PERENNIAL GRASSES AS SUSTAINABLE BIOENERGY CROPS FOR MARGINAL LANDS

BY

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DISSERTATION

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ABSTRACT

To minimize energy dependence on foreign petroleum imports and reduce fossil fuel consumption, the US Government has mandated the annual use of 136 billion liters of biofuels by 2022. Of the 136 billion liters, 61 billion liters should be produced from cellulosic biofuels. This will likely lead to increased production of dedicated energy crops for renewable biofuels. Since an ideal biomass crop should not compete with food crops for land use, biomass crop production on marginal lands can reduce land-use competition between energy and food crops. Chapter 1 of this dissertation provides an introduction and rationale of the research with a general discussion of the marginal lands and comparative potential of the various feedstock crops used in the experiments.

As a biomass crop in the US, switchgrass has been studied extensively and has been identified as a model biomass crop by the US Department of Energy (DOE). Cultivars of this species are generally considered as drought tolerant and are able to grow in a wide range of environments including some marginal lands. *Miscanthus* x *giganteus* has a high biomass yield potential and has been extensively evaluated for biomass in EU and recently in US. They also have moderate tolerance to heat, cold, drought, salinity, and flooding. Prairie cordgrass is native to North America and can be grown on lands that are too wet for corn, switchgrass, and big bluestem. They are highly tolerant to flooding and salinity. Big bluestem was primarily evaluated for forage purpose, but recently it has also been evaluated for bioenergy feedstock potential.

In Chapter 2, the biomass yield and performance of four perennial grass species: *Miscanthus* x *giganteus*, big bluestem (*Andropogan gerardii* Vitman), 'Kanlow' switchgrass (*Panicum virgatum* L.) and four natural populations of prairie cordgrass (*Spartina pectinata* Link.) were investigated in wet marginal land. A three-year study from 2011 to 2013 investigated

the biomass yield and tissue lignocellulosic composition among the compared species in 45 cm and 90 cm row spacing treatments. Biomass yield at 45 cm spacing was significantly higher than that at 90 cm spacing for all the populations compared during the first three years in this experiment. Switchgrass had the greatest biomass yield during all three years in 45 cm spacing, but it was not significantly different from two of the prairie cordgrass populations and *Miscanthus* x *giganteus* by the end of the experiment in 2013. There was no significant difference found among the grasses for cellulose and hemicellulose concentration in tissue.

Waterlogging in poorly drained soils can cause flooding that can delay early-season planting and also, cause difficulties in field operation in late season. High salinity is also a problem causing potentially arable land to be classified as marginal in the US. In Chapter 3, I investigated the potential of two natural populations of prairie cordgrass and a switchgrass in Illinois locations including poorly drained soils in Urbana and Pana, and a highly saline soil in Salem. A three-year study showed a reliable amount of biomass yield for all three grass populations by the end of the third year. All three locations produced acceptable amounts of lignocellulosic contents even when considering that these crops were produced in marginal settings. This research concludes that the evaluated grass populations have a good potential to be grown in a poorly drained, as well as salt-affected marginal land.

In Chapter 4, I evaluated and screened 17 populations of prairie cordgrass (*Spartina pectinata*) along with Kanlow switchgrass (*Panicum virgatum* ‘Kanlow’) in poorly drained soil in Urbana, IL, and plots irrigated with saline water in Pecos, TX. A two-year study from 2012 to 2014 showed a great variation among the prairie cordgrass populations for biomass productivity. However, a Kansas-originating population produced the greatest biomass yields averaged over two years in both locations. Averaged over two years, Kanlow switchgrass was the top producer

in both locations. We also found much variation in tissue mineral concentration among the populations in the two locations. Our study demonstrates that genetics and environment can have a great influence on grass performance.

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Chapter 1.

INTRODUCTION AND RATIONALE

The Renewable Fuel Standard (RFS) established by US Congress mandated the annual use of 36 billion gallons of biofuels by 2022, of which 16 billion gallons per year should come from cellulosic biofuels (Schepf & Yacobucci, 2013). How this will be accomplished is an ongoing topic of great interest. Population growth, finite fossil fuel resources, and increasing living standards are the three major driving forces of renewable energy (Darmstadter, 2004). It is important to that bioenergy development be expedited to enhance energy security and mitigate climate change from greenhouse gases (Hoekman, 2009). Sustainable bioenergy systems within the US can provide a domestic renewable energy supply and enhance national security by serving as an alternative to imported fossil fuels, while simultaneously addressing environmental issues (Milliken et al., 2007). Biomass based energy as an alternate resource has become increasingly important to achieve energy independence from foreign imports of transportation fuel (Heaton et al., 2008). Therefore research efforts and production expansion of the biomass-based energy industry is critical to meet the goals of replacing a significant portion of current petroleum consumption with biofuels in the coming decades (Perlack et al., 2005).

As we plan to increase the production of bioenergy crops within the US, more land area under production will be required, which would have a great impact on land availability for premium food crops (Gallagher, 2008; Tilman et al., 2009). Under the baseline yield assumption by the ‘Billion-Ton’ study (USDOE, 2011), approximately 22 million acres of cropland and 41 million acres of pastureland will shift into energy crop production by 2030 for a simulated farmgate price of dry biomass at $60 per ton. The question now is, where we can find the additional land area? Moreover, can such land be utilized without competing with food crops? Milbrandt and Overend (2009) report that there is a significant area of such land that is considered marginally productive for agricultural crops. Such areas are usually termed as marginal lands (FAO, 1997), however they are also referred to as waste lands, unproductive lands, idle lands, under-utilized lands, abandoned or degraded lands (Lal, 1991; 2008; Wiegmann et al., 2008, Tilman et al., 2009; Shortall, 2013). Currently, about 11% of the US mainland area, approximately 86.5 million hectares, is considered marginal land (Milbrandt et al., 2014). Identifying and utilizing those marginal lands that are suitable for the production of second-generation bioenergy crops (i.e., lignocellulosic biomass crops) would avoid the competition between first generation bioenergy crops (e.g. corn and sugarcane) and food crops for prime agricultural land (Gallagher, 2008; Tilman et al., 2006; Tilman et al., 2009).

Land can be classified as marginal for agricultural production for many reasons including unfavorable climate and physical characteristics, high salinity, waterlogged and marshy lands, or glacial and rocky barren areas (Milbrandt and Overend, 2009). The USDA-NRCS has developed a land capability classification based on the soils ability to produce common cultivated crops and pasture plants without any long-termed deterioration. This classification divides land from I to VIII capability classes (USDA-NRCS, 2010). Land capability classes from IV to VIII are generally categorized as marginal lands (Hamdar 1999; Kang et al, 2013). Tenerelli and Carver (2012) indicated that perennial energy crops can be allocated in less productive land or marginal land with the land capability class from III to V.

Deterioration of land, resulting in marginal status, can result from natural and/or artificial forces (Milbrandt et al., 2014). Some anthropogenic factors like clearing forests, planting shallow-rooted crops, and irrigation can cause changes in water table depth which can consequently lead to excess water evaporation from the soil surface and salt accumulation in the root zone causing land degradation (Abrol et al., 1998; Sim, 2012). There are more than 800 million hectares of salt-affected land globally (Munns, 2005; Rengasamy, 2010) and about 8.5 million hectares of land area affected in US (Pearson, 2009). Irrigation of fields with saline groundwater has been a common practice in the Southwest US in the areas of Salt River Valley of AZ, the Arkansas River Valley of CO, and the Rio Grande and Pecos River Valleys of West TX and NM (Erickson, 1980; Rhoades et al., 1989; Rhoades et al., 1992). Salinity levels in soil, as well as in irrigation water, can undermine the growth and yield of many crops (Fipps, 2003; Bauder et al., 2011).

Other than salinity, poor drainage is another issue that reduces productivity in much US arable land. Poor drainage can cause flooded fields which can eventually reduce soil oxygen availability to plants and limit root respiration and hinder plant growth (Caudle & Maricle, 2012). Early season wet soil conditions in spring can cause a delay in planting and consequently yield losses in crops due to loss of yield potential and diseases associated with wet and cool soil (Bockus and Shroyer, 1998; Stoof et al., 2015). Late season waterlogging can also be problematic due to the difficulty in field operations in standing water (Stoof et al., 2015). Farmlands in the Midwest US were considered unsuitable for crop production due to poorly drained soils until tile drainage systems were installed in the late 19th and early to mid-20th century (Gopalkrishnan et al., 2011). About 47.8% of the total cropland in IL had sub-surface drainage based on 1992 USDA ERS estimates (Sugg, 2007). However there is no comprehensive information available on the recent and accurate status of the extent of tile drained agricultural lands (Sugg, 2007) and there are still many areas with poor drainage issues.

How can biomass for bioenergy be produced on land that is otherwise considered unsuitable for agricultural production? Preliminary work has identified a group of perennial grasses that may be capable of producing economic yields of biomass on marginal lands. For example, some perennial grasses have the ability to tolerate wet soils (Stoof et al., 2015), which would enable them to be used as bioenergy feedstocks in wet marginal lands. An ideal biomass crop must be able to efficiently capture solar energy and convert it into harvestable biomass with low input and minimal environmental impact and/or have positive environmental aspects (Johnson et al., 2007; Heaton et al., 2008). Perennial grasses have low input and maintenance, as well as low production cost, and many are well adapted in marginal lands (Samson and Girouard, 1998). Utilizing perennial grasses as bioenergy feedstocks for production on marginal land can potentially avoid land use competition with food crops and enhance the environment through carbon sequestration and improved water quality (Kim et al., 2011).

We will need a diverse set of bioenergy feedstocks growing sustainably with cost-effective processing to develop economically appealing second-generation biofuels (Simmons et al., 2008) in order to reach the goal of 16 billion gallons of biofuels from cellulosic biomass by 2022. The focus of Chapter 2 of this dissertation is on the evaluation of four perennial grasses for their potential use as bioenergy feedstock in wet marginal land. All four perennial grasses, miscanthus (*Miscanthus* x *giganteus*), switchgrass (*Panicum virgatum*), big bluestem (*Andropogan gerardii*), and prairie cordgrass (*Sparting pectinata*), have been the subject of extensive research as biofuel crops (Mitchell et al., 2008; Boe et al., 2009; Heaton et al., 2010; Propheter et al., 2010; Evers et al., 2013). Although high biomass yield is a very important trait for bioenergy crops (McKendry, 2002; Wright, 2007; Boe and Beck, 2008; Anderson-Teixeira et al., 2012), identifying crops with abiotic stress tolerance is equally important given the limited availability of prime agricultural land for biofuel crops (Quinn et al., 2015).

Miscanthus x *giganteus* is a warm-season perennial grass originated from East Asia (Greef et al., 1997; Hodkinson & Renvioze, 2001). It has a very high biomass yield potential (Heaton et al., 2010) with the biomass yield ranging approximately from 22 to 35 Mg ha^{-1} from Northern to Southern IL (Pyter et al., 2007). Besides its high biomass yield, miscanthus is also moderately tolerant to abiotic stresses such as heat, cold, drought, salinity, and flooding (Quinn et al., 2015). It has been extensively evaluated as a high-yielding biomass feedstock in the EU (Greef et al., 1997; Hodkinson & Renvioze, 2001) and more recently in US. Normally *Miscanthus* x *giganteus* requires three years to become fully productive (Miguez et al., 2008), and therefore, is not usually harvest during the establishment year. Similarly, switchgrass is not harvested in the establishment year, but can sometimes be fully productive in two growing seasons (Grassini et al., 2009; Schmer et al., 2010), or may take three years (Parrish and Fike, 2005; Heaton et al., 2004; Schmer et al., 2010). Generally considered as a drought tolerant grass (Barney et al., 2009; Sanderson et al., 1999), switchgrass is also considered moderately tolerant to heat, cold, salinity, and flooding (Quinn et al., 2015). Switchgrass has been the subject of much research in US, and has been identified by the US DOE as a 'model' bioenergy feedstock (Wright and Turhollow, 2010). It is able to grow on marginal lands, requires low nutrient and moisture inputs, and can produce reliable biomass yields in a wide range of environments (Wright and Turhollow, 2010; Parrish and Fike, 2005). Prairie cordgrass, on the other hand, is native to marshes, drainage ways and moist prairies in North America (Backworth et al., 2007; Boe and Lee, 2007) and can be grown in land that is too wet for corn, switchgrass, and big bluestem (Boe et al., 2013; Boe et al., 2009; Boe and Lee, 2007). It also grows more rapidly and grows taller than switchgrass or big bluestem (Boe et al., 2009). Prairie cordgrass not only has a low tolerance to drought (Quinn et al., 2015), but also has high tolerance to flooding, salinity, and cold (Bonilla-Warford and Zelder, 2002; Skinner et al., 2009; Kim et al., 2011; Quinn et al., 2015). Big bluestem is another potential perennial bioenergy grass with high tolerance to drought, heat, and, salinity (Quinn et al., 2015). It is a dominant species of Midwestern tallgrass prairie and can be used for conservation purposes such as mine reclamation and erosion control (Wennerberg, 2004). Big bluestem has been primarily evaluated as a forage crop, but has recently been considered for bioenergy use (Griffith et al., 2011; Hong et al., 2013). To compare stress-tolerance and production qualities when grown under environmental stresses, these grasses were grown in field research studies.

In Chapters 3 and 4, the research focused on the response of different genotypes of perennial grass bioenergy crops to multiple environmental stresses. Evaluating a common group of prairie cordgrass and switchgrass genotypes, derived from different source populations, may quantify the biomass yields and tissue chemical composition of these potential cultivars. As bioenergy researchers attempt to increase biomass yields to meet the annual production goal of 36 billion gallons of biofuel by 2022, various feedstock species and cultivars will require evaluation for best adaptability and productivity in various marginal land situations. Direct field comparison of perennial grass species in marginal settings offers a better understanding of the species differences in the performance in the particular environment. A particularly important emphasis is in the wet marginal and salinity affected areas of IL and one area with saline water irrigation in TX where the marginal situation is the result of a common practice in agriculture in the area. Testing and evaluating these perennial species in environments in different locations can provide information about the adaptability and potential for use in these areas. The results can help us identify and develop a perennial species with potentials to be used as a bioenergy crops for biofuels in selected marginal lands.

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Chapter 2.

PERENNIAL GRASSES FOR SUSTAINABLE BIOMASS PRODUCTION ON WET MARGINAL LAND

ABSTRACT

Bioenergy crop production on marginal lands can help reduce land-use competition between energy and food crops and have a positive impact on the environment. A diverse set of bioenergy feedstocks is essential to develop a second-generation biofuel with sustainable and cost effective production of bioenergy crops. Ideal perennial energy crops should be well adapted to marginal lands with low production cost. A comparison study of biomass yield and performance trials was established on poorly drained soil (Land Capability Class 4W) in Urbana, IL. *Miscanthus* x *giganteus* (Mxg), big bluestem (*Andropogan gerardii* Vitman), 'Kanlow' switchgrass (*Panicum virgatum* L.) (SW), and four natural populations of prairie cordgrass (17-109, 17-014, 20-107, and 46-102) were planted with 45 and 90 cm row spacing during the spring of 2010. Biomass yield at 45 cm spacing was significantly higher than that compared to 90 cm spacing for all the populations compared during 2011-2013. There was no interaction found between the spacing and harvest year. Switchgrass (29.76 Mg $ha^{-1)}$ had the greatest biomass yield during all three years, but was not significantly greater than 17-109 (24.88 Mg ha^{-1}), 20-107 (24 Mg ha^{-1}), and Mxg (28.81 Mg ha^{-1}) in 2013 for 45 cm spacing. There was no significant difference found among the grasses for cellulose and hemicellulose concentration in tissue. Big bluestem and 46-102 were among the lowest yielding grasses in this study. With comparable biomass yields, tissue lignocellulosic composition in SW, Mxg, 17-109, and 20-107 showed a promising potential to be used as dedicated energy crops in wet marginal lands, while 17-104 was not far behind 17-109 and 20-107.

INTRODUCTION

The US is the largest consumer of petroleum and about 27% of its total consumption relies on imports from foreign countries (EIA, 2014). Because of increasing energy dependency and deterioration of the environment due to greenhouse-gas emissions, the US government mandated the annual production of 136 billion liters of biofuels by 2022 (Schepf & Yacobucci, 2013). Of the 136 billion liters, 61 billion liters should come from cellulosic biofuels (Schepf & Yacobucci, 2013). The use of food crops to produce ethanol has raised concern about food security and environmental degradation (Mitchell, 2008; Pimentel et al., 2009). Cellulosic ethanol is increasingly being considered as a source of energy used to meet growing energy demands (Dwivedi et al., 2009). Federal mandates require the use of novel bioenergy feedstocks to reduce negative impact on environment from greenhouse gases produced by fossil fuels, as well as global food supply, which led to increased production of second-generation biomass crops in the US (United States Congress, 2007; EISA, 2007; Schnepf and Yacobucci, 2013).

Sustainable production of bioenergy crops with end-use technology that is energy efficient is important to a sustainable energy economy (Samson and Girouard, 1998). A diverse set of bioenergy feedstocks that grow sustainably and cost effectively is needed in order to develop the second-generation biofuels (Simmons et al., 2008) necessary to produce 61 billion liters of biofuel per year from cellulosic feedstocks. An ideal biomass crop must be capable of efficiently capturing solar energy and converting it into harvestable biomass with low input and minimal environmental impact (Johnson et al., 2007; Heaton et al., 2008). Also, in terms of land use, an ideal bioenergy crop should not compete with food crops because it will directly reduce potential food supplies (Babcock, 2008; Gonzalez-Hernandez et al., 2009). This definitely limits the land availability for biomass production.

There are marginal lands that are generally not suited to row crop production, but can grow biomass crops, avoiding land-use competition between food crops and biomass crops (FAO, 1997; Tilman et al., 2009). There is much marginal land area worldwide; 11% of the US mainland, approximately 86.5 million ha, is considered as marginally productive for agricultural crops (Milbrandt and Overend, 2009, Milbrandt et al., 2014). Biomass resource potential on those marginal lands in US is 377,413,000 Mg yr^{-1} and ethanol potential is 148 billion l, which is 19% of the current gasoline consumption (Milbrandt and Overend, 2009). In addition, using degraded or abandoned lands from agriculture will minimize the food versus fuel conflict, as well as potential land-clearing from biofuel expansion (Tilman et al., 2009).

Perennial grasses often have low inputs and maintenance costs and some are adapted to marginal lands (Samson and Girouard, 1998). Prairie cordgrass (*Spartina pectinata* Link.) is tall, rhizomatous perennial grass and is indigenous to many of the regions throughout US and Canada (Mobberley, 1956; Stubbendieck et al., 1982). It is native to marshes, drainage ways and moist prairies in North America (Mobberley, 1956; Stubbendieck et al., 1982) and can be grown in land that is too wet for corn (scientific name), switchgrass (*Panicum virgatum* L.), or big bluestem (*Andropogan gerardii* Vitman) (Weaver, 1954). Prairie cordgrass grows more rapidly and taller than switchgrass and big bluestem (Weaver, 1954). Due to its tolerance to salt, alkaline conditions (Kim et al., 2011), flooded condition (Bonilla-Warford and Zelder, 2002), and its adaptability to both dryland and wet soils (Mobberley 1956), it may have potential to be grown on variety of soils. It is tolerant to. Prairie cordgrass produced consistently higher biomass than switchgrass with the mean of 12.7 Mg.ha^{-1} during the 9 year study (Boe et al., 2009). When treated with flooding in different frequencies, it showed no differences in biomass production and showed its high potential for use in stormwater wetlands (Bonilla-Warford and Zelder, 2002). A study by Skinner et al. (2009) involving different grass cultivars, showed that 'Red River' prairie cordgrass provided superior performance in four different field locations when subjected to flooding and soil saturation. Studies have shown that prairie cordgrass has great potential for use as a biomass crop in wet marginal lands (Bonilla-Warford and Zelder, 2002; Skinner et al., 2009).

Miscanthus x *giganteus* is a warm-season perennial grass from East Asia (Greef et al., 1997; Hodkinson & Renvioze, 2001). With a high biomass yield potential (Heaton et al., 2010) it has been reported to have yields ranging from 22 to 35 Mg ha^{-1} from Northern to Southern IL (Pyter et al., 2007). Mature stands of high yielding *Miscanthus x giganteus* has even yielded about 40 Mg ha^{-1} in Europe (Miguez et al., 2008; Lewandowski et al., 2000). Besides high biomass yield it is also moderately tolerant to heat stress, cold stress, drought, salinity, and flooding (Quinn et al., 2015), which makes it a promising feedstock for marginal environment. *Miscanthus* x *giganteus* and switchgrass both take approximately three years to become fully productive, however switchgrass can sometimes reach full production in two years (Miguez et al., 2008; Grassini et al., 2009; Parrish and Fike, 2005; Schmer et al., 2010). Switchgrass can grow in a wide range of environments and requires little moisture and nutrient inputs, but still produce a reliable biomass yield (Wright and Turhollow, 2010; Parrish and Fike, 2005). Some lowland switchgrasses have shown tolerance to flooded conditions (Barney et al., 2009; Porter, 1966). With good drought tolerance and moderate tolerance to heat, cold, salinity and flooding (Barney et al., 2009; Sanderson et al., 1999; Quinn et al., 2015) switchgrass has good potential to be grown as a biomass feedstock in marginal environments. Big bluestem, on the other hand, has a high tolerance to drought, heat, and salinity (Quinn et al., 2015). Although big bluestem was primarily evaluated for forage purposes, it has been evaluated for bioenergy purposes more recently (Griffith et al., 2011; Hong et al., 2013). It can be used for mine reclamation and erosion control and has been a dominant species of Midwestern tallgrass prairie (Wennerberg, 2004). With all the good qualities in terms of different stress tolerances that big bluestem has, it can be a potential bioenergy feedstock for marginal environment.

Waterlogging due to poor drainage and flooding in poor soils can be responsible for the existence of marginal landscapes (Gopalkrishnan et al., 2011). Early spring wet soil can delay planting and can cause yield losses due to diseases associated with wet and cool soil condition (Bockus and Shroyer, 1998; Stoof et al., 2015). In addition, flooding reduces the soil oxygen available to plants and reduces root respiration (Caudle & Maricle, 2012) making conditions unfavorable for plant growth. Later season waterlogging can cause difficulties in field operations due to standing water in fields (Stoof et al., 2015). These wet soils can be utilized by producing flood tolerant bioenergy crops.

Miscanthus x *giganteus*, big bluestem, and switchgrass have been studied as dedicated energy crops and have potential for biomass production for biofuel purposes (Greef et al., 1997; Hodkinson & Renvioze, 2001; Wright and Turhollow, 2010; Griffith et al., 2011). However, no studies have been conducted that compared the biomass potential of these grasses with that of prairie cordgrass when grown in the same environment under the same culture. In addition, the optimum establishment row spacing for prairie cordgrass is not known. Therefore, the objectives of this research are to 1) determine the biomass yield performance and tissue lignocellulosic composition of Kanlow switchgrass, *Miscanthus x giganteus*, prairie cordgrass, and big bluestem grass species in wet marginal land and 2) determine the effect of row spacing on yield performance of the compared grass species on such land. We hypothesize that as a native to wet prairies, prairie cordgrass will tolerate the wet marginal condition better and thus perform better than the other three grasses. Our results will help us identify the best performers in the given environmental conditions in this experiment and further assist biomass crop breeding and development programs.

MATERIALS AND METHODS

A field comparison trial was established for this study in May 2010 on the University of Illinois Energy Farm in Urbana, IL (40° 4'5.82"N and 88°11'26.93"W). The soil on the site is a Flanagan silt loam (Land Capability Class 4W, fine, smectitic, mesic Aquic Argiudolls). The biomass feedstocks in the comparison trial included *Miscanthus* x *giganteus* (Mxg), IL ecotype big bluestem (BB) (seed collected from Decatur, IL), Kanlow switchgrass (SW), and four natural populations of prairie cordgrass, 17-104, 17-109, 46-102, and 20-107, which were collected from Sidney, IL (N 40°00'; W 88°01'), Urbana, IL (N 40°03'; W 88°12'), Sioux Falls, SD (N 43°32'; W 96°49'), and Manhattan, KS (N 39°02'; W 96°31'), respectively. The experimental plots were arranged in a split plot in randomized complete block design with the four blocks representing four replications. The whole plots were two spacing treatments: 0.45 m row spacing with plot size 1.8 m by 2.7 m and 0.90 m row spacing with plot size 3.6 m by 5.4 m. Grass populations were arranged as sub plots within each spacing treatment. Each of the plots in both spacing treatments consisted of four rows with 6 plants in each row which made it altogether 24 plants in the plot. This allowed the higher population density in the 45 cm spaced plots to be compared to the 90 cm spaced plots. The plots were fertilized with Urea at the rate of 112 kg N ha^{-1} annually each spring, 2011 – 2013. Irrigation was only applied during the planting year in 2010 to ensure successful establishment.

Light interception data were collected by measuring twice each week to catch the initial emergence and active growth pattern of different species and once weekly starting in mid-June when plant growth slowed in 2014. Light interception was measured using the AccuPAR LP-80 PAR/LAI ceptometer (Decagon Devices, Inc.2365 NE Hopkins Ct. Pullman, WA 99163 - USA) to compare grass growth patterns as the canopy closed in 2014. The ceptometer sensor was placed between plants in the canopy at ground level in order to measure the portion of light that reached the ground. Another sensor was set outside the canopy in an open area where there was no shade interference to measure the incoming PAR (Photosynthetically Active Radiation) at that time of day. The PAR measurements were taken from two difference places for each plot between 10:00 am and 2:00 pm on clear days to avoid any cloud interference.

The plots were harvested after the killing frost in late fall (November – December) during 2011, 2012, and 2013, and the biomass yields were determined each year. Harvesting was done using a plot combine (Wintersteiger Cibus S harvester mounted with a Kemper forage chopper, Ames, IA) at a 10 cm stubble height. Approximately 1 kg plant sample was collected and placed in a paper bag from each plot at each harvest, which were dried at 60 ^{o}C for 96 hours to determine dry biomass yield. The same samples were also used for tissue composition analysis.

The dried samples were ground using a cutting mill (Retsch SM2000, Haan, Germany), to pass through a 1mm screen size for all the further processes needed for tissue analysis. Samples were stored in airtight plastic bags until further analyses. Approximately 5 to 10 g of ground samples were sent to Brookside Laboratories (Brookside Laboratories, Inc. 200 White Mountain Drive, New Bremen, Ohio 45869) for tissue mineral composition analysis. Tissue ash content was determined using Neycraft Pro furnace (Undersander et al., 1993). The fiber analysis of tissue samples were determined sequentially using Ankom ^{200} Fiber Analyzer (Ankom

Technology, 2052 O'Neil Road, Macedon, NY 14502) for neutral detergent fiber (NDF) and acid detergent fiber (ADF). Acid detergent lignin (ADL) was determined by using Ankom Daisy II incubator (Ankom Technology, 2052 O'Neil Road, Macedon, NY 14502). The data collected from fiber and lignin content were utilized to estimate the lignocellulosic concentrations (cellulose, lignin, and hemicellulose) in the tissue sample. Cellulose was estimated by calculating the difference between ADF and ADL, while hemicellulose content was estimated by calculating the difference between NDF and ADF.

Biomass yield, lignocellulosic composition, and mineral composition data were analyzed by using PROC MIXED procedure in SAS (SAS 9.4. SAS Institute Inc., Cary, NC, USA). Significance was determined at the $P<0.05$ level. Homogeneity of variance was determined using Brown Forsythe's test. Analysis of Variance (ANOVA) was conducted to determine the variance among the main effects of grass populations, spacing, and year as well as their interaction effects for biomass yield and tissue compositions. Grass populations and spacing as well as year were independent variables and were treated as fixed variables, whereas biomass yield, tissue lignocellulosic content and mineral content were the response variables or dependent variables. All the interaction effects (spacing x population, year x population, year x population, year x spacing, and, year x population x spacing) were treated as fixed variables. Tukey's studentized range (HSD) test was done for mean separation of biomass yield and tissue compositions with a significant difference (P=0.05).

RESULTS

The weather data for Urbana showed variations among years throughout the study period. Precipitation in 2012was much lower than the 30-year average from early growing season until August, while it was closer to the average in 2011 and 2013, except when it was lower in July both years (Fig 2.1). The temperature was also higher than normal during July in 2011 and 2012 (Fig. 2.2), which may have caused drought condition in 2012. As the growth pattern among the four species was compared in 2014, all four populations of prairie cordgrass emerged earlier in the season (first week of April) than Mxg, SW, and BB (last week of April to first week of May) in 2014. The growth pattern was recorded in the field as the plants started to emerge in the spring in 2014.

The analysis of variance showed a significant difference for biomass yield for the main effects of spacing treatments, as well as the main effects of the populations across the three-year study period (Table 2.1). Year was considered as a fixed variable and was also significantly different. The interaction effects of population x spacing, as well as the interaction effects of population x year were significant for biomass yield whereas the interaction effect of spacing x year was not significant (Table 2.1).

Biomass yield at the 45 cm spacing was greater than that of the 90 cm spacing for all populations (P<0.0001). Switchgrass was the most productive during all three years, but was not greater than 17-109, 20-107, and Mxg in 2013 for 45 cm spacing (SW= 29.8 Mg ha^{-1}, 17-109= 24.9 Mg ha^{-1}, 20-107= 24 Mg ha^{-1}, and Mxg= 28.8 Mg ha^{-1}) (Table 2.2). Among the PCG populations, 46-102 was the least productive population (13.4 Mg ha^{-1} in 2013) (Fig. 2.4, Table 2.2). Big bluestem productivity (15.5 Mg ha^{-1}) was not different from 46-102 or 17-104 by the end of 2013 (Fig. 2.4). Biomass yield for all of the grass populations were higher in 45 cm spacing in all three years except for 46-102, which was similar from the beginning (Table 2.2). Biomass yield for switchgrass did not increase much going from the second year in 2012 to the third year in 2013 as it did for *Miscanthus* x *giganteus* and prairie cordgrass (Fig. 2.4).

There was little variation among the populations for lignocellulosic concentration in the tissue samples. The main effect of population was significant for cellulose ($P<0.05$) and year was different for hemicellulose and lignin ($P<0.05$) (Table 2.1). The interaction effects for population x spacing, year x population, year x spacing, and year x spacing x population were not significant (Table 2.1). Cellulose concentration was well above 35% of the biomass for all of the species compared (Fig. 2.5). However, BB had significantly higher cellulose concentration in its tissue as compared to that of 46-102 (minimum significant difference value = 33.9) but not different from the rest of the populations (Fig. 2.5). The tissue cellulose concentration among the species ranged from 372 g kg^{-1} in 46-102 to 406 g kg^{-1} in BB. There were no differences among either the species or spacing treatments for hemicellulose concentration (Fig. 2.6). The tissue hemicellulose concentration among the species ranged from 291 g kg^{-1} in Mxg to 317 g kg^{-1} in 20-107. Similarly, there was no difference among species or spacing for tissue lignin concentration (Fig. 2.7). The lignin concentration among the species ranged from 49.8 g kg^{-1} in 17-109 to 63.07 g kg^{-1} in Mxg.

Ash concentration in the biomass showed some differences among the populations (Table 2.1). The ash concentration ranged from 36.2 g kg^{-1} in Mxg to 67.4 g kg^{-1} in 46-102 (Fig. 2.9). 46-102 had significantly higher ash content than only Mxg and SW (45.1 g kg^{-1}) and these two were not significantly lower in ash than the rest of the grass populations.

Tissue phosphorus concentration showed variations among the grass populations; the phosphorus concentrations in the tissue of all four accessions of PCG (46-102=1.1 g kg^{-1}, 17-

104=0.9 g kg^{-1}, 17-109=0.8 g kg^{-1}, and, 20-107=0.8 g kg^{-1}) were higher than in Mxg (0.4 g kg^{-1}) and SW (0.5 g kg^{-1}). The phosphorus concentration in BB tissue (0.6 g kg^{-1}) was not lower than the 17-104, 17-109, or 20-107 PCG accessions.

DISCUSSION

As the results of spring emergence and light interception of the compared grasses showed that all four prairie cordgrass populations emerged earlier in the season as compared to other grass species. A field study in Quebec, Canada showed that prairie cordgrass produced higher biomass due to its earlier spring emergence and extended growing season (Madakadze et al., 1998). This may give an advantage to prairie cordgrass over others in terms of light and water resource exploitation since there will be no competition until before the other grasses emerge. Yield is an important trait when it comes to producing bioenergy crops for biofuels (McKendry, 2002; Wright, 2007; Boe and Beck, 2008; Anderson-Teixeira et al., 2012). There were dry biomass yield differences among the grasses in the study with big bluestem producing 15.5 Mg ha^{-1}, switchgrass 29.8 Mg ha^{-1}, *Miscanthus* x *giganteus* 28.8 Mg ha^{-1}, and 24.9 Mg ha^{-1} for 17-109, the highest yielding prairie cordgrass population at the 45 cm spacing treatment in the end of the study in 2013.

Biomass yields reported for switchgrass in the US ranged from 0.9 Mg ha^{-1} to 34.6 Mg ha^{-1} (Pfeifer et al., 1990; Lewandowski, 2003) and 6.8 Mg ha^{-1} to 11.9 Mg ha^{-1} for big bluestem (Cherney et al., 1990; Lewandowski, 2003). *Miscanthus* x *giganteus* has a very high biomass yield potential (nearly 40 Mg ha^{-1} reported in some European locations) (Heaton et al., 2010, Lewandowski et al., 2000). However, the yield of *Miscanthus* x *giganteus* from Northern to Southern IL ranged from approximately 22 to 35 Mg ha^{-1} (Pyter et al., 2007). Guo et al. (2015) reported the biomass yields of ‘Kanlow’ switchgrass and ’17-109’ prairie cordgrass to be 16.5

Mg ha^{-1} and 16.0 Mg ha^{-1} respectively, in IL. For wet marginal land, the biomass production in this study showed a promising potential for all species. As the purpose of the study, we evaluated the potential of the selected grass populations on a wet marginal land. In 2012, there was an early season drought condition through July that may have dried soils conditions. There was, however, normal precipitation in both 2011 and 2013, which led to early season waterlogging in the field plots resembling the wet marginal situation. Given the yields in those years, PCG populations 17-109 and 20-107, Kanlow switchgrass, and Mxg all have a good potential to produce high biomass yields on wet marginal lands.

However, these four grass species vary in their maturity age in terms of getting into a full production stage. *Miscanthus* x *giganteus* normally takes about three years to become fully productive (Miguez et al., 2008) and are not usually harvested during the establishment year. This is also true for switchgrass when it comes to harvesting in the establishment year, however switchgrass can become fully productive in two years (Grassini et al., 2009; Schmer et al., 2010). Similarly big bluestem also is not harvested during the year of establishment, and it takes about three years for it to become fully productive (Doxon, et al. 2012). Boe et al. 2009, selected a mature stand of prairie cordgrass for their nine year study in which they considered at least a 4 year old stand as mature stand. Boe and Lee, 2007, also found that after 4 years of single annual harvest, prairie cordgrass formed a dense and highly productive sods. As we could see from our results, switchgrass seems to reach its maximum biomass yield earlier than other grass as the biomass yield did not change much from 2012 to 2014 but all the prairie cordgrass and *Miscanthus* x *giganteus* seemed to yield much more in 2013 as compared to that in 2012. A long term experiment, extended beyond 4 years could probably shed some light on the biomass yield trend differences between these species.

The current recommendation for planting *Miscanthus* x *giganteus* in Midwest is 76 cm rows and 76 cm spacing between the plants (Heaton et al., 2011, Williams & Douglas, 2011). With vegetative propagation from rhizomes, prairie cordgrass is generally spaced between 24 – 300 cm (USDANRCS). Big bluestem and switchgrass are also planted in rows with different row spacing, however, Foster et al. (2012), found that row spacing did not have an effect on biomass yield. Our results showed that the biomass yield was significantly higher in 45 cm spacing treatments as compared to 90 cm spacing for all of the grasses compared. However, while 45 cm spacing produced higher yields than 90 cm spacing in this study, this may not be the case in normal, prime cropland. Even though biomass yield in 90 cm spacing continuously increased from 2011 to 2013, lower yield penalty in wider spacing was not overcome during the first 4 years of establishment period. As Boe and Lee, 2007, found that after 4 years, cordgrass seemed to grow much denser it can be speculated that 90 cm spacing may produce more biomass in a long run.

For a good bioethanol conversion quality, it is important to maintain cellulose and hemicellulose content for achieving target yields (Wyman, 1999). Feedstock biomass typically contains 35% - 50% (350 g kg^{-1} to 500 g kg^{-1}) cellulose, 20% - 35% (200 g kg^{-1} to 350 g kg^{-1}) hemicellulose, and 12% - 20% (120 g kg^{-1} to 200 g kg^{-1}) lignin (Wyman, 1999). Over all grasses in this study, cellulose content ranged from 37.2% - 40.6%, (372 g kg^{-1} to 406 g kg^{-1}) hemicellulose content from 29.1% - 31.7% (291 g kg^{-1} to 317 g kg^{-1}), and lignin content from 4.98% - 6.31% (49.8 g kg^{-1} to 63.1 g kg^{-1}). Cellulose, hemicellulose, and lignin concentrations can be determined using two common methods: insoluble dietary fiber and acid detergent fiber. The insoluble dietary fiber method overestimates the lignin concentration (Klason lignin), while the acid detergent fiber method underestimates it (acid detergent lignin) (Godin et al., 2015).

Results here found that the lignocellulosic composition among the grasses in this study were in the reasonable range.

The water use rate of warm-season C_4 grasses are usually lower than that of cool-season C_3 grasses (Black, 1971). This consequently reduces the uptake of silicic acid in C_4 grasses, the main mechanism of accumulating silica (the major portion of ash) in perennial grasses (Samson and Mehdi, 1998). Reported ash contents of prairie cordgrass, switchgrass, big bluestem, and miscanthus were 1.6%, 1.7%, 1.8%, and 2.0% respectively (Radiotis et al., 1996, Samson and Mehdi, 1998). However, it varies within the species depending upon the soil type, rainfall to evaporation ratio and area region (Samson et al., 1993; Samson and Chen, 1995; Samson and Mehdi, 1998). McLaughlin et al. (1996) explains that the combination of these conditions may be the cause of higher ash content (2.8% - 7.6%) in switchgrass. Typically, general biomass feedstocks contains 3-10% ash (switchgrass = 6%, big bluestem = 6%, prairie cordgrass = 6%, and miscanthus = 2%) (Lee et al., 2007). Our results for ash content in tissue ranged from 3.6% in *Miscanthus* x *giganteus* to 6.7% in 46-102. Considering that our study was conducted in a wet marginal land, our results show that the grass species compared in this study had a reasonable range of ash concentration in their tissues. As the literature suggests that miscanthus had the lowest ash concentrations as compared to the other compared species, our results agree with it.

Mineral concentration in soil can create problems making them unsuitable for farming and leading to subsequent abandonment. In our study, we found that grass species have differential ability to remove minerals from the ground. Boateng et al. (2015) suggested that switchgrass and miscanthus can be used for phytoremediation of phosphorus-contaminated soils because these grasses accumulated high phosphorus content in their tissue. However, our study suggested that prairie cordgrass has a potential to accumulate greater amounts of phosphorus in its tissue than any of the grasses in the study. Further studies that examine the phytoremediation potential of these grasses in phosphorus-contaminated areas are recommended.

CONCLUSION

In this study, *Miscanthus* x *giganteus*, switchgrass, and prairie cordgrass were screened to identify their potential as dedicated energy crops in wet marginal lands with all grasses producing acceptable biomass yields. Biomass quality, specifically lignocellulose and ash composition, was not affected by the marginal conditions of the study sites. The four prairie cordgrass populations showed some variations in biomass yield, but given that all of these were unselected natural populations, there appears to be opportunities for further breeding and development. With biomass yields comparable to Kanlow switchgrass and *Miscanthus* x *giganteus*, prairie cordgrass should be further studied and developed as a biofuel feedstock. Studying management practices, such as row spacing, can have a major impact on the biomass yield performance of these grass species. Due to varying nutrient removal from the soil, the use in phytoremediation should be also studied further. Additional research should be directed towards testing these promising perennial feedstocks in various marginal settings with different stresses. Answering the difficult question of food versus fuel becomes easier if perennial energy feedstocks are identified that are capable of economical production on marginal lands where food crops cannot be grown.

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Table 2.1. Analysis of variance for biomass yields, cellulose, hemicellulose, lignin and ash content of four prairie cordgrass populations (17-104, 17-109, 20-107, and 46-102), big bluestem, *Miscanthus* x *giganteus*, and Kanlow switchgrass during 2011, 2012, and 2013.

Source	DF	Biomass Yield	Cellulose	Hemicellulose	Lignin	Ash
Species	6	***	**	NS	NS	NS
Spacing	1	***	NS	NS	NS	NS
Species*Spacing	6	**	NS	NS	NS	NS
Year	2	***	NS	**	**	NS
Year*Species	12	***	NS	NS	NS	NS
Year*Spacing	2	NS	NS	NS	NS	NS
Year*Species*Spacing	12	NS	NS	NS	NS	NS

*** =Significance at $P<0.0001$

** =Significant at $P<0.05$

NS = Not Significant

Table 2.2. Mean biomass yields of four prairie cordgrass populations (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), *Miscanthus x giganteus* (Mxg), and Kanlow switchgrass (SW) during 2011, 2012, and 2013.

Species	Biomass Yield					
	2011		2012		2013	
	45 cm	90 cm	45 cm	90 cm	45 cm	90 cm
17-104	13.61	7.94	14.42	10.57	19.94	13.21
17-109	15.54	8.99	16.08	11.83	24.88	16.66
20-107	15.35	7.04	15.22	10.28	24.00	17.14
46-102	4.39	2.96	6.09	6.07	13.36	11.57
BB	11.51	6.09	14.09	8.02	15.5	8.49
Mxg	13.60	6.61	19.93	13.26	28.82	19.71
SW	23.39	12.29	26.24	13.33	29.76	16.31

Fig. 2.1. Average monthly precipitation for Urbana, IL, during 2011 – 2013 with the 30-year average (1981 – 2010).

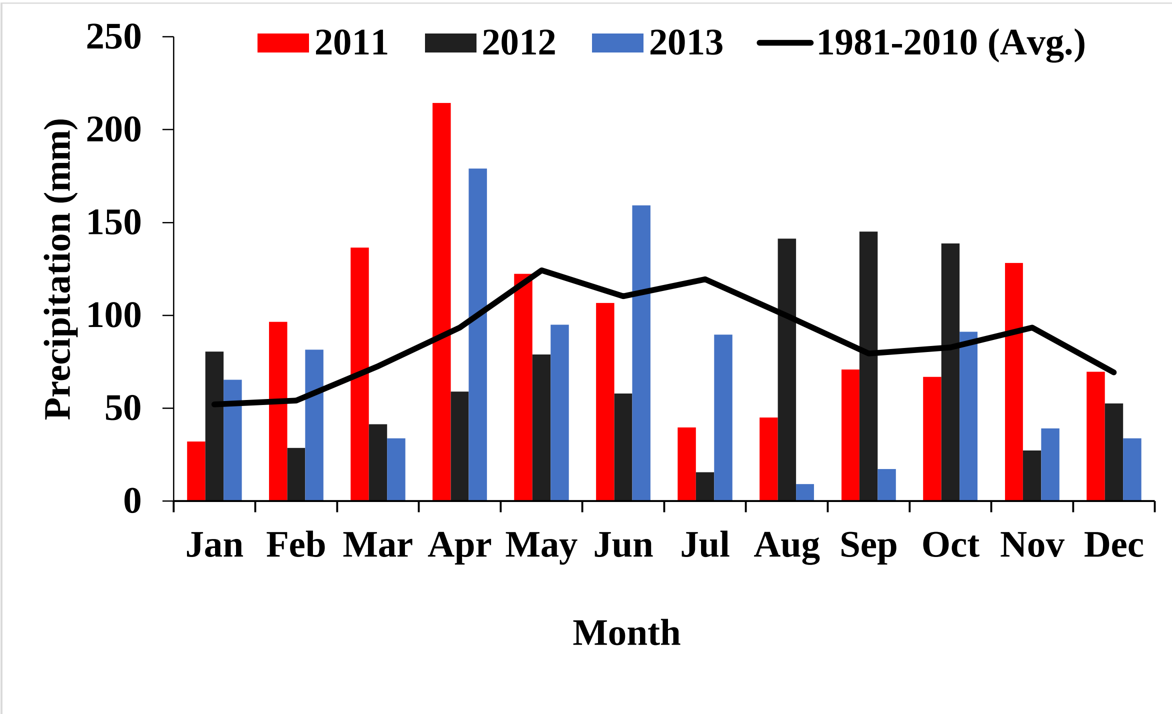


Fig. 2.2. Average monthly temperature for Urbana, IL, during 2011 – 2013 with the 30-year average (1981 – 2010).

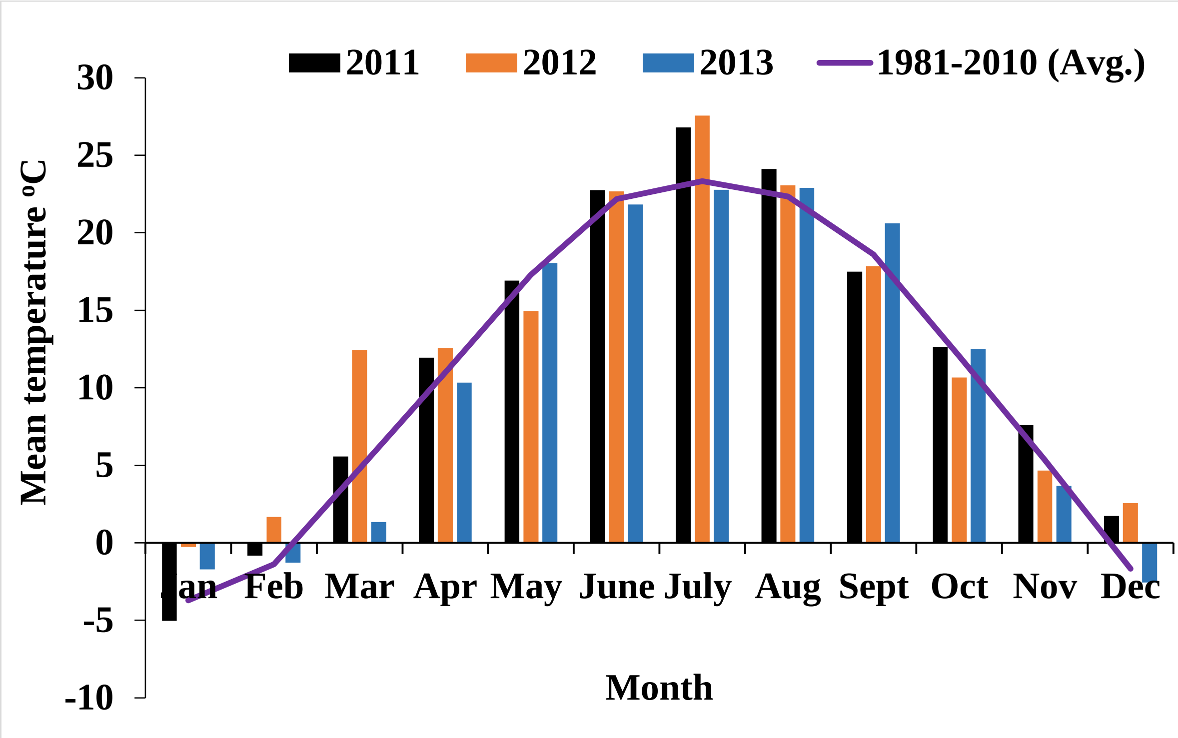


Fig. 2.3. Light intercepted by four populations of prairie cordgrass (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), *Miscanthus* x *giganteus* (Mxg), and Kanlow switchgrass (SW) averaged over two spacings in 2014.

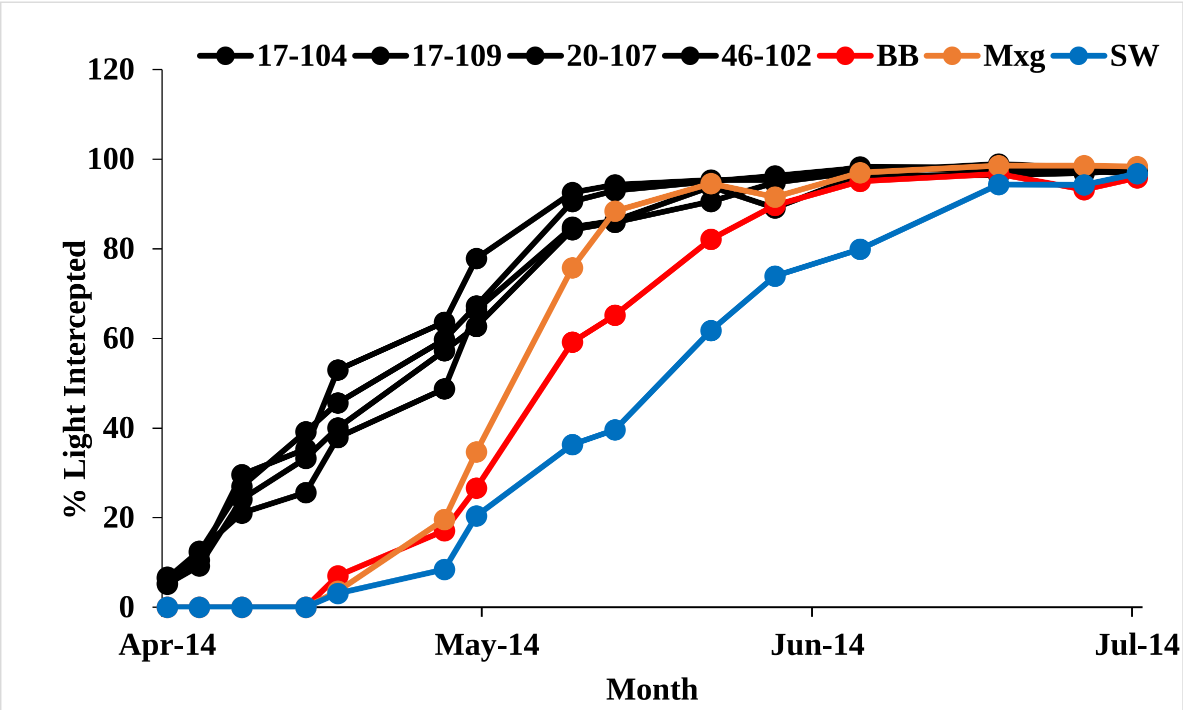


Fig. 2.4. Biomass yield for two spacing treatments averaged over two spacing treatments (45cm and 90cm) from 2011 – 2013 for four populations of prairie cordgrass (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), *Miscanthus* x *giganteus* (Mxg), and Kanlow switchgrass (SW).

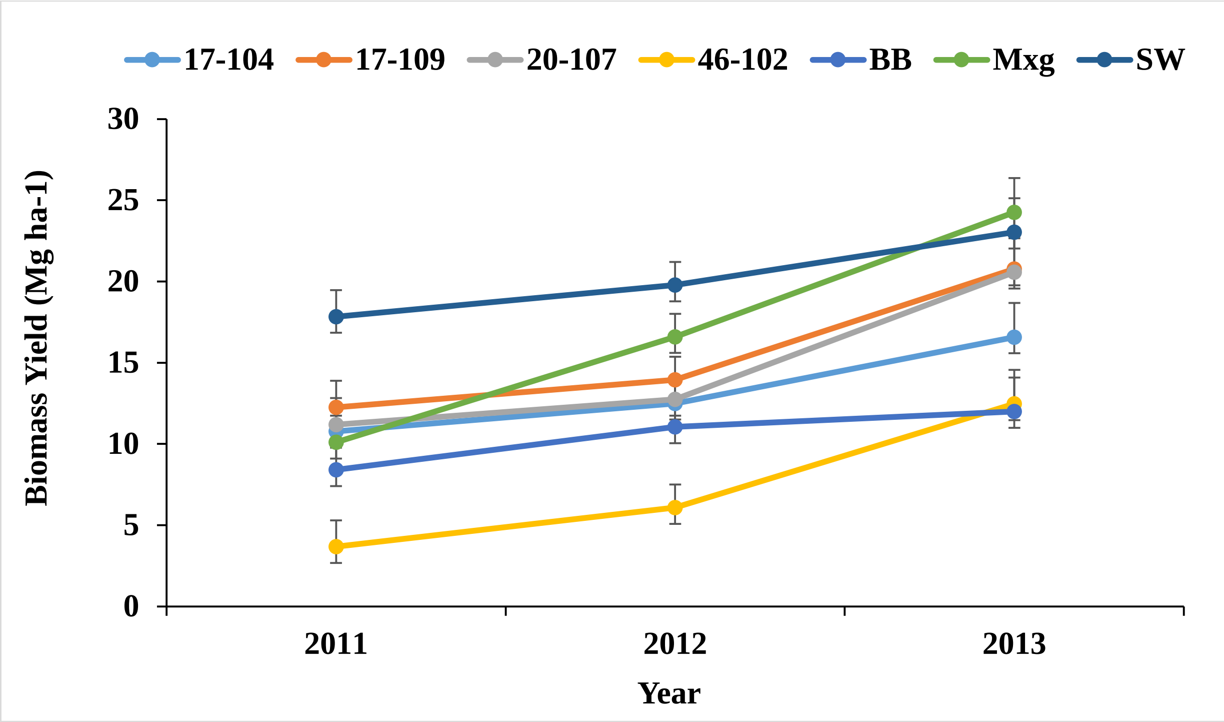


Fig. 2.5. Average biomass yield for two spacing treatments (45 cm and 90 cm) from 2011 – 2013 for four populations of prairie cordgrass (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), *Miscanthus* x *giganteus* (Mxg), and Kanlow switchgrass (SW).

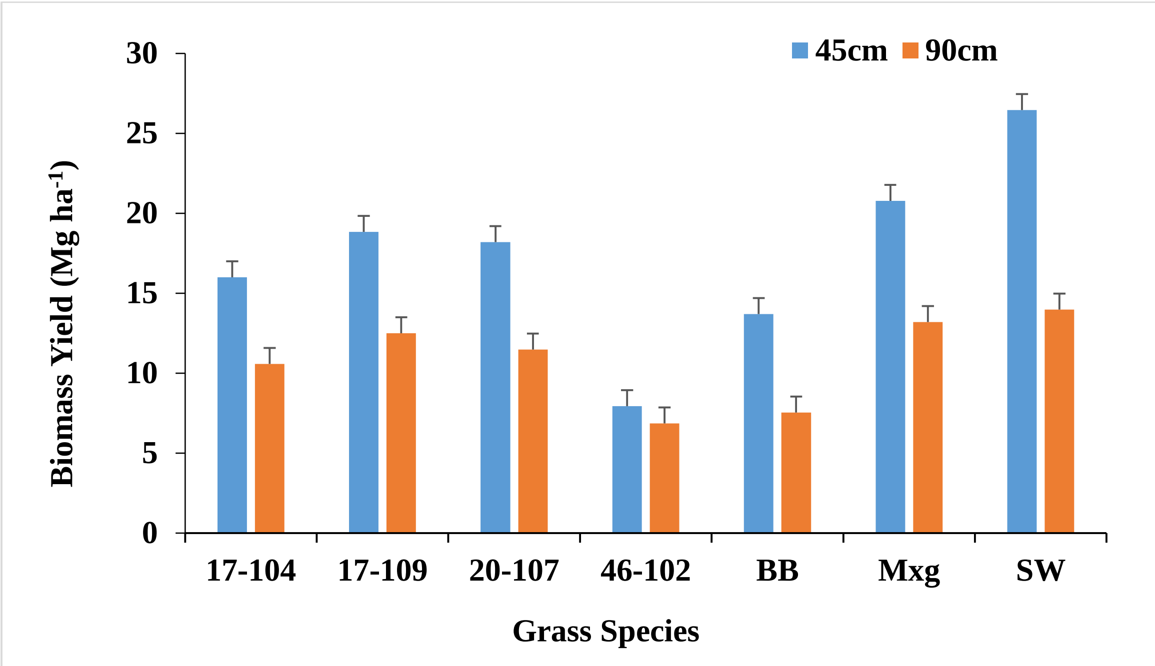


Fig. 2.6. Cellulose concentration in the tissue of four populations of prairie cordgrass (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), *Miscanthus* x *giganteus* (Mxg), and Kanlow switchgrass (SW) from 2011 - 2013.

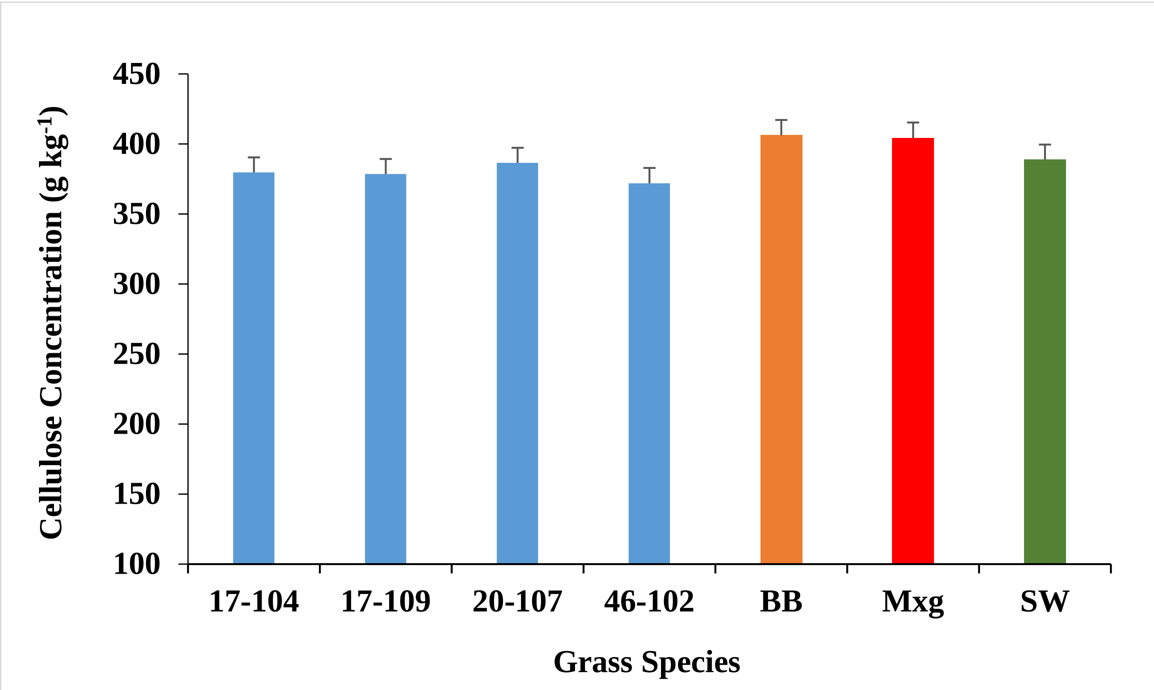


Fig. 2.7. Average hemicellulose concentration in the tissue of four populations of prairie cordgrass (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), *Miscanthus* x *giganteus* (Mxg), and Kanlow switchgrass (SW) from 2011 - 2013

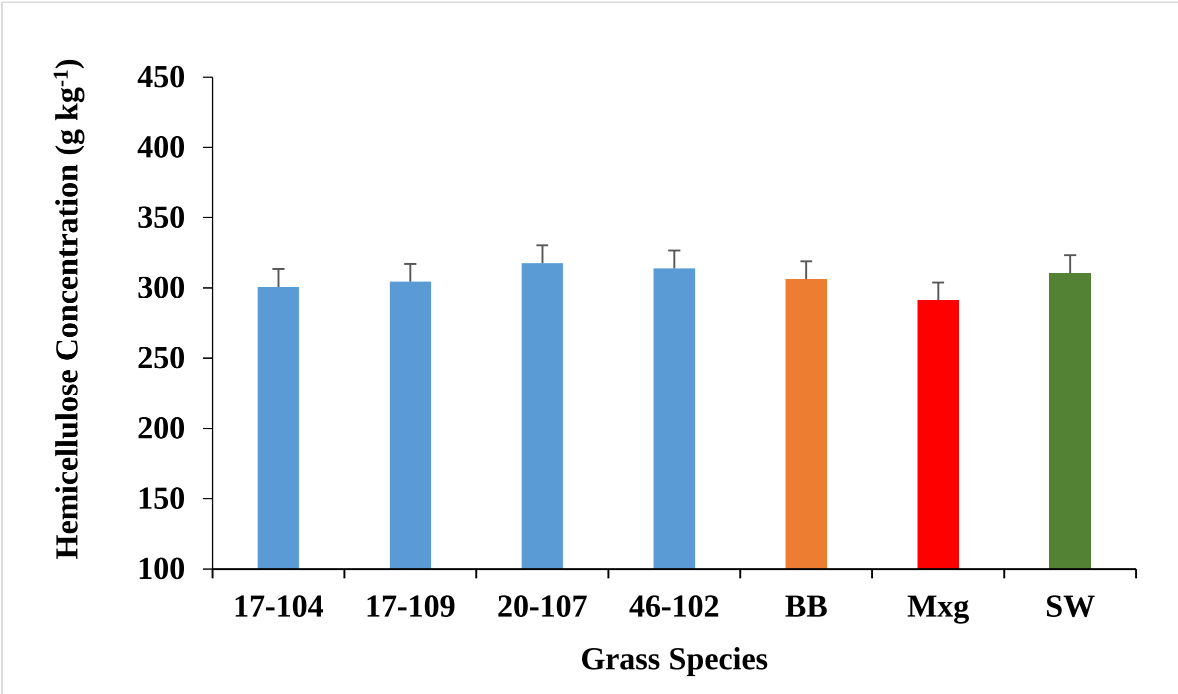


Fig. 2.8. Average Lignin concentration in the tissue of four populations of prairie cordgrass (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), ***Miscanthus*** **x** ***giganteus*** **(Mxg), and Kanlow switchgrass (SW) from 2011 - 2013.**

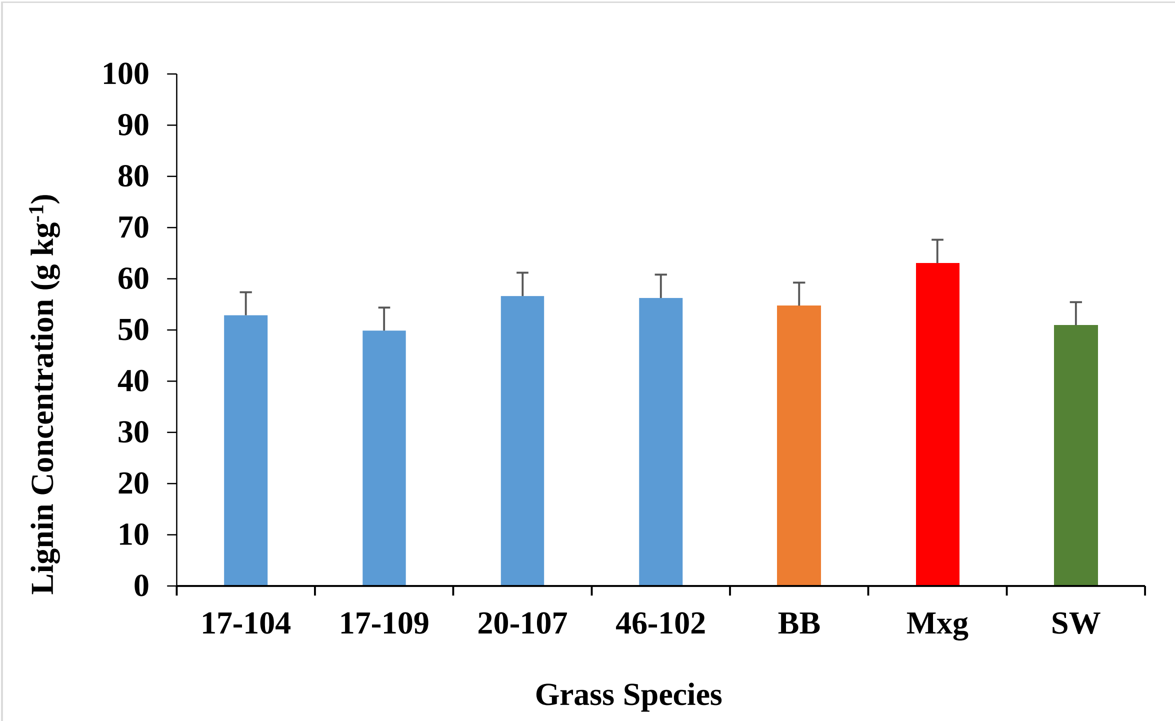


Fig. 2.9. Average ash concentration in the tissue of four populations of prairie cordgrass (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), *Miscanthus* x *giganteus* (Mxg), and Kanlow switchgrass (SW) from 2011 - 2013.

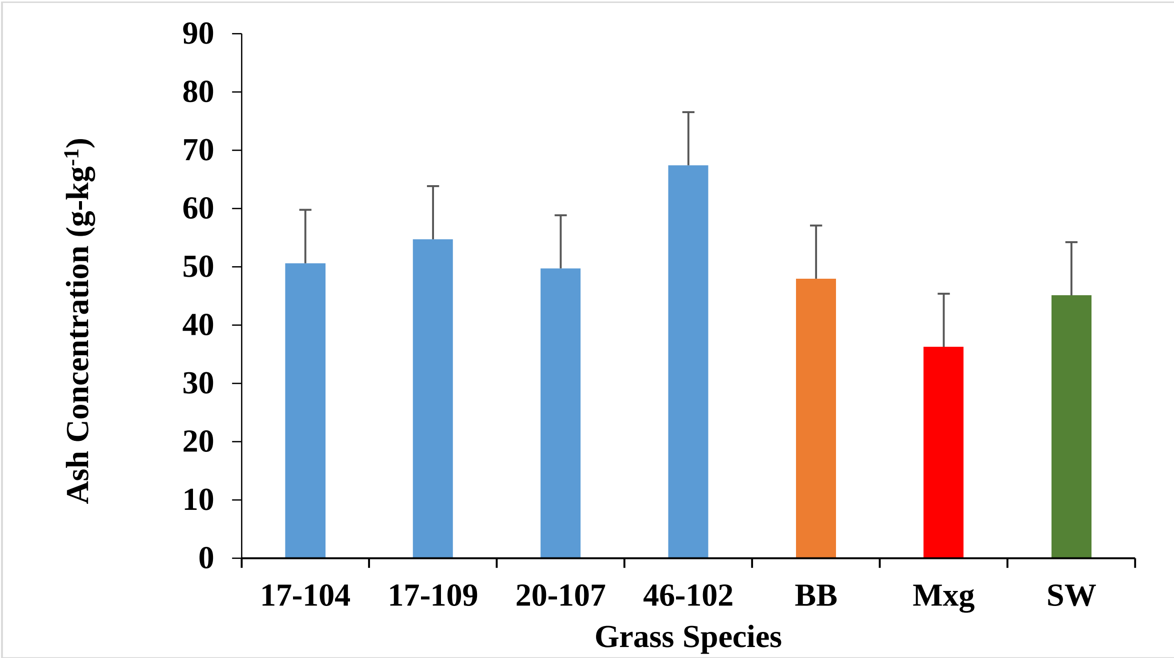
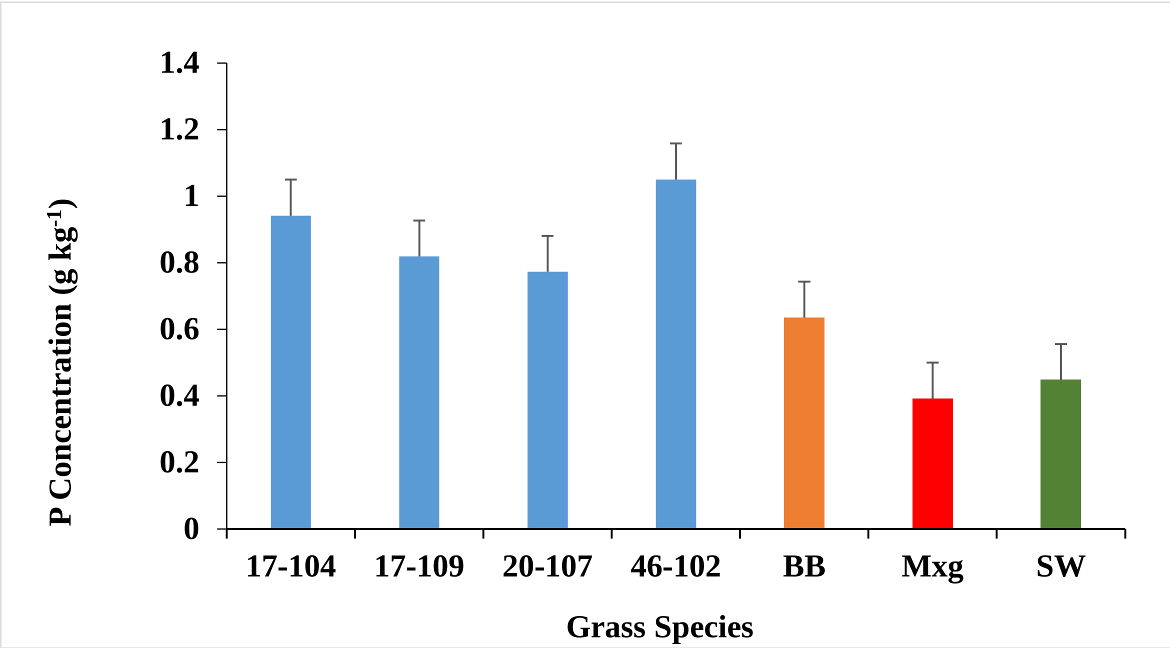


Fig. 2.10. Tissue phosphorus concentration of four populations of prairie cordgrass populations (17-104, 17-109, 20-107, and 46-102), big bluestem (BB), *Miscanthus* x *giganteus* (Mxg), and Kanlow switchgrass (SW) in 2013.



Chapter 3.

SWITCHGRASS AND PRAIRIE CORDGRASS TOLERANCE TO STRESSED ENVIRONMENTS: POORLY DRAINED AND HIGHLY SALINE SOILS

ABSTRACT

In order to have a sustainable energy economy it is important to have a continued and reliable source of bioenergy feedstocks with an efficient end use technology (Samson and Girouard, 1998). Production of second-generation biomass crops is expected to increase due to the federal mandate requiring the use of bioenergy feedstocks to reduce greenhouse gas emissions with minimal impact on the global food supply. Increase in production of second generation lignocellulosic feedstocks will increase demand for arable land. Since it is important for bioenergy production not to compete directly with food crop production, agronomists need to help identify bioenergy crops on marginal lands for sustainable production (i.e. those areas where we cannot grow food crops and thus avoid competition between land-uses). This study evaluates ‘Kanlow’ switchgrass and ‘PCG-109’, & ’17-109’ prairie cordgrass populations on three IL sites, poorly drained sites in Urbana, and Pana and a highly saline site in Salem. A three-year study showed a reliable amount of biomass yield for all three grass populations by the end of the third year. Maximum third-year yields showed an interaction between variety and location: Kanlow produced 14.87 Mg ha^{-1} in Urbana, PCG-109 produced 13.02 Mg ha^{-1} in Salem, and 17-109 produced 12.31 Mg ha^{-1} in Urbana. Grasses at all three marginal sites produced acceptable amounts of lignocellulosic contents. We concludes that the evaluated grass populations have a good potential to be grown in both poorly drained sites and salt-affected marginal land.

INTRODUCTION

A mandate by the US federal government requiring the use of bioenergy feedstocks to reduce the amount of greenhouse gas emissions from fossil fuels, while minimizing the negative impact on global food supply has led to the increased production of second-generation biomass crops (United States Congress, 2007; EISA, 2007; Schnepf and Yacobucci, 2013). Increased production of lignocellulosic bioenergy crops requires additional production area, which can fuel the debate of land use priority between food crops and biofuel crops.

Various biotic and abiotic stresses can limit crop growth and development and subsequently the yield (Venkateswarlu et al., 2012). Land may be classified as marginal for a variety of reasons, including some slightly less severe abiotic stressors which can still produce row crops, but with suboptimal performance (Quinn et al., 2015). An important question is whether marginal lands, which are generally not suitable for row crop production, can be utilized to efficiently produce biomass crops and avoid land-use competition between food crops and energy crops (FAO, 1997; Tilman et al., 2009).

Also termed as degraded lands, marginal lands are unfit for food crop production and often seen as unproductive (Shortall, 2013). Other terms used to refer marginal lands are waste lands, idle lands, under-utilized lands, and abandoned lands (Lal, 1991; 2008; Wiegmann et al., 2008, Tilman et al., 2009; Shortall, 2013). Marginal lands may include areas with poor climate, poor physical characteristics, high salinity, waterlogged and marshy lands, glacial areas and rocky barren areas (Milbrandt and Overend, 2009). Covering approximately 86.5 million hectares, 11% of the US mainland is considered to be marginal land (Milbrandt et al., 2014). Out of the total marginal land, the major portion, 68.25 million hectares (79%), is abandoned croplands, while barren lands and abandoned mine lands cover about 9.7 million hectares and 1.1 million hectares, respectively (Milbrandt et al., 2014).

One factor that causes land marginalization and subsequent abandonment is salt stress commonly found in arid, semi-arid, and irrigated lands around the globe (Flowers & Yeo, 1995; Bot et al., 2000). Change in water table depth caused by anthropogenic factors such as deforestation, planting shallow-rooted crops, and irrigation practices can cause excess water evaporation from soil surface followed by salt accumulation in root zones (Abrol et al., 1998; Sim, 2012). Out of 1.35 billion hectares of total arable land area globally (Anderson et al., 2010), more than 800 million hectares (Munns & Tester, 2008) are affected by high salinity, with approximately 8.5 million of these hectares in the USA (Pearson, 2009).

Other important abiotic factors that can cause marginalization of lands are stresses caused by water scarcity (USDA NRCS, 2014) or excessive water that causes waterlogging resulting from poor drainage and flooding in marginal soils (Gopalkrishnan et al., 2011). Drought is caused from a prolonged period of time with no significant precipitation causing water scarcity, which results in a shortage of water availability (Hale and Orcutt, 1987). Wet soil in spring can delay planting and can cause yield losses due to diseases associated with wet and cool soil condition (Bockus and Shroyer, 1998; Stoof et al., 2015). Similarly, waterlogged conditions in late season can cause difficulties in field operations due to standing water (Stoof et al., 2015). Flooding reduces the soil oxygen availability to plants and reduces root respiration (Caudle & Maricle, 2012) thus making it unfavorable for plant growth. Farmlands in the US Midwest were unsuitable for annual crop production like corn and soybeans due to poor drainage until tile drainage systems were installed (Gopalkrishnan et al., 2011).

In order to utilize these marginal lands for biofuel purposes we need a diverse set of bioenergy feedstocks to develop economically and environmentally sustainable second-generation biofuel industry (Simmons et al., 2008). Perennial grasses commonly require low inputs and have low production costs, and many are well adapted to various marginal lands (Samson and Girouard, 1998). Production of perennial energy crops tolerant of marginal lands can reduce competition with food crops, while simultaneously enhancing environmental benefits of soil and water conservation (Wicke et al., 2011).

Prairie cordgrass (*Spartina pectinata* Link.) is a perennial rhizomatous grass, indigenous to many of the regions throughout United States and Canada (Backworth et al., 2007; Boe et al., 2009) and relatively new to the bioenergy industry. It was shown to be tolerant to flooded conditions (Bonilla-Warford and Zelder, 2002) and can be grown in land that is too wet for corn (Boe et al., 2013) Boe et al., 2009; Boe and Lee, 2007). Studies have shown that prairie cordgrass has good potential for use as a biomass crop in wet marginal lands (Bonilla-Warford and Zelder, 2002; Skinner et al., 2009). It is also tolerant of salt (Kim et al., 2011; Anderson et al., 2014), alkaline conditions, and high water tables (Jensen, 2006); therefore it can be grown on variety of marginal soils. Switchgrass (*Panicum virgatum*), on the other hand, is an established energy crop and has known biomass productivity potential for biofuel purposes. The US Department of Energy identified switchgrass as a ‘model’ bioenergy feedstock as it has been much studied for biomass production (Wright and Turhollow, 2010). Switchgrass is generally considered to be drought tolerant (Barney et al., 2009; Sanderson et al., 1999), however, some lowland switchgrasses also tolerated flooded conditions (Barney et al., 2009; Porter, 1966). In addition, some upland switchgrass cultivars were found to be salinity tolerant (Carson & Morris, 2012; Schmer et al., 2012).

Both prairie cordgrass and switchgrass have a wide range of adaptability to different stressful growing environments. Some switchgrass cultivars have moderate tolerance to flooding and salinity, and prairie cordgrass also has high tolerance to these conditions (Quinn et al., 2015). The problem of high salinity and poorly drained soils still exists in some US farmlands. Considering the biomass potential of these grasses, we wanted to make side-by-side comparisons of switchgrass and prairie cordgrass in field settings having soil salinity and poor drainage issues. Therefore, the objective of this study was to determine the biomass yield performance and tissue lignocellulosic composition of 'Kanlow' switchgrass and two populations of prairie cordgrass ('17-109' and 'PCG-109') on a highly saline soil and in wet marginal lands in IL. Given that these two species can tolerate different environments, we hypothesize that there will be differences in biomass production and lignocellulosic concentrations among the PCG-109 and PC-17-109 prairie cordgrass populations and Kanlow switchgrass and soil conditions. Prairie cordgrass being native to wet prairies we hypothesize that it will tolerate the wet marginal condition better than switchgrass and thus will perform better in wet IL soils. In addition, we hypothesize that the prairie cordgrasses will have better yield performance in the salt affected area. The findings of this experiment will help us understanding the potential of using switchgrass and prairie cordgrass for biomass production in these two marginal settings.

MATERIALS AND METHODS

Field studies were established in three Illinois locations: Urbana (40°4'16.28"N, 88°13'23.09"W), Pana (39°26'28.87"N, 89° 7'13.66"W), and Salem (38°32'52.04"N, 89°1'24.00"W), in July 2011. The soil type in Urbana was a Drummer silty clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquolls), considered as wet marginal soil with seasonal water logging and a Land Capability Class of ‘5W’ without installed drainage. Similarly, the soil type in Pana location was a Virden silty clay loam (Fine, smectite, mesic Aquic Argiudolls), which was also considered as a wet marginal land due to the seasonal water logging and had a Land Capability Class of ‘4W’ without installed drainage. The soil in Salem location was a Cisne-Huey silt loam (Fine, smectic, mesic Mollic Albaqualfs) with a Land Capability Class of ‘3W’. The soil at this location was contaminated with salts due to spilled brine water injected into a nearby oil pump. The contaminated water accumulated in that area leaving a bald patch in the middle of the field. The electrical conductivity of the soil in the Salem study area was greater than 20 dS/m, which is considered extremely high for growing food crops. According to the farmer there was no vegetation in that spot for more than 30 years.

Seeds of PC 17-109, and PCG 109 prairie cordgrass populations and Kanlow and ‘Blackwell’ switchgrasses were sowed in planting trays in May of 2011, and allowed to grow in the University of Illinois Plant Care Facilities’ greenhouse. On July 5, 2011, the seedlings were transplanted in the study plots in Pana, IL, and in Urbana and Salem on July 14, 2011 and July 28, 2011, respectively. Irrigation was applied during the transplanting only to ensure successful establishment. After establishment in 2011, water requirements were entirely dependent on natural precipitation for the remainder of the study period.

The experimental design was a randomized complete block with four replications of each plant population. Four blocks represented the four replications in each site. The plugs were space planted on 90-cm row spacings and 90-cm spacings between plants. Each population consisted of four rows, with four plants in each row making it total of 16 plants per replication in a 2.74 m by 2.74 m square plot. The study period was from 2012 to 2014, and no fertilizer applied to the experiments during the study period. Survival data was collected during the establishment year by making counts of live plants to monitor establishment. Dead plants were removed and replacement grasses planted early in the 2012-growing season. None of the Kanlow or Blackwell switchgrasses survived into the second growing season; the Blackwell was replaced with ‘IL-102’ prairie cordgrass, and the Kanlow switchgrass was replanted. In this study, IL-102 prairie cordgrass was not included in any analysis.

Biomass was not harvested in the 2011 establishment year. The experimental plots in all three locations were harvested after the killing frost in late fall (November – December) during 2012, 2013, and 2014, and the biomass yields were determined in each year. The plots were harvested using a STIHL brand professional trimmer (STIHL FS 90 bike handle trimmer) with a STIHL HL 135^{o} hedge trimmer attachment (STIHL Incorporated 536 Viking Drive Virginia Beach, VA 23452). During each harvest, approximately one kilogram plant samples were collected from each plot and dried at 60 ºC for at least 96 hours to determine dry biomass yield. The dried samples were saved for tissue and mineral composition analysis for later.

The dried samples were ground using a cutting mill (Retsch SM2000, Haan, Germany), to pass through a 1mm screen size which were used for tissue analysis. The ground samples were stored until further analyses. A portion of the samples were sent to Brookside Laboratories (Brookside Laboratories, Inc. 200 White Mountain Drive, New Bremen, Ohio 45869) for tissue mineral composition analysis. Tissue ash content was determined using Neycraft Pro furnace using the methods that was described by Undersander et al. (1993). The fiber analysis of tissue samples were conducted using an Ankom ^{200} Fiber Analyzer (Ankom Technology, 2052 O’Neil Road, Macedon, NY 14502) for neutral detergent fiber (NDF) and acid detergent fiber (ADF). Acid detergent lignin (ADL) was also determined by using an Ankom Daisy II incubator (Ankom Technology, 2052 O’Neil Road, Macedon, NY 14502). The data collected from fiber and lignin content analyses were utilized to estimate tissue cellulose and hemicellulose content. Cellulose was estimated by calculating the difference between ADF and ADL, while hemicellulose content was estimated by calculating the difference between NDF and ADF.

Analysis of variance (ANOVA) was conducted to determine the variance among main effects of grass species and year as well as their interactions for biomass yield and tissue composition. The main effects as well as interaction effect of species and year were treated as fixed variable while replication or block was treated as random variables. The data was analyzed separately for three separate locations. Biomass yield, tissue mineral and lignocellulosic contents were the dependent variables while species and year were independent variables. Dry biomass yield data, as well as tissue composition data, was analyzed using PROC MIXED model procedure in the SAS (SAS 9.4. SAS Institute Inc., Cary, NC, USA). Significance was determined at the $P<0.05$ level. Homogeneity of variance was determined using Brown Forsythe's test. Fisher's least significant difference (LSD) test was conducted to separate the means of biomass yield and tissue lignocellulosic and mineral composition among the grasses in 2012, 2013, and 2014. We used proportion values to analyze the survival data. The proportion values were transformed to arcsine of the square root of the values. Then the data was subjected to mixed model to obtain the estimates and standard error values. Then we transformed back the arcsine of the square root of the values to percentage and presented in a bar chart.

RESULTS

The weather at all three sites showed variations among years during the study period. There was less than average precipitation in 2012 during the early and middle growing season (from April to July) in all three locations (Fig. 3.1), however it was lower than the 30-year average starting from March of 2012 in both of the wet Urbana and Pana sites. The average monthly temperature data for all three locations are shown in Fig. 3.2.

None of the Kanlow and Blackwell switchgrasses survived in Salem during the establishment year (Fig. 3.3), but 70% of the PCG-109 and approximately 47% of the 17-109 prairie cordgrasses in that location survived. Most of the grasses survived in Pana and Urbana. Approximately 97% and 97% of the PCG-109 prairie cordgrasses, 78% and 75% of the 17-109 prairie cordgrasses, and 89% and 97% of the Kanlow switchgrasses survived in Pana and Urbana, respectively (Fig. 3.3).

Analysis of variance showed that there was considerable variation in yield, as well as in lignocellulosic concentration and mineral concentration among species and year for all locations (Table. 3.1). Year was considered as a fixed variable and the year effect was significant in all three locations and its interaction with species was also significant in Urbana and Pana sites. The biomass yield in 2012 was less than in 2013 and 2014, in all three locations (Fig. 3.4). Biomass yield for Kanlow switchgrass did not increase significantly from 2013 to 2014 in either Salem (9 Mg ha^{-1} in 2013 and 9.4 Mg ha^{-1} in 2014) or Urbana (14.8 Mg ha^{-1} in 2013 and 14.9 Mg ha^{-1} in 2014), but was also the same for PC17-109 prairie cordgrass in Salem (10.7 Mg ha^{-1} in 2013 and 10.8 Mg ha^{-1} in 2014) (Fig. 3.4). However, both PC17-109 prairie cordgrass (7.3 Mg ha^{-1} in 2013 and 12.3 Mg ha^{-1} in 2014) and PCG-109 prairie cordgrass (7.3 Mg ha^{-1} in 2013 and 11.6 Mg ha^{-1} in 2014) in Urbana, and PCG-109 prairie cordgrass (8.8 Mg ha^{-1} in 2013 and 13.0 Mg ha^{-1} in 2014) in Salem produced more biomass in 2014 than 2013 (Fig. 3.4). The biomass yield in Pana was lower in 2014 than in 2013 for all three grass populations (Fig. 3.4). The overall yield over three years of study showed that Kanlow switchgrass had higher yield in both Pana and Urbana locations, but was not different in Salem.

The overall cellulose concentration was not different among the grass populations in Salem, whereas it was different in Urbana and Pana (Fig. 3.5). The ANOVA also showed that the year had significant effect on cellulose concentration in Salem and Urbana, while it was not different in Pana. However there was no interaction between year and species in any of the three locations. The cellulose concentration in 2014 ranged approximately 362 g kg^{-1} to 395 g kg^{-1} among the grass populations along the three locations (Fig. 3.5). There were no significant differences in any of the variables for hemicellulose concentration in Salem and Urbana (Table 3.1). However main effects of species and year were significant in Pana for hemicellulose concentration. By the end of the study in 2014, the hemicellulose concentration in all three locations ranged from 322 g kg^{-1} to 374 g kg^{-1} (Fig, 3.6). Although not significantly higher in all locations, PCG-109 prairie cordgrass had slightly higher hemicellulose concentration in plant tissue than the other two grasses in all three locations in 2014 (Fig 3.6).

Tissue lignin concentrations were significantly different for grass populations in Urbana and Salem, but not in Pana (Fig. 3.7). Lignin concentration ranged from 44 g kg^{-1} for Kanlow switchgrass to 50 g kg^{-1} for PC17-109 prairie cordgrass in Pana in 2014. The same year, Kanlow switchgrass contained the lowest lignin concentration at 36 g kg^{-1} in Salem and the highest concentration at 112 g kg^{-1} in Urbana, as compared to the two prairie cordgrass populations (Fig 3.7). However, the ash concentration in 2014 was slightly lower in Kanlow switchgrass in Pana and Urbana, but not significantly lower in Salem. PCG-109 prairie cordgrass had higher ash concentration than the other two grasses in all three locations in 2014 (Fig. 3.8).

For tissue mineral compositions the data was averaged across the years. The phosphorus concentrations were higher in both of the prairie cordgrass populations than in Kanlow switchgrass in all three locations (Fig. 3.9). Similarly, the nitrogen concentration was found higher in both of the prairie cordgrass in Salem and Urbana while it was not significantly different in Pana location (Fig. 3.10). We also examined the sodium concentration in the tissue and found that there were no significant differences among the grass populations in either Salem or in Urbana, whereas, there was numerically higher tissue sodium concentration in Kanlow switchgrass than in the two prairie cordgrass populations (Fig. 3.11. However, the sodium concentration was numerically higher in Salem in both switchgrass and prairie cordgrass populations than in the other two locations, ranging from 0.03 g kg^{-1} to 0.09 g kg^{-1} in Pana, 0.06 g kg^{-1} to 0.08 g kg^{-1} in Urbana, and 0.86 g kg^{-1} to 1.44 g kg^{-1} in Salem.

DISCUSSION

During the establishment year, neither Blackwell nor Kanlow switchgrass cultivars survived, while both natural populations of prairie cordgrass had good survival. Although the stress tolerance level may vary among each genotype and each may behave differently, multiple stresses can often cause damage to plants even if tolerant to one type of stress (Silva et al., 2013). A highly heat-tolerant species, *Jatropha curcas*, is more affected by a combination of heat and salinity stresses than to a single stress (Silva et al., 2013). In our case, the experiment was planted in late July in 2011, heat and salinity stresses probably caused the death of all of Kanlow switchgrasses in 2011 establishment year. When replanted earlier in the growing season in 2012, all of Kanlow switchgrasses survived.

There were differences in performance among the grass populations and locations, however the main objective of this study was not to compare the performance between the locations. Considering that biomass yield is an important trait for bioenergy crops (McKendry, 2002; Wright, 2007; Boe and Beck, 2008; Anderson-Teixeira et al., 2012), all three locations produced a reasonable amount of biomass for a marginal land setting, indicating strong biomass potential for the grass populations tested in this study. The low biomass yield in 2012, as compared to other years, can be attributed to the below average amount of precipitation resulting in early season water scarcity at all three locations. The lower biomass yields in Pana in 2014, compared to 2013, can likely be attributed to lower early season (March through May) precipitation in 2014 than in 2013. The early season is most critical for the plants growth and development.

There were no changes in biomass yields between 2013 and 2014 for Kanlow switchgrass in either Salem or Urbana. Compared to 2013, however, biomass yields for the two prairie cordgrass populations were higher in 2014 in both locations, with the exception of the unchanged yields in the two years in Salem for the 17-109 prairie cordgrass population. Boe and Lee (2007) found large biomass yield variations among different prairie cordgrass populations within and between the years in a four-year period. For the biomass yield in Salem location, which is a salinity affected area, both prairie cordgrass did well on biomass yield which was expected because of their salt tolerance ability. Considering the fact that both of these prairie cordgrass populations are unselected natural populations, there is a tremendous opportunity for their selection and improvement for full exploitation of the salinity affected areas. Our results suggest that Kanlow switchgrass may have reached at full production stage and did not increase yield in 2014, while PCG-109 prairie cordgrass was still increasing and may not have reached full production. We found that Kanlow switchgrass had greater yields in the poorly drained Pana and Urbana sites. This may be because lowland switchgrass ecotypes are well adapted to flood plains and are more robust than upland cultivars (Porter, 1966; Fuentes & Taliaferro, 2002).

Cellulose and hemicellulose content are important for bioethanol conversion quality of a biomass (Wyman, 1999). General feedstocks are 35% - 50% cellulose, 20% - 35%

hemicellulose, and 12% - 20% lignin (Wyman, 1999). Here, we found the cellulose content ranged from 36.2% to 39.5% and hemicellulose ranged from 32.2 % to 37.4 % which is a similar range mentioned above. Since the tissue lignocellulosic composition was different in different locations in different years it is safe to say that lignocellulosic composition is dependent on variety of different factors including the environment. These factors may include plant genotype, plant ecotype, precipitation, fertilizer, harvest time, and different environments (Kim et al., 2011). The most significant factors influencing switchgrass biomass yield and composition were cultivar, location, and harvest time (Casler and Boe, 2003; Casler et al., 2004).

Tissue mineral composition measurements of prairie cordgrass populations showed greater accumulated phosphorus compared to Kanlow switchgrass in all three locations. Boatent et al. (2015) suggests that switchgrass and miscanthus have the potential to be used in phytoremediation on phosphorus contaminated soils. Our results show that prairie cordgrass may have potential to be used for phosphorus remediation in problem soils. In the case of sodium content in the grass tissue, however, there were no differences among the grass populations found in Salem or Urbana, while Kanlow switchgrass had higher sodium content in Pana. However, the tissue sodium content in all three grass populations in Salem, the salt-affected site, were higher. This suggests that all three grass populations performed well in this area and were able to accumulate salt in their tissues which can be a desired trait for plants being produced in areas with salinity issues.

CONCLUSION

From site to site, biomass feedstock choices will likely differ as crop productivity varies, based on soil conditions and marginality as well as local climate (van der Weijde et al., 2013).

Each of the grass species evaluated in this study, Kanlow switchgrass, and PCG-109 and PC17-109 prairie cordgrasses, were able to produce reasonable biomass yield under poorly drained and salt affected marginal land settings. However, weather, soil conditions, and competition with other plant species can play a role in establishment. Tissue composition was not affected for either the switchgrass or the prairie cordgrasses by the marginal situation of the land. Moreover, there is potential for these grasses to be used for phytoremedial purposes in problem soils with salinity and phosphorus contamination. The unselected natural populations of prairie cordgrass used in this experiment can be of great value for further research for breeding and development Overall, the grasses evaluated in this study were found to have a good potential to be used as a biomass crop in poorly drained and salt affected marginal lands.

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Table 3.1. Analysis of variance of biomass yield, lignocellulosic components, and mineral concentrations for Pana, Urbana, and Salem, Illinois, 2012 - 2014.

	df	Yield	Cellulose	Hemicellulose	Lignin	Ash	N	P	Na
					Salem				
Source									
Species	2	NS	NS	NS	**	NS	**	**	NS
Year	2	***	**	NS	**	NS	**	NS	NS
Year*Species	3	NS	NS	NS	NS	NS	NS	NS	**
					Urbana				
Source	df								
Species	2	**	**	NS	**	**	NS	NS	NS
Year	2	***	**	NS	NS	**	NS	**	NS
Year*Species	3	**	NS	NS	NS	NS	NS	NS	NS
					Pana				
Source	df								
Species	2	**	**	**	NS	**	NS	**	**
Year	2	***	NS	**	**	**	***	***	**
Year*Species	3	**	NS	NS	NS	**	NS	NS	**

*** =Significance at P<0.001
*** =Significant at P<0.05
NS = Not Significant

Fig. 3.1. Average monthly precipitation in Pana, Urbana, and, Salem, Illinois, during 2011, 2012, 2013, and 2014, with the 30-year averages (1981 – 2010).

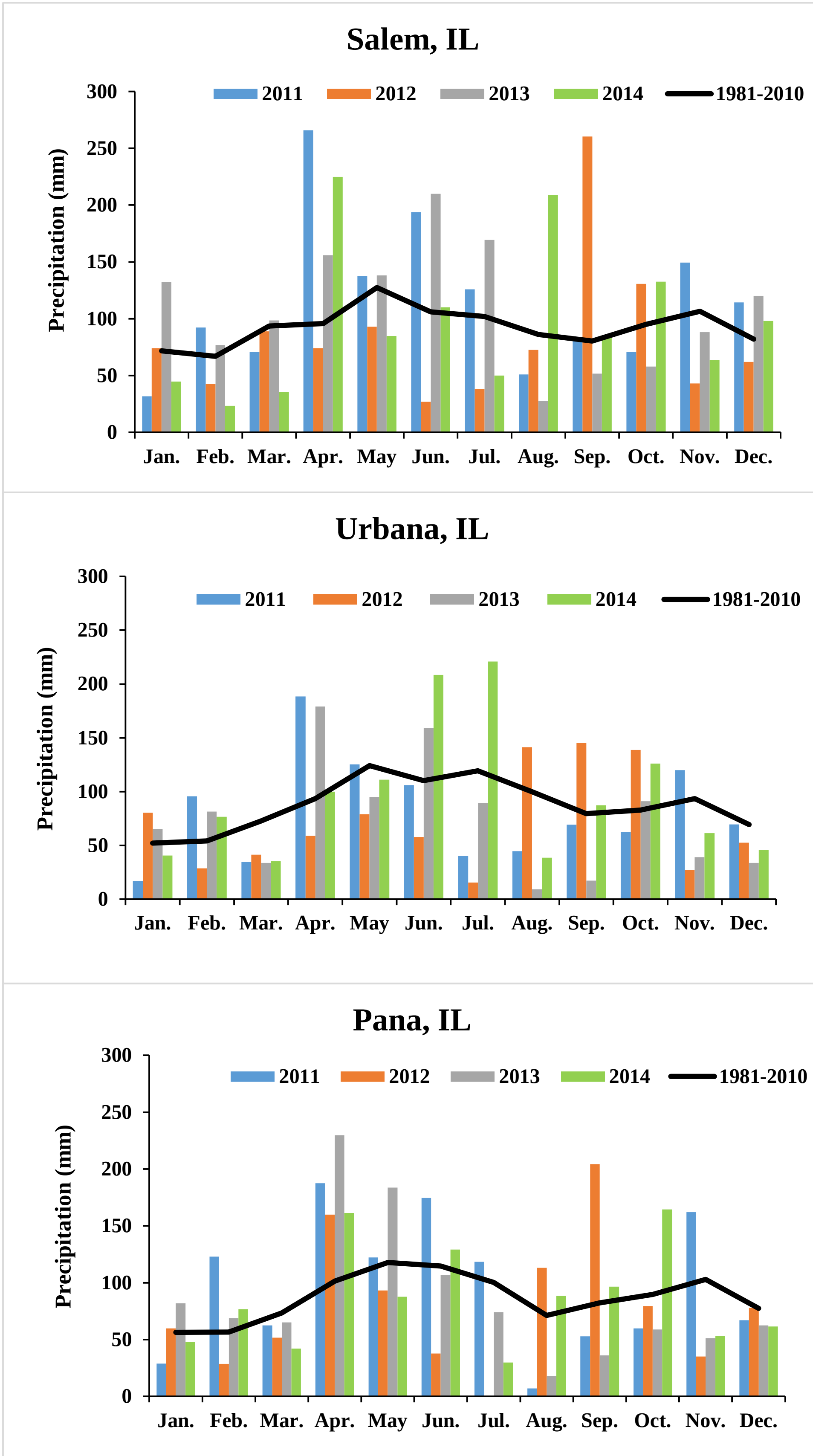


Fig. 3.2. Average monthly precipitation in Pana, Urbana, and, Salem, Illinois, during 2011, 2012, 2013, and 2014, with the 30-year averages (1981 – 2010).

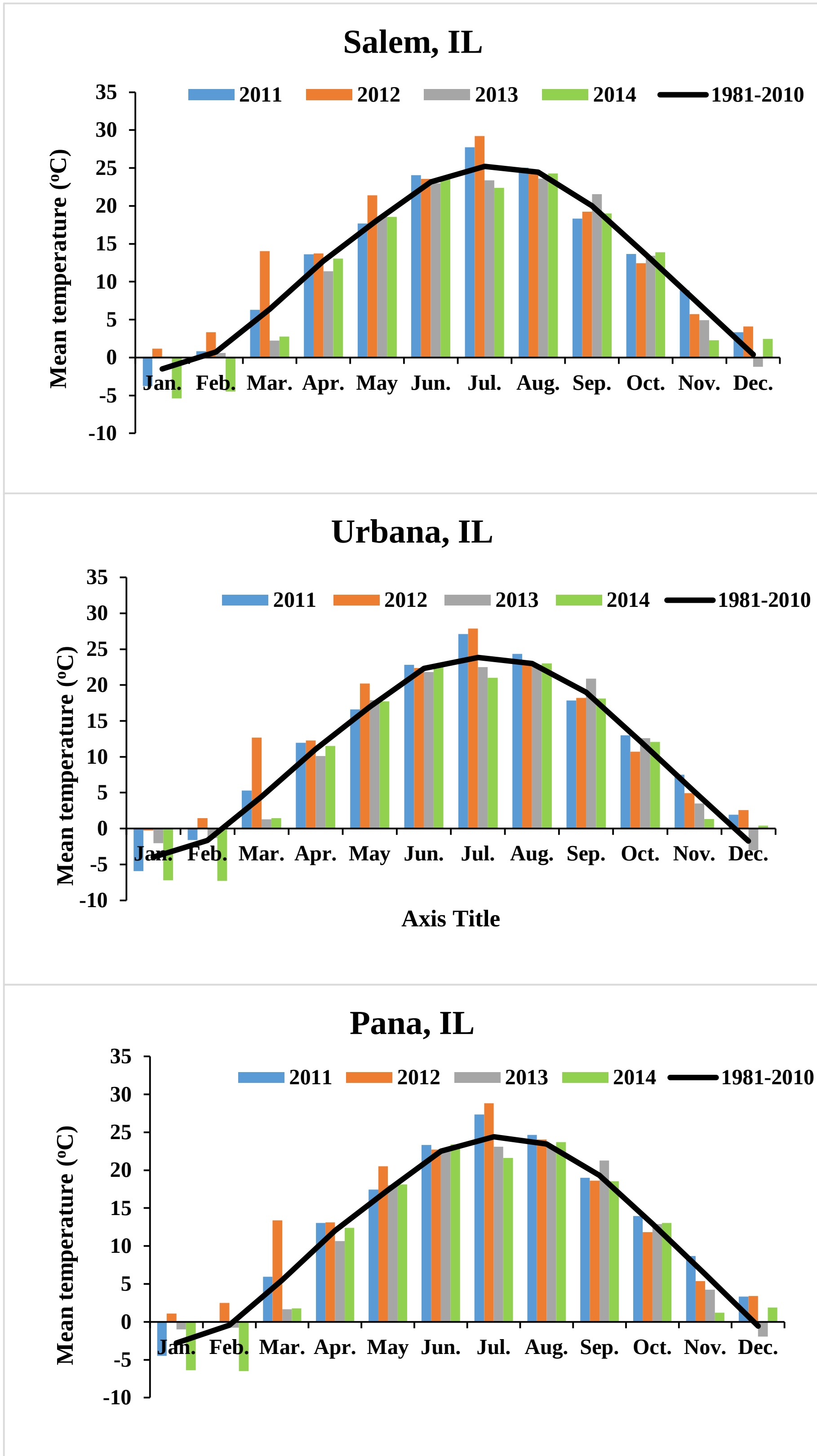


Fig. 3.3. Plant survival percentage for PC17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass during the establishment year (2011) in Pana, Urbana, and Salem, Illinois.

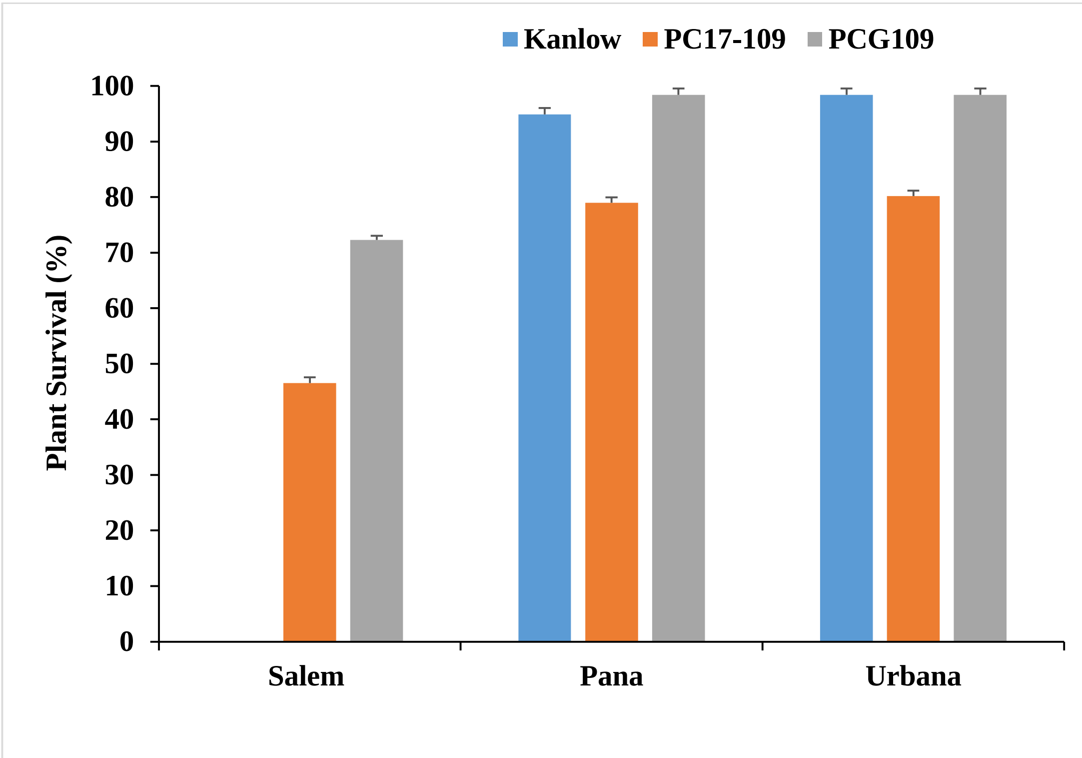


Fig. 3.4. Average 2012, 2013, and 2014 biomass yields for PC17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass in Pana, Urbana, and, Salem, Illinois.

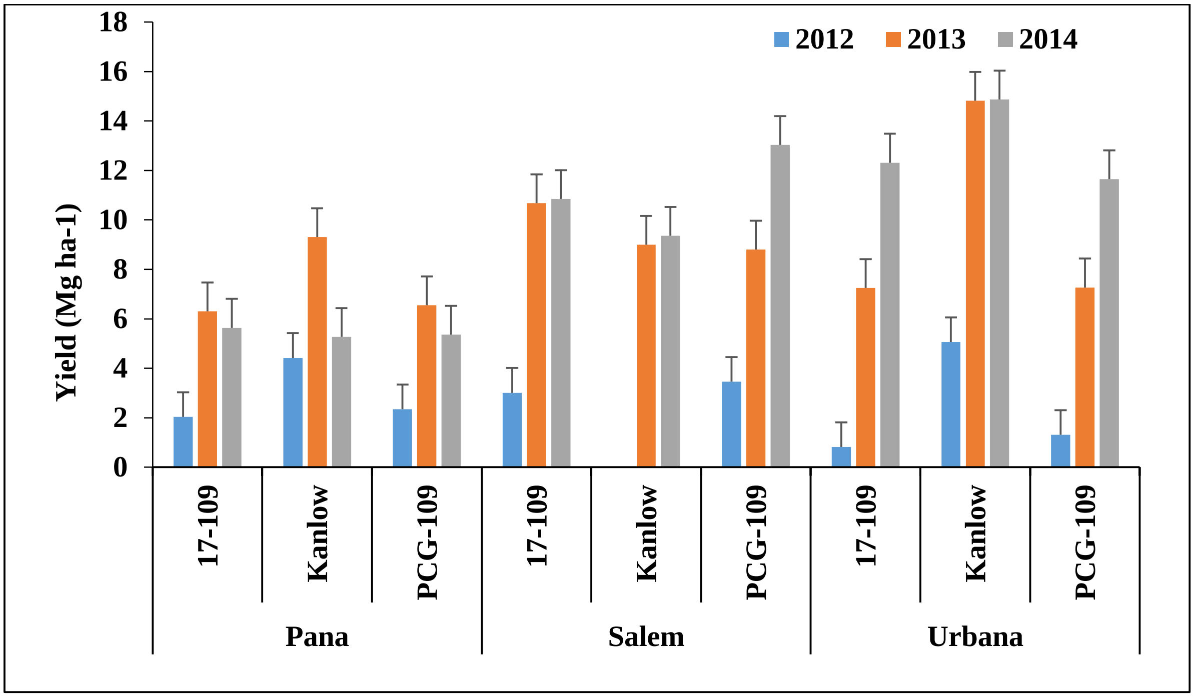


Fig. 3.5. Cellulose concentration for PC17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass in Salem, Urbana, and, Pana, Illinois in 2012, 2013, and 2014.

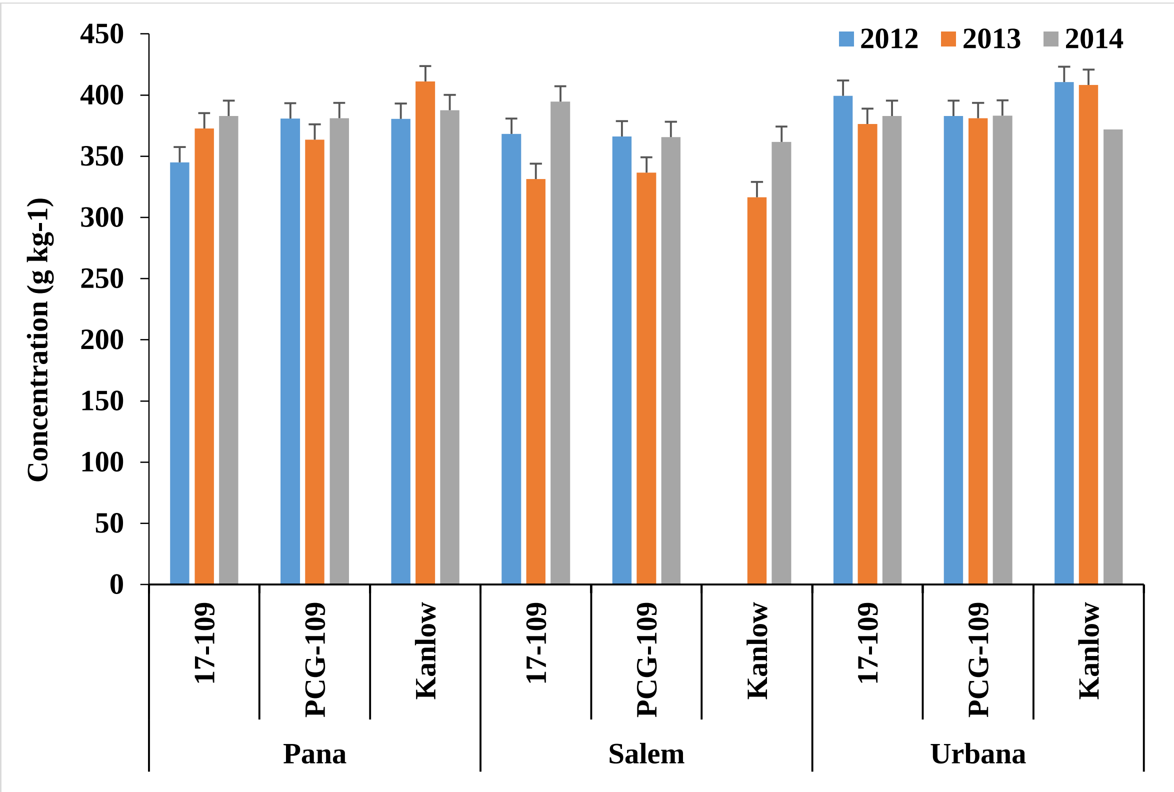


Fig. 3.6. Hemicellulose concentration for 17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass in Salem, Urbana, and, Pana, Illinois in 2012, 2013, and 2014.

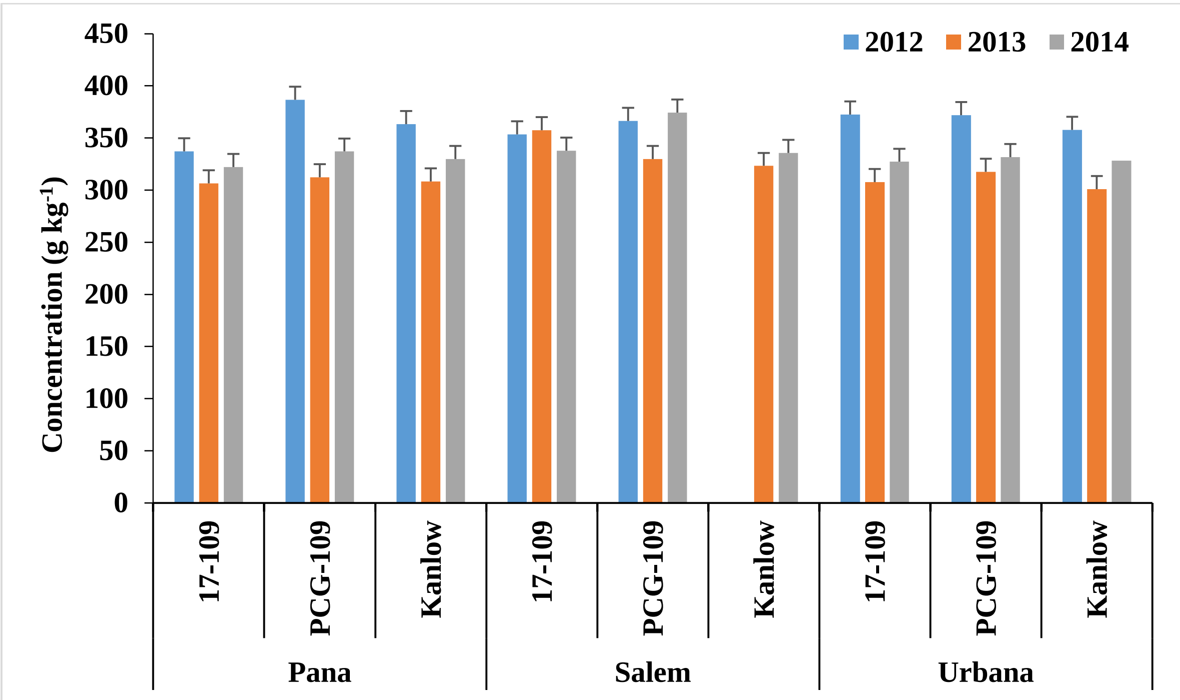


Fig. 3.7. Lignin concentration for PC17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass in Salem, Urbana, and, Pana, Illinois in 2012, 2013, and 2014.

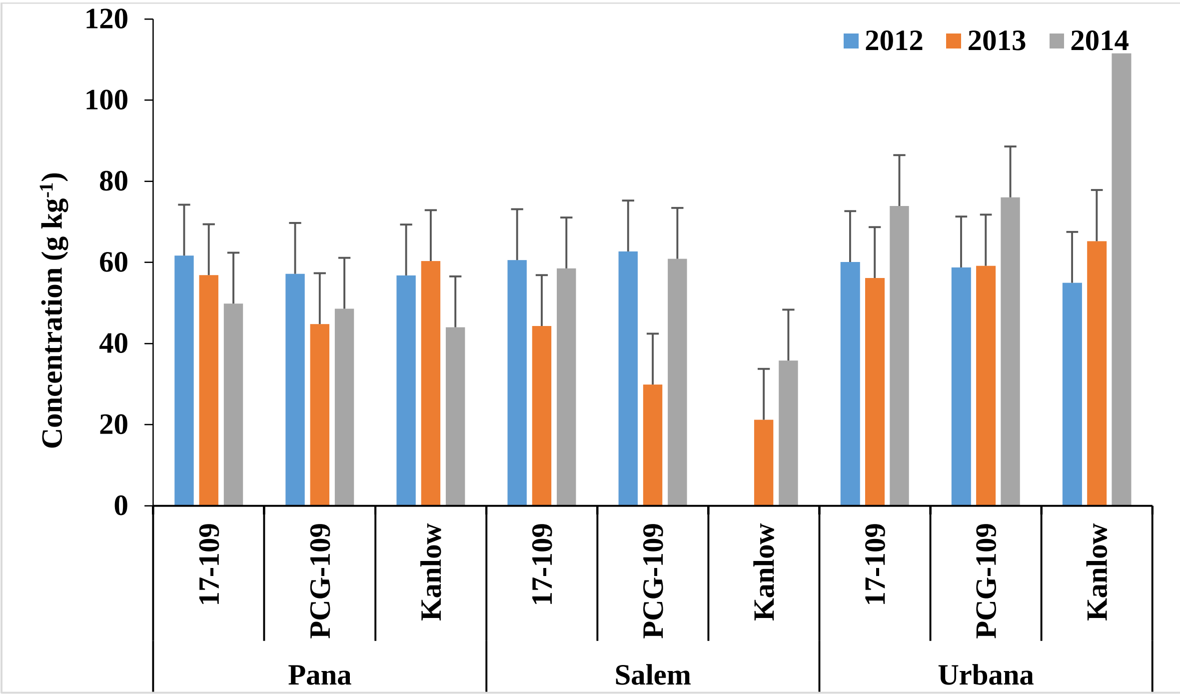


Fig. 3.8. Ash concentration for PC17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass in Salem, Urbana, and, Pana, Illinois in 2012, 2013, and 2014.

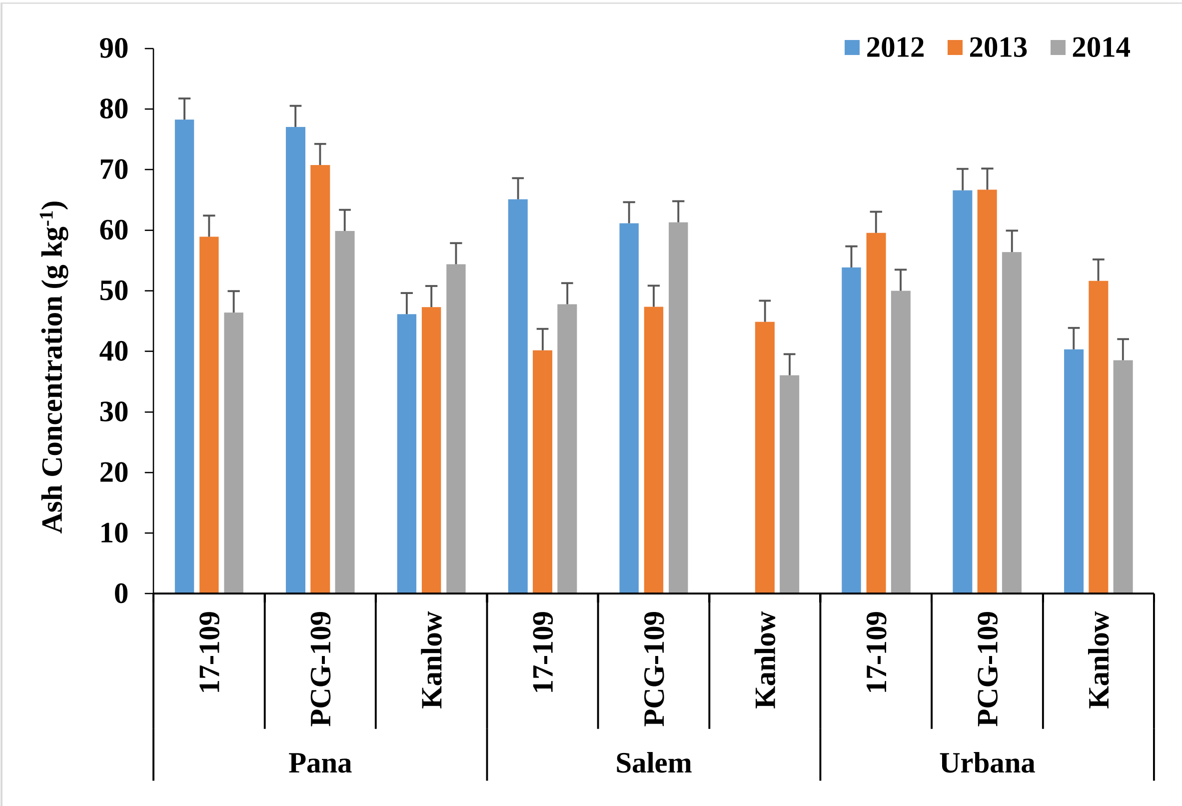


Fig. 3.9. Tissue phosphorus (P) concentration in the 17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass produced in Salem, Urbana, and, Pana, Illinois (averaged over 2012-2014).

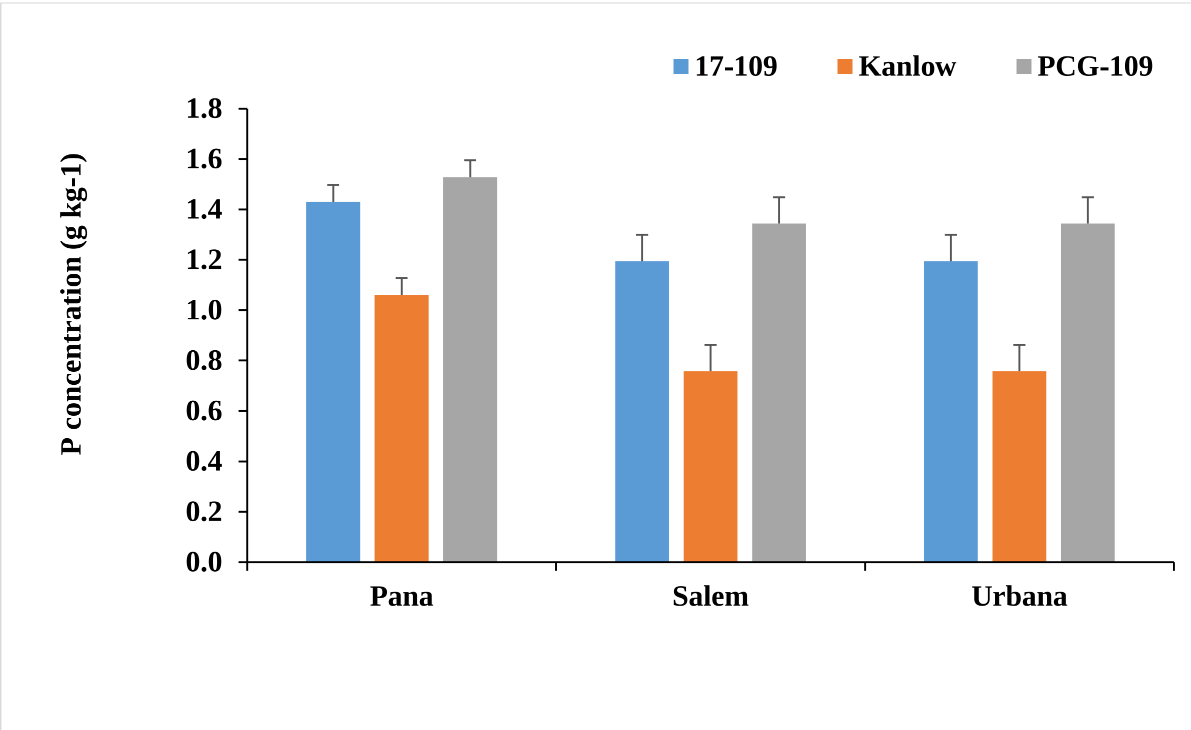


Fig. 3.10. Tissue nitrogen (N) concentration in the 17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass produced in Salem, Urbana, and, Pana, Illinois (averaged over 2012-2014).

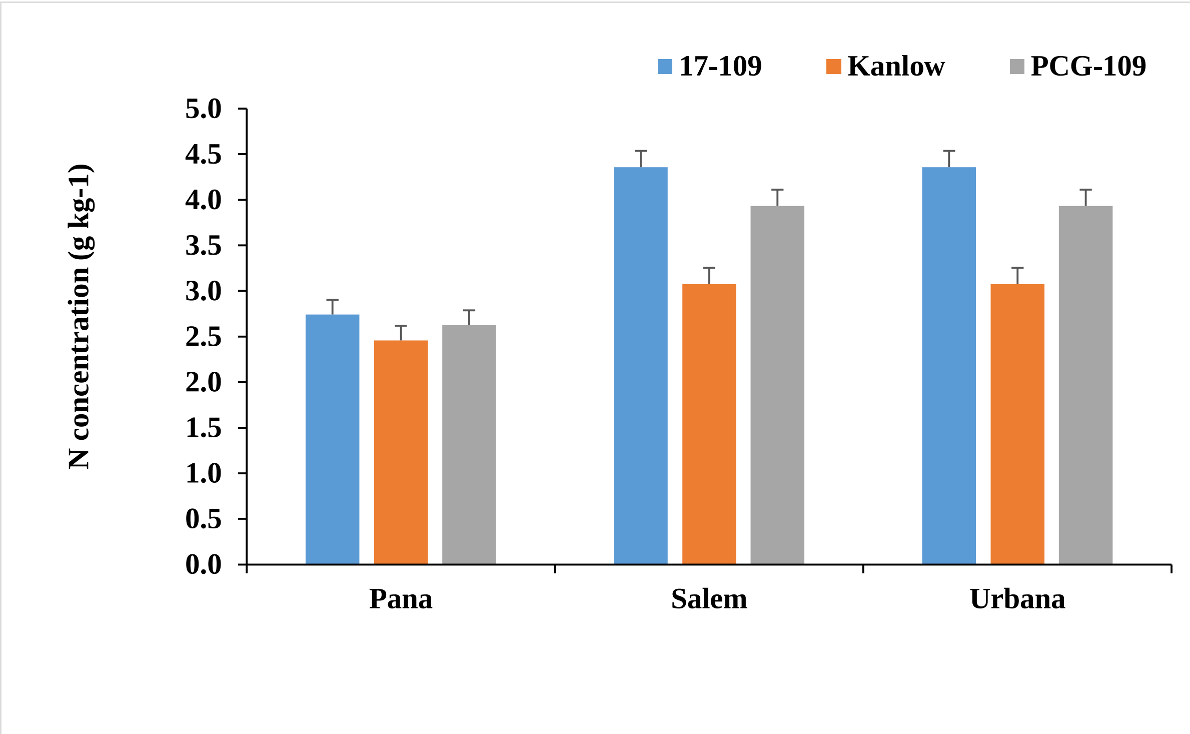
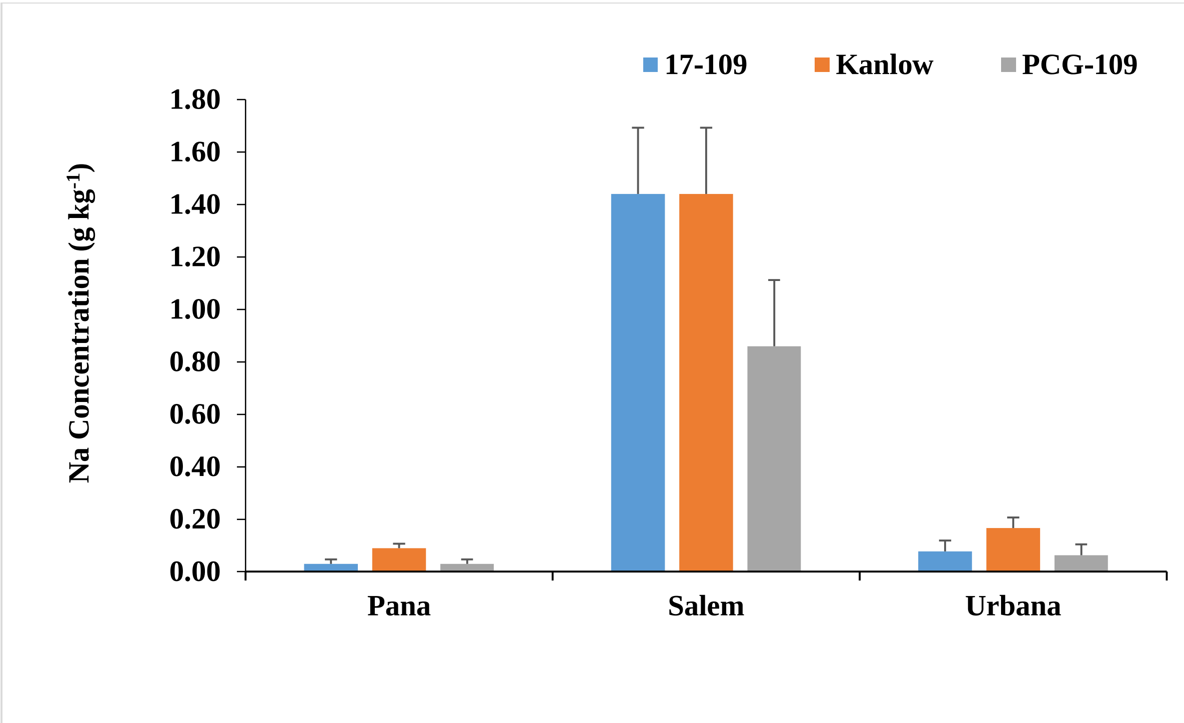


Fig. 3.11. Tissue sodium (Na) concentration in the 17-109 and PCG-109 prairie cordgrasses and Kanlow switchgrass produced in Salem, Urbana, and, Pana, Illinois (averaged over 2012-2014).



Chapter 4.

EFFECTS OF GENETICS AND ENVIRONMENT ON BIOMASS YIELD AND CHEMICAL COMPOSITION OF PRAIRIE CORDGRASS

ABSTRACT

Government policies designed to increase production of renewable biofuels and reduce fossil fuel consumption in US will likely lead to increased need for bioenergy crops. Since bioenergy crops should not compete with food crops, bringing marginal lands into production for bioenergy crops could resolve food versus fuel issues. Salt-affected and waterlogged soils are two of the many factors that can cause land to be considered marginal. Some perennial grasses have potential to be used as bioenergy feedstocks and are well adapted to marginal lands. In this study we evaluated and screened 17 populations of prairie cordgrass (*Spartina pectinata*) along with Kanlow switchgrass (*Panicum virgatum* 'Kanlow') in poorly drained soil in Urbana, IL, and plots irrigated with saline water in Pecos, TX. A two-year study showed variation among the tested populations. Kanlow switchgrass was the top biomass producer when averaged over two years at both sites (14.487 Mg ha^{-1} in IL and 28.449 Mg ha^{-1} in TX). There was great variation in prairie cordgrass biomass productivity, but the southern-originating cultivar, 20-107, produced the greatest biomass yields averaged over two years in both locations (12.351 Mg ha^{-1} in IL and 12.512 Mg ha^{-1} in TX). There was also much variation in tissue chemical composition among the populations. Some variation was due to the origin of the ecotype. Our study concludes that location, species ecotype, and environment can have a great influence on grass performance.

INTRODUCTION

Because the government proposed that 30% of the petroleum used in the US be replaced with renewable biofuels by 2030, the production of lignocellulosic bioenergy feedstocks is expected to increase (Milliken et al., 2007; Heaton et al., 2008). To reach this goal, the production of bioenergy crops will require increased production area, directly affecting land availability for premium food crops (Gallagher, 2008). Identifying and utilizing land areas that are not suitable for row crops, generally termed as marginal lands (Nelson et al., 1997), to produce bioenergy crops can reduce competition between food and bioenergy crops for prime agricultural land (Gallagher, 2008; Tilman et al., 2009). These marginal lands can have unsuitable physical characteristics in a poor climate, and can also include high salinity areas; waterlogged, marshy lands; or barren and glacial areas (Milbrandt & Overend, 2009), as well as be degraded and abandoned agricultural lands (Tilman et al., 2009; Shortall, 2013).

Anthropogenic factors such as irrigating, planting shallowly rooted crops, and clearing woods can cause changes in water-table depths that result in land degradation due to evaporation from the soil surface and salt accumulation in the root zone (Abrol et al., 1998; Sim, 2012). In the US, approximately 8.5 million hectares of land are affected by high salinity (Pearson, 2009). Salt levels in irrigation water are the most commonly occurring salinity-related problem in agriculture (Fipps, 2003) and can be detrimental to both soils and crops (Fipps, 2003; Bauder et al., 2011). Many different salts are commonly found in TX irrigation water (Fipps, 2003).

Poorly drained soils in much of the Midwestern US were unsuitable for crop production until tile drainage systems were installed (Gopalkrishnan et al., 2011). Flooding can reduce soil oxygen availability to plants and reduce root respiration, thus hindering plant growth (Caudle & Maricle, 2012). Wet conditions in spring can delay planting and reduce crop yields (Bockus &

Shroyer, 1998). There are several perennial grasses able to tolerate wet soils (Stoof et al., 2015), potentially making these plants bioenergy feedstocks for use in wet marginal lands not used to produce food crops. In addition, some perennial grasses adapted to marginal lands have low input, maintenance, and production cost requirements (Samson & Girouard, 1998).

Prairie cordgrass (*Spartina pectinata* Link.) is a tall, rhizomatous perennial grass species that is indigenous to many regions of the US and Canada (Boe et al., 2009). Prairie cordgrass has been used for wetland restoration and enhancement, stream bank stabilization, wind strip barrier, filter strip, riparian buffer, prairie landscaping, spillway and dam cover, forage, and wildlife habitat (Jensen, 2006). Since it is native to marshes, drainage ways and moist prairies in North America (Boe & Lee 2007), prairie cordgrass can be grown in the soils that are too wet to grow corn (*Zea mays*), switchgrass (*Panicum virgatum*), and big bluestem (*Andropogan gerardii*) (Boe et al., 2009; Boe and Lee, 2007). It tolerates flooded conditions (Bonilla-Warford & Zelder, 2002), and Skinner et al. (2009) reported that 'Red River' prairie cordgrass was a superior performer in flooded and saturated soil conditions.

High biomass productivity is a desired characteristic of biomass feedstocks (McKendry, 2002; Boe & Beck, 2008). In a seven-year study in southeastern England, prairie cordgrass reported an average yield of 10 Mg ha^{-1} yr^{-1} with production greater than 18 Mg ha^{-1} in two of the study years, and concluded that the grass is an energy efficient biomass producer for that area (Potter et al., 1995). Due to its early spring growth and high tiller density, prairie cordgrass is capable of biomass production, even in short Canadian growing seasons (Madakadze et al., 1998). Red River prairie cordgrass produced 12.7 Mg biomass ha^{-1} from a mature stand on marginal land in a nine-year study conducted by Boe et al. (2009) in eastern South Dakota. In a spaced-plant nursery in IL, prairie cordgrass produced more than 20 Mg biomass ha^{-1} showing high-yield potential as a bioenergy feedstock (Boe et al., 2013). Its high biomass production potential and tolerance to flooded conditions makes prairie cordgrass a potential bioenergy feedstock for wet marginal lands.

When growing in high-salt settings, prairie cordgrass can extrude excess salt through salt glands on the leaf blades (Kim et al., 2012). Prairie cordgrass showed high tolerance to saline and waterlogged peat fields in New Brunswick, Canada, where high salinity and low pH also favored its survival in sloped study areas across a range of soil moisture content (Montemayor et al., 2008). In a study of the effects of sodicity and salinity on switchgrass and prairie cordgrass, Anderson et al. (2015) concluded that several populations of prairie cordgrass and lowland switchgrass cultivars had good emergence and biomass production in moderate to high salt stress conditions and cordgrass has good potential as a biomass feedstock in salt-affected areas.

Given that prairie cordgrass is a salt-tolerant grass and can be used as a bioenergy feedstock on wet marginal land, the objective of this experiment was to compare and evaluate the productivity and lignocellulosic content of ‘Kanlow’ switchgrass (used here as a standard bioenergy check) with17 prairie cordgrass populations in two environments: one in which there is poor drainage and early-season waterlogged soils and the second with a salinity problem. We hypothesize that production and lignocellulosic-content differences among prairie cordgrass genotypes and Kanlow switchgrass exist when they are grown in the different environments.

MATERIALS AND METHODS

Seeds of natural prairie cordgrass populations were collected from throughout the US during 2008 and 2009 (Table 4.1). The seeds were transplanted in trays and pots, respectively and maintained in University of Illinois Urbana-Champaign (UIUC) greenhouse until 2010, when the resulting grasses were transplanted into the field at the Energy Biosciences Institute (EBI)/UIUC Energy Farm, Urbana, IL (40°04'08.1"N 88°11'26.9"W). The soil at the research site was a Drummer silty clay loam (fine-silty, mixed, super-active, mesic typic Endoaquolls). During the winter of 2011, seeds, F1 bulk population, collected from each of 17 prairie cordgrass populations transplanted in 2010, along with ‘Kanlow’ switchgrass, a lowland cultivar, were planted in a 96-cell trays using Sunshine SB300 Universal potting mix (American Plant Products & Services, OK, USA). The seedlings were grown in the UIUC greenhouse under 14 hrs of light at 24–26 °C.

The prairie cordgrass and Kanlow switchgrass transplants (Table 4.1) were shipped to the Pecos Station of the Texas AgriLife Research Center and Extension Center, Pecos, TX (31°22'47.85"N and 103°37'39.40"W) and planted in March 2012, at a site irrigated with saline water. The soil at this site is a Hoban silty clay loam (Fine-silty, mixed, thermic Ustollic Calciorthids, LCC: irrigated=1, Non-irrigated=7C), which could have very severe limitations and be unsuitable for cultivation without irrigation. Irrigation water at this site had an electrical conductivity of 4.4 ds/m. The experiment was conducted using a randomized complete block design with four replications represented by blocks for each population. The field plots had ridges and furrows where the seedlings were planted in two rows, 60 cm apart, in the 86 cm wide ridges. The 10 plants for each population were planted in two rows with 5 plants in each row and 90 cm within the rows. The furrows were 106 cm wide between each ridge where the ridges were the blocks or replications. The plots were irrigated by flooding the furrows at 600-700 gallons per minute for two hours every week from early May through the end of August each year. Water samples were collected from the irrigation wells several times for chemical analysis. Each spring from 2012 to 2014, 112 kg N ha^{-1} was applied to the study using urea.

In April 2012, another study was planted in a poorly drained site on the UIUC Energy Farm (40° 4'4.1"N and 88°11'26.7"W) in Flanagan silt loam (LCC 4W, fine, smectitic, mesic Aquic Argiudolls) soil. The experiment was conducted using a randomized complete block design with four replications of each feedstock population. The four blocks represented the three replications for each population. The 17 prairie cordgrass populations (Table 4.1) and Kanlow switchgrass were planted in single row plots. Each plot contained 10 plants in a row spaced 60 cm apart within the row. The distance between the centers of two plots in a block was 90 cm and the distance between the edges of one block to another block was 152 cm forming an alleyway between blocks. The study was fertilized each spring from 2012 to 2014 with 112 kg N ha^{-1} using urea, and only irrigated in 2012 (the planting year) as needed to ensure successful establishment.

Plots were not harvested during the establishment year in 2012. Harvests in 2013 and 2014 were conducted after killing frosts in the late fall (November – December), and biomass yields were determined. Biomass in TX was harvested using a STIHL professional trimmer (FS 90 bike handle trimmer) with a STIHL HL 135º hedge trimmer attachment (STIHL Inc., 536 Viking Drive Virginia Beach, VA 23452). A plot combine (Wintersteiger Cibus S harvester with a Kemper forage chopper, Ames, IA) set at a 10 cm stubble height was used to harvest the IL plots. Biomass from each plot was weighed in the field immediately after harvest and a subsample of approximately one kg was placed in brown paper bags. The samples collected in TX were shipped to UIUC for processing. Subsamples from each location were dried at 60 ºC for a minimum of 96 hours to determine dry biomass yield, and a portion of the dried samples used for tissue and mineral composition analysis.

The dried samples were ground in a Retsch SM2000 cutting mill (Haan, Germany) to pass through a 1mm screen, and the ground samples were stored in airtight Ziploc plastic bags for further analyses. Subsamples were sent to Brookside Laboratories (Brookside Laboratories, Inc., 200 White Mountain Drive, New Bremen, Ohio 45869) for tissue mineral composition analysis. Tissue ash content was determined using Neycraft Pro furnace (Undersander et al., 1993). An Ankom ^{200} Fiber Analyzer (Ankom Technology, 2052 O'Neil Road, Macedon, NY 14502) was used on tissue fiber to determine neutral detergent fiber (NDF) and acid detergent fiber (ADF). Acid detergent lignin (ADL) was determined using an Ankom Daisy II incubator (Ankom Technology, 2052 O'Neil Road, Macedon, NY 14502). Fiber and lignin content data were used to estimate the cellulose and hemicellulose content in the samples. Cellulose and hemicellulose content were estimated by calculating the difference between ADF and ADL and between NDF and ADF, respectively.

The data was analyzed separately for IL and TX locations. Dry biomass yield, lignocellulosic composition, and mineral composition data were analyzed by using PROC MIXED procedure in SAS (SAS 9.4. SAS Institute Inc., Cary, NC, USA). Homogeneity of variance was determined using Brown Forsythe's test. Analysis of Variance (ANOVA) was conducted to determine the variance among main effects of grass populations, and year as well as their interactions for biomass yield and tissue composition. Grass populations and year were independent variables and were treated as fixed variables, whereas, block was treated as random variables. Biomass yield, lignocellulosic composition, and tissue mineral compositions were all dependent variables. A least significance difference was used to separate the mean effects using Fisher's LSD test when F test was significant (P=0.05). The mean separation was done for only the prairie cordgrass populations in separate locations. A Dunnett's *t* test was done to separate significantly different means of 17 populations of prairie cordgrass with that of Kanlow switchgrass as we included Kanlow as a check in this study.

RESULTS

Grass parental populations with their state of origin with geographical locations are shown in Table 4.1. There were biomass yield differences among the populations ($P<0.0001$), and between years 2013 and 2014 in IL location (Table 4.2). Population main effect was significant in TX as well ($P<0.05$), and so was the main effects of year (Table 4.3). There was no interaction between the year and populations for the biomass yield in either IL or TX Kanlow switchgrass had the highest biomass yields averaged over two years in both TX (28.4 Mg ha^{-1}) and IL (14.5 Mg ha^{-1}) (Table 4.6). The KS-collected 20-107 was the most productive prairie cordgrass in both the TX (16.6 Mg ha^{-1}) and IL (14.4 Mg ha^{-1}) locations by 2014 (Tables 4.4 and 4.5) although it was not significantly different from few others. Overall biomass yield was higher in 2014 as compared to 2013 in both locations, however it was not significantly different for few of the cordgrass populations (Tables 4.4 and4.5). Biomass yield significantly changed for 20-107 and 17-109 in both locations whereas for 40-104 it only changed in TX location. In 2014 the lowest yielding prairie cordgrasses were 46-105 (7 Mg ha^{-1}) in TX and RR (7.7 Mg ha^{-1}) in IL although few others were not significantly higher than these in both locations. Generally, the IL-originating prairie cordgrass populations were more productive in IL than in TX. When compared with the two year average biomass yield of Kanlow switchgrass, the 17 prairie cordgrass populations in TX location were significantly lower (Table 4.6). However, the biomass yield of Kanlow was not significantly higher than 20-107 (KS-originating) at 12.4 Mg ha^{-1}, 17-109 (IL-originating) at 10.5 Mg ha^{-1}, 40-104 (OK-originating) at 10.43 Mg ha^{-1}, IL-102 (IL-originating) at 11.7 Mg ha^{-1}, and, IL-104 (IL-originating) at 10.3 Mg ha^{-1} (Table 4.5) in IL location.

There were no differences among the populations for cellulose, hemicellulose, lignin or ash content in tissue, and no interactions were found between year and population for cellulose, hemicellulose, or ash content in IL (Table 4.2). However the main effects of year was different for hemicellulose, lignin, and ash content (Table 4.2). The main effect of population was only significantly different for hemicellulose and ash content while not for cellulose and lignin content in TX (Table 4.3). The year effect was significant for hemicellulose, lignin and ash content. However, there was no significant interaction between year and populations for any of the variables in TX (Table 4.3). Generally the tissue cellulose and lignin concentration for IL prairie cordgrass populations was greater than that for the TX populations (Table 4.7). Kanlow had the most tissue lignin concentration in IL (93.1 g kg^{-1}) while it was not significantly different from few others. The lignin concentration in the tissues from TX location was not significantly different among the populations. The ash concentration was generally higher in TX than in IL (Table 4.7), however it was not significantly different among the populations in IL. The ash concentration for Kanlow switchgrass was lowest in TX (91.5 g kg^{-1}). The highest ash concentration among the prairie cordgrass populations was in RR in both locations (87.5 in g kg^{-1}) in IL and (227.1 g kg^{-1}) in TX, while the lowest ash concentrations were produced by 17-109 (60.4 kg^{-1}) in IL and 40-104 (135.4 g kg^{-1}) in TX (Table 4.6).

In IL, cellulose concentrations for all populations were above 350 g kg^{-1}, with IL-106 (419.2 g kg^{-1}) the greatest and IL-99 (359.7 g kg^{-1}) the least (Table 4.7). In TX, the 20-107 population had the greatest cellulose concentration with 340.6 g kg^{-1} and the least in ND with 268.1 g kg^{-1} (Table 4.7). Tissue hemicellulose concentration in IL ranged from approximately

290 g kg^{-1} to 310 g kg^{-1}. with the highest content in 19-108 (313 g kg^{-1}) and the least in IL-99 (29 g kg^{-1}). There were no differences among the populations for hemicellulose concentration in IL location. Lignin concentration among the prairie cordgrass populations was greatest in IL-105 (80.3 g kg^{-1} and IL-104 (49.4 g kg^{-1}) (≈ 49%) in TX. The least tissue lignin was produced by the IL-99 population (62.3 g kg^{-1}) in IL and the 46-106 population (38.2 g kg^{-1}) in TX (Table 4.7).

The sodium concentrations were greater in TX than in IL; TX plant tissues ranged between 1.55 g kg^{-1} for IL105 to 0.338 g kg^{-1} for 20-107, while the concentrations for prairie cordgrasses in IL ranged between 0.1407 g kg^{-1} for IL104 and 0.0144 for 46-106 (Table 4.7). Similarly, chloride concentrations were also greater in the TX populations; prairie cordgrass populations tissues from TX ranged from 2.94 g kg^{-1} for IL99 to 1.15 g kg^{-1} for 40-104, while ranging between 0.74 g kg^{-1} for 17-109 and 0.31 g kg^{-1} for ND in IL plant tissues (Table 4.7).

DISCUSSION

High biomass productivity is a critical trait for promising biomass feedstocks (McKendry, 2002; Wright, 2007; Boe and Beck, 2008; Anderson-Teixeira et al., 2012). As our research plots were established in marginal conditions, the yields are not comparable to those of prime cropland. A seven-year study (Potter et al., 1995) reported that prairie cordgrass had an average biomass yield of 10 Mg ha^{-1} yr^{-1} in New Haven, CT. In two years from the same study, the grass produced >18 Mg ha^{-1} (Potter et al., 1995). However these yields were on well-drained, arable lands. A 2000-to-2008 study (Boe et al., 2009) reported that Red River prairie cordgrass produced 12.7 Mg ha^{-1} of biomass from a mature stand on a marginal land. Our results from this two-year study showed the biomass yield for prairie cordgrass ranged between approximately 7 Mg ha^{-1} for PCG 109 in TX and 17 Mg ha^{-1} for 20-107 in TX as well in 2014. Additionally, Boe et al., (2013) found that prairie cordgrass was able to produce three times as much biomass as switchgrass on wet marginal land. Our results are similar to the biomass yields produced in wet marginal land. These biomass yields illustrate the promising potential of prairie cordgrass on marginal sites. However, the study period lasted only two years, so the prairie cordgrass could not have been reached its full biomass potential.

Although it was not the case for all of the tested prairie cordgrass populations, some IL-originating populations (IL102, IL106, and 17-109) produced more biomass (11.6 Mg ha^{-1}, 10.6 Mg ha^{-1}, and 10.5 Mg ha^{-1} respectively) in IL than in TX except for 17-109 which performed similarly in TX (10.23 Mg ha^{-1}). This likely occurred because each plant ecotype has an adaptation zone where it performs well, but can have a negative effect if moved further from its adaptation zone (Vogel et al., 2005). Similarly the populations originating from OK (40-104) and KS (20-107) were reasonably productive in both TX and IL, likely because these populations were not moved far from their origins. This variation in biomass yields showed the potential of prairie cordgrass as widely adapted to a range of abiotic stress conditions. Boe et al. (2009), also wrote that prairie cordgrass can be sustainably productive on wet or saline marginal land.

Casler and Boe (2003) and Casler et al. (2004) reported that cultivar, location, and harvest time are the most significant factors for biomass yield. Since C4 grasses usually have a lower water use rate than C3 grasses (Black, 1971), warm-season grasses tend to take up less silicic acid, the main mechanism of accumulating silica, which is comprises the major portion of ash in perennial grasses (Samson and Mehdi, 1998). Ash concentration varies within the species depending upon different factors such as soil type, rainfall-to-evaporation ratio, and the region (Samson et al., 1993; Samson and Chen, 1995; Samson and Mehdi, 1998). Combinations of the above mentioned factors may cause high ash content in plant tissue (2.8% - 7.6% in switchgrass reported in US) (McLaughlin et al., 1996). In our study, water was not limited in either location, and therefore an abundant water supply may have resulted in the great amounts of ash. Some of the stressor factors like growing region, salt stress, and flood irrigation to evaporation ratio, may have played a role in higher number of ash in TX location as compared to that of IL location. The variation in cellulose, lignin, and ash concentrations in our study can be explained from the differences in genetic, environment, and management factors and supported by various studies (Samson et al., 1993; Samson and Chen, 1995; McLaughlin et al., 1996; Samson and Mehdi, 1998). Here, the populations with high ash concentration originated from SD and ND, while the populations originating in KS, OK, and IL had lower ash concentration. This result again confirms the fact that Vogel et al. (2005) explained about the adaptation zone for each ecotype.

Cellulose and hemicellulose content are important bioethanol conversion quality factors (Wyman, 1999). Feedstock biomass typically contains 35% - 50% cellulose, 20% - 35% hemicellulose, and 12% - 20% lignin (Wyman, 1999). In our study we found the tissue cellulose content ranged from approximately 27% to 42%, hemicellulose content from approximately 27% to 36%, and lignin content from 3.8% to 9.3%. Our results show that the lignocellulosic content was in the ranges described by Wyman (1999).

We found similar variations among the populations in IL and TX locations for tissue mineral composition. There were variations among the tested populations of prairie cordgrass in terms of sodium removal from the ground. The populations that accumulated the most tissue sodium were IL105, IL99, and IL104. The tissue accumulation of sodium ranged from 0.33 g kg^{-1} to 1.55 g kg^{-1} and the tissue accumulation of chloride ranged from 1.38 g kg^{-1} to 3.07 g kg^{-1} in TX. Prairie cordgrass populations 19-108 and 19-110 were among the highest accumulators of chloride (2.47 g kg^{-1} and 2.85 g kg^{-1} respectively) in TX. A study conducted in three Florida locations found that sodium concentrations in giant miscanthus tissue ranged from 1.7 g kg^{-1} to

2.7 g kg^{-1}, while chloride concentrations ranged from 1.8 g kg^{-1} to 4.1 g kg^{-1} (Singh et al., 2015). This range is also similar to our prairie cordgrass sodium and chloride removal findings, showing potential for these populations as an alternate use for phytoremediation in a problem soils.

CONCLUSION

Prairie cordgrass populations tested in this study varied greatly in biomass yield and composition. Location and genetics can have a significant influence on biomass yield and biomass composition (Casler and Boe, 2003; Casler et al., 2004). Tissue lignocellulosic composition can vary depending on plant genotype, plant ecotype, and different environments (Kim et al., 2011). The 20-107 population showed promising yields in both the poorly drained IL site and the saline-water irrigated TX site. Choosing prairie cordgrasses for biofuel production should be made with the full knowledge of the adaptation zone of the specific ecotype. The results of this study show that prairie cordgrass has wide adaptability and has potential to be used as a bioenergy crop in wet and salinity affected marginal lands.

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Table 4.1. Grass populations and their origin state with geographical locations planted the IL and TX study sites.

POP ID	State	Longitude	Latitude	Collection by
17-109	IL	88° 12′ 16″W	40° 3′ 17″ N	seed
19-108	IA	96° 19′ 37″W	42° 19′ 48″ N	seed
19-110	IA	96° 2′ 33″W	41° 47′ 34″ N	seed
20-106	KS	96° 22′ 54″W	39° 3′ 38″ N	seed
20-107	KS	96° 31′ 30″W	39° 0′ 9″ N	seed
29-106	MO	94° 18′ 56″W	37° 51′ 13″ N	seed
40-104	OK	97° 4′ 3″W	36° 49′ 44″ N	seed
46-105	SD	96° 49′ 35″W	43° 23′ 17″ N	seed
46-106	SD	96° 49′ 33″W	43° 10′ 35″ N	seed
IL99	IL	88° 42′ 3″W	39° 44′ 60″ N	seed
IL102	IL	88° 14′ 19″W	40° 3′ 55″ N	seed
IL104	IL	88° 44′ 31″W	40° 10′ 45″ N	seed
IL105	IL	87° 56′ 36″W	40° 54′ 41″ N	seed
IL106	IL	88° 1′ 12″W	40° 39′ 24″ N	seed
PCG109	SD	97° 04′ 50″W	42° 50′ 51″ N	seed
ND	ND	99° 05′ 3″W	47° 27′ 27″ N	seed
RR	SD	Germplasm		seed
Kanlow	OK	Cultivar		seed

Table 4.2. Analysis of variance of biomass yield, cellulose, hemicellulose, lignin and ash contents at the IL location.

Source	DF	Biomass Yield	Cellulose	Hemicellulose	Lignin	Ash
Population	**16**	***	**NS**	**NS**	**NS**	**NS**
Year	**1**	***	**NS**	***	**	***
Year*Population	**16**	**NS**	**NS**	**NS**	**	**NS**

*** =Significance at P<0.001

** =Significant at P<0.05

NS = Not Significant

Table 4.3. Analysis of variance of biomass yield, cellulose, hemicellulose, lignin and ash contents at the TX location.

Source	DF	Biomass Yield	Cellulose	Hemicellulose	Lignin	Ash
Population	**16**	**	**NS**	**	**NS**	***
Year	**1**	***	**NS**	***	***	***
Year*Population	**16**	**NS**	**NS**	**NS**	**NS**	**NS**

*** =Significance at P<0.001

** =Significant at P<0.05

NS = Not Significant

Table 4.4. Biomass yields of 17 prairie cordgrass populations with their state of origin at the IL study site in 2013 and 2014.

POP ID	Origin	Biomass Yield (Mg ha^{-1})	
		2013	2014
17-109	IL	8.35 e-k	12.73 a-d
19-108	IA	7.24 ijk	10.50 b-i
19-110	IA	6.26 jk	9.78 b-j
20-106	KS	8.12 e-k	10.70 a-h
20-107	KS	10.35 b-i	14.35 a
29-106	MO	5.78 k	8.10 f-k
40-104	OK	8.91 d-k	11.95 a-e
46-105	SD	8.35 e-k	9.34 c-k
46-106	SD	7.67 h-k	9.24 c-k
IL102	IL	10.11 b-i	13.28 ab
IL104	IL	7.83 g-k	12.80 abc
IL105	IL	7.91 f-k	11.23 a-h
IL106	IL	8.64 e-k	11.68 a-f
IL99	IL	7.61 h-k	11.55 a-h
ND	ND	7.24 ijk	9.26 c-k
PCG109	SD	7.43 h-k	10.09 b-i
RR	SD	6.03 jk	7.79 g-k

~ Means having a letter in common are not significantly different at the P=0.05 level using Fisher's Protected Least Significant Difference (LSD) test. A dash appearing between two letters means all the letters between those two letters are included.

Table 4.5. Biomass yields of 17 prairie cordgrass populations with their state of origin at the TX study site in 2013 and 2014.

POP ID	Origin	Biomass Yield (Mg ha^{-1})	
		2013	2014
17-109	IL	6.94 d-g	13.53 abc
19-108	IA	3.70 g	7.24 d-g
19-110	IA	4.77 efg	8.66 c-g
20-106	KS	6.63 d-g	10.94 a-d
20-107	KS	8.45 c-g	16.58 a
29-106	MO	6.48 d-g	10.17 b-e
40-104	OK	7.02 d-g	14.73 ab
46-105	SD	3.77 fg	7.02 d-g
46-106	SD	4.01 fg	8.19 c-g
IL102	IL	4.68 efg	7.96 c-g
IL104	IL	5.25 d-g	9.08 c-g
IL105	IL	8.55 c-g	10.19 b-e
IL106	IL	5.69 d-g	9.66 b-g
IL99	IL	5.38 d-g	9.21 b-g
ND	ND	5.76 d-g	9.78 b-f
PCG109	SD	4.54 efg	6.99 d-g
RR	SD	5.46 d-g	9.42 b-g

~ Means having a letter in common are not significantly different at the P=0.05 level using Fisher's Protected Least Significant Difference (LSD) test. A dash appearing between two letters means all the letters between those two letters are included.

Table 4.6. Biomass yields averaged over 2013 and 2014 of 17 prairie cordgrass populations compared with that of Kanlow switchgrass, with their state of origin, at the IL and TX study sites.

POP ID	Origin	Biomass Yield (Mg ha^{-1})	
		IL	TX
Kanlow	OK	14.49	28.45
17-109	IL	10.54 NS	10.24 ***
19-108	IA	8.87 **	5.47 ***
19-110	IA	8.02 **	6.71 ***
20-106	KS	9.41 **	8.78 ***
20-107	KS	12.35 NS	12.51 ***
29-106	MO	6.94 **	8.32 ***
40-104	OK	10.43 NS	10.87 ***
46-105	SD	8.84 **	5.39 ***
46-106	SD	8.45 **	7.49 ***
IL102	IL	11.69 NS	6.95 ***
IL104	IL	10.31 NS	7.16 ***
IL105	IL	9.57 **	9.37 ***
IL106	IL	10.16 **	8.63 ***
IL99	IL	9.58 **	7.29 ***
ND	ND	8.25 **	7.77 ***
PCG109	SD	8.76 **	5.76 ***
RR	SD	6.91 **	7.44 ***

*** =Significance at $P<0.0001$

** =Significant at $P<0.05$

NS = Not Significant

Table 4.7. Lignocellulosic concentration of 17 prairie cordgrass populations and Kanlow switchgrass averaged over two years in IL and TX study sites.

Population	Cellulose		Hemicellulose		Lignin		Ash	
	Concentration (g kg^{-1})							
	IL † (44.2)	TX (30.2)	IL (29.7)	TX (23.5)	IL (13.5)	TX (11.8)	IL (32.4)	TX (32.5)
17-109	410.64 a	318.31 ab	311.78 a	315.31 b	75.80 bcd	45.01 a	60.42 a	144.81 efg
19-108	399.53 ab	293.79 bcd	313.01 a	282.01 efg	71.41 bcd	41.41 a	64.04 a	179.14 bcd
19-110	385.43 ab	280.22 cd	304.33 a	291.75 c-f	70.84 bcd	44.83 a	71.14 a	177.91 bcd
20-106	390.71 ab	299.32 bc	293.93 a	319.19 b	70.82 bcd	40.42 a	80.32 a	139.76 fg
20-107	394.05 ab	340.57 a	312.58 a	310.46 bc	67.17 bcd	46.82 a	68.88 a	148.34 d-g
29-106	382.36 ab	316.76 ab	293.75 a	318.45 b	76.33 bc	46.88 a	74.38 a	149.49 d-g
40-104	389.62 ab	319.29 ab	301.94 a	309.02 bc	70.23 bcd	40.35 a	70.08 a	135.36 g
46-105	378.91 ab	317.35 ab	312.33 a	301.91 b-e	77.98 bc	43.66 a	76.62 a	179.72 bcd
46-106	386.24 ab	297.98 bcd	304.54 a	285.44 d-g	74.53 bcd	38.25 a	72.24 a	177.23 b-e
IL102	382.54 ab	312.43 ab	291.57 a	305.55 bcd	79.58 bc	43.08 a	63.70 a	135.95 g
IL104	396.39 ab	319.91 ab	295.97 a	310.29 bc	69.92 bcd	49.43 a	62.24 a	160.75 c-g
IL105	387.48 ab	300.04 bc	309.70 a	298.47 b-e	80.30 ab	40.72 a	84.5 a	172.18 b-f
IL106	419.15 a	300.05 bc	299.77 a	303.05 b-e	80.12 ab	45.22 a	62.37 a	161.65 c-g
IL99	359.67 b	315.4 ab	290.70 a	303.96 b-e	62.37 d	42.48 a	76.95 a	156.46 d-g
Kanlow	414.41 a	310.82 ab	312.13 a	363.6 a	93.11 a	40.44 a	56.95 a	91.48 h
ND	386.33 ab	268.13 d	297.90 a	265.09 g	70.43 bcd	45.44 a	69.38 a	195.66 ab
PCG109	416.04 a	300.78 bc	307.61 a	273.33 fg	76.90 bc	43.70 a	72.36 a	192.67 bc
RR	380.72 ab	290.14 bcd	297.57 a	271.41 fg	66.24 cd	43.76 a	87.49 a	227.12 a

~ Mean values within the same column having a letter in common are not significantly different at the P=0.05 level using Fisher's Protected Least Significant Difference (LSD) test. A dash appearing between two letters means all the letters between those two letters are included.

† LSD values

Table 4.8. Tissue sodium and chloride concentration of 17 prairie cordgrass populations and Kanlow switchgrass from IL and TX study sites averaged over two years.

Population	Sodium		Chloride	
	Concentration (g kg^{-1})			
	IL † (0.08)	**TX** (0.47)	**IL** (0.25)	**TX** (1.23)
17-109	0.05 b	1.00 b-e	0.75 a	2.45 abc
19-108	0.05 b	0.65 def	0.345 c	2.85 ab
19-110	0.04 b	1.19 abc	0.43 bc	2.42 abc
20-106	0.04 b	0.53 f	0.37 bc	2.34 abc
20-107	0.04 b	0.34 f	0.40 bc	2.02 abc
29-106	0.05 b	1.20 ab	0.50 b	2.17 abc
40-104	0.07 ab	0.72 c-f	0.33 c	1.65 c
46-105	0.04 b	0.36 f	0.35 bc	1.77 c
46-106	0.14 a	0.44 f	0.34 c	1.59 c
IL102	0.05 b	0.37 f	0.35 bc	1.38 c
IL104	0.03 b	1.23 ab	0.45 bc	2.02 abc
IL105	0.04 b	1.55 a	0.36 bc	1.77 c
IL106	0.04 b	1.07 bcd	0.38 bc	1.65 c
IL99	0.04 b	1.27 ab	0.39 bc	2.44 abc
Kanlow	0.14 a	0.48 f	0.70 a	3.06 a
ND	0.05 b	0.44 f	0.31 c	1.83 bc
PCG109	0.05 b	0.41 f	0.35 bc	1.66 c
RR	0.04 b	0.58 ef	0.37 bc	1.60 c

~ Mean values within the same column having a letter in common are not significantly different at the P=0.05 level using Fisher's Protected Least Significant Difference (LSD) test. A dash appearing between two letters means all the letters between those two letters are included.

† LSD values

CONCLUDING REMARKS

Feedstock productivity varies from site to site, usually based on crop responses to soil type and local climate, and as a result, biomass feedstock selection will differ based on the environment. Due to the significant amount of land in US that is considered to be marginally productive for food crops, it is very important to select the right feedstock species and cultivars for the specific type of environment for the best outcome.

In Chapter 2, we investigated four different perennial grass species (prairie cordgrass, switchgrass, big bluestem, and, *Miscanthus* x *giganteus*) in a wet marginal soil. In our 2011 to 2013 study, we found that biomass production varied considerably among the species. Since the experiment was set up in 45 cm and 90 cm spacing treatments, we also learned that row spacing had an effect on the biomass yield of these grass species. Biomass tissue cellulose and hemicellulose composition was not affected by the marginal land.

The spring emergence of these grasses showed that all of the prairie cordgrasses emerged earlier than the other three species. However, we evaluated spring germination only in 2014. Although the same pattern was seen every year throughout the study that was a missed opportunity and a lesson was learned. Spring emergence record for each year would have provided more useful findings. Another important lesson we learned was that early spring weed control was important in order to avoid weed invasions later in the season.

Another interesting thing we found in the field plots was that during the spring green-up, there was frost damage in most of the switchgrass, *M.* x *giganteus*, and big bluestem plots. There was no damage was seen in any of the four prairie cordgrass populations from the frost.

However, whether the frost damage affected the yields and performance of the affected species was not determined. This could be an interesting aspect to include in the future experiments.

This side-by-side study of these grasses was a very important for evaluating and comparing different species in the same environment. However, since we did it only in a wet, marginal setting, it would be interesting to see the experiment repeated simultaneously in prime cropland to compare the two studies. Because this is the first comparison of these species in wet, marginal conditions, our results are of great interest. Again, this study should be repeated in prime cropland.

We also noticed during the study period that there was an insect problem, particularly in prairie cordgrasses, which caused the leaves to have sticky substance on the surface which turned black at the end of the season. We suspect that aphids were the cause and it seemed that these insects preferred one population of prairie cordgrass in particular. Additionally, lodging occurred in the late season and during harvest. A future study should examine the relationships between insect damage and lodging in prairie cordgrass.

Salinity was another issue that we wanted to investigate in terms of biomass production. In Chapter 3, we evaluated the yield performance of two prairie cordgrass species and a switchgrass species in Pana and Urbana, IL, two poorly drained sites, and also a high salinity area in Salem, IL. Both of the species produced a reliable amount of biomass. There were variations found in the biomass yield for species in Urbana and Pana locations each year in all locations. The interaction effects of year x species was also found in Urbana and Pana. The marginal conditions affected the tissue composition of either grass species. Both species accumulated considerable amounts of sodium in their tissues at the salt-affected site.

The salt affected research site in Salem belonged to a farmer. One problem of conducting research on private property is that we have to rely on the farmer and trust his supervision and monitoring of the research plots. Management practices on the adjacent farm (e.g., fertilizer and chemical applications and mechanical operations) may have had a direct effect on our research. It may have been more desirable for researchers to have more control over the experiment plots.

There were two poorly drained soils and only one salt affected soil in this study. If possible, at least three replications of both types of soils would give us stronger comparisons and more accurate conclusions. The choice of feedstock was dependent on the size and availability of the research sites. In the future studies, it is recommended several more species be compared in these problem soils to learn more about additional biomass crops.

In Chapter 4, Kanlow switchgrass and 17 populations of prairie cordgrass were screened and evaluated in a poorly drained soil In Urbana, IL, and saline water irrigated plots in Pecos, TX. We found considerable variations among the grass populations for biomass yield. Location and species ecotype influenced biomass yield and composition. Tissue composition can vary depending on plant genotype, plant ecotype, and different environments. The information on adaptation zone of a specific ecotype is very valuable for choosing biomass crop grass species for specific environments.

We found large amounts of sodium and chloride accumulations in the plant tissue grown in TX as compared to IL. There were variations among the species for the mineral accumulation. In the previous study (Chapter 3), we found that there was a large amount of sodium accumulated in the plant tissues harvested in the salt affected site in Salem as compared to those of Pana and Urbana locations. However all grass species produced acceptable amounts of biomass. As we look at Chapter 2, we found that tissue phosphorus concentration of prairie cordgrass was higher than those of switchgrass, big bluestem, and *M.* x *giganteus*.

All of these results showing tissue mineral accumulations can assist in identifying biomass feedstocks for different types of marginal soils. For example, prairie cordgrass can be used in phytoremediation of farm soils with excess phosphorus. Similarly, some prairie cordgrasses, as well as Kanlow switchgrass, can be used to improve soils with high sodium and chloride. Given that the prairie cordgrass populations included in all the studies above came from unselected natural populations, the grass performed well and was comparable to established bioenergy crops such as switchgrass and *M.* x *giganteus*. This shows the great potential for prairie cordgrass for further selection and improvement for biofuel purposes. Finally, future studies should include multiple species in each specific environment for screening and evaluating.