variation in performance of switchgrass cultivars depending on the quality of the soil they are grown. In this study it was shown that plant height differences between prime and marginal soil were statistically significant ($P < 0.05$) for all the cultivars evaluated, with cultivars always having taller plant heights in prime soil vs. marginal soil (146 vs. 115, on average) (Table 10). These results are consistent with the results of the analysis of variance which suggests that soil quality may play a major role in the variation of plant heights of switchgrass cultivars (Table 6). Additionally to improve plant height of switchgrass in marginal land the selection process may have to include trials in environments with marginal soil types.

Table 7. Plant height (cm) of 14 switchgrass cultivars evaluated at four locations in marginal vs. prime farmland with 0 or 100 Kg of N Ha⁻¹.

Switchgrass stands were established in NY and WI in 2008; in NJ and PA in 2009.

		Plant Height (cm)																Mean Across Location
		NJ				NY				PA				WI				
		Adelphia		Somerset		Syracuse				Rock Springs				Columbia County				
		Prime		Marginal		Prime		Marginal		Prime		Marginal		Prime		Marginal		
Cultivars	Type	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	
Kanlow	NL	158.44	165.22	64.56	50.33	182.60	183.73	157.20	154.09	149.44	150.11	136.00	147.44	124.00	172.00	146.00	141.67	142.68 a ¹
Timber	NL	176.56	155.33	64.32	55.82	151.67	200.10	164.82	140.97	138.78	149.78	132.56	151.22	116.67	163.33	120.00	152.33	139.64 ab
Cave-In-Rock	CU	147.56	140.22	55.11	51.44	154.66	185.14	148.17	152.12	133.00	133.44	122.33	139.00	123.67	185.33	129.67	182.67	136.47 abc
Shawnee	CU	137.11	127.00	66.00	44.22	160.58	183.16	149.01	150.99	129.33	135.56	122.56	133.22	129.00	179.00	132.33	168.33	134.21 abcd
Carthage	CU	16.67	157.78	54.89	44.89	158.89	175.82	135.47	133.35	134.00	139.89	124.56	142.56	129.67	161.33	126.33	140.67	131.67 bcd
Blackwell	CU	147.44	129.67	49.18	55.22	160.58	184.01	146.47	148.45	132.33	132.33	125.00	128.56	113.67	156.67	127.00	164.67	131.33 bcd
Summer	NU	143.22	142.33	61.67	40.31	153.53	165.10	142.24	127.28	116.78	117.78	120.56	126.44	131.00	171.33	113.33	169.33	127.64 cd
Sunburst	NU	144.44	128.00	45.11	39.00	160.30	169.90	131.52	128.98	116.33	117.22	123.56	123.56	136.67	158.67	115.67	187.00	126.62 d
Pathfinder	CU	138.33	124.89	43.89	51.11	166.65	172.44	124.74	112.75	117.00	123.72	116.22	120.89	115.33	171.67	129.00	182.00	125.66 d
KY 1625	CU	122.00	126.56	37.64	36.33	116.84	125.02	113.74	110.63	105.33	114.22	10.44	115.00	117.00	184.00	125.67	163.67	113.63 e
Alamo	SL	166.20	155.78	-	-	163.72	171.59	136.88	129.54	138.22	130.67	148.22	142.67	117.67	144.67	113.00	133.00	142.27 *
BoMaster	SL	177.22	162.56	-	-	163.26	182.03	153.53	132.36	128.33	134.07	135.22	138.33	140.00	153.67	133.00	154.67	149.16 *
High Tide	CU	144.22	147.22	44.61	-	-	134.34	139.70	117.69	114.33	132.67	124.11	121.78	117.67	162.00	129.00	139.00	126.31 *
Performer	SL	163.33	157.89	-	-	-	167.08	145.91	127.56	130.67	140.44	135.56	143.00	121.67	168.67	138.67	155.33	145.83 *
Mean		150.91	144.32	53.36	46.87	157.77	171.39	142.10	133.34	127.42	132.28	126.49	133.83	123.83	166.60	127.05	159.60	
		BC ²	CD	++	++	++	A	CD	DE	E	DE	E	DE	E	A	E	AB	

¹ Cultivar means with the same lower case letter were not statistically different at 0.05 level of probability.

² Location means with the same upper case letter were not statistically different at 0.05 level of probability.

- Means were not calculated due to missing values.

* Cultivars means were not separated by LSMEANS due to missing values.

++ Location means were not separated by LSMEANS due to missing values.

Table 8. Means of plant height (cm) separated by location, site and nitrogen treatment. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.

				Mean Plant Height (cm) per Location
NJ	Adelphia	Prime	0	146.18 cd ¹
			100	139.70 cdef
	Somerset	Marginal	0	54.28 g
			100	47.06 g
NY	Syracuse	Prime	0	156.72 bc
			100	174.44 a
		Marginal	0	141.34 cde
			100	135.96 def
PA	Rock Springs	Prime	0	127.23 ef
			100	131.47 def
		Marginal	0	122.78 f
			100	132.79 def
WI	Columbia County	Prime	0	123.67 ef
			100	170.33 ab
		Marginal	0	126.50 ef
			100	165.23 ab

¹ Location means with same lower case letter were not statistically different at 0.05 level of probability.

Table 9. Means of plant height (cm) separated by genotype. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without the marginal site at NJ and prime at NY due to missing values in the data set.

Cultivars	Type	Mean Plant Height (cm)
Kanlow	NL	150.14 a¹
Timber	NL	146.86 ab
BoMaster	SL	145.25 abc
Cave-In-Rock	CU	144.76 abcd
Performer	SL	144.06 abcd
Shawnee	CU	141.12 abcde
Carthage	CU	139.36 abcde
Alamo	SL	138.04 bcde
Blackwell	CU	137.69 bcde
Summer	NU	135.14 cbef
Sunburst	NU	134.30 def
High Tide	CU	132.45 ef
Pathfinder	CU	131.37 ef
KY 1625	CU	125.19 f

¹ Cultivar means with same lower case letter were not statistically different at 0.05 level of probability.

Table 10. Comparison of plant height (cm) means by cultivar in marginal vs. prime farmland sites across four locations. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.

Cultivars	Type	Mean Plant Height (cm)		
		Prime	Marginal	
Kanlow	NL	160.39	124.66	*
Timber	NL	156.64	122.66	*
Carthage	CU	150.51	112.84	*
Cave-In-Rock	CU	150.38	122.56	*
Shawnee	CU	147.59	120.83	*
Blackwell	CU	144.59	118.05	*
Summer	NU	142.63	112.81	*
Sunburst	NU	141.44	111.80	*
Pathfinder	CU	141.33	110.08	*
KY 1625	CU	126.37	101.14	*

Cultivar means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

Tiller Density

Tiller density has been identified as an important trait for determining dry biomass yield as well as an important criteria for selecting improved biomass yield in switchgrass genotypes (Boe and Beck, 2008; Das et al., 2004). Path coefficient analysis has indicated that both tillers·plant⁻¹ and tillers·m⁻² have the highest positive direct effect on biomass yield (Boe and Beck, 2008; Das et al., 2004). In this study, the analysis of variance for tiller density (tillers·m⁻²) suggested that the location was the most influential factor affecting the variation observed in tiller density, followed by location x soil quality interaction and genotype which were influential but accounted for a smaller portion of the variance (Table 11). Soil quality and nitrogen treatments were also found statistically significant ($P < 0.05$) but the interaction between these two factors was not (Table 11). The two factor interactions location x nitrogen, location x cultivar, and soil quality x cultivar were found significant ($P < 0.05$) as well as a three factor interaction that contained location, soil quality and cultivar (Table 11). These results indicate that the genotype x environment interactions may be influential in the amount of tillers switchgrass plants produce. Casler et al. (2004) found that upland types produced more tillers in northern latitudes while lowland types produced less tillers in northern latitudes. While, Cassida et al. (2005a) observed the opposite in southern environments upland types produced progressively fewer tillers compared to lowland types. This indicates the importance of determining the extent of the influence of the genotype x environment interaction on tiller production to identify specific factors that may be the most important so that breeders can maximize selection and growers can maximize productivity on poor quality soils.

Table 11. Analysis of variance of tiller density (tillers·m⁻²) of 14 switchgrass cultivars evaluated at three locations in marginal vs. prime farmland at each location with 0 or 100 kg of N·ha⁻¹. Switchgrass stands were established in WI in 2008; in NY and PA in 2009 due to missing values in the data set.

Source	Df	F-value	Pr > F
Location (State)	2	142.75	< 0.0001
SQ (Soil Quality)	1	5.01	0.0449
Loc*SQ	2	41.22	< 0.0001
N (Nitrogen)	1	7.76	0.0165
Loc*N	2	9.96	0.0028
SQ*N	1	0.07	0.7967
Loc*SQ*N	2	1.49	0.2652
Cv (Cultivar)	13	14.85	< 0.0001
Loc*Cv	26	4.60	< 0.0001
SQ*Cv	13	2.36	0.0070
Loc*SQ*Cv	22	1.70	0.0338
N*Cv	13	0.85	0.6083
Loc*N*Cv	26	1.50	0.0739
SQ*N*Cv	13	0.73	0.7289
Loc*SQ*N*Cv	22	0.69	0.8428

The highest tiller density was observed in PA, 639.15 tillers·m⁻² on average (Table 13). In WI, the average tiller density was 368 tillers·m⁻², the lowest in the study (Table 13). Tiller density was higher in the prime site compared to marginal site in NJ and WI but not in PA (Table 13). In NJ, plots in the prime site had 33% more tillers·m⁻² than in the marginal site (Table 13). In contrast, in PA it was observed that the marginal site had 17% more tillers·m⁻² than in the prime site (Table 13). Fertilized plots in the prime site in PA had more tillers·m⁻² than unfertilized plots, 648 vs. 508 tillers·m⁻², respectively (Table 13). These results showed a marked difference in tiller density by state compared to the other factors (nitrogen treatment and soil quality).

Although the analysis of variance indicated that soil quality affected tiller density, the only distinct difference between marginal and prime soil was observed in NJ (Table 11 and 13). Additionally, ANOVA results indicated that nitrogen treatment influenced tiller density however this was only significant ($P < 0.05$) in the prime site in PA (Table 11 and 13). The lack of significance may be attributed to the fact that only three locations reported data for tiller density (NJ, PA and WI). There may be stronger evidence for significant effects if other locations had collected tiller density data. These results also indicate that other conditions related to the location of the trials (i.e. rainfall, temperature, latitude, etc.) could be influencing tiller density to a larger extent than just soil quality or nitrogen (Boe and Beck, 2008; Boe and Lee, 2007; Casler et al., 2004; Madakadze et al., 1998; Mulkey et al., 2008).

Upland type cultivars had higher tiller densities than most of the lowland types across locations (Table 14). The central upland type cultivars KY 1625 (623 tillers·m⁻²), Pathfinder (570 tillers·m⁻²) and Carthage (567 tillers·m⁻²) had the highest tiller

densities (Table 14). Timber had the highest tiller density among all lowland types and was better than some upland types with 544 tillers·m⁻² (Table 14). The three switchgrass cultivars of the southern lowland type, Alamo, BoMaster and Performer, had the lowest tiller density across locations with 352, 358 and 319 tillers·m⁻², respectively (Table 14). As mentioned previously, this study was conducted in northern regions of the U.S. which may have environmental conditions that favor the performance of upland cultivars or may be closer to the latitude-of-origin of some cultivars (Casler and Boe 2003; Casler et al., 2004). In contrast, Cassida et al. (2005a) reported higher tiller densities for lowland switchgrass populations, Alamo and the selections, SL931, SL932 and SL941 in southern states, such as, Arkansas, Louisiana and Texas. The results of this study suggested that tiller density may be more influenced by climatic conditions (e.g. temperature, rainfall, etc.) and latitude (day length) than soil conditions. The same cultivar did not rank the same in all the site-by-location combinations (Table 12 and 15). The top three cultivars with the highest tiller density in prime sites performed significantly different in marginal sites but unlike the other agronomic traits reported, cultivars weren't always better in prime sites (Table 15). KY 1625 actually had higher tiller densities in marginal sites than prime sites (Table 15). These results may indicate that multi-environment selection that include marginal land trials may be necessary to effectively identify the best performing cultivars, but it also indicates that genotypes exist that can have higher tiller densities in marginal sites than prime sites. These results indicate that cultivars may be developed with superior tiller density in marginal soil types.

Table 12. Tiller density (tillers per m²) of 14 switchgrass cultivars evaluated at three locations in marginal vs. prime farmland at each location with 0 or 100 Kg of N Ha⁻¹. Switchgrass stands were established in WI in 2008; in NY and PA in 2009.

		Tiller Density (tillers per m²)												Mean Across Location
		NJ				PA				WI				
		Adelphia		Somerset		Rock Springs		Columbia County						
Cultivars	Type	Prime		Marginal		Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	
		0	100	0	100	0	100	0	100	0	100	0	100	
KY 1625	CU	786.68	643.21	558.94	616.70	497.35	896.67	779.50	1011.44	401.33	387.00	415.67	415.67	617.51 a¹
Carthage	CU	755.59	603.76	442.36	359.86	564.30	931.34	585.82	729.29	358.33	372.67	415.67	358.33	539.78 ab
Pathfinder	CU	762.76	652.77	362.25	397.55	697.01	575.48	801.02	655.16	415.67	372.67	372.67	401.33	538.84 ab
Blackwell	CU	636.04	644.40	421.58	506.92	467.46	565.50	779.50	780.70	401.33	372.67	387.00	430.00	532.76 ab
Summer	NU	602.56	486.59	474.64	441.17	503.33	704.18	638.43	883.52	415.67	430.00	401.33	401.33	531.90 ab
Sunburst	NU	752.00	809.39	447.14	514.09	504.52	669.51	565.50	478.22	401.33	430.00	387.00	415.67	531.20 ab
Timber	NL	670.71	679.08	351.78	303.28	459.09	540.39	812.98	724.51	430.00	358.33	387.00	387.00	508.68 bc
Cave-In-Rock	CU	590.60	533.22	460.29	417.25	493.76	485.40	575.06	795.04	401.33	430.00	387.00	415.67	498.72 bc
Shawnee	CU	634.84	585.82	380.19	493.76	450.72	560.72	453.12	650.38	415.67	415.67	430.00	430.00	491.74 bc
Kanlow	NL	597.78	547.56	416.05	302.48	450.72	555.93	570.28	724.51	329.67	315.33	215.00	169.65	432.91 c
Alamo	SL	609.73	656.36	-	-	437.57	353.88	558.32	681.47	71.67	60.28	57.33	38.24	352.49 *
BoMaster	SL	540.38	669.51	-	-	325.19	331.85	600.17	747.22	124.19	146.38	57.33	40.78	501.11 *
High Tide	CU	616.91	655.16	-	-	589.41	863.19	747.22	814.17	329.67	315.33	114.67	127.11	753.50 *
Performer	SL	548.76	640.82	-	-	217.59	401.71	496.16	432.79	157.67	167.40	88.01	43.00	387.06 *
Mean		650.38	629.12	431.52	435.31	475.57	602.55	640.22	722.03	332.40	326.70	293.98	290.98	
		A²	A	++	++	B	A	A	A	C	C	C	C	

¹ Cultivar means with the same lower case letter were not statistically different at 0.05 level of probability.

² Location means with the same upper case letter were not statistically different at 0.05 level of probability.

- Means were not calculated due to missing values.

* Cultivars means were not separated by LSMEANS due to missing values.

++ Location means were not separated by LSMEANS due to missing values.

Table 13. Means of tiller density (tiller per m²) separated by location, site and nitrogen treatment. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.

				Mean Tiller Density (tillers per m²)
NJ	Adelphia	Prime	0	678.96 a ¹
			100	618.58 ab
	Somerset	Marginal	0	431.54 c
			100	435.42 c
PA	Rock Springs	Prime	0	508.83 bc
			100	648.37 a
		Marginal	0	656.12 a
			100	743.28 a
WI	Columbia County	Prime	0	397.03 c
			100	388.43 c
		Marginal	0	379.83 c
			100	381.48 c

¹ Location means with same lower case letter were not statistically different at 0.05 level of probability.

Table 14. Means of tiller density (tillers per m²) separated by genotype. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without the marginal site at NJ and prime at NY due to missing values in the data set.

Cultivars	Type	Mean Tiller Density (tillers per m²)
KY 1625	CU	623.45 a ¹
Pathfinder	CU	570.59 ab
Carthage	CU	567.51 ab
Summer	NU	546.69 abc
Blackwell	CU	546.46 abc
Timber	NL	544.91 abc
Sunburst	NU	541.32 abc
High Tide	CU	517.25 abc
Cave-In-Rock	CU	510.71 bc
Shawnee	CU	502.69 bc
Kanlow	NL	447.65 cd
BoMaster	SL	358.27 de
Alamo	SL	352.43 de
Performer	SL	319.40 e

¹ Cultivar means with same lower case letter were not statistically different at 0.05 level of probability.

Table 15. Comparison of tiller density (tillers per m²) means by cultivar in marginal vs. prime farmland sites across three locations. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.

Cultivars	Type	Mean Tiller Density (tillers · m ⁻²)		
		Prime	Marginal	
KY 1625	CU	602.04	633.36	*
Carthage	CU	597.66	481.89	*
Sunburst	NU	594.46	467.94	*
Pathfinder	CU	579.16	498.34	NS
Summer	NU	523.72	540.13	NS
Timber	NL	522.93	494.31	NS
Blackwell	CU	514.57	550.83	NS
Shawnee	CU	510.57	472.91	NS
Cave-In-Rock	CU	489.05	508.39	NS
Kanlow	NL	466.17	399.68	*

Cultivar means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

Dry Biomass Yield

According to the ANOVA table the nitrogen treatment accounted for the largest portion of the variance for dry biomass yield (Table 16). Switchgrass has been described as a thrifty user of nitrogen because this species has a common strategy of tall prairie grasses for conserving nitrogen. At the end of the season senescent leaves translocate nitrogen to the perennial parts of the plant mainly to the underground biomass (Hargrave and Seastedt, 1994). However it is well known that nitrogen management is an important factor in switchgrass cropping systems where legumes are not included (Bredja et al., 1994; McLaughlin et al., 1999; Mulkey et al., 2006; Vogel et al., 2002). There are many studies reporting a wide range of recommendations for nitrogen fertilization on switchgrass but there is not a consistent trend (Parrish and Fike, 2005). Additionally location, location x soil quality interaction and location x nitrogen treatment interaction were also influential factors and several other interaction effects with two and three factors were significant enough to affect the variation of the response variable dry biomass yield (Table 16). Soil quality was a statistically significant ($P < 0.05$) source of variation; however it accounted for a small portion of the variance of dry biomass yield (Table 16). The results indicated that cultivar, although significant ($P < 0.05$), had one of the lowest effects on biomass yield compared to nitrogen, location and soil quality. The strong effect of environment on biomass yield supports the idea that switchgrass cultivars will need to be evaluated in marginal lands where they will be utilized rather than relying on genetic differences observed on prime farmland of research farms (Atlin and Frey, 1990; Bänziger et al., 1997; Ceccarelli, 1987).

Poor stand establishment of four switchgrass cultivars Alamo, BoMaster, Performer and High Tide resulted in low seedling survival and missing values (Table 17). Across locations and soil types biomass yield was greatest in WI ($7.6 \text{ Mg}\cdot\text{ha}^{-1}$, on average) (Table 17). However, highest dry biomass yields were observed in NJ in prime soil ($8.9 \text{ Mg}\cdot\text{ha}^{-1}$). Switchgrass cultivars had greater yields in the prime soil compared to marginal soil in SD (7.9 vs. $1.7 \text{ Mg}\cdot\text{ha}^{-1}$) and in NJ (8.9 vs. $2.3 \text{ Mg}\cdot\text{ha}^{-1}$) (Table 18). These sites also had the best stand establishment (Table 4). In this study it was observed a positive correlation between stand establishment and dry biomass yield (Table 19). There are studies suggesting that stands with good initial establishment will also have optimal dry biomass yields in post-establishment years (Masters and Vogel, 2001; Schmer et al., 2006; Vogel, 1987).

Even though nitrogen treatment accounted for the largest portion of the variance in dry biomass yield, according to the letter grouping differences for this response were only found in SD prime site (fertilized 9.2 vs. $6.69 \text{ Mg}\cdot\text{ha}^{-1}$ unfertilized), in WI prime site (fertilized 10.3 vs. $5 \text{ Mg}\cdot\text{ha}^{-1}$ unfertilized) and in WI marginal site (fertilized 10.3 vs. $4.7 \text{ Mg}\cdot\text{ha}^{-1}$ unfertilized) (Table 18). In fact, in WI fertilized plots in the marginal site were similar in biomass yields to fertilized plots in the prime site.

Soil type (i.e. either prime or marginal) seemed to have more of an influence on biomass yield than nitrogen in SD and NJ, indicating that in some types of marginal soil, nitrogen amendments may not increase biomass yields enough to compete with prime soil conditions (Table 18). It is not known if higher amounts of nitrogen could improve yields, because only a rate of $100 \text{ kg of N ha}^{-1}$ was tested in

this study. However, Vogel et al. (2002) found that maximum biomass yields of switchgrass were obtained when it was fertilized with 120 kg of N ha⁻¹ and Mulkey et al. (2006) found no additional advantage of fertilizing with rates greater than 56 kg of N ha⁻¹ on nine year old switchgrass grown on conservation reserve land. Switchgrass recommended N rates in the Central Plains and Midwest states range from 50 to 120 kg of N ha⁻¹ (Brejda, 2000). Therefore it is unlikely that much higher yields could be attained with additional amounts of fertilizer.

Table 16. Analysis of variance for dry biomass yield (Mg·ha⁻¹) of 14 switchgrass cultivars evaluated at six locations in marginal vs. prime farmland at each location with 0 or 100 kg of N·ha⁻¹. Switchgrass stands were established in NY, SD and WI in 2008; in MD, NY and PA in 2009.

Source	Df	F-value	Pr > F
Location (State)	5	55.85	< 0.0001
SQ (Soil Quality)	1	28.56	< 0.0001
Loc*SQ	5	56.41	< 0.0001
N (Nitrogen)	1	163.47	< 0.0001
Loc*N	5	35.58	< 0.0001
SQ*N	1	7.41	0.0119
Loc*SQ*N	5	5.46	0.0017
Cv (Cultivar)	13	7.61	< 0.0001
Loc*Cv	63	5.65	< 0.0001
SQ*Cv	13	0.92	0.5307
Loc*SQ*Cv	55	1.06	0.3763
N*Cv	13	2.81	0.0009
Loc*N*Cv	63	2.49	< 0.0001
SQ*N*Cv	13	2.20	0.0099
Loc*SQ*N*Cv	55	1.16	0.2159

Table 17. Dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) of 14 switchgrass cultivars evaluated at six locations in marginal vs. prime farmland at each location with 0 or 100 $\text{kg of N}\cdot\text{ha}^{-1}$. Switchgrass stands were established in NY, SD and WI in 2008; in MD, NJ and PA in 2009.

		Dry Biomass Yield (Mg·ha ⁻¹)																								Mean Biomass (Mg·ha ⁻¹)
		MD				NJ				NY				PA				SD				WI				
		Snow Hill		Adelphia		Somerset		Syracuse		Rock Springs		Brookings		Pierre		Columbia County										
		Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal			
Cultivars	Type	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	
Shawnee	CU	2.99	3.97	6.21	6.72	6.60	10.62	2.52	2.08	2.41	3.67	2.74	6.38	3.87	6.21	3.34	5.74	6.43	13.49	1.98	2.38	6.52	12.39	4.86	12.56	5.70 A¹
Carthage	CU	2.33	4.00	6.63	5.11	9.64	10.00	1.77	2.51	4.54	1.30	2.55	3.63	5.49	7.38	4.19	9.02	10.79	12.47	1.53	1.86	5.43	9.26	4.70	8.17	5.60 A
Blackwell	CU	2.63	4.93	7.08	5.17	8.94	8.44	2.21	2.89	5.04	4.18	3.75	5.73	4.25	6.40	5.33	6.29	8.04	11.55	2.04	1.82	4.37	7.24	4.52	10.96	5.57 A
Cave-In-Rock	CU	2.45	3.97	3.82	2.51	9.95	9.14	2.34	2.49	1.56	2.31	3.52	4.44	5.09	5.34	3.37	6.55	7.00	8.84	1.64	0.87	7.82	13.54	5.63	14.25	5.35 A
Sunburst	NU	4.66	4.63	6.96	6.99	6.38	6.03	2.41	2.68	3.15	1.82	3.95	4.55	4.77	4.30	3.59	3.84	7.48	9.21	1.84	1.33	5.83	13.38	5.61	12.81	5.34 A
Pathfinder	CU	2.48	4.48	4.87	4.54	7.04	8.45	1.80	2.56	2.72	2.64	5.42	4.36	4.28	4.95	3.33	5.25	8.43	11.67	2.53	2.25	4.10	13.00	4.83	11.08	5.29 A
Summer	NU	4.69	3.01	5.41	5.89	6.91	7.02	1.89	2.27	3.47	4.68	4.58	5.18	4.51	5.13	3.83	5.65	6.61	8.37	1.29	1.74	4.33	10.17	4.57	11.34	5.11 A
Timber	NL	2.96	4.09	5.56	6.63	11.27	12.34	2.63	2.65	2.26	2.53	4.38	2.94	6.30	6.89	4.35	9.08	2.41	6.81	1.72	1.28	3.66	6.17	5.37	7.48	5.07 A
Kanlow	NL	3.07	5.77	6.30	7.47	10.45	11.43	2.17	2.95	3.56	1.60	4.46	3.91	5.57	7.47	4.19	8.07	6.71	4.04	2.53	1.64	3.97	5.88	2.66	4.43	5.01 A
KY 1625	CU	3.52	7.05	6.99	5.38	8.44	8.98	2.21	2.17	1.41	2.38	3.38	2.49	3.69	5.07	4.06	5.60	3.01	6.00	1.50	1.19	4.66	12.46	4.97	10.30	4.87 A
High Tide	CU	1.91	4.36	4.90	4.84	6.28	8.54	-	-	1.63	3.78	3.43	2.69	3.57	5.97	3.83	5.09	-	-	1.11	0.92	4.20	9.75	2.87	4.26	4.20 *
BoMaster	SL	2.87	3.22	4.54	2.39	8.38	10.04	-	-	3.22	4.26	4.34	2.58	4.21	5.15	5.55	5.91	-	-	1.69	1.46	2.25	3.81	3.24	3.73	4.14 *
Alamo	SL	1.73	2.12	3.94	2.27	9.62	11.91	-	-	2.76	2.52	3.00	1.71	5.83	5.06	6.15	10.16	-	-	0.96	0.84	1.89	3.34	2.42	2.92	4.06 *
Performer	SL	2.48	5.05	5.28	5.08	4.90	9.74	-	-	1.86	1.91	2.78	1.68	3.66	4.58	4.40	5.36	-	-	0.78	0.87	2.37	4.65	1.74	3.52	3.63 *
Mean		2.91	4.33	5.61	5.07	8.20	9.48	2.19	2.52	2.83	2.83	3.73	3.73	4.65	5.71	4.25	6.54	6.69	9.24	1.65	1.46	4.39	8.93	4.14	8.42	
		fgh ²	defg	cd	cde	ab	a	++	++	gh	gh	efg	efg	def	cd	defg	bc	++	++	h	h	defg	a	defg	a	

¹ Cultivar means with the same upper case letter were not statistically different at 0.05 level of probability.

² Location means with the same lower case letter were not statistically different at 0.05 level of probability.

- Means were not calculated due to missing values.

* Cultivars means were not separated by LSMEANS due to missing values.

⁺⁺ Location means were not separated by LSMEANS due to missing values.

Table 18. Means of dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) separated by location, site and nitrogen treatment. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.

				Mean Dry Biomass Yield ($\text{Mg}\cdot\text{ha}^{-1}$)
MD	Snow Hill	Prime	0	3.18 fghij ¹
			100	4.59 cdefg
		Marginal	0	5.98 cde
			100	5.64 cde
NJ	Adelphia	Prime	0	8.57 ab
			100	9.25 a
	Somerset	Marginal	0	2.19 hij
			100	2.52 ghij
NY	Syracuse	Prime	0	3.01 fghij
			100	2.71 ghij
		Marginal	0	3.87 efghi
			100	4.36 defgh
PA	Rock Springs	Prime	0	4.78 cdefg
			100	5.91 cde
		Marginal	0	3.96 efghi
			100	6.51 bcd
SD	Brookings	Prime	0	6.69 bc
			100	9.24 a
	Pierre	Marginal	0	1.86 ij
			100	1.63 j
WI	Columbia County	Prime	0	5.07 cdef
			100	10.35 a
		Marginal	0	4.77 cdefg
			100	10.34 a

¹ Location means with same lower case letter were not statistically different at 0.05 level of probability.

Table 19. Pearson correlation coefficients for dry biomass yield (DBY) and secondary traits: stand establishment (SE), plant height (PH) and tiller density (TD), of 14 switchgrass cultivars evaluated at six locations in marginal vs. prime farmland at each location with 0 or 100 kg of N·ha⁻¹. Switchgrass stands were established in NY, SD and WI in 2008; in MD, NY and PA in 2009.

Traits	SE	PH	TD	DBY
Stand Establishment (SE) (% Coverage)	—	-0.58	0.35	0.96*
Plant Height (PH) (cm)	-0.58	—	-0.69*	-0.43
Tiller Density (TD) (tillers·m⁻²)	0.35	-0.69*	—	0.19
Dry Biomass Yield (DBY) (Mg·ha⁻¹)	0.96*	-0.43	0.19	—

(*) Statistically significant at 0.05 level of probability.

The central upland types Shawnee, Blackwell and Cave-in-Rock had the highest dry biomass yields across locations ($5.9 \text{ Mg}\cdot\text{ha}^{-1}$) (Table 20). Similarly these three cultivars had ground coverage percentages higher than 70% (Table 4). Among the lowland type cultivars, Timber had the highest biomass yield ($5.7 \text{ Mg}\cdot\text{ha}^{-1}$) and was not significantly ($P<0.05$) different from the best central upland cultivars. Kanlow, although lower in yield at $5.6 \text{ Mg}\cdot\text{ha}^{-1}$, was also not significantly ($P<0.05$) different than the highest yielding cultivars. These two lowland cultivars are from northern origin and had ground coverage percentages above 60% (Table 4). The three lowland switchgrass cultivars, Alamo, BoMaster and Performer, of southern origin exhibited the poorest stand establishment as well as the lowest dry biomass yield across locations (Table 4 and 20). This indicates that some northern lowland types can establish well and produce high biomass yields comparable to that of upland types in northern climates (Casler et al., 2004). It also indicates that there is potential to exploit northern lowland germplasm for future cultivar development for marginal soils in the regions where this study was conducted.

Previous studies have not looked at the effect of soil quality on the agronomic performance of switchgrass cultivars. On average, all the available cultivars had higher dry biomass yields in prime sites compared to marginal sites (Table 21). However, there were no statistical differences ($P<0.05$) between prime and marginal sites for Blackwell, Sunburst and Summer (Table 21). Similar results were observed for the northern lowland cultivar Timber. These results indicate that some cultivars are more influenced by genotype x environmental interaction than other cultivars. These results indicate that direct selection in marginal land may be necessary for

some germplasm sources but also suggests that for the germplasm sources less affected by the genotype x environmental interaction that selection may be possible on prime farm land. In any event, what is clear is that germplasm sources need to be evaluated individually in multi-environment trials because not all germplasm sources responded consistently to the environmental variables.

Table 20. Means of dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) separated by genotype. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without the marginal site at NJ and prime at NY due to missing values in the data set.

Cultivars	Type	Mean
		Dry Biomass Yield ($\text{Mg}\cdot\text{ha}^{-1}$)
Shawnee	CU	5.99 A¹
Blackwell	CU	5.85 A
Cave-In-Rock	CU	5.85 A
Timber	NL	5.79 AB
Carthage	CU	5.75 AB
Sunburst	NU	5.74 AB
KY 1625	CU	5.61 ABC
Kanlow	NL	5.58 ABC
Summer	NU	5.58 ABC
Pathfinder	CU	5.44 ABC
High Tide	CU	4.55 BCD
BoMaster	SL	4.43 CD
Alamo	SL	4.41 CD
Performer	SL	3.95 D

¹ Cultivar means with same lower case letter were not statistically different at 0.05 level of probability.

Table 21. Comparison of dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) means by cultivar in marginal vs. prime farmland sites across three locations. Statistical analysis was conducted without cultivars Alamo, BoMaster, High Tide and Performer due to missing values in the data set.

Cultivars	Type	Mean Dry Biomass Yield ($\text{Mg}\cdot\text{ha}^{-1}$)		
		Prime	Marginal	
Carthage	CU	6.89	4.31	*
Shawnee	CU	6.60	4.80	*
Cave-In-Rock	CU	6.42	4.29	*
Blackwell	CU	6.34	4.82	NS
Pathfinder	CU	6.19	4.41	*
Sunburst	NU	5.97	4.72	NS
Kanlow	NL	5.80	4.24	*
Summer	NU	5.75	4.47	NS
Timber	NL	5.64	4.51	NS
KY 1625	CU	5.56	4.19	*

Cultivar means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

Conclusions

The results of this study are consistent with previous research that suggested a relationship between stand establishment and dry biomass yield. Cultivars with ground coverage percentages above 60% had good dry biomass yield. However it was not possible to determine the highest yielding cultivars just by looking at the establishment data. Central upland cultivars Shawnee and Blackwell had better establishment compared to lowland cultivars of northern origin, Timber and Kanlow but the yields of these two types were not significantly different. Southern lowland types had the worst performance in both traits compared to upland and northern lowland ecotypes.

Cultivars Kanlow and Timber established the best, had the highest tiller densities, the tallest plants and the most biomass yield among lowland cultivars. Both of these cultivars originated in central to northern regions which may explain why they grew better than southern lowland cultivars, such as, Alamo, BoMaster and Performer. Additionally some southern lowlands cultivars produced taller plants compared to several upland cultivars across all the locations. The two northern lowland cultivars showed remarkable adaptation to the locations in this study compared to the southern lowlands. The results of this study may suggest using northern lowland cultivars and germplasm in the selection process to improve other traits such as plant height, tiller density and dry biomass.

Secondary traits have been considered an important tool in breeding programs for direct and indirect selection in species such as maize (*Zea mays* L.), tall fescue

(*Festuca arundinaceae* Schreb.) and smooth brome grass (*Bromus inermis* Leyss). In switchgrass, plant height and tiller density have been identified as significant secondary traits for determining biomass yield as well as a valuable criteria for selecting improved switchgrass genotypes. However in this study the relationship between plant height and biomass yield was only observed for cultivars Cave-in-Rock and Timber, both genotypes were ranked in the top five for both traits. A similar relation was observed between tiller density and biomass yield where only cultivars Blackwell and Carthage ranked in the top five for both traits. Our results did not permit us to identify the highest yielding cultivars by looking at these traits. The results of this research suggests that environmental conditions significantly affected these secondary traits among the switchgrass cultivars tested which may imply that selecting individual genotypes based only on plant height and/or tiller density in one environment may not result in improved switchgrass plants ready to be utilized in marginal soils or other locations.

Various research studies in other crops, such as, barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and maize (*Zea mays* L.) have suggested that conducting breeding research programs in environments that differ greatly from the target environments are not the most efficient method to obtain significant genetic improvement. Switchgrass genotypes are expected to perform well in marginal or low productivity environments; however previous research has not looked at the effect of poor soil quality on the agronomic performance of switchgrass cultivars. The agronomic traits measured in this study seemed to be largely affected by soil conditions and interaction between soil and environmental conditions (location, soil

quality and nitrogen treatment). Soil quality greatly affected plant height. All cultivars grew shorter in marginal soils and they were not ranked the same as in prime vs. marginal soil. For traits such as stand establishment and biomass yield, approximately 50% of the cultivars showed differences related to the soil quality. For tiller density, only 40 % of the cultivars presented differences due to the quality of the soil; however some cultivars had higher tiller density in marginal soils than in prime soils. According to the ANOVA location and the interaction of location x soil quality accounted for the largest portion of variation in stand establishment and tiller density while soil quality and nitrogen treatment were the most influential factors in plant height and dry biomass yield, respectively. Genotype was a statistically significant source of variation for all traits; however these results indicate that the soil and environment may play an important part in the performance of some switchgrass cultivars.

Over all these results indicate that some cultivars are more influenced by environmental conditions than other cultivars. These results indicate that direct selection in marginal land may be necessary for some cultivars; but it also suggests that for cultivars less affected by the environment, selection may be possible on prime farm land. Nevertheless it is important to individually evaluate sources of germplasm in multi-environment trials to determine the extent of the influence of the environment as well as to identify specific factors that may be the most important so that breeders can maximize selection and growers can maximize productivity on poor quality soil.

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**CHAPTER 2: A Comparative Study of the Agronomic Performance of
Switchgrass Clones Selected from Different Environments in Marginal vs. Prime
Farmland in New Jersey and South Dakota**

Abstract

Switchgrass (*Panicum virgatum* L.) has several desirable attributes for biomass production: it has the potential for high biomass yield with low agrochemical input compared to other herbaceous crops and it has great adaptability to different environments. However genotype x environment interaction has been identified as an influential factor in the variability of switchgrass biomass yield. Currently the majority of the selection and research of switchgrass genotypes used for biomass production on low productivity environments has been conducted in Universities' research farms with prime farmland. This research study sought to determine the effects of marginal land on the performance of 45 clones selected for high biomass potential from two-year-old breeding nurseries of switchgrass improvement programs in New Jersey (NJ), South Dakota (SD), and Wisconsin (WI). The clonal material was planted 2009 in two locations (New Jersey and South Dakota) in paired fields (marginal vs. prime land). Each paired location also had a nitrogen treatment of 0 or 100 kg of nitrogen·ha⁻¹·year⁻¹. In the year after establishment the data collected included heading and anthesis date (Julian date); plant height (cm); tiller density (tillers·plant⁻¹) and dry biomass yield (kg·plant⁻¹). In addition to these measurements, visual ratings for disease presence were recorded. In NJ, anthracnose (caused by *Colletotrichum nativas*) severity was assessed while in SD, rust (*Puccinia emaculata*) symptoms were evident during the growing season. The results of this study were

consistent with previous research suggesting that delay in flower initiation may favor higher biomass production in switchgrass. Genotypes 5300-4NJ and 5312-2NJ yielded the most biomass (1.6 and 1.52 kg·plant⁻¹, respectively). Additionally it was observed that soil quality may influence a delay of the reproductive cycle and in secondary agronomic traits such as plant height and tiller density in switchgrass. Overall NJ clones performed the best but were very variable while SD and WI clones showed broad adaptation across location. In this study it was also observed that genotype may have been the most influential factor in switchgrass resistance to anthracnose (*Colletotrichum nativas*) and rust (*Puccinia emaculata*). Genotypes x environment interactions effects were present according to the analysis of variance, but they may have been marginally influential on the response of the germplasm to the diseases.

Introduction

The economic value of switchgrass (*Panicum virgatum*), in soil conservation, as a forage crop and currently as a bioenergy crop has long been recognized (Porter, 1966; Parrish and Fike, 2005). Research efforts are emphasizing improvement of switchgrass biomass yield because this species has the potential for high biomass production with low agrochemical inputs compared to other herbaceous crops (Moser and Vogel, 1995; Parrish and Fike, 2005). As a species, it has broad adaptability and tolerance to unfavorable edaphic factors (Sanderson et al., 1996; McLaughlin et al., 1999). These characteristics make switchgrass suitable for bioenergy production on low productivity environments or marginal land; however there has been little to no extensive research to evaluate its performance on marginal land. To develop an efficient breeding program for increasing switchgrass biomass yield on marginal land, more information is needed on the variation in performance of the available germplasm, which may include the total amount of phenotypic variation, the proportion due to genetic differences, the proportion due to distinct environmental influences (e.i. soil, climate, etc.) and the effect of genotype by environment interactions (Newell and Eberhart, 1961; Porter, 1966; Simmond 1991).

Biomass yield is considered a complex trait regulated by many genes and influenced by the environment (Sleper and Poehlman, 2006). Falconer (1989) suggested that yield in low productivity environments and high productivity environments may be considered as separate traits which may not be regulated by the same sets of alleles. The interaction of the genotype with the environment may result

in different biomass yields depending on the environmental conditions (Falconer, 1989; Sleper and Poehlman, 2006).

It has been well documented that phenotypic variation among switchgrass populations may occur at regional, landscape and neighborhood levels in the northern USA (Casler, 2005). Similarly, switchgrass genotypes from different ecotypes and morphological type combinations differed in performance across the southern US (Cassida et al., 2005). Extensive studies have been conducted to demonstrate that switchgrass cultivars vary widely in their adaptation zones (Casler, 2005; Casler et al., 2004; Casler and Boe, 2003; Cassida et al., 2005; Fuentes and Taliaferro, 2002). Scientists have learned that some cultivars of switchgrass cannot survive and/or have low biomass yields if taken too far from their latitude-of-origin (Casler et al., 2004; Casler and Boe, 2003).

Furthermore there are two different ecotypes of switchgrass, upland and lowland, which have different growth habits and are adapted to different types of habitats (Cornelius and Johnston, 1941; Eberhart and Newell, 1959; Porter, 1966). Upland types are typically octaploids (Hopkins et al., 1996) have shorter, finer stems and are better adapted to drier habitats (Cornelius and Johnston, 1941; Eberhart and Newell, 1959; Porter, 1966; Lewandowski et al., 2003). Upland types are also typically earlier maturing than lowland types (Benedict, 1940; Cornelius and Johnston, 1941; Eberhart and Newell, 1959; Porter, 1966). Lowland types are generally tetraploid (Hopkins et al., 1996), taller and more robust than upland types. They have coarser stems, a more bunch type growth habit and are adapted to wetter sites (Cornelius and Johnston, 1941; Eberhart and Newell, 1959; Porter, 1966;

Lewandowski et al., 2003). They typically mature later than upland types and require a longer growing period (Cornelius and Johnston, 1941; Porter, 1966).

Additionally, day length is one factor that partially controls the adaptation of individual plants or populations of switchgrass to certain environments (Casler et al., 2004). This control is such that most populations when moved north or south from their latitude-of-origin may be affected in their vigor, survival and flowering (Benedict, 1940; Cornelius and Johnston, 1941; Porter, 1966; Casler et al., 2004). In addition to day length, climate, habitat, soil or slope may increase the potential for additional phenotypic variation (Casler, 2005).

The two types have also been found to have different biomass yields in different environments (Sanderson et al., 1999). Upland cultivars are better adapted to mid-northern latitudes and lowland types are better adapted to lower latitudes (Casler et al., 2004; Parrish and Fike, 2005). Comparisons of agronomic performance among switchgrass cultivars selected in high yielding environments suggests that the most promising cultivars for biomass production are ‘Alamo’ for the deep South, ‘Kanlow’ for mid-latitudes and ‘Cave-in-Rock’ for the central northern states (Lewandowski et al., 2003). However it is possible that if switchgrass genotypes are taken off deep, nutrient-rich prairie soils and grown on marginal soils, these genotypes may have inferior agronomic performance. It is also expected there may be a decline in biomass yield and/or survival on marginal soils. It would be important to determine if the decline is the same for all genotypes or if some genotypes are more/less broadly adapted to a range of soil conditions and if new cultivars should be

selected in environments with similar growing conditions than the target environments where they are going to be cultivated.

There are several research studies that suggest that indirect selection is not as efficient as selection under appropriate types of stress and non-stress conditions similar to the target environment (Bänziger et al., 1997; Bänziger et al., 1999; Atlin and Frey, 1990). Direct selection for increased yield and for secondary traits, such as, plant height, tiller density, etc., has been shown to be extremely beneficial to improve tolerance to unfavorable environmental conditions and increase yield in maize (*Zea mays* L.) (Arboleda-Rivera and Compton, 1974; Bänziger et al., 1997; Bänziger et al., 1999; Muruli and Paulsen, 1981); oats (*Avena sativa* L.) (Atlin and Frey, 1990); sorghum (*Sorghum bicolor* L.) (Igartua et al., 1996; Zavala-Garcia et al., 1992) and rice (*Oriza sativa* L.) (Guan et al., 2010). Additionally the presence of high genotype-by-environment (GE) interaction may complicate breeding efforts because the performance of the genotype will vary in different environments, decreasing the accuracy of the prediction (Ceccarelli, 1989). Because previous breeding work on switchgrass has not compared its performance under unfavorable soil or environmental conditions, there is a lack of information regarding the adaptation of switchgrass to low productivity environments. This study aims to address the lack of knowledge in this area to develop more effective breeding programs to improve switchgrass biomass yield in marginal or low productivity environments.

There are several fungal pathogens that have been shown to cause disease on switchgrass and with the increase in cultivation of this species as a monoculture for biomass production; disease pressure may also increase (Crouch et al., 2008; Parrish

and Fike, 2005). Rust fungi: *Uromyces graminicola*, *Puccinia graminis* and *P. emaculata* have been identified as pathogens in switchgrass, as well as *Colletotrichum* spp. that cause anthracnose (Cornelius and Johnston, 1941; Crouch et al., 2009; Gravert and Munkvold, 2002). However it has been suggested that wide distribution of this species in the North American prairies may have exposed switchgrass to an array of native pathogens, thus potentially creating a genetically diverse pool of individuals that may be selected to breed resistance to diseases (Mitchell et al., 2008).

Anthracnose incidence in switchgrass has been reported in several states such as Iowa, New York and Pennsylvania (Bergstrom and Waxman, 2011; Gravert and Munkvold, 2002; Sanderson, 2008). Fungi in the genus *Colletotrichum* are thought to cause the disease in switchgrass but the identity of the species was not certain. Crouch et al. (2009) showed molecular and morphological evidence that a novel species, *Colletotrichum nativas*, was the causal organism of anthracnose in switchgrass. There are several studies in other crops suggesting that inheritance of resistance to this disease may be gene-for-gene type: in creeping bentgrass (*Agrostis palustris*) for resistance to anthracnose caused by *Colletotrichum cereal* (Bonos et al., 2009); in corn (*Zea mays*), caused by *Colletotrichum graminicola* (Toman and White, 1993) and in sorghum (*Sorghum bicolor*) caused by *Colletotrichum sublineolum* (Da Costa et al., 2011).

Rust incidence in switchgrass has been observed in Iowa, Kansas and South Dakota (Cornelius and Johnston, 1941; Gravert and Munkvold, 2002; Mankin, 1969). Several studies have determine that lowland and/or southern switchgrass types are resistant to rust (*U. graminicola*); on the other hand genotypes characterized as upland

and/or northern are highly susceptible to the disease (Barnett and Carver, 1967; Cornelius and Johnston, 1941; Eberhart and Newell, 1959). It also has been suggested that there is great potential for selecting rust resistant genotypes in switchgrass because high heritability estimates and significant additive and non-additive genetic variation has been observed in switchgrass (Eberhart and Newell, 1959; Gustafson et al., 2003; Newell and Eberhart, 1961). However it is important to conduct research to determine if environmental or soil conditions are influential in selection for rust resistance and to test the hypothesis of gene-for-gene resistance to anthracnose in switchgrass.

Materials and Methods

Plant Material and Soil Type

Fifteen clones were selected for high biomass yield potential from two year old breeding nurseries from switchgrass improvement programs in New Jersey (NJ), South Dakota (SD), and Wisconsin (WI). Upland ecotypes were provided by both SD and WI breeding programs while NJ provided both upland (U) and lowland (L) ecotypes (Table 1). Each breeding program was responsible for increasing the plant material from their respective switchgrass clones for the two locations. Tillers were planted in 5 gallon containers with Pro-Mix HP (K.C. Shafer, York, PA) and grown in a greenhouse until late spring in 2009.

The study was conducted in paired fields (marginal vs. prime farmland) in 2 states: New Jersey (NJ) and South Dakota (SD). The soil types are: 1) for the marginal site at Somerset, NJ a Class IV with 0-2% slope, Kleinsville shale (limiting factor nutrient deficiency and poor water-holding capacity) and for the prime site at

the Rutgers Plant Science Research and Extension Farm in Adelphia, NJ a Class II, Freehold sandy loam with 2-5% slope; 2) for the marginal site at Pierre, SD a Class VI, Opal-Lakoma clay with 9-15% slope (liming factor shallow over shale) and for the prime site at Brookings, SD a Class II, Lowry silt loam. Each paired location also had a nitrogen treatment of 0 or 100 kg of nitrogen (N) ha⁻¹ year⁻¹. Urea (46-0-0) or ammonium nitrate (33-0-0) was used as N source and the fertilizer was applied during the spring of the second year after establishment. Plants were transplanted to the field into rows, 0.91 m between plants and 0.91 m between rows; arranged in a randomized complete block design with three replications in the spring of 2009.

Table 1. List of 45 switchgrass clones from breeding programs in New Jersey (NJ), South Dakota (SD), and Wisconsin (WI), evaluated in marginal vs. prime farmland in two U.S. states (NJ and SD). Switchgrass ecotypes evaluated: lowland (L) and upland (U).

NJ Clones	Type	SD Clones	Type	WI Clones	Type
5215-6NJ	L	SD1	U	WS08-K1	U
5300-1NJ	U	SD2	U	WS08-K2	U
5300-4NJ	L	SD5	U	WS08-K3	U
5300-8NJ	L	SD6	U	WS08-K4	U
5300-12NJ	L	SD7	U	WS08-K5	U
5302-1NJ	U	SD8	U	WS08-R1	U
5305-1NJ	U	SD9	U	WS08-R2	U
5305-7NJ	U	SD10	U	WS08-R3	U
5305-10NJ	U	SD11	U	WS08-R4	U
5305-12NJ	U	SD12	U	WS08-R5	U
5312-2 NJ	L	SD13	U	WS08-U1	U
9081-12NJ	U	SD14	U	WS08-U2	U
9100-11NJ	L	SD16	U	WS08-U3	U
9137-11NJ	U	SD18	U	WS08-U4	U
9145-11NJ	L	SD19	U	WS08-U5	U

Data Collection

In this research study, secondary agronomic traits, such as, heading date, anthesis date, plant height and tiller density, were collected, in addition to dry biomass yield per plant, to determine the performance of the 45 switchgrass clones. Heading date was recorded when more than 50% of tillers showed signs of panicle development. Anthesis date was recorded as the date of flower opening when the anthers were visible. Heading and anthesis dates were reported only in NJ. Plant height was measured at maturity by placing a measuring stick at the base of the plant and recording the height (cm) to the average panicle. Tiller density was measured at maturity and it was recorded as the number of tillers per plant. In addition to these measurements, visual ratings for disease presence were taken. In NJ, anthracnose (caused by *Colletotrichum nativas*) severity was assessed during the growing season. The disease symptoms observed were elongated leaf lesions with tan colored, sharply tapered ends and reddish brown borders (Crouch et al., 2009). The assessment was conducted in mid-August, a 1 to 9 (9 = least disease) visual rating scale was utilized (Bonos et al., 2009). In SD, rust (*Puccinia emaculata*) symptoms were evident during the growing season, a 0 (0 = least disease) to 9 visual rating scale was utilized (Gustafson et al., 2003).

Dry biomass yield was determined by harvesting the entire switchgrass plant after senescence. The larger plants were tied with twine and tagged with an identification number (Fig. 1). The plants were cut at 30 cm from the soil surface with a hedge trimmer (KHT600, Kawasaki Motor Corp., Irvine, CA) (Fig. 2). Smaller plants were harvested with hand trimmers and placed in paper bags (S-9621) (U-line,

U.S.-22.68kg). All field samples were placed in a tobacco dryer (DeCloet Bulk Curing Systems, Tillonsburg, Canada) for 10 days at 38° C (Fig. 3). After the samples were dried, the weight was recorded, and the weights of the twine and the tag, for the big plants, or the weight of the dry paper bag, for the small plants, were subtracted. The adjusted dry weight was used for the statistical analysis.



Figure 1. Ready for harvest large plant, tied with twine and tagged with an identification number.



Figure 2. Plants cut at 30 cm from the soil surface with a hedge trimmer (KHT600, Kawasaki Motor Corp., Irvine, CA)



Figure 3. Field samples were placed in a tobacco dryer (DeCloet Bulk Curing Systems, Tillonsburg, Canada) for 10 days at 38° C.

Data Analysis

The experiment was arranged in a split block plot design. The locations, sites and the replications within sites were the blocks while the nitrogen treatment was the main plot and the clones were the split plot; however only the replications were considered random effects. The analysis of variance was performed with the MIXED procedure in SAS (SAS Institute, Cary, NC). The means were separated with the LSMEANS option and adjusted with Tukey's method for multiple comparisons. The PDMIX800 macro was utilized to convert mean separation output to letter groupings in the MIXED procedure (Saxton, 2003).

Several upland genotypes from the WI breeding program (WS08K1, WS08K3, WS08K4, WS08K5, WS08R2, WS08R3, WS08R4 and WS08R5) and one upland genotype from the NJ breeding program (9137-11NJ) did not survive in SD and two entries from WI (WS08R1 and WS08U2) did not survive in NJ after planting likely due to environmental and/or soil conditions. This lack of survival resulted in missing values for the agronomic variables measured in this study. Because of the number of variables in the model and the amount of missing values for these previously mentioned genotypes the LSMEAN option was not able to separate the means for the different responses and the model was not informative. Therefore the genotypes that did not survive were eliminated from the dataset: genotype WS08-R1 for heading and anthesis date analysis; genotypes WS08-R1 and WS08-U2 for anthracnose disease analysis; genotypes WS08K1, WS08K3, WS08K4, WS08K5, WS08R2, WS08R3, WS08R4, WS08R5 and 9137-11NJ for rust disease analysis, and WS08K1, WS08K3, WS08K4, WS08K5, WS08R1, WS08R2, WS08R3, WS08R4,

WS08R5 and 9137-11NJ for the plant height, tiller density and dry biomass yield analysis. This modification allowed for the appropriate statistics to be used for the comparison between available genotypes and environments.

Results and Discussion

Heading and Anthesis Date

The results of this study suggested that heading and anthesis date were correlated and similarly affected by genetic and environmental factors. This is supported by previous studies which have showed the correlation between these two traits (Table 2 and 3) (Casler et al., 2007; Cornelius and Johnston, 1941; McMillan, 1965). Soil quality had the greatest influence on heading and anthesis dates among switchgrass clones (Table 2 and Table 3). Genotype (clones) and the soil quality x genotype interactions also significantly influenced heading and anthesis dates (Table 2 and Table 3). Nitrogen treatment was not a significant ($P < 0.05$) source of variation and neither were the interaction factors that included this term (Table 2). Switchgrass has been identified as a short day species (Benedict, 1940). Previous research indicates that heading and anthesis date may be a function of ecotype, resulting in lowland types remaining vegetative longer in northern latitude, growing taller than upland types (Casler et al., 2004; Casler et al., 2007; McMillan, 1965, McMill and Weiler, 1959). Nevertheless the results of this study suggest that soil quality may also be an important determinant of flowering time in switchgrass.

The upland clones from the SD breeding program were the earliest to flower (mature) with some entries initiating flowering around June 4 of 2009 (Julian day-155) and reaching anthesis by mid- to end of June (160-181) (Table 4 and 5).

However, the differences in heading and anthesis date among SD clones were not statistically significant ($P < 0.05$) (Table 4 and 5). The upland germplasm from WI initiated flowering from mid-June to early-July, reaching anthesis by the end of July (Table 4 and 5). There were no significant differences ($P < 0.05$) among the WI genotypes for heading and anthesis dates (Table 4 and 5). The earliest NJ clones initiated flowering in mid-July while the latest clones did not flower until early-October (Table 4). This wide range is not surprising because both upland and lowland ecotypes were represented among the NJ clones (Casler, 2005; Casler et al 2004; McMillan, 1965). Consistently, lowland ecotypes reached anthesis later than upland ecotypes (Table 5). The upland NJ clones were not significantly different ($P < 0.05$) in reaching maturity from the SD and WI clones and most of the lowland NJ clones had flowering dates that were not significantly different ($P < 0.05$) than three late maturing upland entries from WI (WI08-K4, WI08-K5 and WI08-R3) (Table 4 and 5). Clone 9100-11NJ was the latest maturing entry in this study (Table 4 and 5). The study was conducted in northern regions of the U.S. and these results are consistent with previous research suggesting that lowland types have longer vegetative cycles in northern latitudes, which can be advantageous because lowlands can grow larger; however it can also be detrimental if vegetative cycle continues throughout the colder months in this region (Casler et al., 2007; Madazake et al., 1998; McMillan, 1965; Porter, 1966).

Switchgrass has been described as a short-day species, it blooms when exposed to shortening days of a specific length, which may explain the early maturity of the upland genotypes selected in SD and WI compare to the lowland genotypes from NJ (Benedict 1940; Cornelius and Johnston 1941). On average the plant material

from SD started the reproductive cycle approximately 80 days earlier than the genotypes from NJ, while the genotypes from WI were 60 days earlier. This hasten transition to reproductive development may have reduced the vegetative growth and biomass yield of this northern germplasm (Casler et al., 2004; Casler et al., 2007).

The ANOVA suggested that soil quality was very influential on flowering in switchgrass. Comparisons of heading and anthesis dates in prime vs. marginal soil suggested that flower development was significantly delayed in the latter only for the NJ clones (Table 6 and 7). In fact, several entries from the NJ breeding program did not flower at all at the marginal sites (Table 4). All of the NJ lowland clones and several of the NJ upland clones had significantly different flowering times in prime vs. marginal soil however, none of the upland clones selected from northern latitudes in WI and SD were affected by soil quality and they flowered at similar times in both environments.

Even though switchgrass has been identified as a short day species by Benedict (1940), Van Esbroeck et al. (2003) suggested that native North American grasses, with a wide geographical distribution (i.e. switchgrass), may vary widely in their response to photoperiod. Switchgrass photoperiodicity is still being researched because it is not clear if reproductive development is a true response to day length or is it dependent on other environmental conditions (Parrish and Fike, 2005). Balasko and Smith (1971) observed delay in flowering when switchgrass was grown under a 21/15°C (day/night) regime and was completely inhibited at a 15/10°C (day/night) regime. Porter (1966) reported that upland cultivars presented a delay in anthesis when grown under soil conditions that sustained excess water because they are

adapted to drier soil conditions. Additionally it has been observed that in other short day species, such as maize, nitrogen and water deficiencies in the soil may affect the initiation of the reproductive cycle (Edmeades et al., 1995; Jacobs and Pearson, 1991). Overall the results of this study suggest that soil conditions may be an important part of the variation in switchgrass reproductive development. Furthermore, genotype x environment interactions may affect heading and anthesis dates in switchgrass indicating that selection for these traits may benefit from including environments with marginal soils in the selection process. It also indicates that selection environment may influence responses in other environments.

Table 2. Analysis of variance of heading date of 45 switchgrass clones from three breeding programs (NJ, SD and WI) evaluated in NJ in marginal vs. prime farmland with 0 or 100 kg of N·ha⁻¹.

Source	Df	F-value	Pr > F
SQ (Soil Quality)	1	50.5	0.0021
N (Nitrogen)	1	3.15	0.1507
SQ*N	1	3.69	0.1270
Clone (Genotype)	43	14.2	<.0001
SQ*Clone	43	6.59	<.0001
N*Clone	43	1.19	0.2343
SQ*N*Clone	43	1.12	0.3105

Table 3. Analysis of variance of anthesis date of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated in NJ in marginal vs. prime farmland with 0 or 100 kg of N·ha⁻¹.

Source	Df	F-value	Pr > F
SQ (Soil Quality)	1	44.02	0.0027
N (Nitrogen)	1	2.07	0.2235
SQ*N	1	3.63	0.1294
Clone (Genotype)	43	19.92	<.0001
SQ*Clone	43	5.17	<.0001
N*Clone	43	1.46	0.0568
SQ*N*Clone	43	1.23	0.187

Table 4. Means of heading date separated by genotype for 45 switchgrass clones evaluated in marginal vs. prime farmland sites in NJ. Means were separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entry WS08-R1.

		Mean Heading Date				Mean Heading Date		Mean Heading Date	
NJ Clones	Type	(day)	SD Clones	Type	(day)	WI Clones	Type	(day)	
9100-11 NJ	L	283 A ¹	SD7	U	176 GH	WS08-K5	U	210 BCDEFGH	
5300-8 NJ	L	281 AB	SD12	U	175 GH	WS08-R4	U	185 FGH	
5300-12 NJ	L	273 AB	SD8	U	168 H	WS08-K4	U	183 CDEFGH	
5300-4 NJ	L	268 AB	SD11	U	162 H	WS08-R3	U	182 DEFGH	
5215-6 NJ	L	263 ABC	SD16	U	159 H	WS08-R5	U	178 FGH	
9145-11 NJ	L	259 ABCD	SD10	U	159 H	WS08-K2	U	175 FGH	
5312-2 NJ	L	257 ABCD	SD2	U	157 H	WS08-U2	U	174 FGH	
5305-1 NJ	U	254 ABCDE	SD6	U	157 H	WS08-U3	U	174 FGH	
5300-1 NJ	U	239 ABCDEF	SD18	U	157 H	WS08-K3	U	174 FGH	
9137-11 NJ	U	235 ABCDEFG	SD14	U	156 H	WS08-K1	U	172 FGH	
5305-12 NJ	U	234 ABCDEFG	SD13	U	156 H	WS08-U1	U	170 H	
5305-7 NJ	U	231 ABCDEFG	SD5	U	156 H	WS08-U4	U	170 EFGH	
9081-12 NJ	U	203 CDEFGH	SD9	U	155 H	WS08-U5	U	169 FGH	
5302-1 NJ	U	196 EFGH	SD1	U	155 H	WS08-R2	U	167 H	
5305-10 NJ	U	193 FGH	SD19	U	155 H	WS08-R1	U	-	

¹ Cultivar means with the same upper case letter were not statistically different at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Table 5. Means of anthesis date separated by genotype for 45 switchgrass clones evaluated in marginal vs. prime farmland sites in NJ. Means separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entry WS08-R1.

Mean Anthesis Date			Mean Anthesis Date			Mean Anthesis Date		
NJ Clones	Type	(day)	SD Clones	Type	(day)	WI Clones	Type	(day)
9100-11NJ	L	300 A ¹	SD7	U	181 KLMN	WS08-K5	U	229 BCDEFGHIJKL
5300-8NJ	L	297 A	SD11	U	176 KLMN	WS08-K4	U	212 CDEFGHIJKLMN
5300-12NJ	L	289 AB	SD12	U	168 KLMN	WS08-R3	U	208 DEFGHIJKLMN
5300-4NJ	L	287 ABC	SD8	U	165 LMN	WS08-R4	U	207 GHIJKLMN
5215-6NJ	L	283 ABCD	SD14	U	164 KLMN	WS08-U2	U	200 GHIJKLMN
9145-11NJ	L	278 ABCDE	SD9	U	163 KLMN	WS08-R5	U	199 HIJKLMN
5312-2NJ	L	276 ABCDE	SD10	U	163 MN	WS08-U5	U	192 GHIJKLMN
5305-1NJ	U	272 ABCDEF	SD16	U	162 MN	WS08-U3	U	190 IJKLMN
5300-1NJ	U	261 ABCDEFG	SD13	U	161 MN	WS08-K2	U	189 JKLMN
9137-11NJ	U	253 ABCDEFGH	SD2	U	161 MN	WS08-U1	U	186 KLMN
5305-12NJ	U	251 ABCDEFGHI	SD5	U	161 LMN	WS08-U4	U	185 GHIJKLMN
5305-7NJ	U	247 ABCDEFGHIJ	SD18	U	161 MN	WS08-K3	U	184 HIJKLMN
9081-12NJ	U	222 EFGHIJK	SD6	U	161 MN	WS08-K1	U	182 HIJKLMN
5302-1NJ	U	217 FGHIJKLM	SD1	U	160 MN	WS08-R2	U	181 KLMN
5305-10NJ	U	214 GHIJKLMN	SD19	U	160 N	WS08-R1	U	-

¹ Cultivar means with the same upper case letter were not statistically different at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Table 6. Heading date means of 45 switchgrass genotypes in marginal vs. prime farmland sites evaluated in NJ. Statistical analysis was conducted without entry WS08-R1.

NJ Clones [§]	Type	Heading Date (day)			SD Clones	Type	Heading Date (day)			WI Clones	Type	Heading Date (day)		
		Prime	Marginal				Prime	Marginal				Prime	Marginal	
5215-6NJ	L	198	365	*	SD1	U	154	156	NS	WS08-K1	U	171	172	NS
5300-1NJ	U	189	289	*	SD2	U	156	158	NS	WS08-K2	U	167	182	NS
5300-4NJ	L	197	365	*	SD5	U	155	157	NS	WS08-K3	U	173	175	NS
5300-8NJ	L	196	365	*	SD6	U	156	157	NS	WS08-K4	U	180	187	NS
5300-12NJ	L	204	365	*	SD7	U	195	158	NS	WS08-K5	U	179	241	NS
5302-1NJ	U	175	216	NS	SD8	U	163	174	NS	WS08-R1	U	-	-	
5305-1NJ	U	184	365	*	SD9	U	155	156	NS	WS08-R2	U	164	171	NS
5305-7NJ	U	178	284	*	SD10	U	158	159	NS	WS08-R3	U	173	191	NS
5305-10NJ	U	173	213	NS	SD11	U	160	164	NS	WS08-R4	U	175	195	NS
5305-12NJ	U	180	288	*	SD12	U	158	193	NS	WS08-R5	U	171	185	NS
5312-2NJ	L	191	365	*	SD13	U	155	158	NS	WS08-U1	U	170	170	NS
9081-12NJ	U	179	228	NS	SD14	U	156	156	NS	WS08-U2	U	180	169	NS
9100-11NJ	L	202	365	*	SD16	U	159	159	NS	WS08-U3	U	171	177	NS
9137-11NJ	U	184	286	*	SD18	U	157	157	NS	WS08-U4	U	170	170	NS
9145-11NJ	L	199	365	*	SD19	U	154	156	NS	WS08-U5	U	163	174	NS

Heading date means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

[§]NJ clones with 365 for heading date did not produce flower.

(-) Means were not calculated due to missing values.

Table 7. Anthesis date means of 45 switchgrass genotypes in marginal vs. prime farmland sites evaluated in NJ. Statistical analysis was conducted without entry WS08-R1.

NJ Clones [§]	Type	Anthesis Date (day)			SD Clones	Type	Anthesis Date (day)			WI Clones	Type	Anthesis Date (day)		
		Prime	Marginal				Prime	Marginal				Prime	Marginal	
5215-6NJ	L	229	365	*	SD1	U	161	160	NS	WS08-K1	U	171	172	NS
5300-1NJ	U	221	302	NS	SD2	U	162	161	NS	WS08-K2	U	167	182	NS
5300-4NJ	L	229	365	*	SD5	U	161	161	NS	WS08-K3	U	173	175	NS
5300-8NJ	L	230	365	*	SD6	U	161	160	NS	WS08-K4	U	180	187	NS
5300-12NJ	L	232	365	*	SD7	U	200	161	NS	WS08-K5	U	179	241	NS
5302-1NJ	U	199	235	NS	SD8	U	154	176	NS	WS08-R1	U	-	-	NS
5305-1NJ	U	213	365	*	SD9	U	166	160	NS	WS08-R2	U	164	171	NS
5305-7NJ	U	197	297	*	SD10	U	164	162	NS	WS08-R3	U	173	191	NS
5305-10NJ	U	202	227	NS	SD11	U	177	175	NS	WS08-R4	U	175	195	NS
5305-12NJ	U	202	300	*	SD12	U	166	171	NS	WS08-R5	U	171	185	NS
5312-2NJ	L	224	365	*	SD13	U	161	162	NS	WS08-U1	U	170	170	NS
9081-12NJ	U	202	243	NS	SD14	U	165	162	NS	WS08-U2	U	180	169	NS
9100-11NJ	L	237	365	*	SD16	U	162	161	NS	WS08-U3	U	171	177	NS
9137-11NJ	U	207	299	*	SD18	U	162	160	NS	WS08-U4	U	170	170	NS
9145-11NJ	L	231	365	*	SD19	U	160	160	NS	WS08-U5	U	163	174	NS

Anthesis date means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

[§]NJ clones with 365 for anthesis date did not produce flower.

(-) Means were not calculated due to missing values.

Plant Height

The variation in plant height among the clones from the different breeding programs was mainly influenced by the soil quality and the interaction of location x soil quality (Table 8). The genotype and the interaction factors that included genotype, soil quality and location were also influential (Table 8). The nitrogen treatment was not statistically significant ($P < 0.05$) according to the analysis nor were the interactions that contained this term except for the nitrogen treatment x genotype and nitrogen treatment x genotype x location interaction (Table 8). The importance of genotype or ecotype in the variation of plant height is well documented (Casler et al., 2007; Cornelius and Johnston, 1941; McMillan, 1965); however these results suggest the growing conditions (soil and environmental) may also play a large role in plant height variation (Porter, 1966).

Over all, the clonal material that came from the SD breeding program produced the shortest plants in this study, ranging from 86 to 116 cm (Table 9). This is not surprising because all of the SD clones were upland ecotypes, which tend to grow shorter than lowlands (Casler et al., 2007, McMillan, 1965). The clones from the WI breeding program were also upland types yet these plants grew significantly taller than the SD clones except for 'WS08-R1' and plant heights ranged from 123 to 142 cm (Table 9). The clonal material from NJ produced the tallest plants, height ranged from 127 to 175 cm (Table 9). However, the height differences were not statistically significant ($P < 0.05$) between WI clones and all the NJ upland clones as well as five NJ lowland clones (Table 9). The tallest clones were lowland clones 5215-6NJ and 5300-4NJ with 236 and 232 cm, respectively (Table 9). Genotype or

ecotype is known to strongly influence plant height, i.e. lowland ecotypes typically grow taller than upland ecotypes, this trend was observed for the SD upland clones vs. the NJ lowland clones (Casler et al., 2004; Casler et al., 2007). However the WI upland, NJ upland and lowland clones were not clearly distinguished by height which may suggest that selection for tall upland ecotypes that are similar to lowland types is possible. Additionally, there may be other factors, beside genotype or ecotype, affecting plant height, such as, soil quality, rainfall or temperatures.

The interaction between location and soil quality was a significant source of variation in plant height according to the analysis of variance (Table 8). Five of the NJ clones and one SD clone showed significant variation between locations (Table 10). NJ lowland entries 5300-12NJ and 5312-2 NJ were taller in New Jersey compare to South Dakota (170 and 168 cm vs. 125 and 135 cm, respectively) while NJ upland entries 5300-1NJ, 5305-12NJ and 9081-12NJ were taller in South Dakota compared to New Jersey (188, 189 and 167 cm vs. 133, 135 and 127 cm, respectively) (Table 10). SD14, also an upland ecotype, was taller in South Dakota compared to New Jersey (106 vs. 70 cm) (Table 10). The other clones showed no difference between locations. Porter (1966) conducted reciprocal transplanting experiments where upland switchgrasses were grown in lowland environment and vice versa. The results showed that upland switchgrass did not grow well in a lowland location where soils held too much water and lowland types became reduced in size in the drier upland location, but lowlands grew larger in both environments. Additionally it has been observed that lowland genotypes may take advantage of longer summer days in northern latitudes and grow more than uplands (Benedict 1940; Casler et al., 2004; Casler et al., 2007; Cornelius and Johnston 1941). However these genotypes are also more sensitive to

low temperatures than uplands and less likely to survive if vegetative growth extended too long into the colder months of northern regions like South Dakota due to winter kill (Casler et al., 2004; Madakadze et al., 1998)

Significant differences ($P < 0.05$) between prime and marginal soil type were observed for all NJ clones regardless of ecotype (lowland vs. upland), with prime sites always producing taller plants than marginal sites (on average 177 cm vs 127 cm, respectively) (Table 9 and 11). Conversely, only two WI clones (WS08-K2 and WS08-U3) and one SD clone (SD8) showed significant differences in plant height due to soil quality with prime sites producing taller plants than marginal sites (Table 11). These results are similar to those observed for anthesis and flowering time. These results are interesting because it indicates that for NJ genotypes and some SD and WI genotypes selection environment may influence adaptation in different soils. Atlin and Frey (1990) concluded that selection in multi-environments trials were more efficient in determining superior genotypes when the target environments have variable growing conditions (rainfall, temperature, soil quality, etc). Bänziger et al. (1999) reported that direct selection for tolerance to drought stress and N deficiency increase grain yield in maize (*Zea mays* L.) populations of different genetic background when tested in environments with marginal growing conditions. However most of the clones from SD and WI breeding programs were not influenced by soil quality. This suggests that there are genotypes which may be selected with broad adaptation across soil types and minimal influences of soil quality in single environment trials. Shabana et al. (1980) selected among two groups of random oat (*Avena sativa* L.) lines under low, medium and high productivity conditions. They reported that the lines selected under high and low productivity conditions produced virtually equal yield advances,

although it slightly favored the high productivity environment and the yield of lines selected under medium productivity conditions were somewhat inferior to those from the other two conditions. Quisenberry et al. (1980) reported significant genetic advances for lint yield in cotton (*Gossypium hirsutum* L.) at different testing locations when the lines were selected in an environment considered optimal for upland cotton growth in Texas. Even though NJ clones tended to have a more variable performance, these plants were taller in marginal soil compared to SD and WI plants. The goal is to develop plants that are taller or produced more tillers or yield more biomass with minimal influence from genotype x environment interactions. This research indicates that switchgrass improvement programs may benefit from conducting selection in multi-environment trials that include marginal sites to combine broad adaptation with superior performing genotypes.

Nitrogen treatment was not a significant ($P < 0.05$) source of variation for plant height according to the ANOVA table (Table 8 and 9). Consistently no significant differences were found when comparing plant heights of the clonal material by nitrogen treatment (data not shown). There is evidence that nitrogen fertilization may affect agronomic performance of switchgrass. Vogel et al. (2002) reported that optimum growth was obtained when switchgrass was fertilized with 120 kg of nitrogen·ha⁻¹ in Nebraska. Mulkey et al. (2006) suggested that in switchgrass-dominated Conservation Reserve Program (CRP) land nitrogen application rate of 56 kg of nitrogen·ha⁻¹ would improve vegetative growth without affecting its persistence in South Dakota. Stroup et al. (2003) observed that nitrogen deficiency was greater than water supply as a limiting factor in the agronomic performance of switchgrass plants. However nitrogen availability in the soil is subject to variation due to

environmental and soil conditions. Craswell and Godwin (1984) observed an increase in soil nitrogen losses with an increase in soil temperature. Annual rainfall has also been deemed an influential factor for nitrogen availability (Sala et al., 1988). Ammonium fixation increases with soil liming and decreases as soil pH decreases (Bohn et al., 1979). Staley et al. (1991) studied the response of switchgrass to increasing rates of nitrogen on soils with different water-holding capacities. They observed that nitrogen uptake was better in plants grown in soils with better water-holding capacity. The results from this study may suggest that more attention has to be paid to the accessibility of roots to the nitrogen applied or to soil factors, such as, pH, soil temperature, etc. that may bind nitrogen to the soil particles and reduce availability.

Table 8. Analysis of variance of plant height (cm) of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated on marginal vs. prime farmland in NJ and SD with 0 or 100 kg of N·ha⁻¹.

Source	Df	F-value	Pr > F
Location (State)	1	0.65	0.4448
SQ (Soil Quality)	1	450.72	<.0001
Loc*SQ	1	320.59	<.0001
N (Nitrogen)	1	0	0.9860
Loc*N	1	0.29	0.6025
SQ*N	1	0.34	0.5775
Loc*SQ*N	1	1.22	0.3017
Clone (Genotype)	44	63.79	<.0001
Loc*Clone	44	10.95	<.0001
SQ*Clone	44	9.98	<.0001
Loc*SQ*Clone	37	11.06	<.0001
N*Clone	44	1.56	0.0199
Loc*N*Clone	39	1.88	0.0024
SQ*N*Clone	44	1.15	0.2525
Loc*SQ*N*Clone	34	1.16	0.2580

Table 9. Plant height means of 45 switchgrass clones from SD, NJ and WI evaluated under marginal and prime soil types in NJ and SD. Mean separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

		Plant Height (cm)									
		NJ				SD					
		Adelphia		Somerset		Brookings		Pierre		Mean	
		Prime		Marginal		Prime		Marginal		Plant Height	
NJ Clones	Type	0	100	0	100	0	100	0	100	(cm)	
5215-6NJ	L	236.33	233.67	120.67	104.00	181.81	182.00	168.05	175.87	175.34	A ¹
5300-4NJ	L	232.33	229.67	112.00	104.00	179.96	188.14	162.58	146.22	169.36	A
9145-11NJ	L	223.33	187.67	121.33	120.00	162.31	181.58	177.34	169.60	167.84	AB
9100-11NJ	L	224.33	216.00	130.45	122.67	160.05	132.47	158.49	162.41	163.40	ABC
5300-8NJ	L	205.00	195.33	93.72	107.00	186.09	192.23	152.71	170.76	162.90	ABC
5305-12NJ	U	188.00	182.33	91.67	79.67	185.07	194.27	191.21	185.07	162.16	ABC
5300-1NJ	U	176.33	175.67	89.67	94.00	202.45	182.00	186.09	182.00	161.03	ABCD
5312-2NJ	L	232.67	220.67	125.00	94.33	128.83	132.92	139.06	142.00	151.93	BCDE
WS08-U4	U	210.72	169.87	139.57	136.06	123.72	140.08	129.86	142.48	148.80	BCDEFGH
5300-12NJ	L	231.33	225.67	103.33	122.00	123.68	133.95	114.09	129.16	147.82	CDEFG
9081-12NJ	U	182.00	173.00	73.67	83.00	167.69	157.46	173.82	170.76	147.68	CDEF
WS08-U5	U	171.85	187.67	121.10	154.06	124.74	133.80	130.07	139.41	143.24	DEFGHI
WS08-U1	U	169.67	113.04	128.67	140.33	129.86	138.04	131.90	131.74	140.96	EFGHI
WS08-U2	U	189.85	165.87	139.57	111.80	130.88	146.07	127.81	127.14	135.68	EFGHI
WS08-U3	U	177.67	168.04	107.83	107.30	119.63	132.27	128.83	137.87	134.63	FGHI
5305-10NJ	U	179.67	172.67	94.67	80.33	122.70	142.13	138.04	138.04	133.53	FGHI

5305-1NJ	U	188.67	183.00	86.00	84.33	117.83	153.74	122.04	129.73	133.14	FGHI
5302-1NJ	U	162.67	147.67	90.67	99.00	120.61	156.44	142.13	135.99	131.87	GHIJ
WS08-R1	U	141.01	142.67	103.57	146.54	116.56	129.86	129.86	130.30	128.35	HIJK
5305-7NJ	U	169.67	172.67	77.33	78.67	117.59	122.70	140.08	137.87	127.09	IJK
SD8	U	132.33	122.67	84.00	85.67	135.99	129.86	126.79	114.52	116.48	JKL
SD11	U	113.33	116.00	104.33	99.33	117.59	118.47	123.72	124.74	114.68	KLM
SD16	U	108.67	107.00	94.22	96.00	139.46	92.02	107.36	101.23	105.71	LMN
SD10	U	106.00	130.33	95.33	100.00	98.70	100.08	89.92	106.34	103.30	LMNO
SD13	U	104.00	111.00	94.33	96.67	111.45	104.29	96.11	104.29	102.77	LMNO
SD19	U	106.33	112.33	97.00	94.67	98.16	104.29	110.43	97.14	102.54	LMNO
SD2	U	106.00	116.67	90.33	92.67	113.50	104.29	95.09	97.14	101.96	LMNO
SD1	U	114.00	109.33	96.67	94.67	92.85	109.47	94.07	103.27	101.85	LMNO
SD6	U	106.33	114.00	89.33	91.00	111.45	103.27	99.18	91.00	100.70	MNO
SD7	U	97.67	115.87	98.67	103.33	94.54	101.82	89.98	79.75	97.64	NO
SD12	U	100.67	120.67	72.67	90.00	109.41	94.07	92.90	99.18	97.45	NO
SD18	U	109.00	110.67	87.33	86.00	105.32	88.96	97.14	95.09	97.44	NO
SD5	U	96.67	121.98	86.72	87.00	77.80	93.90	87.93	81.80	91.87	NO
SD14	U	77.29	102.98	43.45	57.73	116.56	104.29	102.10	103.27	88.44	NO
SD9	U	97.00	98.15	80.95	83.00	86.71	89.32	82.25	73.62	86.37	O
9137-11NJ	U	193.33	184.00	83.67	96.00	134.97	141.68	-	117.85	+	
WS08-K2	U	152.35	161.00	101.33	98.80	120.65	141.47	127.00	123.98	+	
WS08-K1	U	153.72	143.87	90.57	95.80	108.47	-	-	-	+	
WS08-K3	U	174.85	153.87	129.57	104.06	133.01	-	-	-	+	
WS08-K4	U	156.85	124.04	88.57	91.06	108.78	-	115.54	-	+	
WS08-K5	U	140.72	139.00	107.83	82.80	137.77	-	-	-	+	
WS08-R2	U	153.33	168.87	101.33	77.33	104.29	110.43	-	-	+	
WS08-R3	U	153.72	183.87	7.57	81.06	150.04	130.76	-	-	+	
WS08-R4	U	190.35	173.37	128.83	94.30	102.34	-	-	-	+	
WS08-R5	U	174.67	157.33	73.33	121.80	111.85	97.02	-	-	+	
Mean		156.58a²	153.13a	98.93c	99.44c	129.03b	132.05b	126.74b	127.17b		

¹ Cultivar means with the same lower case letter were not statistically different at 0.05 level of probability.

² Location means with the same upper case letter were not statistically different at 0.05 level of probability.

(-) Means were not calculated due to missing values.

+ Cultivars means were not separated by LSMEANS due to missing values.

Table 10. Means of plant height separated by genotype for 45 switchgrass clones evaluated in NJ and SD. Means separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

		Plant Height (cm)					Plant Height (cm)					Plant Height (cm)	
NJ Clones	Type	NJ	SD		SD Clones	Type	NJ	SD		WI Clones	Type	NJ	SD
5215-6NJ	L	173.67	177.02	NS	SD1	U	103.67	100.03	NS	WS08-K1	U	-	-
5300-1NJ	U	133.92	188.14	*	SD2	U	101.42	102.51	NS	WS08-K2	U	128.38	128.32 NS
5300-4NJ	L	169.50	169.22	NS	SD5	U	98.22	85.52	NS	WS08-K3	U	-	-
5300-8NJ	L	150.35	175.46	NS	SD6	U	100.17	101.23	NS	WS08-K4	U	-	-
5300-12NJ	L	170.58	125.05	*	SD7	U	103.84	91.44	NS	WS08-K5	U	-	-
5302-1NJ	U	125.00	138.74	NS	SD8	U	106.17	126.79	NS	WS08-R1	U	-	-
5305-1NJ	U	135.50	130.79	NS	SD9	U	89.74	83.01	NS	WS08-R2	U	-	-
5305-7NJ	U	124.58	129.60	NS	SD10	U	107.92	98.69	NS	WS08-R3	U	-	-
5305-10NJ	U	131.83	135.22	NS	SD11	U	108.25	121.12	NS	WS08-R4	U	-	-
5305-12NJ	U	135.42	188.91	*	SD12	U	96.00	98.90	NS	WS08-R5	U	-	-
5312-2NJ	L	168.17	135.69	*	SD13	U	101.50	104.04	NS	WS08-U1	U	149.00	132.92 NS
9081-12NJ	U	127.92	167.43	*	SD14	U	70.31	106.57	*	WS08-U2	U	138.36	133.00 NS
9100-11NJ	L	173.32	153.48	NS	SD16	U	101.56	109.86	NS	WS08-U3	U	139.58	129.68 NS
9137-11NJ	U	-	-		SD18	U	98.25	96.63	NS	WS08-U4	U	163.54	134.07 NS
9145-11NJ	L	163.08	172.60	NS	SD19	U	102.58	102.51	NS	WS08-U5	U	154.43	132.05 NS

Plant height means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Table 11. Comparison of plant height means in marginal vs. prime farmland sites by genotype. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11 NJ.

NJ Clones	Type	Plant Height (cm)			SD Clones	Type	Plant Height (cm)			WI Clones	Type	Plant Height (cm)		
		Prime	Marginal				Prime	Marginal				Prime	Marginal	
5215-6NJ	L	208.53	142.15	*	SD1	U	106.53	97.17	NS	WS08-K1	U	-	-	
5300-1NJ	U	184.11	137.94	*	SD2	U	110.11	93.81	NS	WS08-K2	U	143.91	112.80	*
5300-4NJ	L	207.52	131.20	*	SD5	U	97.80	85.95	NS	WS08-K3	U	-	-	
5300-8NJ	L	194.66	131.14	*	SD6	U	108.76	92.63	NS	WS08-K4	U	-	-	
5300-12NJ	L	178.60	117.03	*	SD7	U	102.35	92.93	NS	WS08-K5	U	-	-	
5302-1NJ	U	146.79	116.95	*	SD8	U	130.21	102.74	*	WS08-R1	U	-	-	
5305-1NJ	U	160.77	105.52	*	SD9	U	92.86	79.88	NS	WS08-R2	U	-	-	
5305-7NJ	U	145.65	108.52	*	SD10	U	108.74	97.86	NS	WS08-R3	U	-	-	
5305-10NJ	U	154.29	112.77	*	SD11	U	116.33	113.03	NS	WS08-R4	U	-	-	
5305-12NJ	U	187.42	136.90	*	SD12	U	106.20	88.69	NS	WS08-R5	U	-	-	
5312-2 NJ	L	178.77	125.08	*	SD13	U	107.69	97.85	NS	WS08-U1	U	148.72	133.19	NS
9081-12NJ	U	170.04	125.31	*	SD14	U	100.22	76.66	NS	WS08-U2	U	144.91	126.44	NS
9100-11NJ	L	183.31	143.50	*	SD16	U	111.63	99.79	NS	WS08-U3	U	148.80	120.46	*
9137-11NJ	U	-	-		SD18	U	103.49	91.39	NS	WS08-U4	U	160.64	136.97	NS
9145-11NJ	L	188.66	147.02	*	SD19	U	105.28	99.81	NS	WS08-U5	U	150.06	136.43	NS

Plant height means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Tiller Density

The analysis of variance suggests that the interaction of location x soil quality had the largest influence on tiller density (Table 12). Location and soil quality as single variation terms were also significant ($P < 0.05$) (Table 12). Genotype and the interactions effects that contained location x genotype, soil quality x genotype as well as a three-way interaction with these terms were also significant ($P < 0.05$) sources of variation (Table 12). Nitrogen treatment did not have a significant ($P < 0.05$) effect on tiller density nor did the interaction effects that contained this term except for location x nitrogen x genotype (Table 12). Das et al. (2004) also found that location affected tiller density. However their study did not include the soil quality and nitrogen treatment components.

The clones from SD and WI had the lowest tiller density, SD clones produced 27 to 65 tillers·plant⁻¹ and WI clones produced 34 to 76 tillers·plant⁻¹, but the differences between these two germplasm sources were not statistically significant ($P < 0.05$) (Table 13). The clonal material from NJ had tiller densities that ranged from 45 to 97 tillers·plant⁻¹ (Table 13). Lowland types produced more tillers than upland types except for 5300-1NJ an upland clone that was ranked in the top three, producing 96 tillers·plant⁻¹ (Table 13). However most of the differences in tiller densities were not statistically significant ($P < 0.05$) among NJ clones compared to two WI clones WS08-U5 and WI08-U4 (Table 13). Generally upland types had higher tiller densities than lowlands in northern latitudes (Casler et al., 2004). Alexopoulou et al. (2008) conducted adaptability and productivity trials in two locations in the Mediterranean region: a southern location in Greece, Aliartos and a northern location in Italy,

Trisaia. They observed that in the northern locations of Trisaia the upland germplasm produced plots with significantly higher tiller density than the lowland germplasm. The results from this study showed that NJ lowland ecotypes produced more tillers in New Jersey and South Dakota compared to SD and some WI upland ecotypes (Table 13 and 14). This indicates that lowland ecotypes with comparable and/or higher tiller density than upland ecotypes may be selected and grown in northern latitudes.

The analysis of variance showed that location and soil quality as well as the interaction between these two factors accounted for the majority of the variance in tiller density in switchgrass (Table 12). On average all the clones had higher tiller density in New Jersey, especially on the prime sites, compared to South Dakota; however this differences in tiller density were significant ($P<0.05$) only for seven genotypes (Table 13 and 14). For NJ lowlands entries 5215-6NJ, 5300-12NJ, 5312-2NJ, 9145-11NJ tillers density ranged from 111 to 130 tillers·plant⁻¹ in New Jersey compared to 43 to 70 tillers·plant⁻¹ in South Dakota (Table 14). The plants from the NJ upland genotype 5300-1NJ also grew more tillers in New Jersey than in South Dakota (123 vs. 69 tillers·plant⁻¹, respectively) (Table 14). Entries SD10 and WS08-U3, both upland types, also had better tiller density in New Jersey than in South Dakota (84 and 78 tillers·plant⁻¹ vs. 26 and 27 tillers·plant⁻¹, respectively) (Table 14). Madakadze et al. (1998) conducted an evaluation of 12 switchgrass cultivars in South-Western Quebec. They observed that switchgrass genotypes originated from lower latitudes were later flowering and produced greater amount of tillers than genotypes originated in Canada; however these genotypes tended to be more susceptible to winter kill, because the vegetative cycle extended too long and the freezing temperatures in this region damaged the plants. The results from this study suggest

that some clones may have responded better to the longer summer days in NJ therefore they grew for a longer period of time and produced more tillers, while this same longer vegetative cycle may have been affected by freezing temperatures in South Dakota. On the other hand there are genotypes, especially SD and WI upland types, which were not affected by location or day length and may have broad adaptability.

When tiller density was compared across soil quality it was observed that on average plants grew more tillers in sites with prime quality soils (Table 15). However the differences in tiller density due to soil quality, were only statistically significant ($P < 0.05$) for five of the NJ clones (Table 15). The NJ lowlands clones 5215-6NJ, 5300-4NJ, 5312-2NJ and 9145-11NJ had tillers densities that ranged from 114 to 133 tillers·plant⁻¹ in New Jersey vs. 58 to 70 tillers·plant⁻¹ in South Dakota (Table 15). The NJ upland clone 5300-1NJ also grew more tillers in New Jersey than in South Dakota (118 vs. 74 tillers·plant⁻¹, respectively) (Table 15). The upland clone 5300-1NJ was similarly in agronomic performance and adaptation to lowland clones. Casler et al. (2004) observed that upland populations from southern latitudes improved in performance and survival as they were moved north. This entry is progeny of the central upland cultivar Carthage, released by the Cape May Plant Material Center, NJ in 2006, which originated in North Carolina but is well adapted to growing conditions of northern Mid-Atlantic States (USDA-NRCS Cape May Plant Material Center, 2007). Overall the results for tiller density were similar to the results for plant height, upland clones from SD and WI exhibited less vegetative growth but better broad adaptation to different locations and soil qualities than lowland clones from NJ. It has been observed that even though vegetative growth of lowland plants may be superior

than upland; it is also variable and highly susceptible to effects of genotype x environment interactions (Boe and Beck, 2008; Cassida et al, 2005; Das et al., 2004; Porter, 1966).

Tiller density has been identified as an important component of forage yield in species such as brome grass (*Bromus inermis* L.) (Tan et al., 1977) and big bluestem (*Andropogon gerardii* Vitman) (Smart et al., 2004). In crops such as wheat (*Triticum aestivum* L.) it has been reported that tiller density is a moderately heritable trait, but selection can be affected by genotype x environment interaction (Comstock and Moll, 1963; Van Sandford and Utomo, 1995). Redfearn et al. (1997) suggested that in switchgrass forage yield was affected mainly by the growth and development of tillers. Several studies have concluded that using higher tiller density as a selection criterion is effective and that there is adequate genetic variability within switchgrass populations to improve biomass yield (Boe, 2007; Boe and Casler, 2005; Das et al., 2004; Redfearn et al., 1997). However the genotype x environment interaction may affect the selection process (Redfearn et al., 1997). Our results showed there are genotypes, especially from SD and WI, with broad adaptation to locations and soil qualities and that the better performing NJ genotypes were affected by genotype x environment interactions. This may indicate in order to achieve greater yields in low productivity environments, selection may benefit from multi-environment trials that include marginal sites to combine broad adaptation with superior genotypes.

Table 12. Analysis of variance of tiller density (tillers·plant⁻¹) of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated at two locations in marginal vs. prime farmland at each location with 0 or 100 kg of N·ha⁻¹.

Source	Df	F-value	Pr > F
Location (State)	1	16.43	0.0037
SQ (Soil Quality)	1	32.75	0.0004
Loc*SQ	1	78.88	<.0001
N (Nitrogen)	1	0.61	0.4585
Loc*N	1	0.04	0.8453
SQ*N	1	0.09	0.7767
Loc*SQ*N	1	0.01	0.9256
Clone (Genotype)	44	15.9	<.0001
Loc*Clone	44	5.82	<.0001
SQ*Clone	44	2.83	<.0001
Loc*SQ*Clone	37	5.35	<.0001
N*Clone	44	1	0.4774
Loc*N*Clone	39	2.26	0.0001
SQ*N*Clone	44	1.2	0.1990
Loc*SQ*N*Clone	34	1.18	0.2344

Table 13. Tiller density (tillers·plant⁻¹) means of switchgrass clones from SD, WI and NJ. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

		Tiller Density (tillers·plant ⁻¹)								
		NJ				SD				
		Adelphia		Somerset		Brookings		Pierre		Mean
		Prime		Marginal		Prime		Marginal		Tiller Density
NJ Clones	Type	0	100	0	100	0	100	0	100	(tillers·plant ⁻¹)
5215-6NJ	L	181.00	226.67	36.00	58.67	62.66	41.33	88.64	88.33	97.95 A ¹
5300-1NJ	U	180.00	175.33	67.67	70.67	67.33	52.00	86.67	71.67	96.42 A
5312-2NJ	L	210.00	199.67	57.67	54.00	64.00	59.33	58.33	65.78	96.09 A
5300-4NJ	L	121.00	155.00	49.00	43.33	106.67	93.33	98.33	90.00	94.58 AB
9145-11NJ	L	154.67	216.67	59.67	58.33	32.93	55.65	76.98	75.70	91.24 ABC
5305-12NJ	U	99.67	152.00	35.33	35.67	85.67	72.67	123.33	118.33	90.33 ABC
5300-8NJ	L	122.33	183.33	46.57	51.33	76.00	47.67	81.25	93.33	87.73 ABC
9100-11NJ	L	138.00	211.67	52.59	39.67	39.41	36.67	85.00	86.17	86.28 ABCD
5300-12NJ	L	133.67	193.00	53.33	66.33	25.10	36.00	61.43	52.43	77.60 ABCDE
WS08-U5	U	118.39	165.90	31.85	90.16	58.33	54.90	54.28	33.79	76.29 ABCDEF
9081-12NJ	U	71.00	114.33	29.33	44.00	66.00	80.33	75.00	75.33	69.42 BCDEFG
WS08-U4	U	135.68	136.95	59.89	56.16	60.00	46.67	31.67	26.21	69.27 ABCDEFGHI
SD8	U	116.00	86.00	38.67	39.67	55.00	47.00	73.33	68.33	65.50 CDEFGH
5305-1NJ	U	109.00	68.67	52.67	23.00	23.36	80.39	68.71	80.66	63.19 CDEFGHIJ
SD12	U	125.00	53.67	32.67	34.33	66.67	63.33	36.30	71.67	60.42 DEFGHIJ
SD10	U	134.00	92.67	43.67	66.33	26.29	21.74	19.44	36.67	55.26 EFGHIJK
WS08-U1	U	111.00	73.33	45.00	50.33	52.67	36.33	36.67	33.84	54.90 EFGHIJK

5305-10NJ	U	85.00	93.33	45.67	39.00	26.67	41.00	45.00	63.33	54.88 EFGHLJK
5302-1NJ	U	78.33	95.00	32.00	32.67	17.11	52.67	61.67	63.33	54.10 EFGHLJK
SD18	U	88.00	57.00	35.00	31.67	61.67	47.33	48.33	58.33	53.42 EFGHLJK
WS08-U3	U	91.67	119.43	43.37	58.56	28.00	19.08	40.00	21.49	52.85 EFGHLJK
SD19	U	82.67	39.00	29.33	19.33	35.00	41.67	69.56	76.67	49.17 EFGHLJK
SD1	U	86.00	47.00	35.33	23.33	31.67	53.13	38.33	65.00	47.48 FGHLJK
WS08-U2	U	112.39	14.96	47.93	38.06	55.33	40.04	31.67	23.81	45.74 EFGHLJK
SD13	U	61.67	77.67	15.33	34.33	59.00	27.33	38.33	50.00	45.46 GHLJK
SD2	U	71.00	33.67	22.49	26.00	24.00	61.00	51.67	71.67	45.23 GHLJK
5305-7NJ	U	53.33	113.67	27.00	22.67	24.33	19.13	45.00	56.37	45.20 GHLJK
SD14	U	63.10	43.52	13.08	10.02	41.00	54.18	51.32	78.33	44.18 FGHLJK
SD11	U	72.00	61.33	28.67	29.33	15.33	22.08	33.33	56.67	39.85 HLJK
SD16	U	80.33	35.67	27.56	20.33	23.92	40.00	50.00	33.33	38.78 HLJK
SD7	U	74.67	30.74	26.67	24.67	26.71	48.31	33.33	30.00	36.99 LJK
SD6	U	50.67	37.00	21.67	16.00	44.67	41.67	38.33	31.67	35.21 JK
WS08-K2	U	51.22	68.00	19.87	24.56	28.33	23.10	26.78	33.62	34.49 JK
SD9	U	52.67	39.69	18.01	20.67	31.37	46.08	21.96	16.67	30.97 K
SD5	U	24.33	25.41	11.51	13.33	36.76	56.48	31.67	20.00	27.34 K
9137-11NJ	U	70.33	113.33	17.67	32.33	53.67	43.33	-	23.61	+
WS08-K1	U	54.77	47.90	5.79	30.06	13.76	-	-	-	+
WS08-K3	U	35.39	65.90	40.89	55.16	41.76	-	-	-	+
WS08-K4	U	5.39	35.15	5.89	11.16	36.95	-	25.00	-	+
WS08-K5	U	-	32.00	19.37	13.56	15.36	-	-	-	+
WS08-R1	U	28.95	-	33.89	34.97	20.67	15.67	23.33	8.82	+
WS08-R2	U	80.00	63.33	33.76	30.67	19.00	19.00	-	-	+
WS08-R3	U	14.28	73.40	18.89	8.16	24.36	7.66	-	-	+
WS08-R4	U	51.89	95.40	51.87	37.56	17.76	-	-	-	+
WS08-R5	U	87.33	105.07	12.37	32.06	12.95	2.74	-	-	+
Mean		101.07a²	101.20a	36.90b	39.15b	45.15b	47.42b	54.59b	57.68b	

¹ Cultivar means with the same lower case letter were not statistically different at 0.05 level of probability.

² Location means with the same upper case letter were not statistically different at 0.05 level of probability.

(-) Means were not calculated due to missing values.

⁺ Cultivars means were not separated by LSMEANS due to missing values.

Table 14. Comparison of tiller density (tillers·plant⁻¹) means in NJ vs. SD locations. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

		Tiller Density (tillers·plant ⁻¹)					Tiller Density (tillers·plant ⁻¹)					Tiller Density (tillers·plant ⁻¹)		
NJ Clones	Type	NJ	SD		SD Clones	Type	NJ	SD		WI Clones	Type	NJ	SD	
5215-6NJ	L	125.58	70.32	*	SD1	U	47.92	47.04	NS	WS08-K1	U	-	-	
5300-1NJ	U	123.42	69.42	*	SD2	U	38.37	52.08	NS	WS08-K2	U	40.92	28.06	NS
5300-4NJ	L	92.08	97.08	NS	SD5	U	18.44	36.24	NS	WS08-K3	U	-	-	
5300-8NJ	L	100.98	74.49	NS	SD6	U	31.33	39.08	NS	WS08-K4	U	-	-	
5300-12NJ	L	111.58	43.62	*	SD7	U	39.38	34.60	NS	WS08-K5	U	-	-	
5302-1NJ	U	59.50	48.70	NS	SD8	U	70.08	60.92	NS	WS08-R1	U	-	-	
5305-1NJ	U	63.33	63.04	NS	SD9	U	32.76	29.19	NS	WS08-R2	U	-	-	
5305-7NJ	U	54.17	36.23	NS	SD10	U	84.17	26.36	*	WS08-R3	U	-	-	
5305-10NJ	U	65.75	44.00	NS	SD11	U	47.83	31.86	NS	WS08-R4	U	-	-	
5305-12NJ	U	80.67	100.00	NS	SD12	U	61.42	59.42	NS	WS08-R5	U	-	-	
5312-2NJ	L	130.33	61.85	*	SD13	U	47.25	43.67	NS	WS08-U1	U	69.92	39.89	NS
9081-12NJ	U	64.67	74.17	NS	SD14	U	32.22	56.15	NS	WS08-U2	U	53.76	37.73	NS
9100-11NJ	L	110.42	62.13	NS	SD16	U	41.06	36.51	NS	WS08-U3	U	78.52	27.18	*
9137-11NJ	U	-	-		SD18	U	52.92	53.92	NS	WS08-U4	U	97.39	41.14	NS
9145-11NJ	L	122.33	60.15	*	SD19	U	42.58	55.76	NS	WS08-U5	U	102.20	50.38	NS

Tiller density means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Table 15. Comparison of tiller density (tillers·plant⁻¹) means in marginal vs. prime farmland sites. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

NJ Clones	Type	Tiller Density (tillers·plant ⁻¹)			SD Clones	Type	Tiller Density (tillers·plant ⁻¹)			WI Clones	Type	Tiller Density (tillers·plant ⁻¹)		
		Prime	Marginal				Prime	Marginal				Prime	Marginal	
5215-6NJ	L	128.08	67.83	*	SD1	U	54.45	40.50	NS	WS08-K1	U	-	-	
5300-1NJ	U	118.67	74.17	*	SD2	U	47.42	43.03	NS	WS08-K2	U	42.63	26.34	NS
5300-4NJ	L	119.00	70.17	*	SD5	U	35.47	19.21	NS	WS08-K3	U	-	-	
5300-8NJ	L	107.33	68.13	NS	SD6	U	43.50	26.92	NS	WS08-K4	U	-	-	
5300-12NJ	L	96.95	58.26	NS	SD7	U	45.31	28.67	NS	WS08-K5	U	-	-	
5302-1NJ	U	60.78	47.42	NS	SD8	U	76.00	55.00	NS	WS08-R1	U	-	-	
5305-1NJ	U	70.22	56.16	NS	SD9	U	42.67	19.28	NS	WS08-R2	U	-	-	
5305-7NJ	U	52.62	37.78	NS	SD10	U	68.98	41.55	NS	WS08-R3	U	-	-	
5305-10NJ	U	61.50	48.25	NS	SD11	U	42.69	37.00	NS	WS08-R4	U	-	-	
5305-12NJ	U	102.50	78.17	NS	SD12	U	77.17	43.67	NS	WS08-R5	U	-	-	
5312-2NJ	L	133.25	58.94	*	SD13	U	56.42	34.50	NS	WS08-U1	U	68.33	41.47	NS
9081-12NJ	U	82.92	55.92	NS	SD14	U	50.22	38.14	NS	WS08-U2	U	56.21	35.28	NS
9100-11NJ	L	106.76	65.79	NS	SD16	U	44.68	32.89	NS	WS08-U3	U	64.77	40.93	NS
9137-11NJ	U	-	-		SD18	U	63.50	43.33	NS	WS08-U4	U	94.91	43.62	NS
9145-11NJ	L	114.81	67.68	*	SD19	U	49.58	48.76	NS	WS08-U5	U	99.56	53.02	NS

Tiller density means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Dry Biomass Yield

According to the analysis of variance, soil quality accounted for the largest amount of variation affecting dry biomass yield (Table 16). Dry biomass yield was also significantly affected by location and the interaction location x soil quality (Table 16). Genotype also was an influential factor according to the analysis of variance (Table 16). Although the rest of the variables in the model were statistically significant ($P < 0.05$) they accounted for a small portion of the variance (Table 16). Several agronomic traits, such as, biomass yield, grain and forage yield, etc. are considered quantitative or complex traits that may be controlled by many genes which may interact with the environment (Sleper and Poehlman, 2006). For example, the amount of biomass produced by a certain switchgrass cultivar may vary not only due to the cultivar's genetic composition but also due to the environmental conditions of where it is grown (Casler et al., 2004; Casler et al., 2007; Cassida et al., 2005; Sanderson et al., 1996). The results of the analysis of variance coincided with these previous studies that location, as well as, genotype x environment interactions may influence variation in biomass production. In this study the data also suggested that quality of the soil specifically may have been an environmental factor that greatly affected the variation in biomass yield of switchgrass plants.

The upland clones from SD and WI had the lowest dry biomass yield in this study, dry biomass yields from the SD clones ranged from 0.04 to 0.23 kg·plant⁻¹ and yields from WI clones ranged from 0.29 to 0.61 kg·plant⁻¹. There were no significant differences between SD and WI clones (Table 17). The clonal material from NJ had the highest dry biomass yield ranging from 0.57 to 1.60 kg·plant⁻¹ (Table 17). For the

most part lowland ecotypes yielded more biomass than upland ecotypes; although the differences were not statistically significant ($P < 0.05$) for all the genotypes (Table 17). 5300-4NJ and 5312-2NJ yielded the most biomass among the NJ clones (Table 17). These two genotypes were not the best performing in relation to secondary traits but they reached maturity very late in the season and they kept growing; this may explain why they produced plants over 160 cm tall with over 90 tillers·plant⁻¹ (Table 4, 5, 9 and 13). The NJ genotypes also grew larger and yielded more biomass than SD and WI genotypes in marginal soils where the initiation of flowering was delayed (Table 6, 7 and 19). This results support the findings that delay in maturity may be related to superior vegetative growth and higher biomass yield in switchgrass (Table 21) (Casler et al., 2004; Casler et al., 2007; Cornelius and Johnston, 1942; McMillan, 1965). Even though switchgrass is considered a short day species, its sensitivity to photoperiod is still being researched because it is not clear if the initiation of reproductive development is a true response to day length or it may also be dependent on other growing conditions such as temperature, soil pH, soil quality, etc. (Balasko and Smith, 1971; Parrish and Fike, 2005; Porter, 1966; Van Esbroeck et al., 2003). Finally breeding programs focused on improving switchgrass biomass yield in marginal soil should select late maturing genotypes but selections should be made and tested in multi-environment trial that include locations with marginal soil to be more effective.

The ANOVA table suggested there is a strong genotype x environment interaction influencing the variation of dry biomass yield (Table 16). However SD clones did not show difference in performance due to location, soil conditions or nitrogen treatment (Table 18, 19 and 20). This clonal material had broad adaptation indicating that agronomic performance of upland types is less variable but biomass

yields were low (Casler et al., 2004; Porter, 1966). Nitrogen treatment was a statistically significant ($P < 0.05$) source of variation; however it accounted for a very small portion of the variance (Table 16 and 17). Differences in biomass yield due to nitrogen fertilization were only significant ($P < 0.05$) for three of the surviving WI clones and only between fertilized and unfertilized plots in the New Jersey prime site (Table 17 and 20). There are several research studies providing evidence that moderate nitrogen fertilization may enhance biomass production in switchgrass (Bredja et al., 1994; Muir et al., 2001; Mulkey et al., 2006; Sanderson and Reed 2000; Vogel et al., 2002). Stroup et al. (2003) reported that nitrogen deficiency was greater than water supply as a limiting factor in the growth of switchgrass plants. However nitrogen availability in the soil is subject to variation due to environmental and soil conditions such as temperature (Craswell and Godwin, 1984); annual rainfall (Sala et al., 1988); soil pH (Bohn et al., 1979), and water-holding capacity (Staley et al., 1991). The results concerning the nitrogen treatment may indicate there are issues with timing of nitrogen applications, availability of nitrogen in the soil or a delay in the response to nitrogen fertilization by switchgrass plants (Bredja, 2000; Jung et al., 1990).

On average dry biomass yield was higher in New Jersey compared to South Dakota (Table 17 and 18). However, the differences in yield due to location were statistically significant ($P < 0.05$) only for NJ clones and for three of the surviving WI clones (Table 18). The five best performing NJ clones in New Jersey were all lowlands (5215-6NJ, 5300-12NJ, 5312-2NJ, 9100-11NJ and 9145-11NJ) and their dry biomass yields ranged from 2.09 to 2.54 kg·plant⁻¹ (Table 18). With exception of 9145-11NJ the best performing NJ clones in South Dakota were not the same as in

New Jersey (5300-1NJ, 5300-4NJ, 5300-8NJ and 9081-12NJ) and their dry biomass yields range from 0.53 to 1.27 kg·plant⁻¹ (Table 18). Additionally two of the best performing NJ clones in South Dakota were uplands (5300-1NJ and 9081-12NJ) (Table 18). The three upland clones from WI yielded more biomass in New Jersey than in South Dakota (on average, 0.65 vs. 0.19 kg·plant⁻¹, respectively) and the best performing WI clone (WS08-U5) in New Jersey also was the best performing in South Dakota (Table 18). It has been well documented that lowland ecotypes may grow larger and produce more biomass than upland even in locations where growing conditions may favor upland development (Benedict 1940; Casler et al., 2004; Casler et al., 2007; Cassida et al., 2005; Cornelius and Johnston 1941; Porter, 1966). However lowlands are more sensitive to low temperatures than uplands and less likely to survive if vegetative growth continues into the cold season in northern regions like South Dakota, due to winter kill (Casler et al., 2004; Das et al., 2004; Madakadze et al., 1998)

The data suggested that on average plants yielded higher dry biomass on prime sites than in marginal sites across locations and the differences in dry biomass yield due to soil quality were not statistically significant ($P < 0.05$) for all SD clones and one of the surviving WI clones (Table 17 and 19). The differences in dry biomass yields due to soil quality were significant ($P < 0.05$) for all the NJ clones and for six of the surviving WI clones, yielding higher dry biomass in the sites with prime soil (Table 19). The five best performing NJ clones in prime quality soils were all lowlands (5215-6NJ, 5300-4NJ, 5300-12NJ, 5312-2NJ and 9145-11NJ) and their dry biomass yields ranged from 2.08 to 2.74 kg·plant⁻¹ (Table 19). With exception of 5300-4NJ and 9145-11NJ the best performing NJ clones in marginal soils were not

the same than in prime soils (5300-1NJ, 5305-12NJ and 9100-11NJ) and their dry biomass yields range from 0.43 to 0.56 kg·plant⁻¹ (Table 19). Additionally two of the best performing NJ clones in marginal soils were uplands (5300-4NJ and 5305-12NJ) (Table 19). The six upland clones from WI affected by soil quality yielded more biomass in prime site than in marginal site (on average, 0.69 vs. 0.15 kg·plant⁻¹, respectively) and the best performing WI clone (WS08-U5) in prime soil also was the best performing in marginal soil (Table 19). The majority of the research on biomass productivity of the different switchgrass genotypes has been conducted on Universities research farms with high productivity environments. Research studies in other crops, such as, barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and maize (*Zea mays* L.) have suggested that conducting crop improvement programs in environments that differ greatly from the target environments are not the most efficient method to obtain significant genetic gains (Atlin and Frey, 1990; Bänziger et al., 1997; Bänziger et al., 1999; Ceccarelli and Grando, 2003; Simmonds, 1991). Overall these results suggest that there may be broad adaptation among SD and WI clones however the majority of these clones were not the best performing. Additionally NJ genotypes may benefit from conducting further selection in multi-environment trials that include marginal sites to combine broad adaptation with superior performing genotypes because of the strong genotype x environment interaction observed in these clones.

All biomass traits evaluated were highly correlated to each other (Table 20) with the highest correlation observed between anthesis and heading date (0.99). Interestingly, dry biomass yield was highly correlated to all traits (heading date (0.93), anthesis date (0.94), tiller density (0.92) and plant height (0.91). This is similar

to several other studies that found biomass traits to be highly correlated (Boe and Beck, 2008; Cortese and Bonos, 2012; Muir et al., 2001). These results indicate that plant height or tiller density measurements may be used as indicators to biomass yield potential. This could dramatically reduce evaluation time and improve selection efficiency.

Table 16. Analysis of variance of dry biomass yield ($\text{kg}\cdot\text{plant}^{-1}$) of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated at two locations in marginal vs. prime farmland at each location with 0 or 100 kg of N ha^{-1} .

Source	Df	F-value	Pr > F
Location (State)	1	480.93	<.0001
SQ (Soil Quality)	1	941.26	<.0001
Loc*SQ	1	727.80	<.0001
N (Nitrogen)	1	62.10	<.0001
Loc*N	1	78.25	<.0001
SQ*N	1	65.02	<.0001
Loc*SQ*N	1	43.94	0.0002
Clone (Genotype)	44	108.58	<.0001
Loc*Clone	44	40.00	<.0001
SQ*Clone	44	54.63	<.0001
Loc*SQ*Clone	37	49.25	<.0001
N*Clone	44	4.73	<.0001
Loc*N*Clone	39	5.46	<.0001
SQ*N*Clone	43	5.42	<.0001
Loc*SQ*N*Clone	34	3.48	<.0001

Table 17. Means of 45 clones dry biomass yield (kg·plant⁻¹) separated by genotype. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

		Dry Biomass Yield (kg·plant ⁻¹)								Mean Dry Biomass Yield (kg·plant ⁻¹)
		NJ				SD				
		Adelphia		Somerset		Brookings		Pierre		
		Prime		Marginal		Prime		Marginal		
NJ Clones	Type	0	100	0	100	0	100	0	100	
5300-4NJ	L	3.37	3.71	0.34	0.33	1.89	1.98	0.54	0.66	1.60 A ¹
5312-2NJ	L	4.86	4.55	0.45	0.30	0.70	0.58	0.36	0.40	1.52 AB
9145-11NJ	L	3.33	4.35	0.40	0.42	0.50	0.66	0.59	0.43	1.33 BC
5215-6NJ	L	3.61	4.11	0.31	0.34	0.69	0.30	0.50	0.43	1.29 C
9100-11NJ	L	3.14	4.51	0.58	0.40	0.22	0.12	0.63	0.63	1.28 C
5300-8NJ	L	2.67	3.43	0.27	0.20	1.05	0.92	0.48	0.58	1.20 C
5300-12NJ	L	3.50	4.23	0.35	0.45	0.22	0.38	0.08	0.15	1.17 C
5305-12NJ	U	2.16	2.35	0.17	0.09	1.45	1.14	0.85	0.97	1.15 C
5300-1NJ	U	2.73	2.72	0.28	0.32	1.18	0.77	0.62	0.50	1.14 C
9081-12NJ	U	1.90	2.21	0.09	0.13	0.76	0.48	0.48	0.42	0.81 D
5305-10NJ	U	2.04	2.14	0.19	0.14	0.25	0.16	0.16	0.21	0.66 DE
5305-1NJ	U	2.10	1.84	0.15	0.06	0.13	0.39	0.19	0.19	0.63 DEF
WS08-U5	U	0.65	2.52	0.20	0.44	0.43	0.34	0.12	0.18	0.61 DEF
5302-1NJ	U	1.87	1.99	0.10	0.10	0.09	0.23	0.21	0.16	0.59 DEF
5305-7NJ	U	1.63	1.93	0.07	0.05	0.21	0.19	0.17	0.27	0.57 EF
WS08-U4	U	0.69	2.06	0.09	0.19	0.34	0.27	0.11	0.11	0.48 EFG
WS08-U1	U	0.49	1.80	0.22	0.20	0.32	0.24	0.14	0.15	0.44 EFG

WS08-U3	U	0.47	2.01	0.11	0.16	0.16	0.08	0.07	0.12	0.40 FG
WS08-K2	U	0.16	1.71	0.07	0.05	0.14	0.09	0.06	0.05	0.29 GH
WS08-U2	U	0.92	-	0.24	0.21	0.46	0.29	0.09	0.13	0.29 GH
SD8	L	0.39	0.29	0.08	0.09	0.37	0.30	0.20	0.13	0.234 GH
SD10	L	0.22	0.22	0.07	0.06	0.12	0.05	0.02	0.18	0.116 H
SD12	L	0.13	0.12	0.04	0.02	0.20	0.19	0.05	0.08	0.105 H
SD1	L	0.12	0.06	0.03	0.02	0.25	0.16	0.03	0.07	0.095 H
SD11	L	0.10	0.13	0.07	0.06	0.06	0.07	0.08	0.07	0.082 H
SD18	L	0.11	0.08	0.03	0.02	0.13	0.09	0.07	0.08	0.078 H
SD19	L	0.09	0.07	0.03	0.02	0.09	0.11	0.07	0.10	0.071 H
SD13	L	0.07	0.13	0.02	0.03	0.13	0.07	0.05	0.05	0.069 H
SD6	L	0.04	0.06	0.02	0.01	0.14	0.07	0.06	0.10	0.061 H
SD14	L	0.01	0.06	0.01	0.003	0.09	0.13	0.04	0.11	0.057 H
SD2	L	0.08	0.04	0.02	0.02	0.06	0.12	0.05	0.06	0.056 H
SD7	L	0.07	0.03	0.03	0.02	0.09	0.10	0.05	0.03	0.052 H
SD16	L	0.09	0.05	0.04	0.02	0.05	0.07	0.06	0.04	0.051 H
SD5	L	0.03	0.03	0.01	0.01	0.08	0.08	0.04	0.02	0.040 H
SD9	L	0.04	0.04	0.01	0.01	0.07	0.03	0.03	0.04	0.035 H
9137-11NJ	U	1.99	2.09	0.06	0.12	0.45	0.14	-	0.04	-
WS08-K1	U	0.27	1.67	0.01	0.07	0.07	-	-	-	-
WS08-K3	U	0.20	1.76	0.16	0.16	0.28	-	-	-	-
WS08-K4	U	0.03	0.12	0.02	0.03	0.05	-	-	0.07	-
WS08-K5	U	0.20	1.05	0.07	0.03	0.08	-	-	-	-
WS08-R1	U	0.13	-	0.11	0.21	0.17	0.09	0.03	0.08	-
WS08-R2	U	0.32	1.73	0.12	0.04	0.11	0.08	-	-	-
WS08-R3	U	0.07	1.90	0.09	-	0.23	0.08	-	-	-
WS08-R4	U	0.53	2.27	0.31	0.19	0.08	-	-	-	-
WS08-R5	U	0.52	2.04	0.03	0.18	0.23	0.003	-	-	-
Mean		1.25a²	1.59b	0.15e	0.14e	0.38cd	0.32c	0.22de	0.22de	

¹ Clone means with the same lower case letter were not statistically different at 0.05 level of probability.

² Location means with the same upper case letter were not statistically different at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Table 18. Comparison of dry biomass yield ($\text{kg}\cdot\text{plant}^{-1}$) means by genotype in NJ vs. SD locations. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

		Dry Biomass Yield ($\text{kg}\cdot\text{plant}^{-1}$)					Dry Biomass Yield ($\text{kg}\cdot\text{plant}^{-1}$)					Dry Biomass Yield ($\text{kg}\cdot\text{plant}^{-1}$)		
NJ Clones	Type	NJ	SD		SD Clones	Type	NJ	SD		WI Clones	Type	NJ	SD	
5215-6NJ	L	2.09	0.48	*	SD1	U	0.059	0.131	NS	WS08-K1	U	-	-	
5300-1NJ	U	1.51	0.77	*	SD2	U	0.039	0.073	NS	WS08-K2	U	0.50	0.087	NS
5300-4NJ	L	1.94	1.27	*	SD5	U	0.025	0.056	NS	WS08-K3	U	-	-	
5300-8NJ	L	1.64	0.76	*	SD6	U	0.031	0.091	NS	WS08-K4	U	-	-	
5300-12NJ	L	2.13	0.21	*	SD7	U	0.036	0.069	NS	WS08-K5	U	-	-	
5302-1NJ	U	1.01	0.18	*	SD8	U	0.216	0.251	NS	WS08-R1	U	-	-	
5305-1NJ	U	1.04	0.22	*	SD9	U	0.025	0.044	NS	WS08-R2	U	-	-	
5305-7NJ	U	0.92	0.21	*	SD10	U	0.140	0.093	NS	WS08-R3	U	-	-	
5305-10NJ	U	1.13	0.19	*	SD11	U	0.091	0.073	NS	WS08-R4	U	-	-	
5305-12NJ	U	1.19	1.10	NS	SD12	U	0.079	0.131	NS	WS08-R5	U	-	-	
5312-2NJ	L	2.54	0.51	*	SD13	U	0.063	0.075	NS	WS08-U1	U	0.68	0.21	NS
9081-12NJ	U	1.08	0.53	*	SD14	U	0.021	0.093	NS	WS08-U2	U	0.33	0.24	NS
9100-11NJ	L	2.16	0.40	*	SD16	U	0.049	0.054	NS	WS08-U3	U	0.69	0.11	*
9137-11NJ	U	-	-		SD18	U	0.063	0.093	NS	WS08-U4	U	0.76	0.21	*
9145-11NJ	L	2.12	0.54	*	SD19	U	0.053	0.090	NS	WS08-U5	U	0.95	0.27	*

Dry biomass yield means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Table 19. Comparison of dry biomass yield ($\text{kg}\cdot\text{plant}^{-1}$) means by genotype in marginal vs. prime farmland sites. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

NJ Clones	Type	Dry Biomass Yield ($\text{kg}\cdot\text{plant}^{-1}$)			SD Clones	Type	Dry Biomass Yield ($\text{kg}\cdot\text{plant}^{-1}$)			WI Clones	Type	Dry Biomass Yield ($\text{kg}\cdot\text{plant}^{-1}$)		
		Prime	Marginal				Prime	Marginal				Prime	Marginal	
5215-6NJ	L	2.18	0.39	*	SD1	U	0.149	0.041	NS	WS08-K1	U	-	-	
5300-1NJ	U	1.85	0.43	*	SD2	U	0.073	0.039	NS	WS08-K2	U	0.52	0.060	*
5300-4NJ	L	2.74	0.47	*	SD5	U	0.058	0.022	NS	WS08-K3	U	-	-	
5300-8NJ	L	2.02	0.38	*	SD6	U	0.075	0.048	NS	WS08-K4	U	-	-	
5300-12NJ	L	2.08	0.26	*	SD7	U	0.070	0.035	NS	WS08-K5	U	-	-	
5302-1NJ	U	1.04	0.14	*	SD8	U	0.340	0.127	NS	WS08-R1	U	-	-	
5305-1NJ	U	1.11	0.15	*	SD9	U	0.045	0.024	NS	WS08-R2	U	-	-	
5305-7NJ	U	0.99	0.14	*	SD10	U	0.153	0.079	NS	WS08-R3	U	-	-	
5305-10NJ	U	1.15	0.17	*	SD11	U	0.093	0.071	NS	WS08-R4	U	-	-	
5305-12NJ	U	1.77	0.52	*	SD12	U	0.160	0.050	NS	WS08-R5	U	-	-	
5312-2NJ	L	2.67	0.38	*	SD13	U	0.101	0.036	NS	WS08-U1	U	0.71	0.18	*
9081-12NJ	U	1.34	0.28	*	SD14	U	0.075	0.039	NS	WS08-U2	U	0.41	0.17	NS
9100-11NJ	L	2.00	0.56	*	SD16	U	0.064	0.038	NS	WS08-U3	U	0.68	0.12	*
9137-11NJ	U	-	-		SD18	U	0.105	0.052	NS	WS08-U4	U	0.84	0.13	*
9145-11NJ	L	2.21	0.46	*	SD19	U	0.089	0.053	NS	WS08-U5	U	0.99	0.23	*

Dry biomass yield means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Table 20. Comparison of dry biomass yield ($\text{kg} \cdot \text{plant}^{-1}$) means by genotype in 0 vs. 100 kg of $\text{N} \cdot \text{ha}^{-1}$. Statistical analysis was conducted without entries WI08-K1, WI08-K2, WI08 K3, WI08-K4, WI08-K5, WI08-R2, WI08-R3, WI08-R4, WI08-R5 and 9137-11NJ.

Dry Biomass Yield					Dry Biomass Yield					Dry Biomass Yield				
		(kg·plant ⁻¹)					(kg·plant ⁻¹)					(kg·plant ⁻¹)		
NJ Clones	Type	0	100		SD Clones	Type	0	100		WI Clones	Type	0	100	
5215-6NJ	L	1.28	1.29	NS	SD1	U	0.110	0.080	NS	WS08-K1	U	-	-	
5300-1NJ	U	1.21	1.08	NS	SD2	U	0.052	0.059	NS	WS08-K2	U	0.10	0.48	*
5300-4NJ	L	1.54	1.67	NS	SD5	U	0.041	0.039	NS	WS08-K3	U	-	-	
5300-8NJ	L	1.12	1.28	NS	SD6	U	0.064	0.059	NS	WS08-K4	U	-	-	
5300-12NJ	L	1.04	1.30	NS	SD7	U	0.059	0.045	NS	WS08-K5	U	-	-	
5302-1NJ	U	0.57	0.62	NS	SD8	U	0.261	0.206	NS	WS08-R1	U	-	-	
5305-1NJ	U	0.64	0.62	NS	SD9	U	0.040	0.030	NS	WS08-R2	U	-	-	
5305-7NJ	U	0.52	0.61	NS	SD10	U	0.108	0.124	NS	WS08-R3	U	-	-	
5305-10NJ	U	0.66	0.66	NS	SD11	U	0.079	0.085	NS	WS08-R4	U	-	-	
5305-12NJ	U	1.16	1.14	NS	SD12	U	0.107	0.103	NS	WS08-R5	U	-	-	
5312-2NJ	L	1.59	1.46	NS	SD13	U	0.068	0.069	NS	WS08-U1	U	0.29	0.60	NS
9081-12NJ	U	0.80	0.81	NS	SD14	U	0.037	0.077	NS	WS08-U2	U	0.44	0.14	NS
9100-11NJ	L	1.14	1.42	NS	SD16	U	0.057	0.045	NS	WS08-U3	U	0.22	0.58	*
9137-11NJ	U	-	-		SD18	U	0.088	0.069	NS	WS08-U4	U	0.31	0.65	NS
9145-11NJ	L	1.20	1.46	NS	SD19	U	0.069	0.074	NS	WS08-U5	U	0.37	0.85	*

Dry biomass yield means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

(-) Means were not calculated due to missing values.

Table 21. Pearson correlation coefficients for dry biomass yield (DBY) and secondary traits: heading date (HD), anthesis date (AD) plant height (PH) and tiller density (TD), of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated at two locations in marginal vs. prime farmland at each location with 0 or 100 kg of N ha⁻¹.

Traits	HD	AD	PH	TD	DBY
Heading Date (HD) (Julian date)	–	0.99*	0.82*	0.81*	0.93*
Anthesis Date (AD) (Julian date)	0.99*	–	0.87*	0.81*	0.94*
Plant Height (PH) (cm)	0.82*	0.87*	–	0.88*	0.92*
Tiller Density (TD) (tillers·plant⁻¹)	0.81*	0.81*	0.88*	–	0.91*
Dry Biomass Yield (DBY) (kg·plant⁻¹)	0.93*	0.94*	0.92*	0.91*	–

Anthrachnose Disease Ratings

Genotype had the greatest influence on anthracnose (*Colletotrichum nativas*) disease reaction (Table 22). Additionally, the interaction of soil quality x genotype was also statistically significant (Table 22). According to the ANOVA table the rest of the independent variables were not statistically significant (Table 22). All clones from the NJ breeding program showed less disease symptoms compared to the SD clones (Table 23). WI clones were in between NJ and SD clones (Table 23). Interestingly, among the NJ clones, there was not a significant difference in anthracnose resistance due to ecotype (Table 23). SD clones had the lowest disease ratings exhibiting more susceptibility to anthracnose except for entries SD2, SD7 SD9, SD10 and SD19 which showed no significant difference compared to some NJ and WI clones (Table 23). The ANOVA table suggested that the interaction genotype x soil quality was also a source of variation for disease resistance; however, only entry 9137-11NJ was more susceptible to anthracnose in marginal soil compare to prime (Table 22 and 24). According to disease ratings, clones from NJ and SD were more susceptible to anthracnose on marginal sites but the differences were not significant (Table 24). The WI clones seemed to have better resistance in marginal sites; however the differences were also not significant (Table 24).

The results of this study suggested a strong genetic control of anthracnose resistance also indicating that environmental conditions may be marginally influential on the incidence of anthracnose in switchgrass. Similar results were observed for anthracnose (*Coletotichum cereale*) resistance in creeping bentgrass (*Agrostis palustris*) (Bonos et al., 2009). Toman and White (1993) studied the inheritance of

resistance to anthracnose stalk rot of corn (*Zea mays*), caused by *Colletotrichum graminicola*. They determined that this trait may be controlled by one or a few genes because of the significant additive and dominance effects observed in a generation mean analysis. Da Costa et al. (2011) investigated the inheritance of resistance to anthracnose, caused by *Colletotrichum sublineolum*, in sorghum (*Sorghum bicolor*). They conducted field crosses between resistant and susceptible parents and backcrosses between F1 plants and the susceptible parents. They suggested that anthracnose resistance in sorghum is controlled by a single gene with complete dominance because the proportions of resistant and susceptible individuals in the segregated populations were similar to the frequencies expected when this type of inheritance is present. The results in our study are beneficial for switchgrass breeding because they indicate that selection could be done in one environment. The differences in genetic variation among clones indicate that tolerant genotypes can be identified and could be used in cultivar development.

Table 22. Analysis of variance of anthracnose (*Colletotrichum nativas*) disease ratings of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated in NJ on marginal vs. prime farmland with 0 or 100 kg of N·ha⁻¹.

Source	Df	F-value	Pr > F
SQ (Soil Quality)	1	4.11	0.1125
N (Nitrogen)	1	0.13	0.7358
SQ*N	1	4.79	0.0938
Clone (Genotype)	42	15.23	<.0001
SQ*Clone	42	3.59	<.0001
N*Clone	42	1.28	0.1523
SQ*N*Clone	42	1.41	0.0769

Table 23. Anthracnose disease (*Colletotrichum nativas*) ratings of 45 switchgrass clones. Means were separation with the LSMEANS option and adjusted with Tukey's method for multiple comparisons.

Mean Anthracnose				Mean Anthracnose				Mean Anthracnose			
NJ Clones	Type	Disease	Rating	SD Clones	Type	Disease	Rating	WI Clones	Type	Disease	Rating
5305-7NJ	U	7	A ¹	SD7	U	4	DEFGIJK	WS08-K4	U	6	ABCDEF
9100-11NJ	L	7	AB	SD19	U	4	EFGIJK	WS08-R4	U	6	ABCD
5305-10NJ	U	7	AB	SD2	U	4	FGIJK	WS08-R3	U	6	ABCDEFG
9137-11NJ	U	7	AB	SD10	U	4	FGIJK	WS08-R2	U	6	ABCD
5300-8NJ	L	7	AB	SD9	U	4	FGIJK	WS08-K3	U	6	ABCDEFGI
5305-12NJ	U	7	AB	SD6	U	4	IJK	WS08-R5	U	6	ABCDEFG
5300-12NJ	L	6	AB	SD5	U	3	IJK	WS08-U1	U	5	ABCDEFGI
5312-2NJ	L	6	AB	SD8	U	3	JK	WS08-K1	U	5	ABCDEFGIJK
5305-1NJ	U	6	AB	SD18	U	3	JK	WS08-U4	U	5	ABCDEFGIJK
9145-11NJ	L	6	AB	SD1	U	3	JK	WS08-U3	U	4	CDEFGIJK
5300-4NJ	L	6	ABC	SD11	U	3	JK	WS08-K2	U	4	EFGIJK
5300-1NJ	U	6	ABCD	SD12	U	3	JK	WS08-U5	U	4	EFGHIJK
5215-6NJ	L	6	ABCDH	SD16	U	3	JK	WS08-K5	U	3	GIJK
5302-1NJ	U	6	ABCDE	SD13	U	3	K	WS08-R1	U	-	
9081-12NJ	U	5	BCDEFGIJ	SD14	U	3	JK	WS08-U2	U	-	

¹ Disease rating means with the same upper case letter were not statistically different at 0.05 level of probability.

(-) Means were not calculated due to missing values.

A 1 to 9 (9 = least disease) visual rating scale was utilized (Bonos et al., 2009).

Table 24. Comparison of anthracnose disease (*Colletotrichum nativas*) ratings means by genotype in marginal vs. prime farmland sites. Statistical analysis was conducted without entries WI08-R1 and WI08-U2.

Anthracnose Disease Rating					Anthracnose Disease Rating					Antracnose Disease Rating				
NJ Clones	Type	Prime	Marginal		SD Clones	Type	Prime	Marginal		WI Clones	Type	Prime	Marginal	
5215-6NJ	L	7	5	NS	SD1	U	4	3	NS	WS08-K1	U	4	6	NS
5300-1NJ	U	6	6	NS	SD2	U	5	3	NS	WS08-K2	U	3	4	NS
5300-4NJ	L	6	6	NS	SD5	U	4	3	NS	WS08-K3	U	5	7	NS
5300-8NJ	L	7	6	NS	SD6	U	4	3	NS	WS08-K4	U	5	8	NS
5300-12NJ	L	7	6	NS	SD7	U	6	3	NS	WS08-K5	U	3	4	NS
5302-1NJ	U	6	5	NS	SD8	U	3	4	NS	WS08-R1	U	-	-	
5305-1NJ	U	8	5	NS	SD9	U	5	3	NS	WS08-R2	U	6	7	NS
5305-7NJ	U	8	6	NS	SD10	U	4	3	NS	WS08-R3	U	5	7	NS
5305-10NJ	U	7	7	NS	SD11	U	3	3	NS	WS08-R4	U	5	7	NS
5305-12NJ	U	8	5	NS	SD12	U	4	3	NS	WS08-R5	U	5	6	NS
5312-2NJ	L	7	6	NS	SD13	U	4	3	NS	WS08-U1	U	5	6	NS
9081-12NJ	U	6	5	NS	SD14	U	4	2	NS	WS08-U2	U	-	-	
9100-11NJ	L	7	7	NS	SD16	U	4	3	NS	WS08-U3	U	3	5	NS
9137-11NJ	U	8	5	*	SD18	U	4	2	NS	WS08-U4	U	5	5	NS
9145-11NJ	L	7	6	NS	SD19	U	5	3	NS	WS08-U5	U	3	4	NS

Disease rating means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

(-) Means were not calculated due to missing values.

A 1 to 9 (9 = least disease) visual rating scale was utilized (Bonos et al., 2009).

Rust Disease Ratings

The analysis of variance suggests that genotype was the most important source of variation for rust (*Puccinia emaculata*) resistance in this study (Table 25). No other factor was found to be a statistically significant source of variation (Table 25). The NJ and WI clones had the lowest ratings suggesting higher resistance among these clones to rust disease (Table 26). There were no significant differences among clones from these two breeding programs (Table 26). Additionally there were no significant differences between upland and lowland NJ ecotypes (Table 26) The SD clones exhibited high susceptibility to rust (Table 26). There were no significant differences between marginal vs. prime sites (data not shown). Cornelius and Johnston (1941) evaluated 34 accessions of switchgrass and concluded that the collections from North Dakota and Nebraska were extremely susceptible to rust fungus *U. graminicola*. They also observed that germplasm from lowland locations in Oklahoma and southern Texas was resistant to this pathogen. Previous studies have reported high heritability estimates and the presence of both additive and non-additive genetic variation for rust resistance in switchgrass (Eberhart and Newell, 1959; Gustafson et al., 2003; Newell and Eberhart, 1961). This indicates that selection programs to increase rust resistance in switchgrass may be conducted in single-environment trials because there is evidence supporting a strong genetic control of this trait in switchgrass (Steffenson et al., 1984).

Table 25. Analysis of variance of rust disease (*Puccinia emaculata*) ratings of 45 switchgrass clones from three breeding programs in NJ, SD and WI evaluated in SD on marginal vs. prime farmland with 0 or 100 kg of N·ha⁻¹.

Source	Df	F-value	Pr > F
SQ (Soil Quality)	1	0.53	0.5061
N (Nitrogen)	1	1.84	0.2462
SQ*N	1	3.66	0.1284
Clone (Genotype)	35	14.81	<.0001
SQ*Clone	35	1.3	0.1487
N*Clone	35	0.67	0.9108
SQ*N*Clone	35	1.07	0.3850

Table 26. Means rust disease (*Puccinia emaculata*) ratings of 45 switchgrass clones evaluated in South Dakota. Means were separated with LSMEANS option and adjusted with Tukey's method for multiple comparisons.

Mean Rust			Mean Rust			Mean Rust		
NJ Clones	Type	Disease Rating	SD Clones	Type	Disease Rating	WI Clones	Type	Disease Rating
5300-12NJ	L	1 EFGHI	SD9	U	7 A	WS08-K2	U	1 DEFGHI
5305-1NJ	U	1 EFGHI ¹	SD16	U	6 AB	WS08-U5	U	1 DEFGHI
5312-2NJ	L	1 GHI	SD2	U	6 AB	WS08-U1	U	1 EFGHI
9081-12NJ	U	0 EFGHI	SD1	U	5 AB	WS08-U3	U	1 EFGHI
9100-11NJ	L	0 GHI	SD5	U	5 ABC	WS08-U4	U	1 EFGHI
9145-11NJ	L	0 HI	SD7	U	5 AB	WS08-U2	U	1 FGHI
5215-6NJ	L	0 I	SD12	U	5 ABCD	WS08-R1	U	0 GHI
5300-1NJ	U	0 I	SD18	U	4 ABCD	WS08-K1	U	-
5300-4NJ	L	0 I	SD19	U	4 ABCD	WS08-K3	U	-
5300-8NJ	L	0 I	SD10	U	4 ABCDEFG	WS08-K4	U	-
5302-1NJ	U	0 I	SD13	U	4 ABCDE	WS08-K5	U	-
5305-7NJ	U	0 I	SD6	U	4 ABCDEF	WS08-R2	U	-
5305-10NJ	U	0 I	SD14	U	3 BCDEFGH	WS08-R3	U	-
5305-12NJ	U	0 I	SD8	U	2 CDEFGHI	WS08-R4	U	-
9137-11NJ	U	-	SD11	U	1 EFGHI	WS08-R5	U	-

¹ Disease rating means with the same upper case letter were not statistically different at 0.05 level of probability.

(-) Means were not calculated due to missing values.

A 0 to 9 (0 = least disease) visual rating scale was utilized (Gustafson et al., 2003).

Conclusions

Switchgrass has been described as a short-day species and there is plenty of evidence showing that the initiation of the reproductive cycle may be a function of genotype or ecotype, e.g., lowland types tend to remain vegetative longer in northern latitudes, thus growing taller and producing more biomass than upland types which tend to mature early. Lowland ecotypes from NJ were the latest to mature, thus these plants produced the most biomass. In this study it was also observed that environmental and soil conditions affect the initiation of flowering in switchgrass. Interestingly in marginal soils late maturing NJ clones performed better than the SD and WI clones.

Additionally, results from these studies also suggest that growing conditions, e.g., soil quality may also be an important factor in agronomic characteristics affecting biomass production. NJ clones exhibited significant differences in height and tiller density in prime vs. marginal soils, while WI and SD clones were not as affected by the environmental conditions. Even though the vegetative growth of NJ clones was significantly reduced in marginal soils compared prime soils, they still exhibited acceptable and occasionally above average growth and yields compared to SD and WI clones. The identification of superior clones in marginal environments and clones that exhibit broad adaptation across environments in this study indicates that cultivars can be developed with these characteristics. These results also indicate that crop improvement programs for switchgrass may benefit from conducting selection in multi-environment trials that include marginal sites and harsh environments that provide broad adaptation.

In this study it was observed that genotype was the most influential factor in switchgrass resistance to anthracnose (*Colletotrichum nativas*) and rust (*Puccinia emaculata*). Environmental conditions may be marginally influential on the incidence of anthracnose in switchgrass. This indicates that selection should be effective in improving anthracnose and rust resistance in switchgrass. However since these diseases were only evaluated at one site, anthracnose in NJ and rust in SD, it is unknown whether exposure to different isolates would result in a similar outcome. Additionally, the diversity of these two pathogens in switchgrass growing areas is not known so the variability in isolate response is unclear. Further research on the variability in genotype performance to these two pathogens is necessary to avoid fungicide applications and yield losses in the future.

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**CHAPTER 3: Bioenergy Characteristics of Native Warm-season Grass
Monocultures and Multi-Species Mixtures in Marginal vs. Prime Farmland in
New Jersey, New York and Pennsylvania**

Abstract

Several studies have pointed out the benefits of increasing the biodiversity of natural plant communities or agricultural systems to maintain stability and increase biomass yields. Additionally other studies have observed a reduction in fertilizer application when grass and legumes are cultivated in a mixture. The current study compares the biomass production of low, medium and high biodiversity communities (treatments) in marginal vs. prime farmland and aims to determine whether legumes may improve productivity of switchgrass without the addition of synthetic fertilizers. Three tall grass prairie species, big blue-stem (BB) (*Andropogon gerardii* Vitman) (Niagara), prairie cordgrass (PC) (*Spartina pectinata* Bosc ex Link) (Red River), and coastal panicgrass (PG) (*Panicum amarum* Elliott) (Atlantic) were compared in monocultures (low biodiversity treatment) to switchgrass (SG) (Cave-in-Rock). Three-way grass combinations (medium biodiversity treatment) that included switchgrass and grass/legume combinations (high biodiversity treatments), containing three grasses and one legume [showy ticktrefoil (DsC) *Desmodium canadense* L. or purple prairie-clover (DaP) *Dalea purpurea* Vent.], were also compared at all the locations. Overall 13 biodiversity treatments were seeded in 2008 or 2009 in three locations (New Jersey, New York and Pennsylvania) in paired fields (marginal vs. prime land). Each location had a nitrogen treatment of 0 or 100 kg of N·ha⁻¹·year⁻¹. In the year after establishment measurements of stand establishment (% coverage), plant height (cm) and dry biomass yield (Mg·ha⁻¹) to compare the performance of the

different biodiversity treatments. Switchgrass and big bluestem dominated the high biodiversity treatments plots in this study. The showy ticktrefoil had better establishment than the purple prairie-clover. Location and soil quality were the most influential factors in the establishment, plant height and dry biomass yields of the biodiversity treatments. Monoculture plots were not significantly different than the yields of high diversity treatment plots. These results suggest that increasing the number of species may not be detrimental to biomass yield and also indicate that there are environmental factors (soil and climate) that may influence the performance of low and high diversity plant communities and that multi-environmental trials should be conducted to determine the best combination of species that yield more biomass.

Introduction

Native perennial grasses have been utilized extensively as an energy source in the U.S., since the 1920s as feed for traction animals in farms and nowadays these species are cultivated as biomass feedstock for conversion into ethanol, heat and electricity (Lewandowski et al., 2003; Vogel, 1996). One of the advantages of cultivating perennial C₄ grasses, for biomass production, relies on their efficient photosynthetic pathways which enables them to produce more biomass with higher cellulose contents than annual crops (Clifton-Brown and Jones, 1997; Christian, 1994). Another advantage is that soil tillage is not required after the establishment year, enabling the development of extensive root systems which may reduce risks of soil erosion (Tilman et al., 2006). Additionally sequestering of carbon from the environment and confining it into soil organic matter is another important advantage in C₄ grass cultivation (Ma et al., 1999).

In 1979 the U.S. Department of Energy, through the Oak Ridge National Laboratory, established the Herbaceous Energy Crop Research Program (HECRP) for research on perennial and annual herbaceous plant material as energy crops that could be economically cultivated on a wide array of locations and without difficulty incorporated into conventional farming operations (Lewandowski et al. 2003). From the initiative of the HECRP several perennial C₄ species, native to North America, have been studied as energy crops, among them are big bluestem (*Andropogon gerardii* Vitman), and switchgrass (*Panicum virgatum*), which now is considered as a model crop for biomass production. Other C₄ species that are important in habitat restoration programs, such as, coastal panicgrass (*Panicum amarum* Elliott) and prairie cordgrass (*Spartina pectinata* Bosc ex Link) have been researched to a lesser

extend as energy crops (Boe and Lee, 2007; Christian et al., 2002; Gonzalez-Hernandez et al., 2009). There are studies suggesting that in monocultures some of these C₄ species, especially switchgrass, can produce high amounts of biomass but more research is required to understand if biomass production could benefit from multi-species plantings (Lewandowski et al., 2003).

There is plenty evidence that increasing plant species diversity is necessary to maintain a multifunction agricultural system that not only may provide biomass for energy but it may be a sustainable system (Groom et al., 2007; Hector and Bagchi, 2007; Johnson and Runge, 2007; Sanderson et al., 2004). An increase in the number of species in grassland plant communities has been linked to stability in response to environmental disturbance (McNaughton, 1977; Frank and McNaughton, 1991; Tilman and Downing, 1994); reduction in the amount of unwanted species through the better utilization of available resources by the established species (Naeem et al., 2000; Tilman, 1997; Tracy and Sanderson, 2004a), and enhanced nutrient retention (Tilman et al., 1996). However if improving biomass yield for energy production is the primary objective, functional diversity must be achieved and the utilization of a select group of plant species that have been studied to work well together is imperative (Tracy and Sanderson 2004b).

Jung et al. (1990) studied the effects of N fertilization (0 or 75 Kg·Ha⁻¹) on yield and plant morphology of warm-season grasses in Pennsylvania. Big bluestem and switchgrass were among the species studied. The result suggested that stand establishment was highly variable among cultivars of these two species. They also reported that, under northeastern hill environments, big bluestem cultivar Niagara and switchgrass cultivar Carthage (NJ-50) persisted well for 9 years without addition of

soil amendments; however it was observed that fertilization of this plant material increased biomass yield. Additionally mixtures of these two C₄ grasses harvested once a year after a killing frost have been shown to produce great amounts of biomass with high concentration of lignocellulose and low concentrations of total nitrogen and ash (Mulkey et al., 2008).

Coastal panicgrass (*Panicum amarum* Elliott) is similar in morphology and adaptability to switchgrass (*Panicum virgatum* L.) (Aldreson and Sharp, 1994). Christian et al. (2002) tested the suitability of these two C₄ species as energy crops for the UK. Switchgrass and coastal panicgrass grew successfully in Southern England and the biomass yields were comparable; however maximum yields had not been reached by the end of the study. They also reported that both species were susceptible to lodging and that a delay in harvest resulted in reduced concentration of certain minerals in the harvested material as well as less biomass production. In northern regions, such as, South Dakota in the U.S. and South-Western Quebec in Canada, prairie cordgrass have been shown to produce comparable amount of biomass to lower-latitudes switchgrass populations with the advantage of tolerance to the winter temperatures of those regions (Boe and Lee, 2007; Madakadze et al., 1998). Further research should concentrate in determining if multi-species planting with these C₄ species could favor higher biomass production.

The cultivation of legumes along with grasses for forage has also been shown to provide several advantages over grass monocultures. Baylor (1974) observed that incorporating legumes generally resulted in increased yield and higher quality forage. Grass/legume mixtures have led to greater stand longevity than grass or legume monocultures, which may be related to the nitrogen fixation characteristics of

legumes, as well as reduced weed invasions and soil erosion (Drosom and Smith, 1976). However, the level of success of grass/legume mixtures depends on the compatibility of the associated species, not only with respect to the aboveground biomass growth habit, but also with respect to the root system interrelationships (Papadakis, 1941; Virtanen and Hausen. 1935). Harmful root interrelationships may be due to various phenomena, such as, competition for nutrients and water as well as the excretion of toxic substances detrimental to other species growth. (Ahlgren and Aamodt, 1939; Loehwing, 1937). Successful grass/legume mixture may suppress weed growth and improve biomass yield by up to 73% (Picasso et al., 2008). Purple prairie-clover (*Dalea purpurea* Vent. syn. *Petalostemon purpureum*), native to the Midwest, have been shown to enhance switchgrass biomass yield but did not influence *in vitro* dry matter digestibility (IVDMD) (Posler et al., 1993). Showy ticktrefoil (*Desmodium canadense* L.), also a legume native to North America, which has been used during meadow restoration efforts but has been less studied as a companion to energy crops, may also have characteristics that could improve soil conditions to increases switchgrass biomass production (Olszewski et al., 2010). However, the ability of grass/legume mixtures to persist stably in combination is a factor that must be considered in these systems because at times dominant species tend to outcompete and displace weaker species which may lead to renovation of the pasture and additional costs for the grower (Springer et al., 2001).

Tilman et al. (2006) conducted a decade long study comparing the bioenergy productions of monocultures (1 species), low-diversity (2, 4 and 8 species) and high-diversity (16 species) mixtures of native grassland perennials with low-agrochemical input and on agriculturally degraded and abandoned nitrogen-poor sandy soils. They

reported that high-diversity mixtures consistently outperformed monocultures in bioenergy productions. They also observed that the bioenergy produced from high-diversity mixtures plots were carbon negative because net ecosystem carbon dioxide sequestration exceeded fossil carbon released during conversion to biofuel.

The current study compares the biomass production of native warm-season grass monocultures and multi-species mixtures than include native legumes in marginal vs. prime farmland. The information obtained through this experiment may assist us in identifying optimum grasses mixtures or grass/legume mixture for biomass production in different environments. Additionally we aim to determine whether legumes may improve productivity without the addition of synthetic fertilizers.

Materials and Methods

Plant Material and Soil type

Three tall grass prairie species, big blue-stem (BB) (*Andropogon gerardii* Vitman) (Niagara), prairie cordgrass (PC) (*Spartina pectinata* Bosc ex Link) (Red River), and coastal panicgrass (PG) (*Panicum amarum* Elliott) (Atlantic) were compared in monocultures to switchgrass (SG) (Cave-in-Rock) stands (Table 1). In addition to monocultures, three-way grass biodiversity treatments were also compared, all three-way combinations included switchgrass (Table 1). Finally grass/legume biodiversity treatments, containing three grasses and one legume, were also compared at all the locations (Table 1). The native legume species utilized in this study were: showy ticktrefoil (DsC) (*Desmodium canadense* L.), common in the upper Midwest and can perform well in the east, and purple prairie-clover (DaP)

(*Dalea purpurea* Vent. syn. *Petalostemon purpureum*) also common in the upper Midwest.

Table 1. Native grass and legume biodiversity treatments evaluated in marginal vs. prime farmland in three U.S. states New Jersey (NJ), New York (NY) and Pennsylvania (PA).

Grass Monocultures	3-Grass Mixtures	Grass/Legume Mixtures[§]
Switchgrass ‘Cave-in-Rock’	SG + BB + PC	SG + BB + PC + DsC
Big bluestem ‘Niagara’	SG + BB + CP	SG + BB + CP + DsC
Prairie cordgrass ‘Red River’	SG + PC + CP	SG + PC + CP + DsC
Coastal panicgrass ‘Atlantic’		SG + BB + PC + DaP
		SG + BB + CP + DaP
		SG + PC + CP + DaP

Abbreviation of species: SG, switchgrass; BB, big blue-stem; PC, prairie cordgrass; PG, coastal panicgrass; DsC, showy ticktrefoil and DaP, purple prairie-clover.

The study was conducted in paired fields (marginal vs. prime farmland) in three states: New Jersey (NJ), New York (NY), and Pennsylvania (PA). The soil types were: 1) the marginal site at Somerset, NJ was a Class IV, Kleinsville shale with 0-2% slope (limiting factor nutrient deficiency and poor water-holding capacity) and the prime site was located at the Rutgers Plant Science Research and Extension Farm in Adelphia, NJ which was a Class II, Freehold sandy loam with 2-5% slope; 2) the marginal site at Syracuse, NY was a Class IV, Honeoye and Lasing gravelly silt loam with 15-25% slope (limiting factor the slope steepness and erosion) and the prime site in NY was a Class II Conesus gravelly silt loam; 3) the marginal site at Rock Springs, PA was a Class IV, Andover channery silt loam with 0-15% slope (limiting factor restrictive layer in fragipan, poor water-holding capacity and soil acidity) and the prime site was a Class I,II, Hagerstown silt loam with 0-8% slope. Each paired location also had a nitrogen treatment of 0 or 100 kg of nitrogen (N)·ha⁻¹·year⁻¹. Urea (46-0-0) or ammonium nitrate (33-0-0) was used as a nitrogen source and the fertilizer was applied during the spring of the second year after establishment.

Stratified and non-stratified seed lots from each entry were sent to the Ohio Seed Improvement Association (Co-PI) for germination tests. Based on those results grass seeding rates were calculated and standardized to approximately 11.2 kg of Pure Live Seed·ha⁻¹ (PLS) or (10 lb. of PLS·acre⁻¹). Three-way grass mixtures were seeded at equal amounts of each species by seed and for the grass/legume mixtures the two native legume species were added to the grass mixtures at 2.2 kg of PLS·ha⁻¹ or (2 lb. of PLS·acre⁻¹). Grass and legume seeds were provided by Ernst Conservation Seeds (Meadville, PA).

The research plots were 1.8 x 1.8 m and were arranged in a randomized complete block design with three replications. The plots were seeded during the spring of 2008 at all the locations. Satisfactory establishment was obtained at the NY prime and marginal sites; however because of poor establishment at the NJ and PA at the marginal sites new plots had to be seeded in 2009 in both paired fields. Poor germination of the prairie cordgrass plots followed by other species contamination resulted in removing the mono-stand prairie cordgrass plots from the analyses.

Data Collection

Stand establishment was recorded by species the following spring after seeding using a 0.75 x 0.75 m frequency grid (Vogel and Masters, 2001). For this study, the frequency grid was a metal frame with 25 squares (5 x 5) made out of re-bar and painted orange for better visibility (Chap.1; Fig. 1). The frequency grid was systematically placed within the research plot. After placing the grid on the plot, the researcher counted the number of cells containing 1 or more seedlings of the species included in the biodiversity treatment, flipped the frame end-over-end and counted again. The process was repeated until a total of 100 cells were counted within the seeded plot area. The counts were converted into a percentage of plot coverage by dividing the total number of cells that contained at least 1 seedling by 100. These percentages of coverage were utilized in the statistical analysis.

In the year after establishment plant height (cm) and dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) were recorded to compare the performance of the monocultures vs. the species combination treatments. Plant height was not recorded by species. It was measured at maturity by placing a measuring stick in three random spots within the seeded plot. The researcher recorded the height from the soil surface to the top of the

panicles in each random spot (Chap. 1; Fig 2). The three measurements were averaged per plot. This average was used for the statistical analysis. Plant height data using the method described above was reported from NJ and NY. The plant height data from PA was not utilized because it was not taken from all the biodiversity treatments.

Dry biomass yield was determined by harvesting a 0.30 x 1.8 m strip from the 1.8 x 1.8 m research plot with a two-wheel tractor with a sickle bar mower attachment (BCS, Hector, NY) (Chap1; Fig. 4). The fresh weight of the field sample was recorded and subsamples were placed in a paper bags (S-9621) (U-line, U.S.). The fresh weight subsamples were recorded and then they were placed in a tobacco dryer at 43°C for 10 days (Chap.1; Fig 6). The fresh and dry weights of the subsamples were used to calculate moisture content which was then subtracted from the fresh weight of the field samples. The adjusted weight of the field samples was converted to $\text{Mg}\cdot\text{ha}^{-1}$ and used for the statistical analysis.

Data Analysis

The experiment was arranged in a split block plot design. The locations, sites and the replications within sites were the blocks while the nitrogen treatment was the main plot and the biodiversity treatments were the split plot; however only the replications were considered random effects. The analysis of variance was performed with the MIXED procedure in SAS (SAS Institute, Cary, NC). The means were separated with the LSMEANS option and adjusted with Tukey's method for multiple comparisons. The PDMIX800 macro was utilized to convert mean separation output to letter groupings in the MIXED procedure (Saxton, 2003).

The biodiversity treatments: prairie cordgrass monoculture; coastal panicgrass monoculture, and the 3-grass mixture that contained both species, had poor establishment in NJ which translated into missing values for the rest of agronomic traits measured. Therefore, these combination treatments were not included in the statistical analysis.

Results and Discussion

Stand Establishment

Stand establishment was measured before the nitrogen fertilizer treatments were applied, thus the nitrogen treatment was not included in the experimental design to analyze this response variable. According to the ANOVA table, all the sources of variation for stand establishment were statistically significant ($P < 0.05$); however the location and the location x soil quality interaction accounted for the largest portion of the variance in stand establishment (Table 2). Generally warm-season perennial grasses are difficult to establish (Coukos, 1944; Moser, 2000; Robocker et al., 1953). The germination and establishment potential of warm-season perennial grasses can be affected by factors inherent in the species, such as, seed size (Aiken and Springer, 1995; Smart and Moser, 1999); seedling morphology (Elbersen et al., 1999; Rischler and Monk, 1980; Tischler and Voigt, 1981) and degree of seed dormancy in the species (Coukos, 1944; Mullen et al., 1985); as well as, environmental conditions e.g., soil texture, temperature and moisture (Hsu and Nelson, 1986; Robocker et al., 1953; Stout et al., 1986; Vassey et al., 1985). The results in this study indicate that genotype x environment interactions may have played a significant role in the variation of stand establishment.

Table 2. Analysis of variance of stand establishment (% coverage) of 13 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location. Research plots were established in 2008 in NY and in 2009 in NJ and PA.

Source	Df	F-value	Pr > F
Location (State)	2	1025.74	<.0001
SQ (Soil Quality)	1	84.56	<.0001
Loc*SQ	2	548.15	<.0001
Tmt (Species)	12	69.47	<.0001
Loc*Tmt	24	22.25	<.0001
SQ*Tmt	12	12.24	<.0001
Loc*SQ*Tmt	24	9.24	<.0001

Establishment was better in prime land sites in NJ (69% prime vs. 25% marginal) and PA (72% prime vs. 69% marginal) but not in NY (12% prime vs. 36% marginal) (Table 3). NY prime site had the poorest stand establishment and PA prime site had the best establishment, 12% and 72% respectively (Table 3). The NY prime site had significant weed competition during the establishment year which accounted for the poor establishment at this location. Within locations, the differences between marginal vs. prime sites were significant only in NJ and NY (Table 3). Monocultures of big bluestem and switchgrass had the best establishment across locations (Table 3). According the means of stand establishment calculated by species, the big bluestem monoculture plots established better than the switchgrass plots (74% vs. 65.7%); however there was a lot of variation in the raw data as noted by the high standard deviations (SE) (Table 4). Switchgrass and big bluestem performed similarly when cultivated in 3-grass and grass/legume treatment combinations and were the dominant species compared to the other grasses and legumes (Table 4). Switchgrass and big bluestem were well-established species across in the U.S. northeast before European settlers arrived, which may indicate the genotypes of these species may have adapted without difficulty to the growing conditions in this trials (Jung et al., 1985; Jung et al., 1988).

Coastal panicgrass did not establish well in NY and prairie cordgrass did not establish at all in NJ, thus percent coverage of the monocultures, 3-grass mixture and grass/legume mixtures that included both species simultaneously were 40% or lower. According the means of stand establishment calculated by species the panic grass monoculture plots established better than the prairie cordgrass plots (28.63% vs. 19.54%); however there was a lot of variation in the raw data as noted by the high

standard deviations (SE) (Table 4). Slow germination and slow seedling development are characteristic of C₄ grass species such as, prairie cordgrass and panic grass (Knapp, 2000). This combined with weed pressure may have been a major reason for poor stand establishment at both locations NJ and NY. Weed competition have been shown to significantly affect stand establishment of warm-season grasses (Cox and McCarty, 1958; Martin et al., 1982; Masters et al., 1996; Moser, 2000).

In the grass/legume biodiversity treatments, plots with showy tick-trefoil established better than the plots with purple prairie clover with total ground cover percentages between 45 to 48% vs. 4 to 6%, respectively (Table 5). The legumes utilized in this study are native of the Midwest and are used during land restoration efforts (Olszewski et al., 2010; Posler et al., 1993). However like many perennial grassland legumes these two species are also susceptible to weed competition, due to their slow rate of seedling growth compared with that of annual weeds (Beran et al., 1999; Linscott and Hagin, 1974; Moyer et al., 1989; Olszewski et al., 2010). The results of this trial may indicate that the even though the switchgrass and big bluestem had a slow development ultimately environmental and/or soil conditions may have favor them when competition for resources occurred in the stand, thus leading to higher coverage percentages compared to the legumes (Table 3, 4 and 5) (Blanchet et al., 1995; Springer et al., 2001). This was not the case for prairie cordgrass and panic grass which performed poorly (Table 3 and 4).

The ANOVA table indicated that soil quality may have been an influential factor on stand establishment (Table 2). It was observed that seven of the 13 biodiversity treatments were affected by the differences in soil quality: two of the monoculture stands (switchgrass and panic grass); one of the 3-grass mixture

(switchgrass + big bluestem + coastal panicgrass), and 4 of the grass legume mixtures (2 that contained showy tick-trefoil and 2 that contained purple prairie-clover) (Table 6). Only switchgrass monoculture established better in marginal than in prime land (Table 6). This species is well known for its wide adaptation; tolerance for unfavorable soil conditions, and for its use in trials focused on recovering difficult-to-vegetate sites (Parrish and Fike, 2005; Zak, 1977).

For most of the combination treatments that included prairie cordgrass, differences in establishment between marginal and prime land, were not significant (Table 6). However, this species had poor establishment in NY prime site and did not establish at all in NJ, this most likely affected the ground cover percentage data (Table 3 and 4). Four grass/legume biodiversity treatments were affected by soil quality and had coverage percentages lower than 45% in marginal land (Table 6). These results may indicate that biodiversity treatments that include grasses and legumes may need to be tested further in marginal soils because these two species may be susceptible to competition from the grasses and/or weeds (Beran et al., 1999; Olszewski et al., 2010). On the other hand, big bluestem monocultures were not affected by soil quality suggesting that this species may have wide adaptation to different soil types comparable to switchgrass (Table 5) (Jung et al., 1990; Moser and Vogel, 1995).

Table 3. Stand establishment (% coverage) of 13 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.

		Stand Establishment (% coverage)						
		NJ		NY		PA		Mean
		Adelphi	Somerset	Syracuse		Rock Springs		Across
Species	Biodiversity Treatment	Prime	Marginal	Prime	Marginal	Prime	Marginal	Location
Big bluestem	Monoculture	97.50	65.50	52.67	81.67	76.33	78.83	75.42 A ¹
Switchgrass	Monoculture	98.50	56.50	10.50	97.33	71.17	65.17	66.53 B
SG/BB/PG	3-Grass mixture	86.28	35.67	16.83	31.22	76.00	73.67	53.28 C
SG/BB/PG/DaP	Grass/legume mixture	84.89	27.94	12.78	31.61	76.67	74.33	51.37 CD
SG/BB/PG/DsC	Grass/legume mixture	88.33	26.06	12.83	25.56	78.33	74.67	50.96 CDE
SG/BB/PC	3-Grass mixture	60.22	26.89	10.28	31.28	74.33	71.67	45.78 DEF
SG/BB/PC/DsC	Grass/legume mixture	58.00	36.22	8.56	23.28	77.67	68.83	45.43 DEF
SG/BB/PC/DaP	Grass/legume mixture	59.56	22.56	12.89	31.11	71.50	67.33	44.16 EF
Panic grass	Monoculture	95.17	12.17	0.50	6.67	64.83	65.50	40.81 F
SG/PC/PG	3-Grass mixture	56.61	6.11	10.83	28.06	68.83	69.83	40.05 F
SG/PC/PG/DaP	Grass/legume mixture	57.44	8.33	5.50	22.00	74.17	71.17	39.77 F
SG/PC/PG/DsC	Grass/legume mixture	56.72	9.22	6.50	22.94	74.00	67.00	39.40 F
Prairie cordgrass	Monoculture	0.00	0.00	3.67	41.67	60.33	52.33	26.33 G
Mean		69.17a ²	25.63c	12.64d	36.49b	72.63a	69.26a	

¹ Cultivar means with the same upper case letter were not statistically different at 0.05 level of probability.

² Location means with the same lower case letter were not statistically different at 0.05 level of probability.

Table 4. Calculated means and standard deviations (SE) of stand establishment of 13 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location.

Species	Biodiversity Treatment	SG		BB		PC		PG		DsC		DaP	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Switchgrass	Monoculture	65.71	±8.16										
Big bluestem	Monoculture			74.33	±10.12								
Prairie cordgrass	Monoculture					19.54	±11.71						
Panic grass	Monoculture							28.63	±4.89				
SG/BB/PC	3-Grass mixture	48.13	±3.61	47.75	±11.50	0.63	±0.45						
SG/BB/PG	3-Grass mixture	54.58	±8.41	46.63	±10.45			22.79	±1.62				
SG/PC/PG	3-Grass mixture	53.33	±9.65			1.46	±1.33	21.33	±1.43				
SG/BB/PC/DsC	Grass/legume mixture	46.96	±5.56	46.71	±3.93	0.83	±0.79			39.58	±4.36		
SG/BB/PG/DsC	Grass/legume mixture	47.96	±3.69	43.75	±7.96			22.88	±2.46	38.38	±7.46		
SG/PC/PG/DsC	Grass/legume mixture	47.29	±5.34			1.50	±1.44	22.75	±1.08	35.17	±8.18		
SG/BB/PC/DaP	Grass/legume mixture	46.58	±5.56	47.50	±6.16	0.50	±0.32					4.29	±0.55
SG/BB/PG/DaP	Grass/legume mixture	47.79	±5.47	48.75	±12.41			21.42	±2.72			5.42	±2.89
SG/PC/PG/DaP	Grass/legume mixture	46.42	±6.40			1.46	±1.38	22.08	±2.29			5.08	±2.28

Table 5. Stand establishment (% coverage) of 6 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.

Biodiversity Treatment		Stand Establishment (% coverage)						Mean Across Location
		NJ		NY		PA		
		Adelphia	Somerset	Syracuse		Rock Springs		
Species		Prime	Marginal	Prime	Marginal	Prime	Marginal	
SG/BB/PC/DsC	<i>Desmodium canadense</i>	81.50	42.00	18.17	11.83	67.83	67.50	48.14 A ¹
SG/BB/PG/DsC	<i>Desmodium canadense</i>	76.83	57.00	11.83	12.67	66.00	57.00	46.89 A
SG/PC/PG/DsC	<i>Desmodium canadense</i>	80.33	31.50	16.17	15.33	64.00	65.50	45.47 A
SG/BB/PC/DaP	<i>Dalea purpurea</i>	18.50	3.17	0.00	0.00	20.50	4.17	7.72 B
SG/BB/PG/DaP	<i>Dalea purpurea</i>	19.33	1.00	0.00	0.00	17.17	6.33	7.31 B
SG/PC/PG/DaP	<i>Dalea purpurea</i>	14.83	0.67	0.17	0.00	11.83	4.00	5.25 B

¹ Cultivar means with the same upper case letter were not statistically different at 0.05 level of probability.

Table 6. Comparison of stand establishment (% coverage) means sorted by biodiversity treatment in marginal vs. prime farmland sites across three locations.

Species	Biodiversity Treatment	Mean Stand Establishment (% coverage)		
		Prime	Marginal	
Switchgrass	Monoculture	60.06	73.00	*
Big bluestem	Monoculture	75.50	75.33	NS
Prairie cordgrass	Monoculture	21.33	31.33	NS
Panic grass	Monoculture	53.50	28.11	*
SG/BB/PC	3-Grass mixture	48.28	43.28	NS
SG/BB/PG	3-Grass mixture	59.70	46.85	*
SG/PC/PG	3-Grass mixture	45.43	34.67	NS
SG/BB/PC/DsC	Grass/legume mixture	48.07	42.78	NS
SG/BB/PG/DsC	Grass/legume mixture	59.83	42.09	*
SG/PC/PG/DsC	Grass/legume mixture	45.74	33.06	*
SG/BB/PC/DaP	Grass/legume mixture	47.98	40.33	NS
SG/BB/PG/DaP	Grass/legume mixture	58.11	44.63	*
SG/PC/PG/DaP	Grass/legume mixture	45.70	33.83	*

Cultivar means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

Plant Height

Plant height was not recorded by species but was reported as an average of three measurements in the plots in NJ and NY. Heights were not recorded for all the biodiversity treatments in PA so the data from PA was not included in the statistical analysis. The biodiversity treatments: prairie cordgrass monoculture; coastal panicgrass monoculture, and the 3-grass mixture that contained both species, were not included in the statistical analysis because of poor establishment which translated into missing data (Table 8, 9 and 10).

Soil quality had the largest effect on plant height followed by the interaction effect of location x soil quality (Table 7). The sources of variations such as, location, nitrogen treatment, biodiversity treatment and some two- and three-way interaction effect were statistically significant ($P < 0.05$); however this sources accounted for a small fraction of the variance in the ANOVA (Table 7). These results suggests that genotype x environment interactions may affect the performance of high-biodiversity plant communities cultivated for biomass production and that more research should be conducted in multi-environment trials to have a better understanding of the potential of high-diversity plant communities to supply biomass.

On average switchgrass monoculture plots had the tallest plants (102 cm) in this study while big bluestem monoculture plots had the shortest plants (90 cm) (Table 8). Though, the differences in plant height among several of biodiversity treatments were not statistically significant ($P < 0.05$) (Table 8). According to the letter groupings there was no difference on plant height between the plots with the grass/legume biodiversity treatments (Table 8). Big bluestem had better stand

establishment than switchgrass, however this results may indicate that switchgrass has a more rapid development or it is better adapted to the environmental conditions (Jung et al., 1985; Madakadze et al., 1998).

Only switchgrass monocultures showed a significant difference in plant height due to location (94.8 cm in NJ vs. 109.8 cm in NY), all other treatments were not significantly different in NJ vs. NY (data not shown). On average, plants grew approximately 48% taller in prime land sites compared to marginal sites (Table 9). Tilman et al. (2006) observed that high-diversity plots of native grasses perform better than monocultures in agriculturally degraded and nitrogen poor sandy soil; however the trials were conducted only in one location and the plots were burned down after removing a small area to measure biomass, thus minerals may have been replenished improving the fertility in the soil (Gonzalez-Hernandez et al., 2009; Russelle et al., 2007).

Even though, the ANOVA table suggested that the nitrogen treatment was a statistically significant ($P < 0.05$) source of variation; it only accounted for a very small portion of the variance (Table 7). When the heights of nitrogen-fertilized plots were compared to non-fertilized plots there were no significant ($P < 0.05$) differences found (Table 10). There were no differences observed in plant height between fertilized grass-only plots and unfertilized grass/legume plots (Table 10). There are several research studies providing evidence that moderate nitrogen fertilization may enhance biomass production in perennial native grasses (Bredja et al., 1994; Mulkey et al., 2008). Incorporating legumes into grass dominated land has generally resulted in increased yield and higher quality forage (Baylor, 1974). Grass/legume mixtures have

led to greater stand longevity than grass or legume monocultures, which may be related to the nitrogen fixation characteristics of legumes, as well as reduced weed invasions and soil erosion (Drosom and Smith, 1976). However nitrogen availability in the soil is subject to variation due to environmental and soil conditions such as temperature (Craswell and Godwin, 1984); annual rainfall (Sala et al., 1988); soil pH (Bohn et al., 1979), and water-holding capacity (Staley et al., 1991). The results concerning the nitrogen treatment indicate there may be issues with timing of nitrogen applications and/or nitrogen fixation by legumes, availability of nitrogen in the soil or a delay in the response to nitrogen fertilization by perennial grasses (Bredja, 2000; Jung et al., 1990).

Table 7. Analysis of variance of plant height (cm) of 13 warm-season grass/legume biodiversity treatments evaluated at two locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha⁻¹.

Source	Df	F-value	Pr > F
Location (State)	1	5.78	0.0429
SQ (Soil Quality)	1	911.41	<.0001
Loc*SQ	1	480.02	<.0001
N (Nitrogen)	1	21.51	0.0017
Loc*N	1	31.81	0.0005
SQ*N	1	33.23	0.0004
Loc*SQ*N	1	0.92	0.3663
Tmt (Species)	9	5.81	<.0001
Loc*Tmt	9	3.14	0.0031
SQ*Tmt	9	2.82	0.0069
Loc*SQ*Tmt	9	4.23	0.0002
N*Tmt	9	1.56	0.1478
Loc*N*Tmt	9	0.82	0.5970
SQ*N*Tmt	9	0.87	0.5581
Loc*SQ*N*Tmt	9	1.07	0.3945

Table 8. Means of plant height (cm) of 13 warm-season grass/legume biodiversity treatments evaluated at two locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha⁻¹. Means were averaged across locations, soil quality and nitrogen treatments. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.

		Plant Height (cm)								Mean [§] Across Locations	
		NJ				NY					
		Adelphia		Somerset		Syracuse					
		Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal		
Species	Biodiversity Treatment	0	100	0	100	0	100	0	100		
Switchgrass	Monoculture	145.44	147.56	40.00	46.44	118.25	89.55	115.71	115.71	102.33	A ¹
SG/PC/PG/DaP	Grass/legume mixture	150.67	146.11	30.13	52.13	112.40	103.41	99.48	107.46	100.22	AB
SG/BB/PG/DaP	Grass/legume mixture	148.11	140.78	39.11	42.56	116.56	99.77	99.48	82.97	96.17	ABC
SG/BB/PG	3-Grass mixture	147.33	144.00	42.00	38.96	115.85	96.73	93.84	90.45	96.15	ABC
SG/BB/PC/DaP	Grass/legume mixture	149.78	144.89	36.11	43.33	113.17	100.47	82.03	80.76	93.82	BC
SG/BB/PC	3-Grass mixture	135.00	141.22	40.78	45.56	116.65	98.43	90.59	81.47	93.71	BC
SG/PC/PG/DsC	Grass/legume mixture	142.00	146.33	38.67	50.34	109.36	85.75	79.59	80.10	91.52	C
SG/BB/PG/DsC	Grass/legume mixture	147.89	139.89	39.22	40.56	115.62	91.49	78.27	77.71	91.33	C
SG/BB/PC/DsC	Grass/legume mixture	149.56	128.00	39.67	41.67	111.56	95.44	82.77	76.60	90.66	C
Big bluestem	Monoculture	136.67	133.78	37.11	42.18	110.63	100.75	85.80	77.05	90.50	C
Prairie cordgrass	Monoculture	-	-	-	-	110.07	99.20	90.03	74.51	+	
Panic grass	Monoculture	152.11	151.00	48.00	-	119.23	-	82.42	88.90	+	
SG/PC/PG	3-Grass mixture	150.89	148.67	50.78	-	108.30	87.21	99.32	86.36	+	
Mean [§]		145.24a ²	141.26a	38.28d	44.37d	114.00b	96.18c	90.76c	87.03c		

¹ Cultivar means with the same upper case letter were not statistically different at 0.05 level of probability.

² Location means with the same lower case letter were not statistically different at 0.05 level of probability.

- Means were not calculated due to missing values.

+ Cultivars means were not separated by LSMEANS due to missing values.

Table 9. Means of plant height (cm) of 13 warm-season grass/legume biodiversity treatments evaluated at two locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha⁻¹. Means were averaged across locations and nitrogen treatments and compared by soil quality. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.

Species	Biodiversity Treatment	Mean Plant Height (cm)		
		Prime	Marginal	
Switchgrass	Monoculture	94.86	109.81	*
Big bluestem	Monoculture	87.43	93.56	NS
Prairie cordgrass	Monoculture	-	-	
Panic grass	Monoculture	-	-	
SG/BB/PC	3-Grass mixture	90.64	96.78	NS
SG/BB/PG	3-Grass mixture	93.07	99.22	NS
SG/PC/PG	3-Grass mixture	-	-	
SG/BB/PC/DsC	Grass/legume mixture	89.72	91.59	NS
SG/BB/PG/DsC	Grass/legume mixture	91.89	90.77	NS
SG/PC/PG/DsC	Grass/legume mixture	94.34	88.70	NS
SG/BB/PC/DaP	Grass/legume mixture	93.53	94.11	NS
SG/BB/PG/DaP	Grass/legume mixture	92.64	99.70	NS
SG/PC/PG/DaP	Grass/legume mixture	94.76	105.69	NS

Cultivar means significantly (*) or not significantly (^{NS}) different in marginal vs. prime soil at 0.05 level of probability.

Table 10. Means of plant height (cm) of 13 warm-season grass/legume biodiversity treatments evaluated at two locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of N·ha⁻¹. Means were averaged across locations, soil quality and compared by nitrogen treatment. Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.

Species	Biodiversity Treatment	Plant Height (cm)	
		Nitrogen Treatment (kg of N·ha ⁻¹)	
		0	100
Switchgrass	Monoculture	104.85 A ¹	100.22 ABC
Big bluestem	Monoculture	92.55 ABCD	88.58 BCD
Prairie cordgrass	Monoculture	-	-
Panic grass	Monoculture	100.44	-
SG/BB/PC	3-Grass mixture	95.50 ABCD	91.67 ABCD
SG/BB/PG	3-Grass mixture	99.76 ABC	92.53 ABCD
SG/PC/PG	3-Grass mixture	102.32	-
SG/BB/PC/DsC	Grass/legume mixture	95.89 ABCD	85.43 D
SG/BB/PG/DsC	Grass/legume mixture	95.25 ABCD	87.41 CD
SG/PC/PG/DsC	Grass/legume mixture	92.40 ABCD	90.61 BCD
SG/BB/PC/DaP	Grass/legume mixture	95.27 ABCD	92.36 ABCD
SG/BB/PG/DaP	Grass/legume mixture	100.82 AB	91.52 ABCD
SG/PC/PG/DaP	Grass/legume mixture	98.37 ABCD	101.97 AB

¹ Cultivar means with the same upper case letter were not statistically different at 0.05 level of probability.

Dry Biomass Yield

Dry biomass yield was not recorded by species. The biodiversity treatments: prairie cordgrass monoculture; coastal panicgrass monoculture, and the 3-grass mixture that contained both these species, were not included in the statistical analysis because of poor establishment which translated into missing data. Location x soil quality interaction was the most influential factor in the variation of dry biomass yield (Table 16). According to the analysis of variance nitrogen treatment and the interaction of location x nitrogen treatment were statistically significant ($P < 0.05$), but they accounted for a smaller fraction of the variance (Table 16). The biodiversity treatment was not a significant ($P < 0.05$) source of variation for biomass yield, as there were not significant ($P < 0.05$) differences among species mixtures (Table 16 and 17). There is evidence that increasing biodiversity or species richness in nutrient-poor grassland systems may enhance biomass production (Hector et al., 1999). However, in nature, plant communities in nutrient-rich soils are most productive when there is low species diversity (Grime, 2001). This suggests that biodiversity-productivity relationship may be affected by environmental interactions and more comparative studies should be conducted focused on the effects of marginal and prime soils on biomass production of high-diversity systems.

In New Jersey dry biomass yields were above $6.5 \text{ Mg} \cdot \text{ha}^{-1}$, on average, in the prime sites compared to approximately $2 \text{ Mg} \cdot \text{ha}^{-1}$ in the marginal sites (Table 17). Conversely in New York plots in the prime sites yielded $\sim 2.5 \text{ Mg} \cdot \text{ha}^{-1}$ of dry biomass compared to plots in marginal sites that yielded $\sim 7 \text{ Mg} \cdot \text{ha}^{-1}$ of dry biomass (Table 17). Dry biomass yields did not vary in PA due to soil quality (Table 17). These

results are similar to the results for stand establishment (Table 3). Previous switchgrass studies have observed higher biomass production with better stand establishment (Casler et al., 2007; Cassida et al., 2005; Schmer et al., 2006). Hector et al. (1999) observed high variations in yield responses due to location; however their analysis was not powerful enough to reveal significant location x species richness interaction when each location was analyzed. There is evidence that a range of species may respond differently to different environmental conditions (Hooper et al., 2005; Nyfeler et al., 2009). More experimental trials may be needed to understand the effects of the environment and/or species x environment interactions on biomass production of high biodiversity plant communities.

When comparing prime vs. marginal sites by biodiversity treatment, it was observed that switchgrass monocultures yielded the highest dry biomass ($4.34 \text{ Mg}\cdot\text{ha}^{-1}$) on prime land and big bluestem monocultures yielded the highest dry biomass ($4.74 \text{ Mg}\cdot\text{ha}^{-1}$) on marginal land (data not shown). The lowest yielding biodiversity treatments were grass/legume mixtures (SG/BB/PG/DsC $4.13 \text{ Mg}\cdot\text{ha}^{-1}$ and SG/PC/PG/DaP $3.96 \text{ Mg}\cdot\text{ha}^{-1}$) (data not shown). However, these differences in dry biomass yield due to soil quality were not statistically significant ($P < 0.05$). Soil quality alone was not a significant source of variation but the interaction with location was. These results suggest that soil conditions alone may not affect these high diversity plant communities but in combination with environmental conditions due to location may be cause fluctuations in yield. Further research must be conducted in multi-environment trials to determine the effects of environmental interactions.

In this study dry biomass yields averaged $4.42 \text{ Mg}\cdot\text{ha}^{-1}$ (Table 17). Switchgrass monoculture (cultivar was Cave-In-Rock) yielded, on average, $4.77 \text{ Mg}\cdot\text{ha}^{-1}$ compared to the biomass yields in the cultivar study (Chapter 1) which were $\sim 5.35 \text{ Mg}\cdot\text{ha}^{-1}$. The results of these research indicated that monocultures of switchgrass alone or strictly grass mixtures did not produce more biomass than plots of grass/legume mixtures. This suggests that increasing diversity by adding species from different botanical families into mixture plots does not reduce the biomass yield but further research may be needed to determine the optimal combination of species. Nyfeler et al. (2009) found that fertilized ($50 \text{ kg N ha}^{-1} \text{ year}^{-1}$) high diversity plots that included legumes were able to produce comparable yields to grass monocultures fertilized with $450 \text{ kg N ha}^{-1} \text{ year}^{-1}$ when the proportion of legumes in the mixture were about 50 to 70%; however sward management failed to maintain such high proportions of legumes throughout the duration of the study. Experimental trials using native C_4 prairie grasses have found that an increase in the abundance of these species in conservation grasslands may decrease species richness but may increase the biomass production per unit of land area (Adler et al., 2009). The use of the correct species in combination may produce the same or better biomass yield compare to monocultures because these plant communities are expected to better utilized resources due to niche complementarity and positive interspecific interaction (Hector et al., 1999; Hooper et al., 2005). However, further studies using the correct combinations of species may aid in determining the influences that environmental and/or soil conditions may have on the performance of such combinations.

Even though nitrogen treatment was a statistically significant ($P < 0.05$) source of variation according to the ANOVA it accounted for a smaller portion of the

variance compare to location x soil quality interaction (Table 16). The letter groupings showed no differences in dry biomass yield due to nitrogen treatment in NJ and NY, but in the Pennsylvania prime site fertilized plots yielded more biomass than unfertilized plots ($4.78 \text{ Mg}\cdot\text{ha}^{-1}$ vs. $3.00 \text{ Mg}\cdot\text{ha}^{-1}$) (Table 17). When unfertilized grass/legume plots were compared to fertilized monocultures or fertilized grass only mixtures there were no statistically significant ($P < 0.05$) differences observed (data not shown). It could be argued that the legumes may have provided nitrogen to the unfertilized plots, thus the lack of difference between the treatments. The establishment data showed that the purple prairie clover had poor establishment but the showy tick trefoils had coverage percentages around 40% (Table 3). The level of success of grass/legume mixtures depends on the compatibility of the associated species and the ability of the species to persist stably in the mixture (Papadakis, 1941; Virtanen and Hausen, 1935; Springer et al., 2001). There are studies which have reported difficulties maintaining optimal proportions of legumes in mixtures with grasses (Beran et al., 1999; Blanchet et al., 1995; Guckert and Hay, 2001; Nyfeler et al., 2009; Springer et al., 2001). Visual observation in our study suggests that showy tick trefoil was present in the mixture throughout the trials; this is not the case for the purple prairie clover. However these observations were not quantified. Further research may be able to determine the effect of the legumes in the biomass production.

Table 16. Analysis of variance of dry biomass yield ($\text{Mg}\cdot\text{ha}^{-1}$) of 13 warm-season grass/legume biodiversity treatments evaluated at three locations in marginal vs. prime farmland in NY (established in 2008) and NJ (established in 2009) with 0 or 100 kg of $\text{N}\cdot\text{ha}^{-1}$.

Source	Df	F-value	Pr > F
Location (State)	2	3.52	0.0626
SQ (Soil Quality)	1	0.07	0.7976
Loc*SQ	2	98.09	<.0001
N (Nitrogen)	1	24.01	0.0004
Loc*N	2	8.66	0.0047
SQ*N	1	0.42	0.5302
Loc*SQ*N	2	1.7	0.2234
Tmt (Species)	9	0.77	0.6479
Loc*Tmt	18	1.13	0.3316
SQ*Tmt	9	0.16	0.9971
Loc*SQ*Tmt	18	0.74	0.7655
N*Tmt	9	1.22	0.2934
Loc*N*Tmt	18	1.31	0.1973
SQ*N*Tmt	9	2.17	0.0298
Loc*SQ*N*Tmt	18	1.91	0.0226

Table 17. Dry biomass yield (Mg·ha⁻¹) of 13 biodiversity treatments evaluated at three locations in marginal vs. prime farmland at each location.

Mean separation done with LSMEANS option and adjusted with Tukey's method for multiple comparisons.

		Dry Biomass Yield (Mg·ha ⁻¹)												Mean [§] Across Locations	
		NJ				NY				PA					
		Adelphia		Somerset		Syracuse		Rock Springs		Rock Springs					
Species	Combinations	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal	Prime	Marginal		
		0	100	0	100	0	100	0	100	0	100	0	100		
Switchgrass	Monoculture	7.44	8.31	1.95	2.40	4.16	1.04	6.35	9.82	3.44	5.31	2.68	4.38	4.77 A ¹	
Big bluestem	Monoculture	5.77	6.48	1.50	1.50	3.51	4.27	8.85	4.80	2.14	4.34	3.13	3.96	4.71 A	
SG/BB/PC	3-Grass mixture	6.98	6.19	1.59	2.45	2.96	2.54	8.23	7.27	3.48	4.82	3.93	4.94	4.62 A	
SG/BB/PG	3-Grass mixture	6.14	6.59	1.66	2.17	2.29	4.70	6.16	8.85	3.35	4.99	4.00	4.47	4.61 A	
SG/BB/PC/DsC	Grass/legume mixture	7.37	6.66	2.04	2.05	2.09	2.54	8.04	5.57	2.25	4.66	3.13	4.03	4.42 A	
SG/BB/PG/DsC	Grass/legume mixture	5.78	5.84	2.09	1.78	3.41	3.02	5.48	7.75	3.20	3.50	3.43	5.13	4.27 A	
SG/PC/PG/DsC	Grass/legume mixture	8.55	6.89	1.53	2.49	2.44	1.61	5.26	8.56	2.40	4.25	3.86	5.18	4.25 A	
SG/BB/PC/DaP	Grass/legume mixture	8.38	6.00	1.76	2.12	1.40	3.23	7.02	7.33	2.31	4.47	3.41	3.81	4.20 A	
SG/BB/PG/DaP	Grass/legume mixture	7.39	6.53	2.08	2.05	1.48	2.71	5.92	8.47	4.01	6.50	3.81	5.58	4.20 A	
SG/PC/PG/DaP	Grass/legume mixture	5.09	7.89	1.82	2.04	1.99	2.09	5.25	6.65	3.44	4.97	3.72	5.98	4.19 A	
Prairie cordgrass	Monoculture	-	-	-	-	1.82	1.10	7.69	2.44	1.40	2.79	2.00	2.27	+	
Panic grass	Monoculture	8.19	7.92	2.34	-	3.01	1.68	7.22	2.13	2.94	5.99	3.80	6.35	+	
SG/PC/PG	3-Grass mixture	9.03	8.45	1.57	-	2.44	2.47	4.64	6.50	2.33	5.34	4.25	5.69	+	
Mean [§]		6.89a ²	6.74ab	1.80e	2.11e	2.57e	.78cde	6.66ab	7.51a	3.00de	4.78bc	.51cde	.75bcd		

¹ Cultivar means with the same upper case letter were not statistically different at 0.05 level of probability.

² Location means with the same lower case letter were not statistically different at 0.05 level of probability.

- Means were not calculated due to missing values.

+ Cultivars means were not separated by LSMEANS due to missing values.

Conclusions

Switchgrass and big blue stem monocultures had the best stand establishment among the biodiversity treatments and our data suggest that these two species were the most dominant in the high biodiversity treatment plots. The prairie cordgrass and panic grass had the worst establishment in monoculture and in high biodiversity treatment plots. Among the legumes, the showy tick-trefoil had better establishment than the purple prairie-clover. The results indicated the location and soil quality were the most influential factors in the establishment of the biodiversity treatments. More than 50% of the biodiversity treatments had better establishment in prime quality soil than in marginal quality soil.

Our results showed that switchgrass monocultures had the tallest plants in this study and that big bluestem had the shortest; however the statistical differences among the biodiversity treatments were not clear because there were several sources of variation including several interaction factors. The most noticeable differences were observed only when comparing across soil quality, plants grew 48% taller in prime quality soil sites compare to marginal quality soil sites.

The interaction of location x soil quality was the most influential factor in the variation of dry biomass yield among biodiversity treatments. Monoculture plots were not significantly different than the yields of high diversity plots. These results suggest that increasing the number of species may not be detrimental to biomass yield and also indicate that there are environmental factors (soil and climate) that may influence the performance of low and high diversity plant communities and that multi-

environmental trials should be conducted to determine the best combination of species that yield more biomass.

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CONCLUSIONS OF THE THESIS

The results from the seeded studies (cultivars and biodiversity) were consistent with previous research suggesting a relationship between stand establishment and dry biomass yield. Switchgrass as many other warm-season grasses are known to establish slowly and have significant amounts of dormancy in the seed. Due to the significant correlation between stand establishment and biomass yield, further research should be aimed to better understand switchgrass dormancy and to use the genetic potential in the species to produce genotypes with uniform seed quality and low dormancy.

Cultivars Kanlow and Timber are lowland cultivars which originated in central to northern regions of lowland adaptations. These cultivars showed a broader adaptation to the different environments and produced biomass yields comparable to the best performing upland cultivars. The literature suggests that lowland cultivars may have a comparative advantage over upland cultivars when grown in northern latitudes because they delay flowering and have more time to develop more biomass. However an extended vegetative stage may be detrimental in northern regions with extreme winter temperatures that may cause damage or even senescence to the plant. The combination of lowland vigor and upland winter hardiness may be used in mix plantings to maximize the production of biomass in different U.S. regions. Additionally breeding programs may consider selecting lowlands with improved winter hardiness and uplands for improved vigor.

Marginal soil had a marked effect on the performance of switchgrass in all of the studies. All cultivars were shorter in marginal soils and did not rank the same in

prime vs. marginal soil. For stand establishment and biomass yield approximately 50% of cultivars showed differences due to soil quality. For tiller density only 40% of the cultivars presented differences due to soil quality; however some cultivars had higher tiller density in marginal soils. In the clones study, it was observed that in sites with marginal soil plants had delayed flowering, a reduction in size, tiller density and biomass production. Differences in biomass production were also noticed in the biodiversity study where plots in sites with prime farmland yielded more biomass. For some cultivars and clones the differences in performance due to soil quality were not statistically significant. This indicates that there genotypes adaptable to a broader range of environments may exist; however this study had only one year of data and more data may be able to give statistical significance to some of the differences. As a conclusion while it may be possible to use the cultivars and clones with less variability in performance in this study for breeding programs with indirect or single-environment selection practices, it may be beneficial for these programs to include selection in marginal soils to embrace the greater genetic potential of the species.

In this study it was observed that genotype was the most influential factor in switchgrass tolerance to diseases and environmental conditions may be marginally influential. This suggests that selection in single-environment trials may be effective to improve switchgrass tolerance to disease. However since these diseases were only evaluated at one site, anthracnose in NJ and rust in SD, it is unknown whether exposure to different isolates would result in a similar outcome. Additionally, the diversity of these two pathogens in switchgrass growing areas is not known so the variability in isolate response is unclear. Further research on the variability in

genotype performance to these two pathogens is necessary to avoid fungicide applications and yield losses in the future.

The results for the biodiversity study showed that switchgrass and big bluestem monoculture plots yielded the most dry biomass overall, however, the statistical analysis showed that the yields of monoculture plots were not significantly different than the yields of higher diversity plots with three or four species. This suggests that increasing the number of species in bioenergy production fields may not be detrimental to biomass yield. However due to the dominance of grass species in the plots, the benefits of growing legumes along side were not able to be determined. Further research should consider using cool season legumes which may be able to enhance soil quality for the warm-season grasses used for biomass production.

Curriculum Vitae

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Education

Master of Science in Horticulture from Louisiana State University with minor in Plant Physiology

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Principal Occupations

2009 – 2012 Research Assistant, Rutgers University, New Brunswick, NJ

2004 – 2007 Research Assistant, Ione E. Burden Center, Baton Rouge, LA

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Publications

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