Developmental morphology, biomass yield and compositional differences among upland and lowland switchgrass (*Panicum virgatum* L.) ecotypes grown as a bioenergy feedstock crop

by

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DEDICATION

I dedicate this dissertation to my beloved parents Mian Fazeelat Hussain and Bushra Fazeelat, without whom none of my success would have been possible. Thank you for your unconditional love and believing in me. Thank you for making my dreams come true, and I hope you are as proud of me as I am proud of you.

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ABSTRACT

Sustainable and successful development of the bioenergy industry strongly depends upon the amount and quality of bioenergy feedstock produced. Switchgrass (*Panicum virgatum* L.) has been identified as a model lignocellulosic bioenergy crop in the U.S. Information regarding its growth and development is considered critical for making management decisions, production of high quality feedstock and to support simulation model calibration and subsequent analysis.

In the first study (Chapter 2), we evaluated upland (‘Cave-in-Rock’, ‘Trailblazer’ and ‘Blackwell’) and lowland (‘Kanlow’ and ‘Alamo’) ecotypes of switchgrass for harvest management, morphological (phenology and leaf area index) and biomass yield differences. A field study was conducted near Ames, IA during 2012 and 2013. The experiment design was a randomized complete block design. Six in-season destructive biomass harvests were collected at approximately 2-week intervals each year. In both years, delaying harvest to later maturity increased biomass yield in all cultivars. Averaged over years lowland cultivars ‘Kanlow’ and ‘Alamo’ produced higher dry matter yield (6.31 and 5.98 tons ha^{-1}, respectively) than upland ecotypes ‘Cave-in-Rock’, ‘Trailblazer’ and ‘Blackwell’ (5.89, 4.78 and 4.53 tons ha^{-1}, respectively). Lowland cultivars had delayed reproductive development compared with upland ecotypes. The widely recommended cultivar in Iowa ‘Cave-in-Rock’ had the highest mean stage count and leaf area index at the end of both growing seasons, but did not produce the greatest biomass. There was a nonlinear relationship between MSC and biomass yield. However, the magnitude and form of the response was different between cultivars and from year to year.

In the next study (Chapter 3), the objective was to quantify the chemical composition of switchgrass varieties as influenced by harvest management, and to determine if ecotypic differences exist among them. We found that delaying harvest increased cellulose, hemicellulose and lignin concentrations while decreasing nitrogen and ash concentrations in all varieties. On average, Kanlow had the highest cellulose and hemicellulose concentration (354 and 321 g kg^{-1} DM respectively), and Cave-in-Rock had the highest lignin concentration (33 g kg^{-1} DM). The lowest nitrogen and ash concentrations were observed in Kanlow (14 and 95 g kg^{-1} DM respectively). In general, our results indicate that delaying harvest until fall improves feedstock quality, and ecotypic differences do exist between varieties for important feedstock quality traits.

The objective of the third and final study (Chapter 4), was to develop a new mechanistic model to describe switchgrass phenology with the objective to assist agronomists and on-going breeding programs. Switchgrass is sensitive to photoperiod. However, existing switchgrass phenology models rely on thermal time coupled with ad-hoc empirical modifications to account for the effects of photoperiod when the model is to be applied across a wide range of environments. Our model simulates four phases of switchgrass development (emergence to juvenile, juvenile to elongation, elongation to flowering and flowering to maturity). It uses daily temperature and site latitude as driving variables, contains five cultivar specific biological meaningful parameters, and two model constants (base and optimum temperature of 10 ^{o}C and 30 ^{o}C respectively). Three of the cultivar specific parameters (a_1, a_2, a_3) define the thermal optimum time needed to complete a phase and the other two parameters describe the critical photoperiod (p_{crit}) and the photoperiod sensitivity (p_{sen}). The model matched Iowa's observations with a RMSE (relative mean

square error) of 2.6 days for each cultivar or with 5.1 days error when average parameters by ecotype were used. The next step in the analysis was to generalize the model by providing estimates of the photoperiod effect, and for that we used diverse literature database. We found that p_{crit} was 13.5 h and 12.7 h for upland and lowland cultivars. By using these parameters the overall prediction ability of the model across representative sites in the U.S. had an 8.4 days error. This model can be a helpful tool for improvement and development of calibration protocols for other models and it can also serve as a template for the development of phenology models for other perennial grasses.

CHAPTER 1

GENERAL INTRODUCTION

Global Concern

The world's population is expected to reach 9.6 billion by the first half of this century, an increase of 34 % over where it stands today (United Nations, 2012). This drastic increase in human population is also going to result in an approximately 70 % increased demand for food and fiber by 2050 (FAO, 2009). Human survival mainly depends upon the natural resources present in our environment like water, soil, natural gas and petroleum oil. These resources are limited and are being diminished and deteriorated. Studies have reported that planetary oil and gas reserves have been declining (Campbell, 2006; IEA, 2007). Chapman et al. (1972) described the energy crisis as "our natural gas and oil resources are less than what we desire and in future these supplies might cost us a lot more". This statement is still true today with significant variation in crude oil prices in international markets and with a generally increasing trend.

Furthermore, the use of nonrenewable fuels contribute to global climate change that are disturbing our ecosystems (Ansuategi and Escapa, 2002; Smith and Fang et al. 2008; Gaffney and Marley, 2009). One of the major concerns is the accumulation of greenhouse gasses in the atmosphere (IPCC, 2008), which not only depletes the ozone layer but also leads to increase in global temperature/global warming. This increased atmospheric temperature has been estimated to keep rising throughout 21st century (NRC, 2001). IPCC (2008) also reported that the earth's surface air temperature has increased by 0.5 °C in the

20th Century, and this rise in air temperature will also continue in 21st century with an estimated increase of 1.5 to 4.6 ^{o}C.

One of the solutions for reducing environmental variation related to climate change is to limit the use of conventional energy sources like coal, gas and petroleum oil. All of these concerns are major driving forces directing the world's attention towards biorenewable energy sources such as biofuel. Crops grown for biofuels will be a step towards increased biodiversity, environmental sustainability and energy security. Production of renewable fuels from locally grown crops will also reduce U.S. dependence on foreign petroleum oil for fuel and energy needs. According to the Energy Independence and Security Act (EISA) of 2007, the U.S. is expected to produce 136 billion L of renewable fuels by 2022 to meet energy demand and reduce foreign dependence on fossil fuels. Out of this 136 billion L, about 80 billion L will be derived from non-food sources like lignocellulosic material and rest will be produced from grain crops like corn and soybean (EISA, 2007; Perlack and Wright et al. 2011). Use of food crops to produce biofuel also leads to a competition between food and fuel due to limited availability of fertile lands (Tilman et al. 2006). One of the solutions to this problem is the use of the lands marginal for producing food crops for production of lignocellulosic biomass crops (Cai X, et al. 2011).

Lignocellulosic Feedstocks

It has been estimated that U.S. agriculture and forest lands are capable of supplying 1.3 billion tons of dry lignocellulosic materials per annum for bioenergy purposes (Perlack and Wright et al. 2005; DOE, 2011). Currently, there are two main sources of bioenergy feedstock supply; i.e., (i) agricultural and forestry residues and (ii) dedicated energy crops.

Agriculture and forestry residues are the left over biomass after harvesting the main crop component e.g., corn stover, wheat straw, tree residues from lumber production and silviculture. Lignocellulosic bio refineries can utilize these crop residues as feedstock for biofuel and energy production, but the U.S. biofuel policy goals cannot be achieved without the use of dedicated energy crops (Perlack and Wright et al. 2005).

Dedicated energy crops refers to the lignocellulosic crops grown specifically for bioenergy production; e.g., *Miscanthus*, kenaf, big bluestem, and switchgrass. Recently, warm-season grasses have gained increased attention by the scientific community as a reliable source for lignocellulosic feedstock supply. The Bioenergy Feedstock Development Program at the U.S. Department of Energy has identified switchgrass as a model 2nd generation bioenergy feedstock crop in North America (Missaoui et al. 2006; Boutan J. 2007; Wright and Turhollow, 2010; Perlack et al., 2011).

Switchgrass as a Bioenergy Crop

Switchgrass (*Panicum virgatum* L.) is a C_4 perennial grass native to North America that ranges from Quebec to Central America (Moser and Vogel, 1995; Bransby et al. 1999; Missaoui et al. 2006). This grass has been proven to be an excellent candidate crop to be grown as a bioenergy feedstock (Vogel, 1996; Fike et al. 2006a). Some of its desirable traits include perenniality, high biomass yield production, and its adaptability to lands marginal for food crops (McLaughlin et al. 1996). In addition to these traits, another desirable trait is the seed production for its propagation, therefore, it can be grown with the available forage equipment. The species is polymorphic with two distinct ecotypes classified based on their habitat preferences, morphological characteristics and ploidy level; i.e., upland and lowland ecotypes (Brunken and Estes, 1975). Each ecotype is further sub divided based on its geographic origin i.e., southern and northern ecotypes (Sanderson, 1992; Casler, 2005). Both ecotypes include a great number of genetically varying varieties which has made it possible for this species to possess a wide range of adaptability (Cassida et al. 2005). Generally latitude is thought to be the largest determinant factor of switchgrass productivity and survival (Casler et al. 2004). The lowland ecotypes are heat tolerant and mostly found in wet climates at lower latitudes ranging throughout the lower plains of Midwest into the Southern U.S. Upland ecotypes are cold tolerant, found frequently in dry climates and range through the northern latitudes of North America (Brunken and Estes, 1975; Bransby et al. 1999; Sanderson et al. 1996; Casler et al. 2004).

Switchgrass reproduces through seeds and spreads vegetatively as well. Seedling establishment includes the germination and emergence phase followed by the development of adventitious root system (Newman and Mooser, 1988). Duration and rate of germination and emergence are influenced by many environmental factors including water, soil, air temperature, light etc. In literature the reported base temperature for germination and growth is between 8 and 10 ^{o}C, and optimum temperature is around 30 ^{o}C (Hsu et al. 1985; Seepaul et al. 2011), and maximum temperature can be around 40 ^{o}C, but all of these conditions appear to be cultivar dependent.

Switchgrass is an obligate outcrossing species, and both ecotypes possess high self-incompatibility and produce few or no seeds at all when self-pollinated (Talbert et al. 1983; Taliaferro and Hopkins, 1996). Cross pollination in switchgrass only takes place between cytotypes with analogous ploidy levels (Missaoui et al. 2006). Switchgrass ecotypes have varying ploidy levels. Upland ecotypes are tetraploid and octaploid with 2n = 8x = 72 chromosomes, whereas, the lowland ecotypes are mostly tetraploid with 2n = 4x = 36 chromosomes (Hopkins et al. 1996; Hultquist et al. 1997; Lu et al. 1998; Missaoui et al. 2006; Zalapa et al. 2011). Switchgrass inflorescence is a diffuse panicle with 2 – 20 inches in length, with a two flowered spikelet which disarticulate below the glumes. Leaf number per plant or per tiller in switchgrass vary based on genotype and environmental conditions (Redfearn et al. 1997), and the leaf length can vary anywhere from 4 – 24 inches.

Switchgrass genotypes have caespitose growth habit with short rhizomes, and Panicoid type root system (Newman et al.1988; Tischler et al. 1993). The rooting depth of established plants can reach up to 3m (Weaver, 1954), similarly the plants can reach a height of 0.5 – 3m (Moser and Vogel, 1995; Vogel, 2004).

Biomass Production

The wide natural geographic distribution of switchgrass has divided cultivars into two ecotypes; upland and lowland cultivars (McLaughlin et al., 2005). Both ecotypes possess large genotypic, phenotypic and physiological differences (Stroup et al. 2003; Casler et al. 2004; Das et al. 2004; Gunter et al. 1996; Yang et al. 2009; Cortese et al. 2010). Switchgrass varieties that have demonstrated greater biomass yield potential in Southern U.S. are the lowland cultivars such as Alamo and Kanlow (Douglas et al. 2004; McLaughlin et al. 2005). These cultivars have been selected from lower latitude, have thicker stems, delayed flowering and are adapted to wet conditions (Casler and Boe, 2003; Casler, 2005; Parrish et al., 2008). Upland cultivars such as Cave-in-Rock on the other hand have demonstrated prominent biomass yields in the mid and northern ranges of U.S. (Douglas et al. 2004; McLaughlin et al. 2005). Upland cultivars have been selected from higher latitudes, are thin-stemmed and found in drier conditions (Moser and Vogel, 1995). Upland cultivars have demonstrated greater winter survival potential than the lowland cultivars when grown at the same latitude (Cassida et al. 2005, Parrish et al. 2008).

It has been observed that when lowland ecotypes are moved to northern locations, they produce high dry matter yields due to their delayed flowering, which allows them to accumulate more biomass, but they may be unable to produce seeds and get to physiological maturity (Casler et al. 2014). In perennial grasses in order to survive the winter, reaching adequate dormancy and translocation of metabolites to the below ground plant parts is very important. An extended photoperiod may encourage lowland ecotypes to accumulate more biomass, but their incomplete dormancy and metabolite translocation may risk their survival when moved to northern latitudes (Casler et al. 2004, Casler, 2005). On the other hand when upland ecotypes are moved to southern locations their dry matter yields are reduced because they flower early due to the shorter day length in summer, and they can also be more prone to diseases (Olsen, 1984; Van Esbroeck et al. 2003; Casler et al. 2004, Casler et al. 2005).

Generally, switchgrass stand establishment is slow and requires two to three years to reach maximum biomass yields. However, once established it can be maintained for more than 10 years (Evanylo et al. 2005; Fike et al. 2006b; Perlack et al. 2011). An established stand of switchgrass can yield from 8–15 tons ha^{-1} depending upon the location and cultivar (McLaughlin et al. 2002; Roger and Charles, 2002; Monti et al. 2008; Adler et al. 2008), and with a net energy yield of about 70 GJ ha^{-1} (Schmer et al. 2014).

A number of factors can affect the biomass yield and quality of switchgrass grown as a bioenergy crop such as, cultivar, soil fertility, climatic conditions and field management. It can be cultivated and harvested as a traditional forage crop (Raneses et al. 1998), but crop management techniques are far different when grown as a bioenergy crop due to biomass quality requirement (Lowenberg-DeBoer and Cherney, 1989). As a bioenergy crop, growers will be more concerned about the total biomass yield; whereas, as a forage crop, growers are also concerned about its nutritive value. Therefore, optimum and appropriate management is required for both systems (Sanderson et al. 1999). As a bioenergy crop, varying harvest regimes have been demonstrated in one and two harvest systems. Lowland cultivars are recommended to be harvested once per year at the end of growing cycle to achieve maximum biomass yield (Bransby et al. 1999; Cassida et al. 2002). Upland cultivars on the other hand may be harvested twice per year since they mature faster than the lowland ecotypes (Bransby et al. 1999; Cassida et al. 2002).

Increase in biomass yield of a grass is a result of leaf number and leaf expansion, stem number and stem length and the development of reproductive structures (Frank, 1996). Kalu et al. (1983) and Nelson et al. (1994) reported plant maturity as an important factor affecting the morphological development within a specie. Casler et al. (2012) and Aspinwall et al. (2013) reported that in geographic zones where switchgrass ecotypes overlap, clear morphological differences have been observed between the upland and lowland ecotypes, such as phenological traits. Grass phenology has been observed to play an important role in biomass accumulation. Production of greater biomass yield by lowland ecotypes is also generally attributed towards their delayed maturity and faster stem elongation (Casler et al.

2004). The perennial grass staging developed by Moore et al. (1991) divides grass phenological stages into five distinct stages: 1) emergence, 2) vegetative/leaf development, 3) elongation/stem elongation, 4) reproductive/floral development, and 5) seed development and ripening. In this system each mainstage such as vegetative, elongation etc., is further subdivided into sub-stages which have assigned numeric numbers which range from 0.5 – 4.9. These numbers allow researchers to quantitatively estimate the growth stages and apply statistics to determine differences between plant populations with the help descriptive values of physiological and ecological state of the sward.

During vegetative growth switchgrass phenology is closely related to growing degree days (GDD), but this relationship has been observed to vary with cultivar and the location where it is grown (Sanderson and Wolf, 1995a, Sanderson and Wolf, 1995b, Madakadze et al. 1998). However, reproductive development in switchgrass is closely associated with day-of-year suggesting a photoperiod effect (Cornelius and Johnston, 1941, Sanderson and Wolf, 1995a, Hopkins et al., 1995a, Mitchell et al. 2001). Nevertheless, these relationships are also dependent upon the cultivar × environment interactions, and differences between relative biomass yields are often different from year to year, locations and harvest management (Sanderson et al. 1999; Hopkins et al. 1995a, Hopkins et al. 1995b).

Furthermore, canopy architecture is an important factor describing grass growth. It can be influenced by the interaction between plant and its environment (Welles and Norman, 1991). Moore and Moser (1995) reported that the canopy architecture of a grass sward results due to continuous changes and is a function of tiller morphology at varying developmental stages and developmental stage distribution within a tiller population. Grass canopy architecture can not only affect the plant physiology and light interception, it can also affect its biomass yield production (Nelson and Moser, 1994). One of the important trait of canopy architecture is tiller number. Generally high yielding grass species are associated with the production of greater number of reproductive tillers, and most of the grass dry matter yield comes from the nodes and internodes of these tillers, and a small portion of comes from the leaves (Kalmbacher, 1983).

Generally, a population of grass tillers is referred as grass sward and variability in the structure of a grass sward with time mirror the seasonal variation in developmental morphology of each tiller (Moore and Moser, 1995). Tiller demographic analysis can be used to describe this seasonal variation in developmental morphology of a grass sward. This analysis is usually presented by plotting the number of tillers m^{-2} and categorizing them in vegetative, elongation, reproductive and seed ripening stage over the time. In a tiller demographic study of switchgrass Mitchell et al (1997), reported that switchgrass tiller number decline with the advanced maturity. Thus, morphological and phenological development are considered very important to better understand harvest management practices as a bioenergy crop and to obtain best possible yields. Lemus et al. (2008) reported that switchgrass yields can be improved from 2 to 5 mg ha^{-1} by simply optimizing agronomic practices and harvest management. Most of the switchgrass information available is based on wide geographic zones and as a forage crop; however, information related to locally established field trials to evaluate switchgrass cultivars from both ecotypes for developmental morphology, harvest management and dry matter yield as bioenergy crop will be more useful for cultivar selection and development.

Biomass Composition

Biomass yield potential is the most important characteristic of an ideal lignocellulosic bioenergy crop, however, feedstock quality is also an important attribute. Lignocellulose is the main component of biomass dry matter, which is made up of cellulose, hemicellulose, lignin and minerals. Lignocellulosic biomass feedstocks are currently converted into energy by biochemical conversion for liquid fuel production (Hayn et al. 1993; McKendry, 2002; Hamelinck et al. 2005) and thermochemical conversion (e.g., combustion/direct combustion, pyrolysis and gasification) for heat and electricity generation (Ortiz-Canavate, 1994; McKendry, 2002). Biofuel refineries have different feedstock quality requirements, therefore, feedstock quality is also dependent upon the conversion system being used (McKendry, 2002; Hamelinck et al. 2005; Adler et al. 2006; Sanderson et al. 2007; Cheng et al. 2010).

For biochemical conversion systems, higher cellulose and hemicellulose concentrations are desirable by contributing more fermentable sugars for liquid fuel synthesis (Kurakake et al. 2001; Datar et al. 2004). However, depending upon the biochemical conversion technology being used lignin can become both desirable by adding more fermentable sugars or undesirable by reducing the availability of cellulose and hemicellulose during fermentation. Some advance biochemical conversion technologies can convert lignin contents into fermentable sugars through pretreatment processes (Cheng et al. 2009). However, currently used conversion technologies in the bioenergy industry by hydrolysis and enzymatic systems are not able to convert lignin into liquid fuel (McLaughlin et al. 1996; McKendry, 2002).

In some thermochemical conversion systems, higher nitrogen and ash concentration reduce the system's conversion efficiency by causing slagging (Sanderson et al. 1996). The buildup of mineral elements, N and ash on the inner surface of the boiler are called slagging, which hinders the efficient transfer of heat and can ultimately make a production plant inoperable (Blander et al. 1997). Higher nitrogen concentration in the dry matter feedstock used in thermochemical conversion systems can result in lower hydrocarbon yields (Agblevor et al. 1992; Agblevor et al. 1995), and higher nitrogen oxide (NO_X) emissions to the air (Burner et al. 2008). In literature, the reported critical limit for N concentration in dry matter feedstock is 10 g kg^{-1} DM (Lewandowski et al. 1997). Ash on the other hand is the waste product of thermochemical and biochemical process and its higher concentrations not only decrease the conversion efficiency of the system but can also increase the handling and processing costs (Miles et al. 1993). McKendry (2002) reported that switchgrass feedstock has higher heating value of 17.4 MJ kg^{-1}, which is almost equal to the hard wood (18.5 MJ $kg^{-1}$for poplar and 20 MJ kg^{-1} for willow). In literature it has been reported that every 1 % increase in ash concentration in the dry matter feedstock used can reduce the heating value of thermochemical conversion system by 0.2 MJ kg^{-1} (Cassida et al. 2005).

Higher concentrations of energy dense compounds like lignin, on the other hand are desirable for thermochemical conversion systems due to their higher heating value. Johnson et al. (1995) and Vermerris et al. (2008) reported that in lignin due to the presence of carbon-carbon linkage which can be oxidized, lignin has a large energy contents of about 26 G Mg^{-1} which is equal to the energy of a coal. Lignin is a vital component of the plants cell wall and has an important role in plant vascular transport and by providing structural strength. It has a molecular weight of 10,000. Lignin is a cross linkage of aromatic polymers of phenylpropane units which are referred as monolignols, varying with the numbers of methoxy (OCH_3) group attached to the phenolic units (McKendry, 2002; Keshwani, 2010).

Moreover, the lignin during thermochemical conversion process, can also be converted into many valuable chemicals or materials such as ethylene, propylene, butadiene and aromatics (e.g., benzene, toluene and xylene isomers) (Royal Society of Chemistry, 2015). However, since lignin binds with cellulose and hemicellulose, it can also reduce their availability in biochemical conversion systems, which can result is reduced biofuel production (Trebbi, G. 1993; Adler et al. 2006; Sanderson et al. 2007).

In addition to these structural carbohydrates (lignin, cellulose, hemicellulose), lignocellulosic feedstock also contains nonstructural carbohydrates such as sucrose, glucose, fructose and starch. Even though they are not present in very high quantities as compared to the structural carbohydrates in the feedstock, they are still a source of fermentable sugars for liquid fuel production (Dien et al. 2006; Johnson et al. 2007).

Composition of feedstocks can vary based on the geographic location where they were produced, genetic factors, developmental morphology and agronomic practices (Adler and Sanderson et al. 2006; Royal Society, 2008; Vogel et al. 2002). Most of the past switchgrass compositional and harvest management studies have addressed its use as a forage crop, however, due to tremendous genetic variability understanding of ecotypic variation in switchgrass biomass quality as a bioenergy application is more limited and needs further research.

Modeling Switchgrass Phenology

In the switchgrass life cycle, temperature plays vital role from breaking of bud dormancy to development of reproductive phase (Parrish et al. 2008). Switchgrass cultivars have large genetic variability for their temperature requirements for growth and development (Mcmillan and Weiler, 1959). The development of switchgrass is similar to other perennial grasses and follows the same growth pattern, described by Moore et al. (1991) and as aforementioned in detail i.e., emergence, vegetative, elongation, reproductive and physiological maturity stage. The duration of each phase is mainly cultivar and temperature dependent. Madakadze et al. (1998) reported a strong relationship between leaf development and internode elongation. Sanderson and Wolf (1995), reported that Cave-in-Rock and Alamo when grown near to their zone of origin need 200 and 430 growing degree days (GDD) at a base temperature of 10 ^{o}C to accomplish leaf development and 378 and 1020 GDD for stem elongation stage. Reproductive stage on the contrary is mainly controlled by the photoperiod (Van Esbroeck, 2003), but other factors including temperature cannot be completely ignored.

In another study to analyze the effect of temperature on growth and development of switchgrass, Blasko and Smith (1971), performed an experiment with four day/night temperature regimes of 32/26 ^{o}C, 27/21 ^{o}C, 21/15 ^{o}C and 15/10 ^{o}C. From this study they concluded that at the highest temperature treatment (32/26 ^{o}C) switchgrass had greatest growth and development. They also reported delayed inflorescence in switchgrass plants at 21/15 ^{o}C, and complete inflorescence inhibition at 15/10 ^{o}C. Other studies have also reported temperature effects on switchgrass, and most of them have reported that the optimum temperature for switchgrass growth and development is around 30-33 °C (Bendict, 1941; Masiunas and Carpenter, 1984; Hsu et al. 1985).

Brown et al. (2000) conducted a simulation study to determine the effects of raised temperature in comparison to the present climatic conditions on switchgrass. He reported that a rise of 6-8 °C in the temperature from present climatic temperatures significantly increased switchgrass production, which might be the result of reduced cold stress and prolonged growth season in Minnesota, Iowa, Nebraska and Kansas. He also added that a rise in environmental temperature due to global warming will improve switchgrass biomass yields. This approach seems realistic considering switchgrass is a C_4 grass and its cultivars belong to a wide geographic zones and have a lot of dry matter yield differences among them. Thus, this temperature rise will allow cultivation of high yielding cultivars e.g., lowland ecotypes in the areas where their survival otherwise will be at risk. However, we should also bear in mind that switchgrass growth and development is not only temperature driven but also associated with the other environmental factors. In many annual cereal studies in the past, photoperiod have been reported to significantly affect the duration of vegetative growth (Vince-Prue, 1975; Russell and Stuber, 1983; Miglietta, 1989; Collinson et al. 1992). Although, switchgrass is not an annual grass specie but it has the determinate growth habit which is similar to those grasses (Hyder, 1974).

Many past studies have suggested that switchgrass is sensitive to photoperiod (Hopkins et al. 1995; Sanderson and Wolf, 1995, Van Esbroeck, 1997; Redfearn et al. 1997; Mitchell et al 1997; Madakadze et al. 1998). Van Esbroeck (2003), studied the effect of day length on two widely grown switchgrass cultivars (Cave-in-Rock and Alamo) with 12 h and

16 h photoperiod. He reported that at 16 h photoperiod inflorescence was delayed by 17 days and the time for panicle exertion was increased by 243 % as compared to the 12 h. On the other hand in Alamo at 16 h photoperiod inflorescence was delayed by 15 days and the time for panicle exertion was increased by 136 % as compared to the 12 h photoperiod.

Many in-field trials provide direct information related to environmental, genetics, soil and management practices for improved productivity (Heaton et al. 2004; Jager et al. 2010; Monti, 2012). Similarly, different crop models are widely used as tools to summarize this information and develop better understanding to enhance management practices, estimate potential yields and to determine environmental influences related to the increase in bioenergy crops in our agroecosystem (Zhang et al. 2010). These models are mainly classified into two categories i.e., empirical and mechanistic models. Empirical models are based on statistical approaches to establish relationships between crop yields and environmental and agronomic variables, such as switchgrass biomass yield model developed by Wullschleger et al. (2010). This model takes into consideration climatic, physical and management variables including temperature, precipitation, fertilizer and ecotypes for biomass yield prediction. Similarly, Jager et al. (2010) developed an ecotype specific empirical model that also determines response of many biophysical and management variables on switchgrass biomass production.

In contrast to the above mentioned empirical models, mechanistic models provide insight into physiological and morphological developments and their interaction with biomass yield. Two widely used models for switchgrass are ALMANAC and EPIC models (Kiniry et al. 2005; Thomson et al. 2009). ALMANAC model provides information associated with crop growth and biomass yields across different environmental conditions by taking into account abiotic stresses and crop weed competitions (Kiniry et al. 1992). The EPIC model is process based and predicts switchgrass growth and development by taking into consideration the environmental impacts of agronomic practices along with dry matter yield estimation (Williams et al. 1989; Izaurralde et al. 2006). Although both of these models i.e., ALMANAC and EPIC are different from each other in many ways, inputs for these models are not very different for their field measurement such as leaf area index, radiation use efficiency, carbon partitioning and crop phenology. These models have been very helpful for researchers to understand and simulate switchgrass biomass yield at a specific location (Kiniry et al. 2005; Kiniry et al. 2008) as well as on a regional scale (McLaughlin et al. 2006; Zhang et al. 2010).

However, to our knowledge thorough information regarding switchgrass photoperiodic sensitivity is limited in literature and especially missing from these models. Such information is fundamental and can greatly support researchers to improve switchgrass crop growth models and breeding programs. One of the most important challenges that bioenergy crop modelers face is to precisely model biomass yields of new cultivars being developed. Each new cultivar might require its own parameter estimation as a result of varying phenological development, which also makes crop model output sensitive to precise modeling of its phenology (Van Oort et al. 2011). Generally, the above mentioned models compensate for the photoperiod/day length effects by regulating the growing degree days requirements (of the same cultivar) at different latitudes. Although this approach may work well for these models, it lacks the mechanistic consideration which can provide better assessment of switchgrass adaptation to various geographic locations.

Therefore, more detailed studies are required to determine the effect of temperature and photoperiod on switchgrass growth and development, and more specifically, integration of crop phenological information into the modeling platform by taking into account thermal and photoperiod effects on switchgrass growth and development. This information will also help other researchers to improve the already present models as well as for the development of new models.

OBJECTIVES

Given the wide genetic variability within the switchgrass genus, variability in developmental morphology as well as dry matter yields, this project used five cultivars from two different ecotypes, upland and lowland. The overall objectives of this dissertation work were twofold: (1) Determine the ecotypic differences among switchgrass cultivars for developmental morphology (phenology and LAI) and biomass yield and (2) Determine ecotypic differences among switchgrass cultivars for compositional traits important for its use as a bioenergy feedstock. The objectives of the first study (Chapter 2) were to evaluate ecotypic differences in switchgrass for their harvest management, developmental morphology and dry matter yields. The objectives of the second study (Chapter 3) were to quantify dry matter composition as influenced by harvest date and to determine if ecotypic differences exist for their chemical composition. The objectives of the third study (Chapter 4) were to investigate the combined effect of photoperiod and temperature on switchgrass phenology by combining experimental information from a wide range of environmental conditions.

DISSERTATION ORGANIZATION

This dissertation is organized into five chapters, and focuses on three research papers that are intended for submission to different Journals. The first chapter contains a general introduction, consisting a comprehensive literature review, following by the description of the objectives and dissertation organization. The second chapter contains the first manuscript “developmental morphology and biomass yield differences of upland and lowland switchgrass ecotypes grown in Iowa”. The third chapter contains the second manuscript “compositional differences among upland and lowland switchgrass ecotypes grown as a bioenergy feedstock crop”. The fourth chapter contains the third manuscript “A new model for switchgrass phenology that combines temperature and photoperiod effects”. The fifth chapter contains general conclusion derived from the three manuscripts.

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CHAPTER 2

DEVELOPMENTAL MORPHOLOGY AND BIOMASS YIELD OF UPLAND AND LOWLAND SWITCHGRASS ECOTYPES GROWN IN IOWA

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Abstract

Sustainable and successful development of the bioenergy industry will strongly depend upon the amount and quality of bioenergy feedstock produced. Switchgrass (*Panicum virgatum* L.) has been identified as a model lignocellulosic bioenergy crop in the U.S. Information regarding grass growth and development is considered critical for making management decisions and to support simulation model calibration and subsequent analysis. The objective of this study was to evaluate upland ('Cave-in-Rock', 'Trailblazer' and 'Blackwell') and lowland ('Kanlow' and 'Alamo') ecotypes of switchgrass for harvest management, morphological (phenology and leaf area index) and biomass yield differences. A field study was conducted near Ames, IA during 2012 and 2013. The experiment was laid out as randomized complete block design, and consisted of three upland varieties and two lowland varieties. Six in-season destructive biomass harvests were collected at approximately 2-week intervals each year. In both years, delaying harvest to later maturity increased biomass yield in all cultivars. Averaged over years lowland cultivars 'Kanlow' and 'Alamo' produced higher dry matter yield (6.31 and 5.98 tons ha^{-1}, respectively) than upland ecotypes

‘Cave-in-Rock’, ‘Trailblazer’ and ‘Blackwell’ (5.89, 4.78 and 4.53 tons ha^{-1}, respectively). Lowland cultivars had delayed reproductive development compared with upland ecotypes. At the end of both growing seasons, upland ecotypes had a higher mean stage count than the lowland ecotypes, indicating that upland ecotypes had morphologically more mature tillers than the lowland ecotypes. The widely recommended cultivar in Iowa ‘Cave-in-Rock’ had the highest mean stage count and leaf area index at the end of both growing seasons, but did not produce the greatest biomass. There was a nonlinear relationship between MSC and biomass yield. However, the magnitude and form of the response was different between cultivars and from year to year. The relationship between biomass yields and mean stage count could be useful for developing switchgrass management strategies such as cultivar selection, fertilizer application rates and optimum harvest time.

Keywords: Biomass yield; Mean stage count; Grass morphology; Switchgrass development; Leaf area index.

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1. Introduction

Increasing energy demand, uncertainty in crude oil prices, and environmental impacts of using fossil fuels have increased interest in alternative energy sources, including biofuels. Growing biomass crops that can be converted to fuel is one approach to addressing these concerns (Ragauskas et al., 2006; Farrell A. et al., 2006; CAST 2007). Switchgrass (*Panicum virgatum* L.) is a C_4 perennial grass native to North America (Bransby et al., 1999; Missaoui et al., 2006). It has been selected as a model 2nd generation bioenergy crop as an alternative to fossil fuel by The Bioenergy Feedstock Development Program (BFDP) at the US Department of Energy (Missaoui et al., 2006). Switchgrass has already demonstrated great potential for being an ideal bioenergy feedstock in Central and North America (Bransby et al., 1999; Douglas et al., 2004; Cassida et al., 2005; Bouton J. 2007) through wide geographic adaptability, suitability to grow on marginal lands, high nutrient and water use efficiency and by providing natural habitat for migratory birds (Moser et al., 1995;; Paine et al., 1996; Sanderson et al., 1996).

The wide natural geographic distribution of switchgrass has resulted in the evolution of two broad ecotypes; i.e., upland and lowland ecotypes (McLaughlin et al., 2005). Both ecotypes possess large genotypic, phenotypic and physiological differences (Gunter et al., 1996; Stroup et al., 2003; Casler et al., 2004; Das et al., 2004; Yang et al., 2009; Cortese et al., 2010). Cultivars selected from lower latitudes are generally tall, thick-stemmed, later flowering, adapted to wet conditions and have higher biomass yield potential as compared to the upland ecotypes (Casler and Boe 2003; Casler 2005; Parrish et al., 2008). Upland ecotypes are shorter, thin-stemmed, relatively lower yielding, found in drier conditions

(Moser and Vogel 1995), and have better winter survival than lowland ecotypes when grown at the same latitude (Cassida et al., 2005; Parrish et al., 2008).

In geographic zones where the adaption of these ecotypes overlaps, clear morphological differences have been observed between them (Casler 2012; Aspinwall et al., 2013). These differences have been observed to play an important role in biomass accumulation and composition. Production of greater biomass yields by lowland ecotypes is also generally attributed to their delayed maturity and faster stem elongation (Casler et al., 2004).

There are five distinct stages in the development of most grasses: 1) germination, 2) vegetative/leaf development, 3) Elongation/stem elongation, 4) reproductive/floral development, and 5) seed development and ripening (Moore et al., 1991). Switchgrass development is closely associated with growing degree days (GDD) during its vegetative stage, but the relationship varies with cultivar and location (Sanderson and Wolf, 1995a; Sanderson and Wolf, 1995b; Madakadze et al., 1998). Reproductive development on the other hand is more closely related to the day-of-the-year, suggesting a photoperiod effect (Cornelius and Johnston, 1941; Hopkins et al., 1995a; Sanderson and Wolf, 1995a). However, these relationships are also affected by cultivar × environment interactions and differences between relative biomass yields are often different from year to year, locations and harvest management (Sanderson et al., 1999; Hopkins et al., 1995a, 1995b). Therefore, understanding morphological development is considered very important for quantifying and describing important grass canopy processes which can be affected by the plant × environment interaction (Welles and Norman, 1991), and estimation of grass developmental morphology can potentially be used to predict yield and composition (Sanderson and Wedin, 1989; Hintz and Albrecht, 1991; Sanderson et al. 1995b; Casler and Boe, 2003; Dien et al., 2006).

Most of the information available on morphological development of switchgrass ecotypes is based on comparisons made among cultivars grown in different geographic zones; however, information from side-by-side comparisons at the same location is not extensive. Such information could be very useful for cultivar selection and development and to understand differences between ecotypes. The objectives of this study were (i) to determine the effect of harvest date and switchgrass phenology on biomass yield (ii) to evaluate biomass yield differences between switchgrass ecotypes and, (iii) to determine if switchgrass morphological development, an easy observable trait in the field, can be used as a predictive tool for leaf area index (LAI) and dry matter yield (DMY).

2. Material and Methods

2.1. Experimental Site

To determine the developmental morphology and dry matter yields of upland and lowland switchgrass cultivars, a study was conducted during 2012 and 2013 on a switchgrass cultivar trial established in 2007 at Iowa State University, Sorenson Research Farm, near Ames, IA (42^{o}0’41” N, 93’44’34” W). The experimental field was arranged as a randomized complete block design with six replications. Five switchgrass varieties of two distinct ecotypes, upland (‘Cave-in-Rock’, ‘Blackwell’ and ‘Trailblazer’) and lowland (‘Kanlow’ and ‘Alamo’) were used as treatments. Every year prior to spring growth initiation, standing dead material was mowed at a stubble height of 5 cm, and the plant residue was removed from the field. To control weeds each year a pre-emergence application of atrazine [6-Chloro-N-ethyl-N'-(1-methylethyl)-1, 3, 5-triazine-2, 4-diamine] and Paramount [3, 7-Dichloro-8-quinolinecarboxylic acid] was applied at 2.23 kg and 0.56 kg ha^{-1} a.i.ha^{-1} respectively. Nitrogen (NH_4NO_3, 34-0-0), P (TSP, 0-46-0) and K (KCl, 0-0-62) fertilizer were applied every year in early May at 78 kg ha^{-1}, 67 kg ha^{-1} and 90 kg ha^{-1}, respectively. Total precipitation and mean monthly air temperature for 2012 and 2013 were collected at a site located less than eight km from the experimental site, and the data were downloaded from the Iowa Environmental Mesonet (2014) (Table 2.1).

2.2. Biomass harvest

In 2012, biomass harvest samples were collected from three randomly selected blocks and the remaining blocks were used the following year to collect harvest samples. Each plot was divided into six subplots corresponding to six biomass harvests. Each year, depending on the spring growth, the first harvest occurred at early vegetative growth (approximately two weeks after emergence). In 2012 and 2013, the first harvest samples were harvested on 136th and 149th day of the year (DOY) respectively, and the remaining five harvests were collected at approximately 2-week intervals. At each harvest date, two 0.1-m^2 samples were harvested by placing two quadrats randomly within each subplot. Tillers were carefully hand clipped at ground level and transported to the laboratory for further processing.

2.3. Morphology and development

The LAI data was estimated by using LI-2000 plant canopy analyzer system (LI-COR Biosciences. Lincoln, Nebraska). Measurements were taken biweekly on the same day as the biomass harvest samples were collected and within the first 4 h of daylight on clear sunny days. Each LAI determination consisted of four below canopy measurements interspersed with one above canopy measurement. Below canopy measurements were recorded by placing the LI-2000 bar close to the ground surface and on the non-harvested portion of whole plot.

Mean stage count (MSC) for each switchgrass variety was determined at each harvest date. Biomass harvest samples were hand sorted and morphologically classified according to the Nebraska system for staging perennial grasses described by Moore et al. (1991).

2.4. Dry Matter Yield

Harvested biomass samples were pooled after morphological classification to determine the dry matter yields. Biomass samples were weighed for fresh weight and then dried in a forced-air oven at 60 ^{o}C for 72 h or until a constant dry weight was achieved, and then reweighed.

2.5. Statistical Analysis

Statistical analysis was performed by analysis of variance using the GLIMMIX procedure of SAS 9.4 (SAS Institute, 2013). Switchgrass variety, year and harvest date were considered fixed effects, while blocks and the interactions with blocks were considered as random effects. To determine the relationship between switchgrass development, leaf area index (LAI) and dry matter yield DMY, quadratic regression equations were fitted with MSC as independent variable and LAI and DMY as dependent variables. All statistical tests were made at alpha=0.05.

3. Results

3.1. Temperature and precipitation

Growing season conditions differed between years. Mean monthly air temperature in the first half of 2012 was warmer compared to 2013 and the 20-year mean, while the rest of the growing season temperatures were fairly typical (Table 2.1). In 2013, spring regrowth was about two week delayed as compared to 2012, which was related to cool temperatures in May. Precipitation in both years was lower than the 20-year mean except April and May of 2013. April in both years and May in 2013 was wetter than the 20-year average and then followed by a drier summer (Table 2.1). Year 2012 experienced serious drought as a result of below normal rainfall in spring, and later during the spring and summer temperatures were above long-term normal.

3.2. Morphology and development

Development (MSC) was affected by harvest date, but the effect was different among the varieties (Table 2.2, Fig. 2.1 and 2.2). Averaged over both years, ‘Cave-in-Rock’ which is an upland cultivar, had the highest MSC of 2.52, whereas the two lowland cultivars, ‘Kanlow’ and ‘Alamo’, had the lowest MSC of 2.28 and 2.29 respectively.

Due to significant variety × year interactions, each year was then analyzed separately. In 2012, variety and harvest date affected the MSC and there was a significant interaction between them. However, in 2013 variety and harvest date were found to have an effect on MSC, but the two-way interaction of variety and harvest was not significant. This indicates that switchgrass varieties in 2012 had larger differences between harvests than in 2013.

In both years, MSC for all switchgrass varieties increased gradually from the first harvest date to the final, and the highest MSC for all switchgrass varieties was observed at the final harvest (Fig. 2.1 and 2.2). At the end of each growing season, MSC for upland ecotypes was always greater than lowland ecotypes, suggesting that upland ecotypes matured earlier than lowland (Fig. 2.1 and 2.2). In 2012, there was an interaction between variety and harvest date. At the final harvest in 2012, Cave-in-Rock had the highest MSC of 3.6, whereas Kanlow and Alamo had the lowest MSC of 3.1 and 3.0, respectively (Fig. 2.1). However in 2013, only variety and harvest date affected the MSC and there was no interaction between them. In 2013, at the final harvest, Cave-in-Rock had the highest MSC of 4.6 and Kanlow and Alamo had the lowest MSC of 3.1 and 3.2, respectively (Fig. 2.1 and 2.2).

The LAI for all switchgrass varieties increased with maturity in both years (Fig. 2.3). The LAI of all varieties differed with harvest date (Table 2.2); earlier harvest dates had lower LAI compared to later harvest dates. Averaged over both years, the increase in LAI of switchgrass varieties between first and last harvest ranged from 0.8 for Alamo to 5.6 for Cave-in-Rock.

In 2012, variety and harvest date affected the LAI of switchgrass varieties and there was an interaction between them. In 2012, LAI for all switchgrass varieties increased gradually from the first harvest date to the final. The increase in LAI for switchgrass varieties between first and last harvest ranged from 0.6 for Blackwell to 6.7 for Cave-in-Rock (Fig. 2.3). However in 2013, only variety and harvest date affected the LAI, but there was no interaction between them, suggesting that LAI among switchgrass varieties changed differently in 2013 compared to 2012. In 2013, a gradual increase in LAI was only observed until mid-season and then it decreased (Fig. 2.3). The LAI between first and fourth harvest ranged from 0.93 for Kanlow to 5.1 for Cave-in-Rock (Fig. 2.3). Similar to MSC, at the end of both years, LAI for upland varieties was always greater than the lowland varieties.

3.3. Dry Matter Yield

The DMY of all switchgrass varieties increased gradually with delayed harvest in both years (Fig. 2.4). Year, variety and harvest date significantly affected the DMY (Table 2.2, Fig. 2.4). Averaged over both years, Kanlow had the highest DMY of 6.31 tons ha^{-1} and Trailblazer had the lowest DMY of 4.5 tons ha^{-1}.

In 2012, variety and harvest date influenced DMY, but there was no interaction between them. The highest DMY for all switchgrass varieties were observed at the last harvest; 'Kanlow' accumulated the highest DMY of 14.3 tons ha^{-1} and 'Blackwell' had the lowest DMY of 10.2 tons ha^{-1} (Fig. 2.4). Similarly in 2013, variety and harvest date affected the DMY for switchgrass varieties, but there was no interaction between them. In 2013, DMY for all switchgrass varieties were lower as compared to 2012. However, varieties followed a similar trend as in 2012 with 'Kanlow' producing the highest yield with 10.88 t ha^{-1} and 'Blackwell' with the lowest DMY of 6.7 tons ha^{-1} (Fig. 2.4).

3.4. Predictive relationships

The relationships between MSC, DMY, MSC and LAI were nonlinear. Quadratic equations gave the best polynomial fit in terms of predicting relationships between MSC, LAI and DMY in 2012 and 2013 (Fig. 2.5 and Fig. 2.6). In both years these relationships were positive and with good predictive values (r^2 = 0.6-0.94).

The equation for predicting LAI and DMY using the quadratic MSC model in 2012 generally gave higher r^2 than in 2013. In terms of ecotypes, LAI and DMY were found to be more predictable with the MSC quadratic equation in upland than the lowland ecotypes (Fig. 2.5 and Fig. 2.6), but year to year variation also seems to have an effect on this relationship.

4. Discussion

Results of two years of growth and developmental study of switchgrass ecotypes confirmed significant variation between switchgrass ecotypes for their biomass yield and phenology. This study showed that LAI, MSC and biomass yields were significantly different between switchgrass ecotypes, suggesting that there is substantial scope for enhancing switchgrass genotypes in the U.S. Midwest for improved biomass yield.

The ultimate goal of a biomass crop is to produce high yield of high quality over the growing season and harvest management has the potential to greatly affect biomass yields and quality. Poor harvest management might not only result in reduced biomass quality and yield, it might also decrease stand persistence (Moore and Moser 1995; Mitchell et al., 2001). Harvest timing can also vary with location as well as with cultivar. Results from this study observed significant variation among switchgrass ecotypes for their optimal harvest time. Delayed harvest to later maturity resulted in higher dry matter yields in all cultivars over the growing season. These results are in agreement with previous studies where they also recommended one end-of-season harvest to obtain the maximum switchgrass dry matter yields (Dwyer et al., 1963; Balasko et al., 1984; Trocsanyi et al., 2009; Madakadze et al., 1999; Sanderson et al 1999; Muir et al., 2001; Vogel et al., 2002; Smart et al., 2004). Harvesting at later stages of switchgrass growth and development will also increase lignocellulose and decrease mineral concentration in biomass (Jung and Vogel, 1992; Madakadze et al., 1999; Mitchell et al., 2001; Mulkey et al., 2006; Waramit et al., 2011).

Canopy architecture not only affects plant growth via light interception but it can also affect the composition of the biomass (Nelson and Moser, 1994). Canopy architecture traits related to morphological development have always been of great interest for researchers in order to enhance and optimize economic biomass yields. Results from our study demonstrated significant differences between switchgrass ecotypes for their MSC over the two growing seasons. Generally, upland ecotypes reached higher MSC than the lowland ecotypes at a given day of the year, and this difference between the upland and lowland ecotypes became larger at the start of reproductive development. Upland ecotypes emerged earlier in the spring and matured about 2-3 weeks earlier than the lowland ecotypes. Previous studies have also reported similar findings that upland ecotypes mature earlier than the lowland ecotypes (Sanderson and Wolf, 1995; Cassida et al., 2005; Parrish et al; 2008). Standard deviation of MSC (S_{MSC}) acted in a straight line fashion for all ecotypes as the growing season progressed in 2012, but variability for S_{MSC} in 2013 was greater especially during the mid-season harvest (Fig. 2.1). This indicates that tiller population of switchgrass ecotypes during mid-season harvests were comprised of diverse sub-stages. Reproductive development of switchgrass is thought to be linked with day of year but not completely independent of other factors. In 2012, the onset of reproductive development was about 2 weeks earlier in all varieties than in 2013, which indicates that growing season temperatures also plays an important role in switchgrass phenological development, and year to year variability can be explained by the differences in mean monthly temperatures.

Switchgrass LAI on the other hand increased with advanced maturity and varied across years. In both years, switchgrass ecotypes accumulated LAI in a curvilinear manner. During mid-season, LAI reached its maximum and then declined due to senescence. This pattern of LAI development has been described by the Hernández et al., (1999) as size/density compensation, where tillers number decreases but biomass increases. Results from this study also revealed significant differences between switchgrass ecotypes for LAI. The greater LAI in 2012 might be the result of higher temperatures during early growth periods, which also decreased the LAI differences among upland and lowland ecotypes. These results are in agreement with previous research that switchgrass leaf/stem ratio increases at higher temperatures in both upland and lowland ecotypes (Kandel et al., 2013). However, in 2013 clear differences between upland and lowland switchgrass varieties for their LAI throughout the growing season occurred, clearly indicating an ecotypic effect. Year 2013, being less warm than 2012, resulted in delayed spring emergence and delayed reproductive development in all switchgrass varieties as compared to 2012. Van Esbroeck et al. (1997) reported similar findings that slow leaf area appearance in switchgrass is associated with late panicle emergence. They reported about 60 days earlier panicle emergence in upland ecotypes Cado and Cave-in-Rock than lowland type Alamo.

Biomass yields of all switchgrass ecotypes were inconsistent across years. Year 2012, being warmer than 2013 and with higher LAI and more tillers m^{-2} (Fig. 2.2), also produced higher biomass yields than 2013. This might be explained by the fact that at higher temperatures switchgrass varieties produced greater tiller number in 2012, and resulted in higher DMY than 2013. Kandel et al., (2013) reported similar findings that at higher temperatures root/shoot and leaf/stem ratio increases in both upland and lowland ecotypes. Biomass yields of switchgrass ecotypes increased in a somewhat linear manner as the growing season progressed in both years. Maximum biomass yields in both years were observed post-reproductive development and at the last harvest in all ecotypes. These results are consistent with past findings that maximum switchgrass biomass yields in the US Midwest occurred in mid-August at full panicle emergence to post-anthesis (Vogel et al., 2002). In both years, Kanlow produced the highest biomass yields and Alamo and Cave-in-Rock had the second highest biomass yields after Kanlow. Cave-in-Rock, typically the most recommended cultivar for Iowa, did not produce higher biomass yields than its lowland counterparts, but it performed better than all other upland cultivars.

Switchgrass has a determinate growth habit and as the inflorescence starts developing the vegetative growth is terminated (Dahl et al., 1977; Mitchell et al., 1997). This indicates that maximum biomass yields in switchgrass occur when most of the grass tillers are post-reproductive development. This phenomenon on the other hand also reduces the dry matter yield accumulation in early flowering plants by terminating their vegetative growth. As lowland cultivars with delayed maturity as compared to the upland cultivars, Kanlow and Alamo accumulates more vegetative biomass than upland ecotypes (Van Esbroeck et al., 1997; Casler et al., 2004).

There was a strong quadratic relationship of MSC with LAI and DMY. However, a slight under prediction in both years after the mid-season of growth and development was also observed, which might be the result of faster LAI and dry matter accumulation. These findings are consistent with the previous studies (Kalu and Fick, 1981; Moore et al., 19991; Mitchell et al., 2001). Response of the MSC quadratic equations also varied from year to year which also indicates that this relationship can be affected by environmental factors such as temperature and precipitation (Sanderson et al., 1999).

In conclusion, the results in this experiment show variability among switchgrass ecotypes for biomass yield and important morphological traits. These traits were also observed to have an impact on biomass yield accumulation and harvest management among switchgrass ecotypes. This study also provides support for planting lowland switchgrass varieties in Iowa to obtain greater biomass yields. However, delayed maturity in lowland ecotypes might also reduce their persistence in northern locations in the long term. Winter temperatures during the course of this study were fairly typical and no winter losses were observed. Therefore, we also suggest that lowland ecotypes should be tested for their cold tolerance before commercially grown for biomass production in Iowa. Moreover, the results of MSC relationship to LAI and DMY study can also be useful in development of new and improvement of existing switchgrass morphological models for LAI and DMY prediction. However, this information would work best if the models are cultivar and region specific.

Acknowledgments

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Tables

Table 2.1. Mean monthly air temperature and precipitation for 2012, 2013 and 20 year mean at Ames, IA.

	Temperature (°C)			**Precipitation (Inches)**		
Month	2012	2013	20 Year Mean	2012	2013	20 Year Mean
Jan	-2	-5	-7	7	15	20
Feb	-1	-3	-4	44	20	25
Mar	12	-1	3	60	38	50
Apr	12	8	10	122	148	98
May	19	16	16	62	180	125
Jun	23	21	21	75	76	132
Jul	27	23	23	37	26	107
Aug	22	23	22	74	55	124
Sep	18	19	18	47	50	81
Oct	10	11	12	59	97	61
Nov	6	1	4	23	40	51
Dec	-2	-8	-4	26	8	27

Table 2.2. Analysis of variance for mean stage count (MSC), leaf area index (LAI) and dry matter yield (DMY) and significance in response to switchgrass varieties and harvest dates during growing seasons 2012 and 2013 at Ames, IA.

	MSC	LAI	DMY
Y	3.3	4.1	14.5**
H	992.1**	406.3**	233**
Y × H	14.4**	59.3**	7.3**
V	23**	54.7**	12**
Y × V	5.6**	26.2**	1.1
H × V	3.8**	1.5	1.1
Y × H × V	0.7	0.8	0.8

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

Y Year

V Variety

H Harvest date

Figures

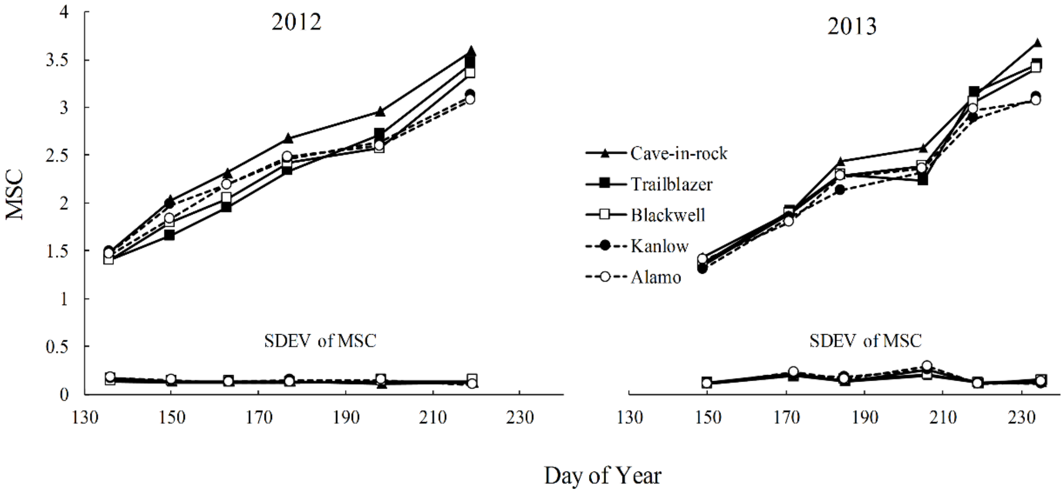

Figure 2.1. Mean stage count (MSC) and standard deviation of mean stage count (S_{MSC}) for five switchgrass varieties grown near Ames, IA in 2012 and 2013. Crop re-growth began on May 1st in 2012 and on May 15th in 2013

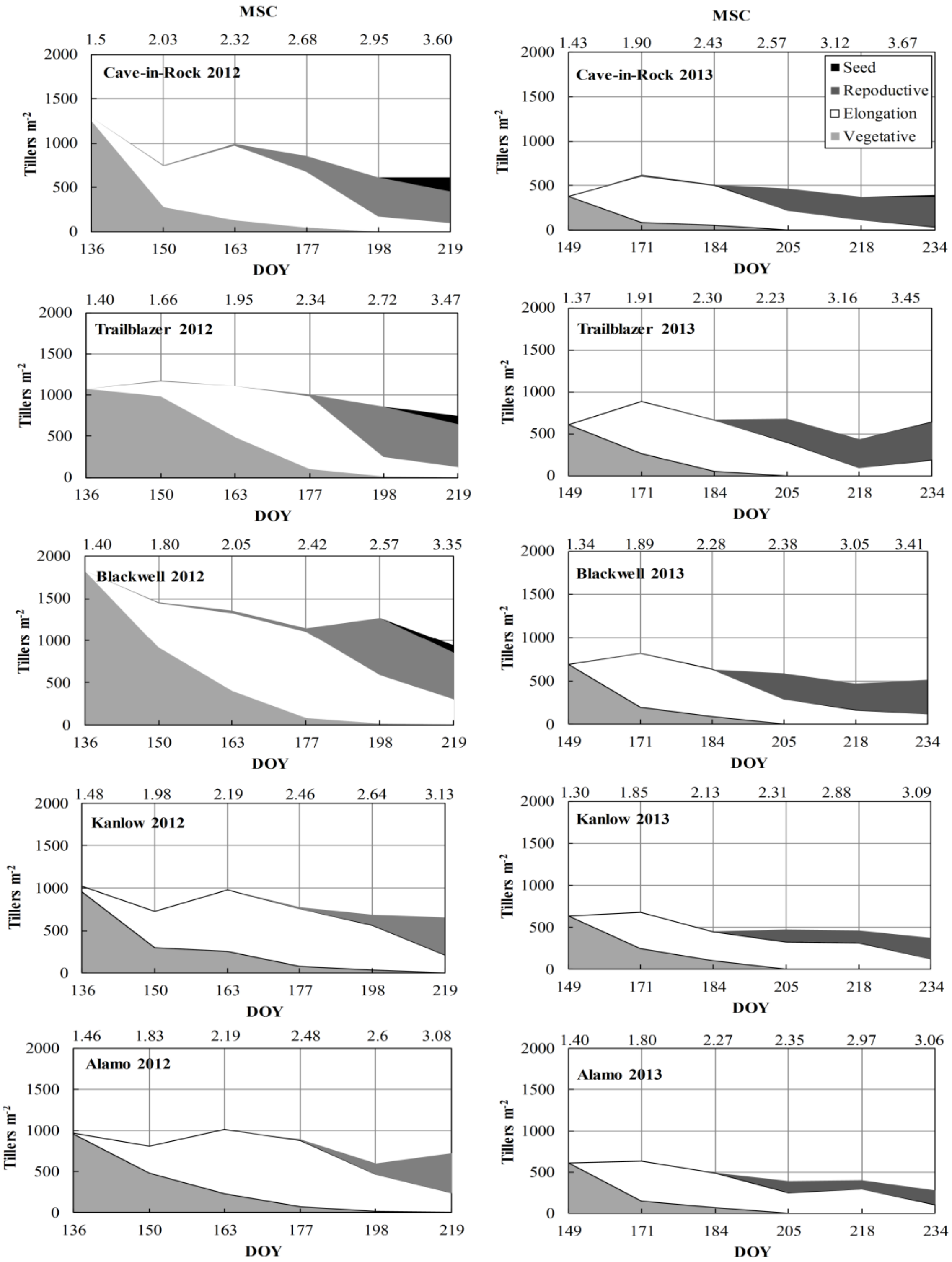

Figure 2.2. Tiller demographics and MSC for five switchgrass varieties grown near Ames, IA. In 2012 and 2013

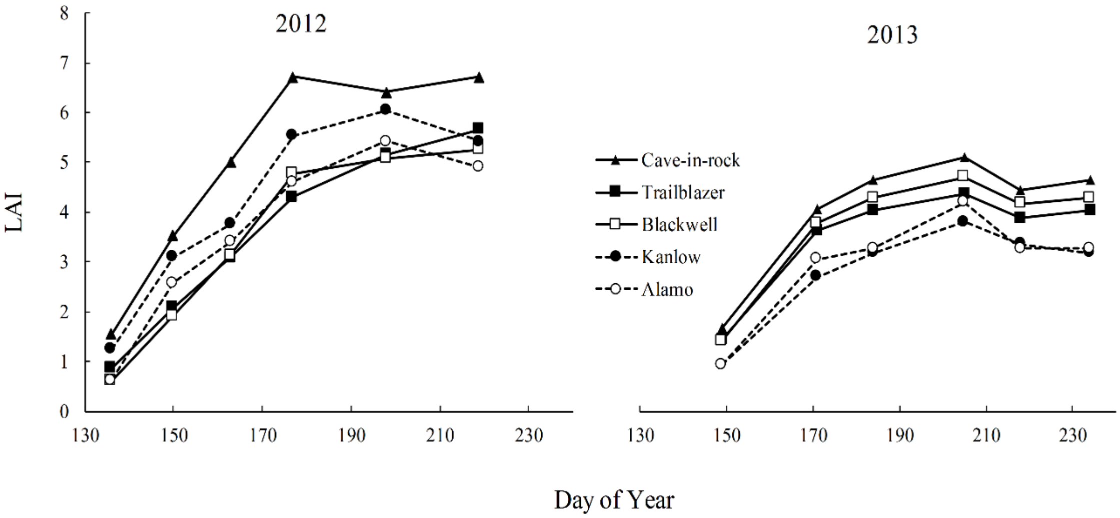

Figure 2.3. Leaf Area Index (LAI) for five switchgrass varieties grown near Ames, IA in 2012 and 2013

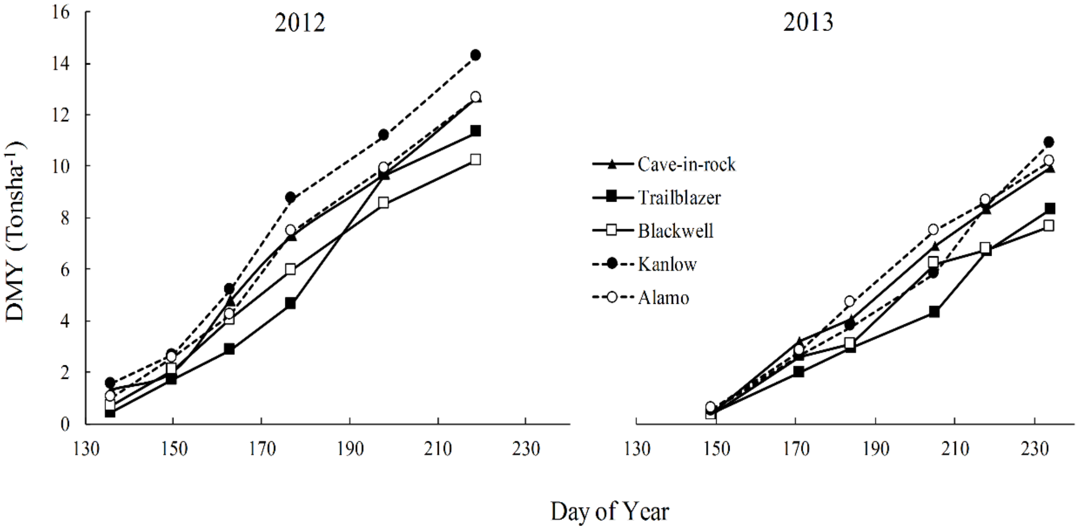

Figure 2.4. Dry Matter Yield (DMY) Tons ha^{-1} for five switchgrass varieties grown near Ames, IA in 2012 and 2013

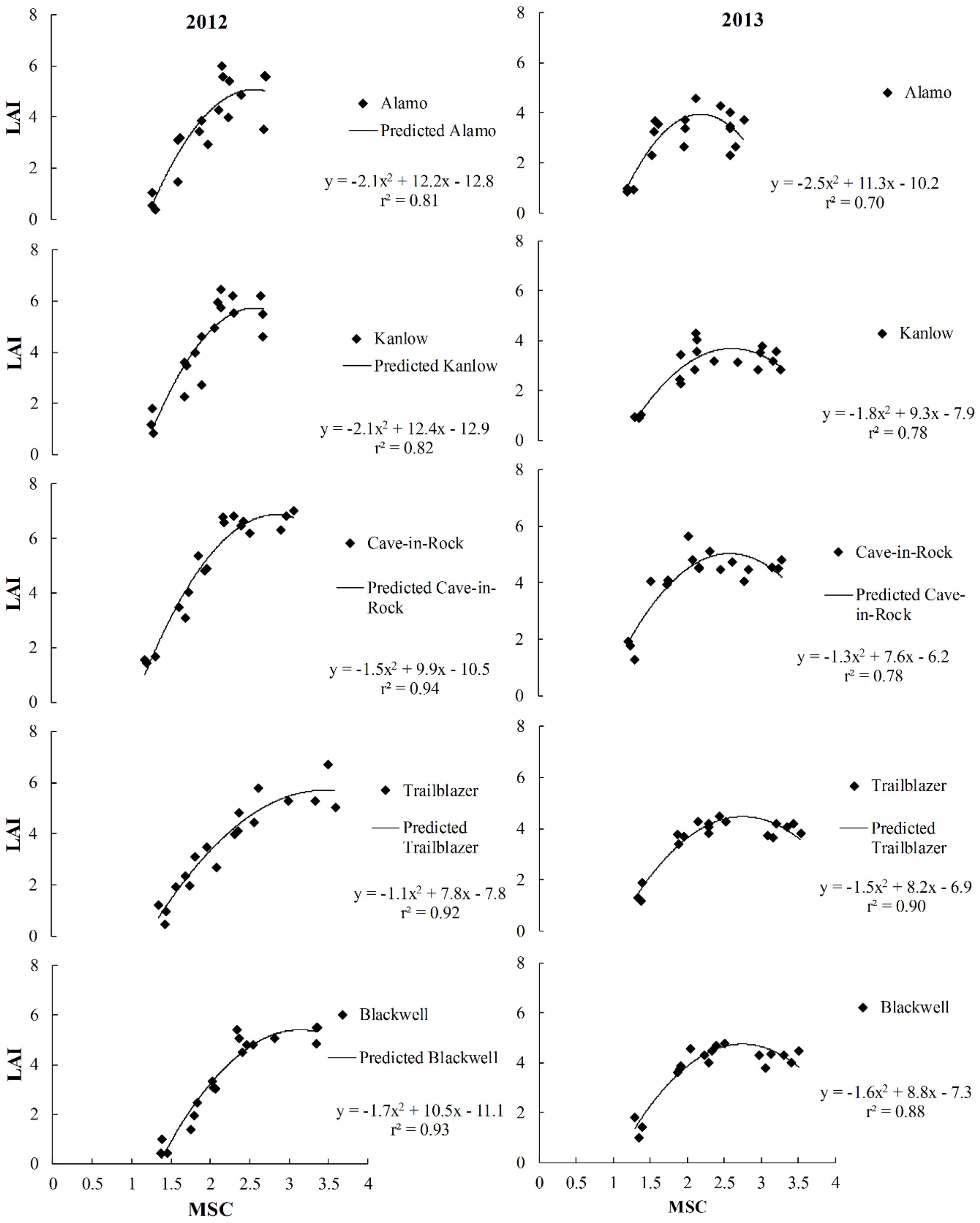

Figure 2.5. Mean stage count (MSC) and Leaf area index (LAI) relationships for switchgrass cultivars grown near Ames, IA in 2012 and 2013

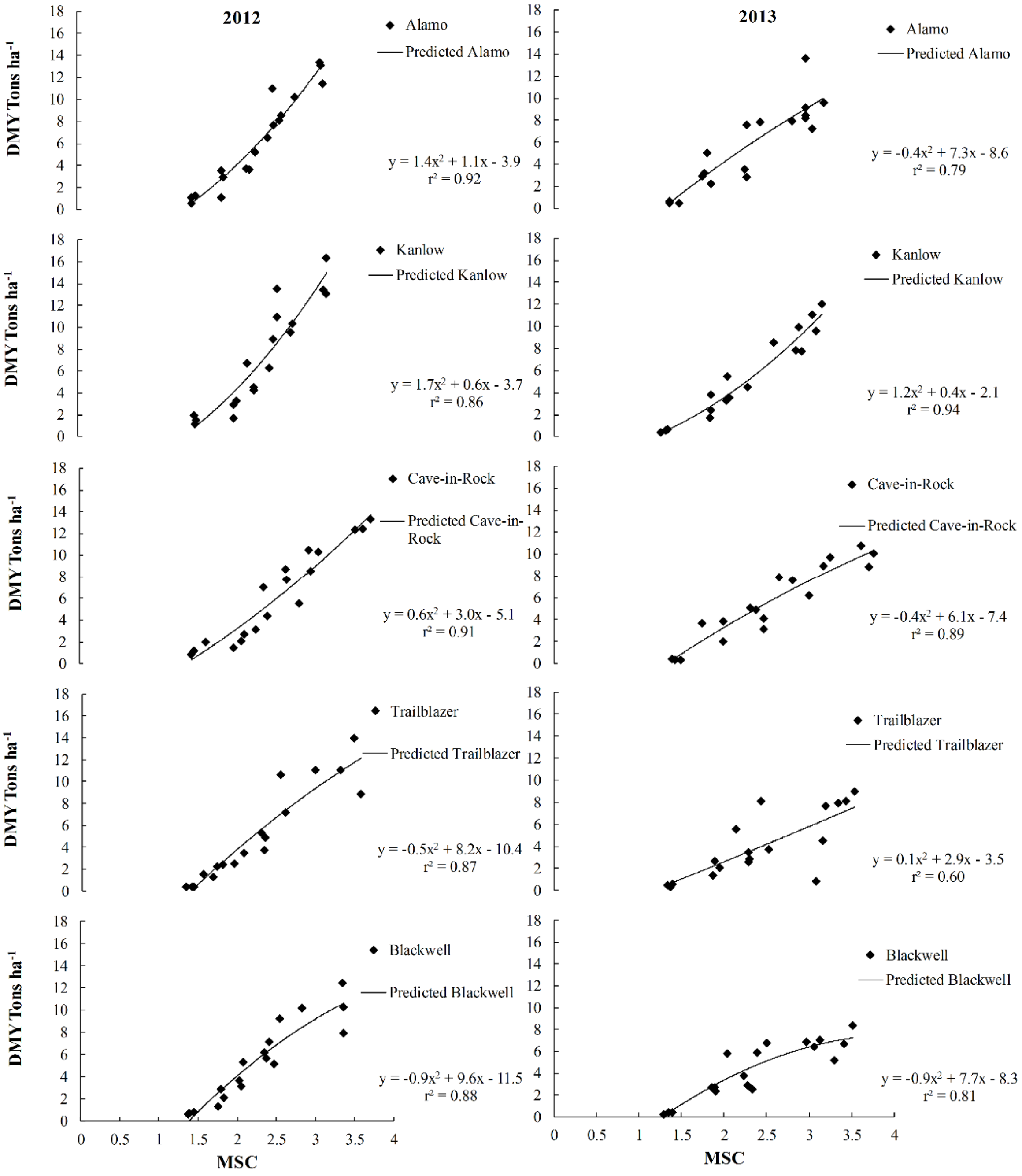

Figure 2.6. Mean stage count (MSC) and dry matter yield (DMY) relationships for switchgrass cultivars grown near Ames, IA in 2012 and 2013

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CHAPTER 3

COMPOSITIONAL DIFFERENCES AMONG UPLAND AND LOWLAND SWITCHGRASS ECOTYPES GROWN AS A BIOENERGY FEEDSTOCK CROP

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Abstract

Feedstock quality mainly depends upon the biomass composition and bioenergy conversion system being used. Higher cellulose and hemicellulose concentrations are desirable for biochemical conversion, whereas higher lignin is favored for thermochemical conversion. The efficiency of these conversion systems is influenced by the presence of high nitrogen and ash concentrations. Switchgrass (*Panicum virgatum* L.) varieties are classified into two ecotypes based on their habitat preferences, i.e., upland and lowland. The objectives of this study were to quantify the chemical composition of switchgrass varieties as influenced by harvest management, and to determine if ecotypic differences exist among them. A field study was conducted near Ames, IA during 2012 and 2013. Upland (‘Cave-in-Rock’, ‘Trailblazer’ and ‘Blackwell’) and lowland switchgrass varieties (‘Kanlow’ and ‘Alamo’) were grown in a randomized block design with six replications. Six biomass harvests were collected at approximately 2-week intervals each year. In both years, delaying harvest increased cellulose, hemicellulose and lignin concentrations while decreasing nitrogen and ash concentrations in all varieties. On average, Kanlow had the highest cellulose and hemicellulose concentration (354 and 321 g kg^{-1} DM respectively), and Cave-in-Rock had the highest lignin concentration (33 g kg^{-1} DM). The lowest nitrogen and ash concentrations were observed in Kanlow (14 and 95 g kg^{-1} DM respectively). In general, our results indicate that delaying harvest until fall improves feedstock quality, and ecotypic differences do exist between varieties for important feedstock quality traits. These findings also demonstrate potential for developing improved switchgrass cultivars as bioenergy feedstock by intermating lowland and upland ecotypes.

Keywords Switchgrass; Ecotypes; Biomass yield; Biomass quality; Harvest management; Feedstock composition

Abbreviations

ADF: Acid detergent fiber

ADL: Acid detergent lignin

NDF: Neutral detergent fiber

TNC: Total nonstructural carbohydrates

N: Nitrogen

DM: Dry matter

1. Introduction

Interest in replacing fossil fuels with alternative biofuels has increased due to instability in oil producing countries, uncertainty of crude oil prices in international markets and environmental concerns. One of the solutions to these problems is the use of alternative energy resources, including biofuels (Ragauskas et al. 2006). The United States produces ethanol mainly from grain crops (Farrell et al. 2006; CAST, 2007), but grain based ethanol has been reported to produce an equal amount of greenhouse gas emissions as production of petroleum based fuels (Farrell et al. 2006). One way to overcome this problem is to produce biofuels from lignocellulosic feedstocks (Farrell et al. 2006; CAST, 2007); therefore, maximizing biomass production of dedicated bioenergy crops through improved genetics and agricultural practices is a necessity.

Recently, warm-season grasses have gained increased attention by the scientific community as a reliable source for lignocellulosic feedstock supply. Switchgrass (*Panicum virgatum* L.), a C_4 grass native to United States, has been identified as a strong candidate for a 2nd generation bioenergy feedstock in Central and North America (Boutan J., 2007). Some of its desirable traits include perenniality, adaptability to marginal land and high biomass yield potential (McLaughlin et al. 1996).

There are two ecotypes of switchgrass classified by their habitat preference i.e., upland and lowland. Upland ecotypes are frequently found in dry climates and at higher latitudes while lowland ecotypes are mostly found in wet climates at lower latitudes (Brunken et al. 1975; Sanderson et al. 1996; Casler et al. 2004). Biomass yields of these ecotypes mainly depend on the origin of cultivar and cultural practices like fertilizer rate and harvest management. Usually, when both ecotypes are grown at the same latitude, cultivars selected from northern latitude tend to have lower biomass yields, early flowering and longer winter dormancy than cultivars that originated in more southern latitudes; whereas, southern ecotypes have delayed flowering, produce thicker stems and more biomass than upland ecotypes (Cassida et al. 2005; Parrish et al. 2008). Generally, establishment of a switchgrass stand is slow and two to three years are required to reach peak biomass yields. However, once established it can be maintained for more than 10 years (Evanylo et al. 2005; Fike et al. 2006; Perlack et al. 2011).

High biomass yield is an important attribute of an ideal bioenergy crop, but the quality of a feedstock for conversion is also important. Lignocellulose is the most important and the largest constituent of biomass dry matter from dedicated bioenergy crops, which is primarily made up of cellulose, hemicellulose, lignin and mineral elements. Feedstock quality, however, depends upon the bioenergy conversion system used to convert the biomass to fuel (e.g., thermochemical, biochemical or direct combustion system (Adler et al. 2006; Sanderson et al. 2007). High mineral concentration, notably nitrogen and ash concentrations, decrease the efficiency of direct combustion and thermochemical conversion systems (Trebbi G., 1993). Lignin, on the other hand, is important for thermochemical conversion processes, but since it also binds with cellulose and hemicellulose, higher concentrations of lignin also limits the availability of cellulose and hemicellulose during biochemical conversion processes, resulting in reduced biofuel yields (Trebbi G., 1993; Adler et al. 2006; Sanderson et al. 2007). In addition to the above-mentioned structural carbohydrates (i.e., cellulose and hemicellulose), switchgrass also contains nonstructural carbohydrates including sucrose, glucose, fructose and starch. These sugars are not present in very high concentrations compared to the structural carbohydrates, but can be used as a source of fermentable sugars for liquid fuel production (Dien et al. 2006; Johnson et al. 2007).

Concentrations of these important feedstock components can vary significantly due to geographic location, genetic factors, plant maturity and agronomic practices (Vogel et al. 2002; Adler et al. 2006; Royal Society, 2008). While many switchgrass compositional and harvest management studies addresses variability in forage quality, understanding of ecotypic variation in switchgrass quality as a bioenergy applications is more limited. To fill this void we conducted an experiment to: (i) quantify the chemical composition of switchgrass cultivars as influenced by harvest date, and (ii) to determine if ecotypic differences exist between upland and lowland switchgrass ecotypes for their chemical composition. This information will help biomass producers to choose the best suited variety for biomass production, and also allow researchers to select and improve current ecotypes for increased adaptability and maximum biomass and biofuel production.

2. Materials and Methods

2.1. Experimental Site

To evaluate the dry matter (DM) composition of upland and lowland ecotypes of switchgrass, a study was conducted during 2012 and 2013 on a pre-existing switchgrass variety trial established in 2007 at Iowa State University, Sorenson Research Farm, near Ames, IA (42^{o}0’41” N, 93’44’34” W). The experiment was arranged as a randomized complete block design with six replications of five switchgrass varieties of two distinct origins, upland (Cave-in-Rock, Blackwell and Trailblazer) and lowland (Kanlow and Alamo). Every year before spring growth initiation, standing dead material was mowed to a stubble height of 5 cm and the plant residue removed from the field. To control weeds each year atrazine [6-Chloro-N-ethyl-N'-(1-methylethyl)-1, 3, 5-triazine-2, 4-diamine] and quinclorac [3, 7-Dichloro-8-quinolinecarboxylic acid] were applied before switchgrass emergence at 2.23 kg a.i. ha^{-1} and

0.56 kg a.i. ha^{-1} respectively. Nitrogen, P and K fertilizer were applied every year in early May at 78, 67 and 90 kg ha^{-1}, respectively. Mean monthly air temperature and total precipitation were measured during 2012 and 2013 at a site located less than eight kilometers from the experimental field, and the data were compiled from the Iowa Environmental Mesonet (2014) (Fig. 3.1 and 3.2).

2.2. Biomass Harvest

Biomass samples were collected from three randomly selected blocks in 2012 and the remaining three blocks were harvested the following year. Each plot was divided into six subplots corresponding to six biomass harvests. Each year depending on the spring growth, the first harvest occurred at early vegetative growth (approximately two weeks after emergence). In 2012 and 2013, the first harvest occurred on the 136th and 149th day of the year (DOY), respectively, and the remaining five harvests were collected at approximately 2-week intervals. At each harvest date, two-0.1 m^2 samples were harvested within each subplot by using a hand clipper at ground level. Each subplot was harvested only once during the growing season.

2.3. Sample Processing

Harvested samples were dried at 60°C in a forced-air oven for 72 h or until a constant sample dry weight was observed. Dried samples were ground to 1 mm with a shear mill (Thomas Scientific, Philadelphia, PA, USA). Ground samples were then mixed thoroughly to obtain subsamples for compositional analysis. These subsamples were kept at ambient humidity for about 48hr and then stored in plastic vials at room temperature.

2.4. Biomass Composition

All chemical analyses were performed in duplicate. Biomass samples were mixed again before weighing samples for chemical analysis. Sequential fiber analysis was used to determine neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) using an ANKOM 200 Fiber Analyzer (ANKON Technology Corp. Fairport, NY). All values were corrected for moisture concentration. The moisture concentration of each sample was determined by drying another subsample in the oven at 105°C for 4 h. Hemicellulose was calculated as the difference between NDF and ADF concentration, cellulose as the difference between ADF and ADL, and lignin as ADL corrected for ash concentration.

Total nonstructural carbohydrates (TNC) were determined using methods described by Murphy et al. (2012) and Guiragossian et al. (1979). In this procedure, a 0.125 g sample was weighed and placed in a test tube to be refluxed for 1 hr in 25 mL of 0.2-N sulfuric acid. After cooling, the mixture was filtered with Whatman # 42 filter paper. A 1-mL subsample was drawn from the filtrate and diluted by a factor of 20 with distilled water. Then, 1 mL of 5% phenol solution and 5 mL of 18-M sulfuric acid were added to the diluted mixture, and the solution's absorbance measured at 490 nm. The absorbance values were then used to determine TNC concentration by using a glucose reference calibration and calculated on the basis of g of glucose kg^{-1} sample dry matter. Nitrogen (N) and carbon (C) were determined using a LECO True Spec^{TM} CN Analyzer (LECO Corp. St. Joseph, MI). Total ash concentration was determined by the method of Undersander et al. (1993) by ashing a 0.5 g subsample in pre-weighed crucibles at 500°C for 4 h.

2.5. Statistical Analysis

Statistical analysis was performed by analysis of variance using the GLIMMIX procedure of SAS 9.4 (SAS Institute Cary, NC USA). Switchgrass variety, year and harvest date were considered fixed effects, while blocks and the interactions with blocks were considered as random effects. Means were compared using *t*-tests and linear contrasts. Differences were considered significant at $P<0.05$.

3. Results

In both years, lowland varieties of switchgrass (Kanlow and Alamo) performed better than the upland varieties (Cave-in-Rock, Blackwell, and Trailblazer) in terms of feedstock quality traits. Climatic conditions were different in both years. The first half of 2012 was warmer compared to 2013 and the 20-year average monthly temperature, while the remaining of the growing season temperatures were close to the 20-year average (Fig. 3.1). Precipitation in both years was lower than the 20-year average except April and May of 2013, which received about 49 and 69 % more rainfall respectively than 20-year average (Fig. 3.2).

3.1. Cellulose

Cellulose concentration for all switchgrass varieties increased continuously with advanced maturity in both years (Fig. 3.3). Year, variety and harvest date significantly affected the cellulose concentration and there was an interaction between them (Table 3.1, Fig. 3.3). Averaged over both years, ‘Kanlow’ had the highest cellulose concentration, which was 354.2 g kg^{-1} DM, and was significantly different from all other varieties. ‘Cave-in-Rock’ had the lowest cellulose concentration, which averaged 330.2 g kg^{-1} DM.

Because there was a significant variety × year interaction, each year was evaluated separately. In 2012, variety and harvest date significantly affected cellulose concentration (Fig.

3.3). However, in 2013 only harvest date was found to have an effect on cellulose concentration (Fig. 3.3), indicating that the cellulose concentration among switchgrass varieties changed differently in 2013 as compared to 2012.

In both years, maximum cellulose concentration for all switchgrass varieties was observed at the late season harvests. Cellulose concentration in 2012 ranged from 225 g kg^{-1} DM for Cave-in-Rock at the first harvest date to 394.1 g kg^{-1} DM and 351.5 g kg^{-1} DM for Kanlow and Cave-in-Rock respectively, at the last harvest date (Fig. 3.3). Similarly in 2013, cellulose concentration ranged from 238.2 g kg^{-1} DM for Cave-in-Rock at the first harvest date to 431.3 g kg^{-1} DM and 398.4 g kg^{-1} DM for Kanlow and Cave-in-Rock respectively, at the final harvest date (Fig. 3.3).

3.2. Hemicellulose

Variety and harvest date affected hemicellulose concentration, but the response to harvest date varied among varieties (Table 3.1, Fig. 3.4). Averaged over both years, Kanlow had the highest hemicellulose concentration of 321 g kg^{-1} DM, which was significantly different from all other varieties, and Cave-in-Rock had the lowest hemicellulose concentration of 306.5 g kg^{-1} DM.

Differences in hemicellulose concentrations among the varieties varied between the two years. In 2012, variety and harvest date affected hemicellulose concentration and there was a significant interaction between them. However, in 2013 variety and harvest date were found to have an effect on hemicellulose concentration, but there was no interaction. This reflects that switchgrass varieties in 2012 had higher variability for hemicellulose concentration between harvests than in 2013.

Generally, in both years, earlier harvest dates typically had lower hemicellulose concentrations than later harvests (Fig. 3.4). In 2012, hemicellulose concentration among switchgrass varieties ranged from 275 g kg^{-1} DM for Blackwell to 335 g kg^{-1} DM for Kanlow for first and third harvest, respectively. Whereas in 2013, hemicellulose concentration for switchgrass varieties did not change greatly between harvests and ranged from 286.3 g kg^{-1} DM for Blackwell and 322.4 g kg^{-1} DM for Trailblazer for the first and third harvest, respectively. This indicates that mid-season harvests usually contain higher concentrations of hemicellulose as compared to early and late season harvests.

3.3. Lignin

Lignin concentration increased with plant maturity in both years. Year, variety and harvest date affected lignin concentration in switchgrass varieties and there was an interaction between year and harvest date. Averaged over both years, Trailblazer and Kanlow had the lowest lignin concentration of 30.3 and 31.1 g kg^{-1} DM respectively, and Cave-in-Rock had the highest concentration of 33.3 g kg^{-1} DM (Table 3.1, Fig. 3.5).

In 2012, variety and harvest date affected lignin concentration, but their respective two-way interaction was not significant. Lignin concentration in 2012 ranged from 14.1 g kg^{-1} DM for Alamo at the first harvest date to 52.2 g kg^{-1} DM and 52.7 g kg^{-1} DM for Alamo and Cave-in-Rock, respectively, at the last harvest date. In 2013, the only factor that affected the lignin concentration among switchgrass varieties was harvest date. Average lignin concentration ranged from 15.2 g kg^{-1} DM for Kanlow to 49.1 g kg^{-1} DM for Cave-in-Rock between the first and last harvest date respectively. This suggests that lignin concentration of each variety vary from one harvest date to another and typically later harvests will have a higher lignin concentration than earlier harvest dates, but the differences among varieties for their lignin at a given day of the year don’t differ significantly from each other.

3.4. Total Nonstructural Carbohydrates (TNC)

Total nonstructural carbohydrate (TNC) concentration for all switchgrass varieties decreased with maturity in both years (Fig. 3.6). The occurrence of a variety and harvest date interaction for TNC concentration (Table 3.1) indicated that changes in amount of TNC concentration were inconsistent among varieties throughout growing season. Averaged over both years, Cave-in-Rock had the highest TNC concentration of 189.3 g kg^{-1} DM, and Blackwell had the lowest of 175.2 g kg^{-1} DM.

There were no significant differences among varieties for their TNC concentration in either year. However, in 2012, an interaction between harvest date and variety were observed for TNC concentration, indicating that differences in TNC concentrations between varieties depended on harvest date. In 2012, average TNC concentration ranged from 157.9 g kg^{-1} DM for Alamo to 142 g kg^{-1} DM for Blackwell. In 2013, variety, harvest and their interaction were all non-significant, suggesting that TNC concentration of varieties did not change a lot over the growing season. The average amount of TNC in 2013 ranged from 208.5 g kg^{-1} DM for Blackwell to 223.4 g kg^{-1} DM for Cave-in-Rock.

3.5. Nitrogen

The nitrogen concentration of all switchgrass varieties decreased curvilinearly with delayed harvest, and this trend was consistent in both years (Fig. 3.7). Varieties differed significantly with harvest date (Table 3.1); earlier harvest dates had higher nitrogen concentrations than later harvest dates. Averaged over both years, the nitrogen concentration of switchgrass varieties between first and last harvest date ranged from 32.5 g kg^{-1} DM for Cave-in-Rock to 5.5 g kg^{-1} DM for Kanlow.

In 2012, an interaction between variety and harvest date occurred for nitrogen concentration. The decrease in nitrogen concentration for switchgrass varieties between first and last harvest ranged from 33 g kg^{-1} DM for Cave-in-Rock to 6.9 and 6.3 g kg^{-1} DM for Alamo and Kanlow, respectively (Fig. 3.7). However in 2013, harvest date was the only factor that affected nitrogen concentration, indicating that nitrogen concentration among switchgrass varieties changed differently in 2013 compared to 2012. In 2013, the decrease in nitrogen concentration between first and final harvest ranged from 32.1 g kg^{-1} DM for Cave-in-Rock to 4.5 and 4.6 g kg^{-1} DM for Kanlow and Alamo, respectively (Fig. 3.7).

3.6. Carbon-to-Nitrogen Ratio (C-N ratio)

Carbon-to-nitrogen ratio (C-N ratio) for all switchgrass varieties increased continuously with advancing maturity in both years (Fig. 3.8). Year, variety and harvest date had significant effects on the C-N ratio (Table 3.1, Fig. 3.8). Averaged over both years, Kanlow had the highest C-N ratio of 44, which was significantly different from all other varieties except Blackwell and Alamo, and Trailblazer had the lowest C-N ratio of 35.

In 2012, varieties differed in C-N ratio across harvest dates; earlier harvest dates in 2012 ranged from 13 for Alamo at first harvest date to 67.7 for Kanlow at the last harvest date (Fig. 3.8). However in 2013, the only factor that affected the C-N ratio among switchgrass varieties was harvest date, and the average C-N ratio ranged from 12.8 for both Kanlow and Cave-in-Rock at the first harvest date to 96.3 for Kanlow at the last harvest date (Fig. 3.8).

This indicates that C-N ratio of switchgrass varieties vary from one harvest date to another and typically later harvests will have higher C-N ratio than earlier harvest dates.

3.7. Ash

The ash concentration of all switchgrass varieties decreased continuously with advancing maturity in both years (Fig. 3.9). Varieties differed significantly with harvest date (Table 3.1); earlier harvest dates had higher ash concentrations compared to the later harvest dates. Averaged over both years, the lowest ash concentration was recorded at the sixth harvest for Alamo and Kanlow; i.e., 65 g kg^{-1} DM and 65.5 g kg^{-1} DM respectively.

In 2012, variety and harvest date affected ash concentration, but there was no interaction between them. Ash concentration in 2012 ranged from 163 g kg^{-1} DM for Blackwell at the first harvest to 66.6 g kg^{-1} DM and 68.8 g kg^{-1} DM for Alamo and Kanlow, respectively, at the last harvest (Fig. 3.9). However in 2013, the only factor that affected the ash concentration among switchgrass varieties was harvest date. Average ash concentration ranged from 132.6 g kg^{-1} DM for Alamo at the first harvest date to 62.2 g kg^{-1} DM and 63.3 g kg^{-1} DM for Alamo and Kanlow, respectively, at the last harvest date (Fig. 3.9). This suggests that ash concentration of varieties vary from one harvest date to another and typically later harvests will have lower ash concentrations than earlier harvest dates, but there will be no significant differences among varieties for their ash concentration at a given day of the year.

4. Discussion

This study provided new data on chemical composition of five switchgrass cultivars and revealed the magnitude of ecotypic differences between upland and lowland cultivars. This information is critical, fills knowledge gaps and has the potential to assist decision making about the proper time of harvest of switchgrass for further industrial processing.

An efficient lignocellulosic feedstock-based bioenergy system requires optimization of desired compositional traits of the feedstock (Cassida et al. 2005). Optimization of compositional traits depends on the conversion process used, since thermochemical and biochemical conversion systems contrast in their requirements of feedstock composition. Generally, for an ideal bioenergy feedstock production a higher concentration of fiber and reduced levels of nitrogen and ash are the goals (Ablevor et al. 1992; Sanderson et al. 2007).

Results from this study demonstrated a significant variation between switchgrass ecotypes for important quality traits. Harvest time was also observed to play an important role in influencing the biomass feedstock composition over the growing season. Delayed harvest to later maturity stages increased the cellulose, hemicellulose and lignin concentration, but a curvilinear decrease was observed for nitrogen and ash concentration in all switchgrass cultivars. These findings are consistent with past studies, which also found that warm season grasses increase in cell wall constituents with advancing maturity (Mitchell et al. 2001; Lemus et al. 2002; Mulkey et al. 2006; Adler et al. 2006; Waramit et al. 2011), and decrease their nitrogen and ash concentration (Madakadze et al. 1999; Wilson et al. 2013a; Wilson et al. 2013b). Jung et al. (1992) and Griffin et al. (1998) and reported that in grasses with advanced maturity stems contain higher fiber concentrations than the leaves (Jung et al. 1992; Griffin et al. 1998). Switchgrass stems contain less nitrogen compared to the leaves (Wilson et al. 2013a). This indicates that variation in these chemical constituents with maturity is likely due to the differences in stem to leaf ratio at later developmental stages in switchgrass (Twidwell et al.

1988; Madakadze et al. 1999; Lemus et al. 2002; Mulkey et al. 2006). However, results from this study have also revealed that ecotypes differentially vary in their fiber concentration with advanced maturity and cultivar. Lowland ecotypes had higher cellulose and hemicellulose concentrations compared to their upland counterparts, and these results are consistent with the earlier findings (Lemus et al. 2002; Bhandari et al. 2014). For biochemical conversion systems, higher cellulose and hemicellulose concentrations are desirable by contributing more fermentable sugars for liquid fuel synthesis (Kurakake et al. 2001; Datar et al. 2004).

Lignin concentrations were higher in Cave-in-Rock, which is an upland ecotype that tended to maintain higher lignin levels in both years. Lemus et al. (2002) and Bhandari et al. (2014) reported similar findings that upland ecotypes contain higher lignin concentration than lowland ecotypes. However, in our results it was also noteworthy that lowland types did not have the lowest levels of lignin, and the concentrations were fairly comparable to the upland ecotypes. Higher lignin concentrations are desirable for thermochemical conversion systems (Hayn et al. 1993); however, because lignin is recalcitrant to degradation, it also reduces biochemical conversion efficiency by reducing the cellulose and hemicellulose availability for fermentation, thus reducing the ethanol yields (Sun et al. 2002; Dien et al. 2006; Fu X., et al. 2011).

The occurrence of high nitrogen concentration in herbaceous bioenergy feedstock has significant implications of reducing the conversion efficiency of biomass to biofuel (Ablevor et al 1992; Bridgwater et al. 1994; Czernik et al. 2004; Boateng et al. 2006). Our results demonstrate that nitrogen concentration in all switchgrass cultivars decreased with advanced maturity. Lowland ecotypes had lower nitrogen concentration compared to the upland ecotypes at the last harvest. This curvilinear decrease in nitrogen concentration as the growing season progressed is similar to that reported in prior studies of nitrogen cycling in grasses (Madakadze et al. 1991; Beale et al. 1997; Sanderson et al. 1999; Wilson et al. 2013a). In addition to the increase in stem to leaf ratio, this decrease in nitrogen concentration might also be partially attributed to the nutrient translocation from aboveground plant parts to the belowground storage organs (Vogel et al. 2002; Wilson et al. 2013a).

In concert with nitrogen concentration, the TNC and C-N ratio changed dramatically over the growing seasons. In both years TNC concentrations decreased early during the growing season, and then a slight increase was observed at the last harvest in all switchgrass cultivars. In contrast to TNC, a continuous increase was observed for C-N ratio in all switchgrass cultivars in both years. At the end of each growing season, lowland ecotypes had the highest C-N ratio compared to the upland ecotypes. This might be the result of reduction in nitrogen concentration and increase in carbohydrates before the onset of winter. Past studies have reported similar increases in carbohydrate levels in cold acclimated *Arabidopsis* plants than in non-acclimated (Cook et al. 2004).

The presence of higher ash concentrations in herbaceous feedstock negatively interfere with conversion processes by reducing hydrocarbon yields (Ablevor et al. 1992), and creating fusible slag that fouls boilers and machinery and also increases maintenance costs (McLaughlin et al. 1996; Jorgensen et al. 1997). In our results, a continuous decrease in ash concentration in all switchgrass ecotypes occurred with advancing maturity and delayed harvest, and this is consistent with past findings (Mulkey et al. 2006; Waramit et al. 2011; Wilson et al. 2013b). However, this study also found that lowland ecotypes contained less ash as compared to the upland ecotypes, and these results are consistent with previous studies, as well (Cassida et al. 2005; Hong et al. 2014).

5. Conclusion

Based on results presented here and elsewhere, we conclude that ecotypic differences do exist between switchgrass cultivars for their fiber, nitrogen and ash concentrations. Lowland ecotypes usually contain higher cellulose, hemicellulose and lower nitrogen and ash contents; whereas, upland ecotypes produce higher lignin, nitrogen and ash concentrations. Moreover, delaying biomass harvest to later maturity stages or until fall will ensure greater fiber and lower nitrogen and ash concentrations.

This study was designed to understand the feedstock compositional differences between upland and lowland ecotypes and their harvest management. Results from this study will also aid in future switchgrass breeding for improved cultivar development as a bioenergy feedstock.

Acknowledgements

The authors thank the CenUSA Bioenergy project and Iowa State University for providing financial support for this study.

Tables

Table 3.1. Analysis of variance for cellulose, hemicellulose, lignin, TNC, nitrogen, C-N ratio and total ash concentrations and significance in response to switchgrass varieties and harvest dates during growing seasons 2012 and 2013 at Ames, IA.

	Cellulose	Hemicellulose	Lignin	TNC	Nitrogen	C-N Ratio	Total Ash
Y	273**	4	25.6**	153**	96.7**	178**	14.43*
H	532.8**	56**	615.1**	16.6**	731.3**	177.8**	176.8**
Y × H	13.8**	3.9**	20.5**	6.2**	20**	17.4**	5.1**
V	18**	22**	5.4**	4.4**	15**	6.0**	5**
Y × V	6.1**	6.4**	1.2	1.2	6.7**	0.27	2.9*
H × V	0.74	2.1**	0.9	1.9*	1.4	0.74	0.91
Y × H × V	1.2	1.3	1.2	1.2	1.7*	0.39	0.95

*	Significant at the 0.05 probability level
**	Significant at the 0.01 probability level
Y	Year
V	Variety
H	Harvest date

Figures

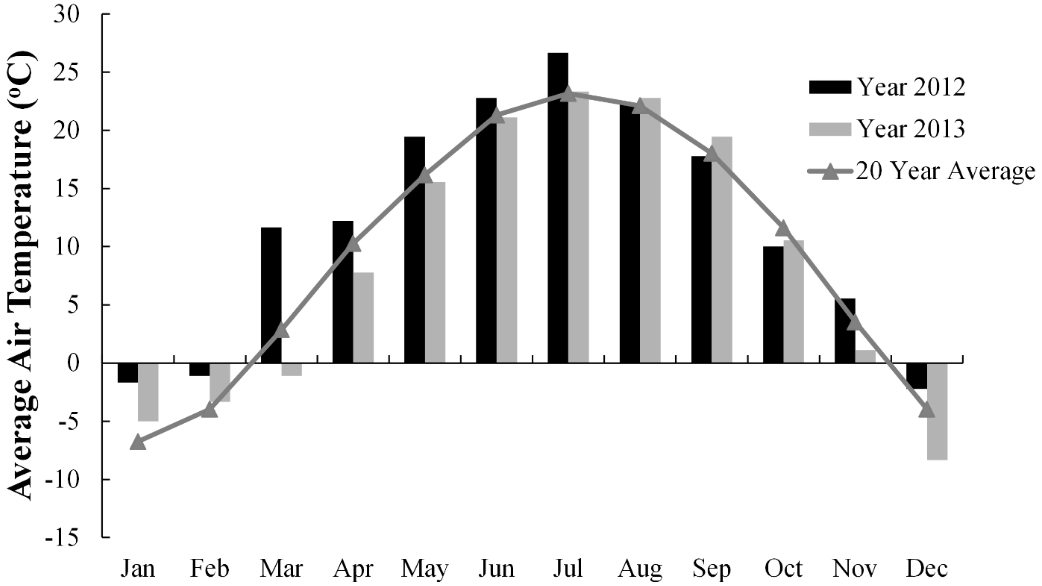

Figure 3.1. Mean monthly air temperature for 2012, 2013 and 20 year average at Ames, IA

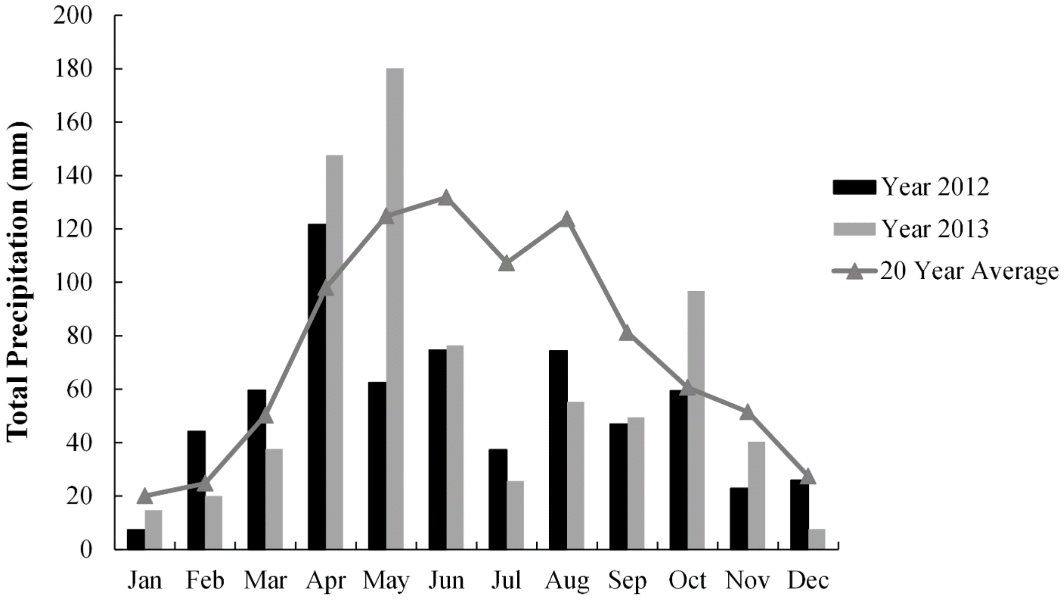

Figure 3.2. Mean monthly precipitation for 2012, 2013 and 20 year average at Ames, IA

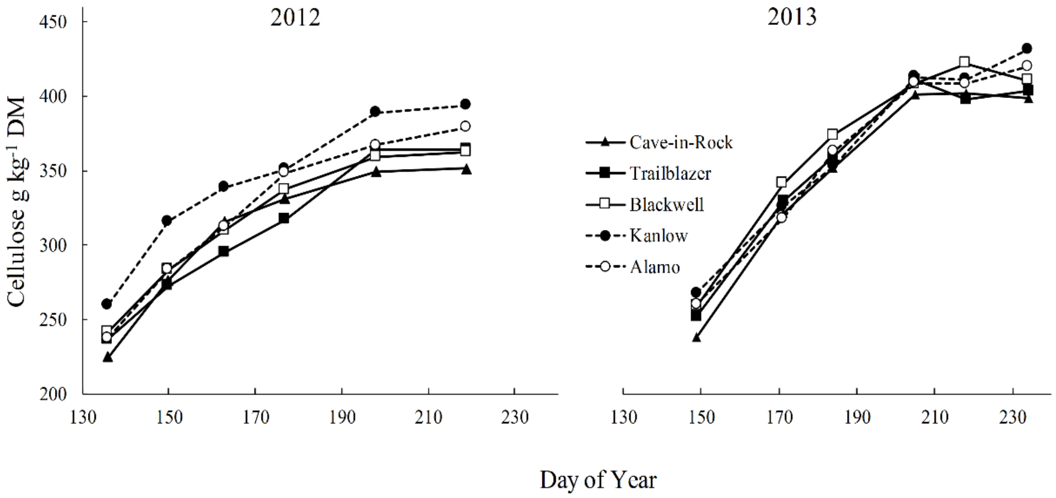

Figure 3.3. Cellulose concentration of switchgrass varieties during year 2012-2013

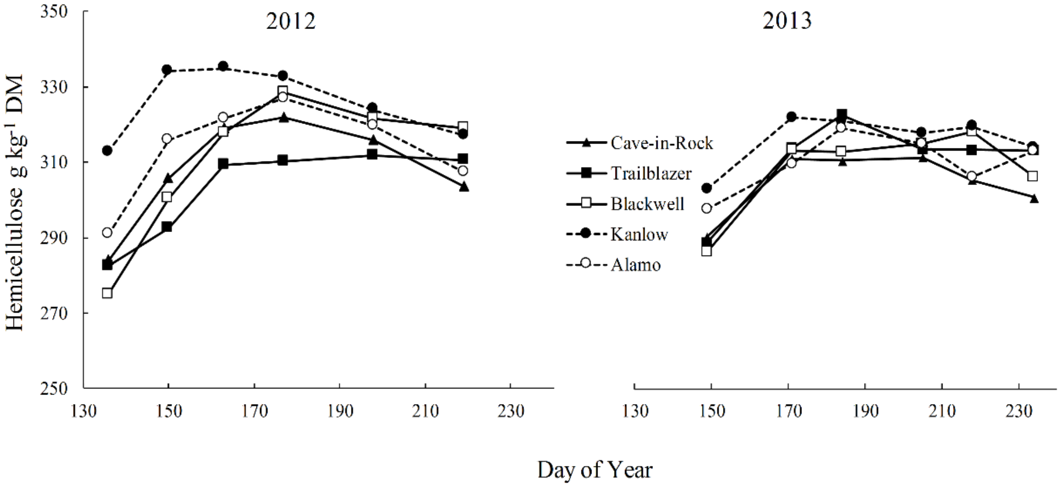

Figure 3.4. Hemicellulose concentration of switchgrass varieties during year 2012-2013

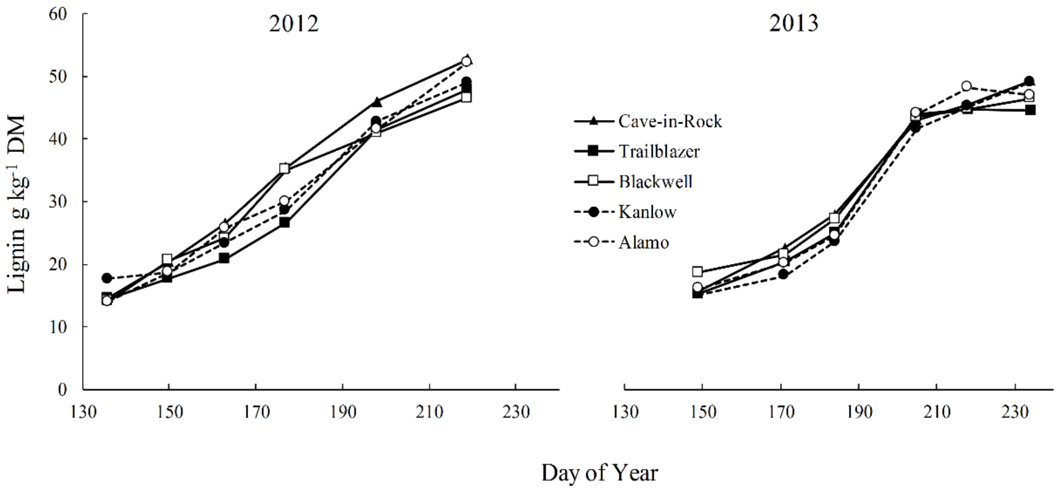

Figure 3.5. Lignin concentration of switchgrass varieties during year 2012-2013

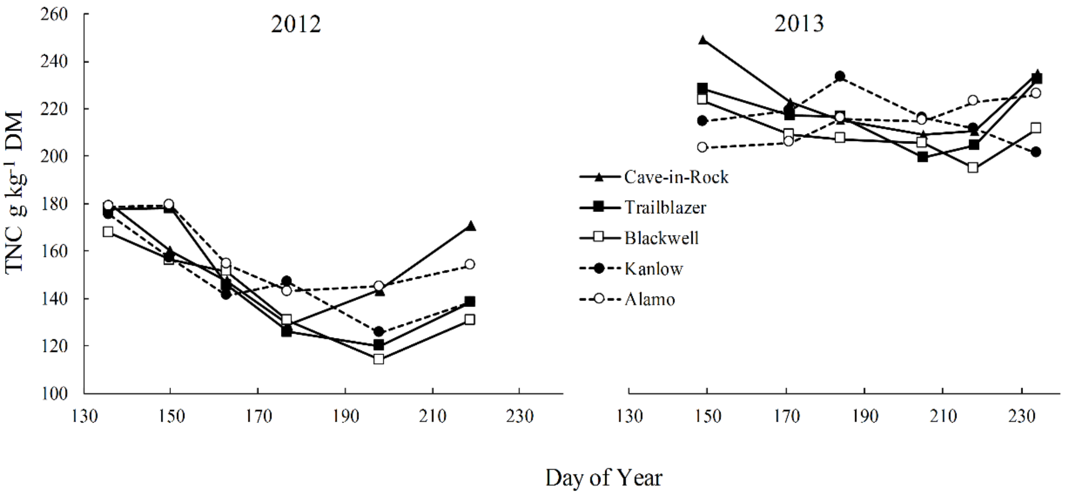

Figure 3.6. Total non-structural carbohydrate (TNC) concentration of switchgrass varieties during year 2012-2013

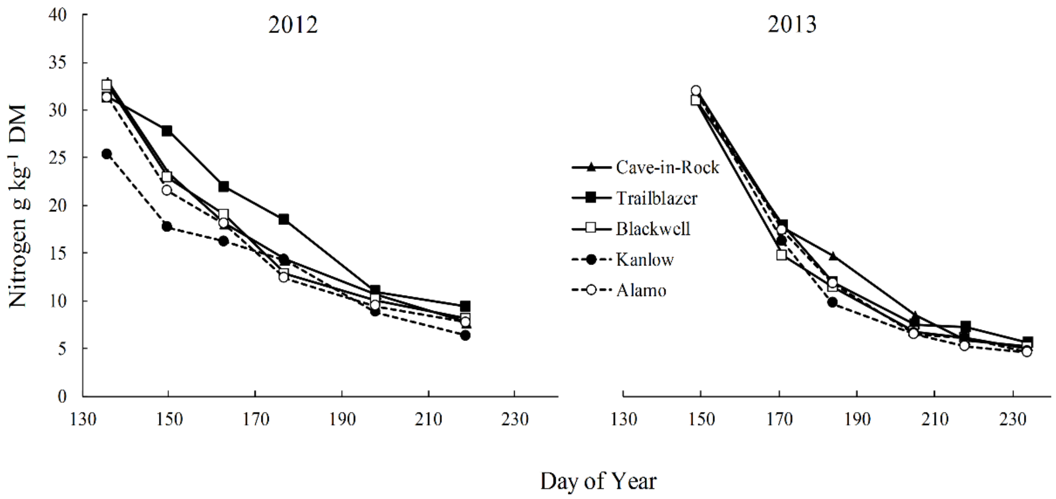

Figure 3.7. Nitrogen concentration of switchgrass varieties during year 2012-2013

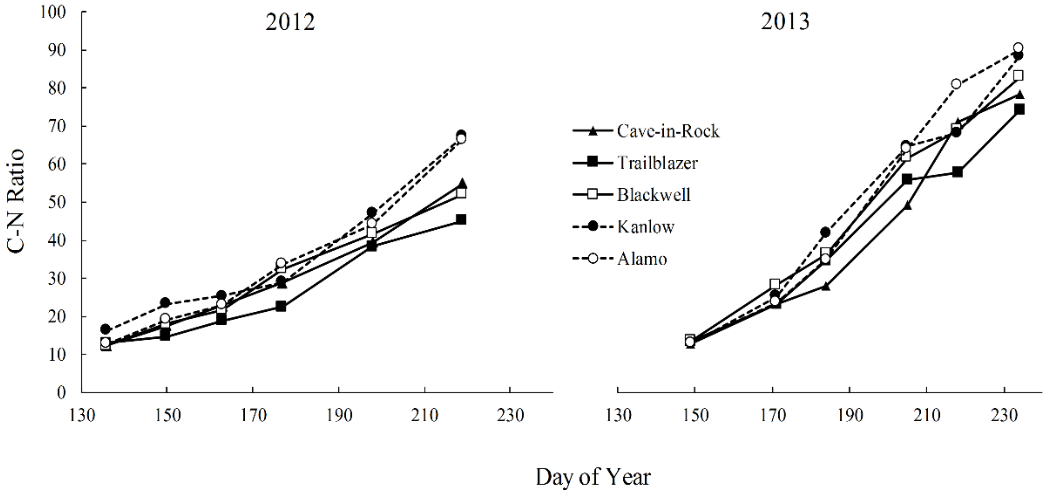

Figure 3.8. Carbon to Nitrogen Ratio (C-N Ratio) of switchgrass varieties during year 2012-2013

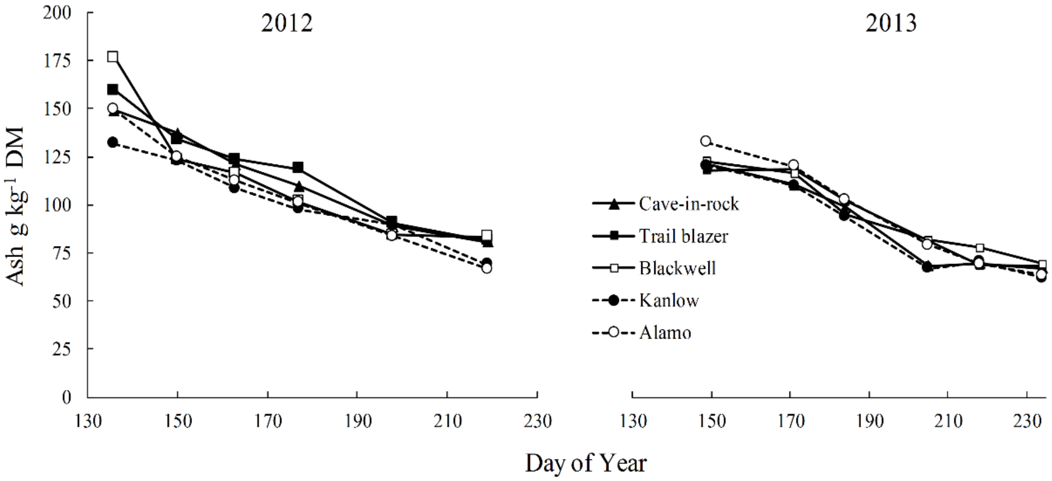

Figure 3.9. Total ash concentration of switchgrass varieties during year 2012-2013

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CHAPTER 4

A NEW MODEL FOR SWITCHGRASS PHENOLOGY THAT COMBINES TEMPERATURE AND PHOTOPERIOD EFFECTS

A manuscript intended to be submitted to GCB-Bioenergy Journal

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Abstract

Switchgrass is a short-day perennial species and its development is affected by day-length. However, existing approaches to model switchgrass phenology rely on thermal time method coupled with ad-hoc empirical modifications to account for the effects of photoperiod when the model is applied across a wide range of environments. Given the large industrial interest in switchgrass as a bioenergy feedstock and the multi-state research efforts to improve switchgrass productivity and adaptability (see CenUsa project), in this study we developed a new mechanistic model to describe switchgrass phenology with the objective to assist agronomists and on-going breeding programs. The model simulates four phases of switchgrass development (emergence to juvenile, juvenile to elongation, elongation to flowering and flowering to maturity), uses daily temperature and site latitude as driving variables, contains five cultivar specific biological meaningful parameters, and two model constants (base and optimum temperature of 10 and 30 ^{o}C, respectively). Three of the

cultivar specific parameters (a_1, a_2, a_3) define the thermal optimum time needed to complete a phase and the other two parameters describe the critical photoperiod (p_{crit}) and the photoperiod sensitivity (p_{sen}). To develop the model we first considered detailed experimental data for Iowa and expert's knowledge, while to estimate photoperiod parameters we used a literature database with 45 observations. The model matched Iowa's observations with a RMSE (relative mean square error) of 2.6 days for each cultivar or with 5.1 days error when average parameters by ecotype were used. The use of parameters by ecotype instead of specific cultivar expands the potential applicability of the model at the cost of losing some prediction power. The next step in our analysis was to generalize the model by providing estimates of the photoperiod effect with the help of diverse literature database. We found that p_{crit} was 13.6 h and 12.6 h for upland and lowland cultivars. By using these parameters the overall prediction ability of the model across representative sites in the US had an 8.8 days error. In conclusion, this study offers a new mechanistic way of evaluating switchgrass adaptability at any location and a framework to quantify genotypic difference in phenology.

1. Introduction

Switchgrass (*Panicum virgatum* L.) has been selected as a model 2nd generation bioenergy feedstock crop by the U.S. Department of Energy (Missaoui et al. 2006; Boutan J. 2007; Wright et al. 2010; Perlack and Wright et al. 2011). Its wide geographic distribution includes a variety of cultivars belonging to two distinct ecotypes i.e., upland and lowland (McLaughlin et al. 2005). These ecotypes have great genetic, phenotypic and physiological diversity (Stroup et al. 2003; Casler et al. 2004; Das et al. 2004; Yang et al. 2009; Cortese et al. 2010). Upland ecotypes are well adapted in the mid and Northern U.S. (Douglas et al.

2004; McLaughlin et al. 2005); whereas, lowland ecotypes are considered best for Southern U.S. conditions (Douglas et al. 2004; McLaughlin et al. 2005).

Morphological development of switchgrass is similar to other perennial grasses and follows the general developmental pattern described by Moore et al. (1991), but when both ecotypes are grown at the same latitude significant differences are observed in terms of phenology and morphology (Casler et al. 2012; Aspinwall et al. 2013). The duration of each phase is cultivar dependent, and it is largely driven by temperature. Temperature also plays a vital role in the vegetative growth such as leaf area index and stem elongation (EL) (Madakadze et al. 1998). In grasses the EL phase is a transition phase; the apical meristem gradually starts changing from a vegetative bud to a floral bud. Hence, this phase is also referred as floral induction. During this transition the culm starts increasing in size resulting in stem elongation. In contrast to vegetative phase, the reproductive development of switchgrass is dependent on both temperature and photoperiod (day length) (Van Esbroeck et al. 2003). Day length is the detrimental factor that triggers the transition from vegetative to reproductive growth in some species.

To our knowledge detailed information on switchgrass photoperiodic sensitivity is missing from the literature. Such information is fundamental and can greatly assist researchers to improve switchgrass crop growth models and breeding programs. One of the most important challenges that bioenergy crop modelers face is to precisely model biomass yields of new cultivars being developed. Each new cultivar might require its own parameter estimation as a result of varying phenological development, which also makes crop model output sensitive to precise modeling of its phenology (Van Oort et al. 2011).

Well known models used in switchgrass modeling are the ALMANAC and EPIC models (Kiniry et al. 2005; Thomson et al. 2009). These are process based models and utilize concepts (routines) like radiation use efficiency, carbon partitioning, leaf area index and morphological development including phenology. Although these models are widely used to predict biomass yields, they use a simplified and temperature driven approach to simulate phenology. Usually they compensate for the photoperiod effects by adjusting the GDD requirement (of the same cultivar) at different geographic locations. Though this might be robust and work well, it lacks the mechanistic consideration that will allow for a better exploration of switchgrass adaptation to various climatic conditions.

Different methods have been reported in the literature to model temperature × day length interaction (Setiyono et al. 2007). Out of these models multiplicative method is mostly used in models like GECROS and DSSAT-CROPGRO. In these models a specific developmental stage is completed when it reaches a target (e.g., 20 physiological days). The alternative to multiplicative method is the thermal time period approach (Carberry et al. 2002). In this approach, daily temperature is used to estimate thermal time and day length to express the target as a function of the day-of-year, and a stage is considered to be complete when it accumulates the required thermal time. Although these approaches are different from each other, both use similar input parameters (Archontoulis et al. 2014).

The overall goal of this study is to investigate the combined effect of photoperiod and temperature on switchgrass phenology by combining experimental information from a wide range of environmental conditions (31° to 46°N latitude). More specifically, our first objective was to develop a switchgrass phenological model that incorporates both photoperiod and thermal effects on the rate of development. The second objective was to calibrate the new model using detailed experimental observations from central Iowa, provide a sensitivity analysis of the input parameters and finally to estimate critical photoperiod values for upland and lowland switchgrass ecotypes. The final objective was to evaluate performance of the calibrated model by conducting a sensibility tests, i.e., investigate switchgrass adaptability in the USA and judge model predictions against expert opinions.

2. Materials and Methods

2.1. Approaches to model temperature × photoperiod effects

In general a phenology model is divided in a number of phases, e.g., sowing to emergence, emergence to elongation etc., which are usually measurable and/or represent well crop's growth stages. The length of each phase is determined by a target, which is generally described as thermal time target (°C-d) or number of optimum days (d). This target is altered by day-length in photoperiod sensitive species such as switchgrass. Thus, in short or long day species the calculation of the target becomes very complicated as it varies a lot with annual day-length variation. Different methods are used to model temperature × photoperiod interaction (Setiyono et al. 2007; Archontoulis et al. 2014). Among these methodologies, the multiplicative method is extensively used in numerous crop models such as DSSAT-CROPGRO, GECROS and SOYDEV. In the multiplicative method, temperature and photoperiod functions are used as 0–1 modifiers to accommodate the optimum rate of development (unit d^{-1}). A phase is considered complete when the daily accumulated rate of development has attained a mark (e.g., 15 physiological days). The second method is the thermal time target approach (Carberry et al., 1992; Holzworth and Hammer, 1996; Carberry et al., 2001) used by the APSIM simulation platform. In this approach, temperature is used to calculate daily thermal time (°C-d) and photoperiod to describe a target as a function of day of the year. A phase is considered complete when the accumulated thermal time has attained a target. Nevertheless the multiplicative and thermal time methods are conceptually different from each other, they both use the similar input parameters (see Fig. 4.1 in Archontoulis et al. 2014).

2.2. Model development for switchgrass phenology

In this work we follow the methodology developed by Archontoulis et al. (2014) to model temperature × photoperiod effect. The duration of switchgrass life cycle (after re-growth thus valid for crops after the 2nd year onwards) can be described by the following equation:

$$\text{Crop cycle} = VE\_JUV + JUV\_EL + EL\_FL + FL\_PM \qquad \text{eq. 1}$$

Where:

$$VE\_JUV = (T_o - Tb) * a_1 \qquad \text{eq. 2}$$

$$JUV\text{- } EL = (T_o\text{-}T_b) * a_2 / (1 - P_{sen} * (P - P_{crit}) \qquad \text{eq. 3}$$

$$EL - FL = (T_o\text{-}T_b) * a_3 / (1 - P_{sen} * (P - P_{crit}) \qquad \text{eq. 4}$$

$$FL\_PM = (To\text{-}Tb) * a4 \qquad \text{eq. 5}$$

Symbols explanations are provided below and for a summary of these see table 4.1. Eq. 1 describes that switchgrass phenology is divided in four major phases: re-growth to end of juvenile (VE_JUV), juvenile to stem elongation (JUV_EL), elongation to flowering (EL_FL) and flowering to physiological maturity (FL_PM). The emergence of tillers following winter dormancy (VE) is dependent upon the climatic conditions such as soil temperature, air temperature and soil moisture. Thus, the prediction of this phase is complicated as it requires soil water and temperature models to quantify the re-growth date. In our model re-growth date is fixed (user input). The VE _ JUV phase (Eq. 2) is mainly dependent upon the air temperature and therefore has a constant thermal time target period set by the parameter a_1. After the end of the basic vegetative phase (juvenile) short day plants becomes sensitive to photoperiod. Thus the JUV _ EL and EL _ FL phases (Eq. 3 and 4, respectively) are driven by both temperature and photoperiod. Therefore, in these stages thermal time targets vary with the annual day length variations. Thus, the equations for these two phases also have two additional terms (P_{crit} and P_{sen}). The physiological maturity phase is included in this general model but not included in our analysis because as a biomass producing crop our main emphasis is from emergence to flowering during which maximum biomass accumulation occurs.

2.3. Estimation of phenological parameters

Equation (1) is a compound non-linear equation with biologically expressive parameters (Table 4.1). Hence, some of the important parameters can be optimized with the use of suitable statistical methods, well-defined initial values and sufficient datasets. Our approach to develop phenological parameters for switchgrass is briefly described as follows: step (1) we compiled a database with switchgrass phenological information from the literature (Table 4.2); step (2) we calibrated parameters for Eq. 1 using detailed experimental data from central Iowa, performed a sensitivity analysis to determine important parameters for optimization and established starting values (Table 4.3); step (3) we used an R optimizer (see Archontoulis et al. 2014) for estimation of critical photoperiod.

2.3.1. Database with phenology and weather data

2.3.1.1. detailed data from central Iowa

A study was conducted during year 2012 and 2013 on a switchgrass variety trial established in 2007 at Iowa State University, Sorenson Research Farm, near Ames, IA (42°0’41” N, 93’44’34” W). The experiment was arranged as randomized complete block design with six replications and five switchgrass varieties of two distinct origins i.e., lowland (‘Kanlow’ and ‘Alamo’) and upland (‘Cave-in-Rock’, ‘Trailblazer’, and ‘Blackwell’). Each year, before spring regrowth, aboveground plant dead material was cut at a stubble height 5 cm, and the residue was taken off the field. Pre-emergence application of “atrazine” [6-Chloro-N-ethyl-N'-(1-methylethyl)-1, 3, 5-triazine-2, 4-diamine] and “Paramount” [3, 7-Dichloro-8-quinolinecarboxylic acid] was applied at 2.23 kg a.i.ha^{-1} and 0.56 kg ha^{-1}, respectively, for weed control.

To estimate the phenology of switchgrass varieties biomass harvest samples were collected from three randomly chosen blocks in the first year, and the remaining three blocks were harvested the following year. Each plot was further subdivided into six subplots to which six biomass harvests collected during the growing season were randomly assigned. The first biomass harvest was collected during the early vegetative phase generally two weeks after the spring regrowth, and the remaining five biomass harvests were collected at approximately two week intervals depending upon weather conditions. Two quadrats of 0.1 m^2 were placed randomly in each subplot for the sample harvest. Aboveground plant material was cut carefully close to the ground surface using the hand clippers.

Harvested samples from each harvest date were analyzed for determining the developmental stage for each variety. Samples were hand separated and morphologically categorized as described by the Nebraska perennial grass staging system (Moore et al. 1991). In this method each set of harvested sample has categorically separated tillers according to their developmental phase. Then, each stage is assigned a numeric number according to the Nebraska staging system, which is then multiplied with the total tillers with in each stage, the resulting numbers are summed and divided by their respective total tiller numbers to obtain mean stage count for each set of harvested sample.

2.3.1.2. Literature data

Table 4.2 illustrates the database utilized in this study (n =10). This database covers a wide range of cultivars and experimental locations across the USA (from 31° to 46°N). Almost all of the datasets included upland switchgrass cultivars except 7 and 8 which only have lowland cultivars. Dataset 4 and 10 have both lowland and upland cultivars. Information related to regrowth/emergence, elongation, flowering and physiological maturity were obtained from figures or tables. We were able to extract almost all of the important developmental staging information like vegetative phase, elongation phase, flowering phase etc., from dataset 2, 3, and 6, rest of the datasets only have flowering data. For detailed information about the datasets please refer to the Table 4.2.

2.3.1.3. Weather data

Daily climatic data (min. max. temp., radiation and precipitation) for Ames, IA experimental site were collected at a weather station situated about 8 km from the experimental field, and the data were downloaded from Iowa Environmental Mesonet (2014).

For all other sites weather data were Weather data for all sites were obtained from “daymet” with the help of coordinates being reported in the literature (http://daymet.ornl.gov/) (Thornton et al., 2012). Daymet is a gallery of algorithms and computer programs developed to interpolate and extrapolate weather data from ground-based climatological stations to generate estimates of daily climatic parameters.

2.3.2. Switchgrass starting parameters, sensitivity study and assumptions

The crop cycle equation (Eq. 1) has many biologically meaningful parameters (Table 4.1). Parameters describing cardinal temperature (Table 4.1) and the nature of the temperature response function (Fig. 3.1c) were taken from the literature (Madakadze et al. 2003; Kiniry et al. 2008). Parameters describing photoperiod effects, P_{crit} and P_{sen} for each ecotype were set within a reasonable range observed in the literature (12-16 h; Van-Esbroeck et al. 2003). Parameters a_1 to a_3 and P_{sen} and P_{crit} were estimated from the central Iowa data. Then a sensitivity analysis was performed to determine the most important parameters for further optimization using the entire database. Among the parameters included in Eq. (1) we initially prioritized for optimization the following: P_{crit}, a_3, P_{sen}, a_1. Also we assumed the other values constant between ecotypes i.e., upland and lowland; a_1 = 5d, a_2 = 5d and a_3 = 10d in order to obtain reasonable number of parameters to be approximated through optimization. Preferably, it would be required to have estimates for all the coefficients but this is not possible due to the limitations in statistical methods and unavailability of sufficient database.

2.3.3. The optimization program

We used the optimization program developed by Archontoulis et al. (2014). The optimizer is written in R program (R Development Core Team, 2005), which is widely used in agronomic research to optimize parameters. The optimizer calculates:

a) daily photoperiod as a function of day of the year, latitude and solar angle
b) daily thermal time using a 3-hour interpolation method and pre-specified temperature/thermal-time relationships (see Fig. 4.1 in Archontoulis et al. 2014)
c) crop phases as described in Eq. 1

Briefly, the R optimization program works as follows. First, it defines the phase targets as a function of daily photoperiod for the JUV_EL and EL_FL phases, and reads constant values for the VE_JUV, and FL_PM phases. The photoperiod driven targets were calculated from the equation presented in Figure 4.1d in Archontoulis et al. 2014. Secondly, starting at re-growth, it calculates and accumulates thermal time until a target has been reached, which signals the end of a phase. Then the same process is repeated in the next phase until physiological maturity has been reached. Thirdly, it counts the number of calendar days needed to complete each phase, and outputs this information as DAE (days after emergence). Finally, it optimizes the desirable parameters by minimizing the differences between predicted and measured values using a stochastic method (see below).

The optimization code is based on a custom Markov chain Monte Carlo algorithm (Givens and Hoeting, 2005) that allowed to optimize the switchgrass phenology model given flexible input data. The algorithm produces candidate parameters from independent normal distributions. The starting values from Table 4.3 were used as the means of the distributions and the standard deviations are derived from the suggested ranges. The standard deviations vary dynamically during the chain progress through a tuning parameter (scale). This scale parameter increases by 10 % when the proposed move is rejected and is reduced when a move is accepted. This usually results in a reasonable acceptance rate. We chose not to include strong priors on the estimation method, but this could be incorporated in a future version. The chains were run for 10000 iterations. For calculating summary statistics we used a burn-in period of 1000 iterations. The convergence of the algorithm was assessed visually and also using the Gelman diagnostics (Gelman et al. 2004) with a value between 1 and 1.2 considered acceptable. In addition, the model efficiency (see statistics) was also used to evaluate the agreement between simulations and observations. The R code is available from the corresponding author upon request.

2.3.6. Switchgrass adaptability – model application study

In total 300 simulations (5 States × 30 years × 2 ecotypes) were conducted in R. Each simulation refers to a particular combination of emergence time, year, cultivar and location. Average of the optimized parameters for upland and lowland ecotypes were used during this procedure. To get better understanding and to test the applicability of these parameters we tested them against wide geographic location i.e., Minnesota, Iowa, Missouri, Arkansas and Louisiana.

2.4. Statistics

The goodness of fit was assessed by calculating the root mean square error (RMSE, the lower the value the better), the relative absolute error (RAE, the lower the value the better) and the modelling efficiency (ME, the higher the value the better). The equations can be viewed in Archontoulis and Miguez et al. (2015) and for more details we are referring to Wallach et al. (2006).

3. Results

3.1. Model calibration for Iowa

The model predicted all the key development stages of switchgrass very well for the calibration dataset (RMSE of 2.6 days; Fig. 4.1a). The residuals were equally distributed along the 1:1 of Fig. 4.1a which shows that the model predictions are robust. The optimized parameter values by cultivars as well as by ecotype are shown in Table 4.3. Analyzing the parameters by ecotype we found that using average ecotype values instead of specific cultivars the predictions were still robust (Fig. 4.1b) and accurately, with an RMSE of 5.1 days. A major difference between the two ecotypes was the a_2 parameter.

3.2. Model validation

We tested the ability of the model to predict time to flowering to a range of literature values from different geographic locations (Table 4.2). We selected to test only time to flowering because this information was available in the switchgrass literature. For this analysis we used parameters averaged over ecotype shown in Table 4.3. This assumption is reasonable given that the parameters did not change much across varieties belonging to an ecotype. The results from this validation are presented in Fig. 4.2a. The model predicted time to flowering with a RMSE of 17 days, showing that the model is not robust enough for application outside the calibration range. Possible reasons for the low prediction power included the uncertainly in time of re-growth as this information was rarely reported in the papers and had to be estimated for each dataset or it had to be extrapolated from figures.

Another important reason is that the locally developed parameter values (Table 4.3) and especially the photoperiod related parameters were not robust enough for the application outside the calibration range. To test the first hypothesis, we re-ran the validation study and assumed that time to re-growth occurs approximately 60 GDD after the last frost (Tmin < 0 ^{o}C). This approach successfully used by Miguez et al. (2012) to model *Miscanthus* re-growth and subsequently biomass production across USA. Thus we re-ran the analysis and the new results are presented in Fig. 4.2b. The results were improved (RMSE from 17 to 15.3 days) but not to a very satisfactory level. At least this way of estimation of re-growth (automatic based on Tmin instead of expert judgement in the lack of specific information) minimizes user uncertainty with the estimation of re-growth date.

3.3. Model sensitivity analysis

To improve further the model predictions is very important to know the sensitivity of each parameter to model output. To examine which of the model parameters had the greatest impact on the simulation output (e.g., time to flowering), we performed a sensitivity analysis, for that, we increased and decreased all of the input parameters (one at a time) by 15% and 30% to study their sensitivity with respect to the flowering time. For this analysis we used Cave-in-Rock, Trailblazer, Blackwell, Kanlow and Alamo cultivars and 2012 Ames, IA weather data (Fig. 4.3).

Among the six (a_1, a_2, a_3, p_{crit}, p_{sen} and regrowth) input parameters, the p_{crit} was found to be the most important in terms of sensitivity to the flowering time followed by the time of re-growth and subsequently all the other parameters followed (Fig. 4.3). These results showed that for a robust estimation of p_{crit} a geographically diverse database was needed.

Therefore to get a better estimation of p_{crit}, we performed a second round of optimization in which we used all of the literature data and optimized only p_{crit}. In this analysis a_1, a_2, a_3 and p_{sen} were fixed at values reported in table 4.3 by ecotype and the re-growth time assumed 2 weeks after the last frost. The results showed that there is an improvement compared to the previous effort (Fig. 4.2b vs. 4.2c; RMSE from 15.3 to 11.9 days), but the optimization diagnostics suggested that this approach needs improvements. This is because the estimation of p_{crit} is sensitive to the value of the parameter a_2 (one parameter compensates for the other), and therefore we performed another round of optimization where we optimized both p_{crit} and a_2. The optimized results (parameter values) for each literature dataset is showed in Fig. 4.4. As it can been seen in Fig. 4.4, that there was a clear difference between the two ecotypes in terms of p_{crit} and a_2 and that within a group there was some genotypic variation as well. Using average values reported in Fig 4.4 for p_{crit} and a_2, the model predicted days to flowering with a RMSE of 8.8 days (Fig. 4.2d). To judge overall model performance, one has to consider also the variability that exists in the observations.

3.4. Scenario study – switchgrass adaptability to different environment

We used the model with the parameter from the last optimization to explore how switchgrass phenology varied from year to year and from one location to another location for two widely used switchgrass ecotypes i.e., upland and lowland (Cave-in-Rock and Alamo, respectively). The time to re-growth was assumed 2 weeks after the last frost. The results from this analysis are presented in Fig. 4.5, which shows that upland and lowland switchgrass ecotype flower around 215th and 230th DOY (day of year) in Minnesota. In Iowa the upland and lowland ecotype flower around 200th and 215th DOY respectively, while in

Arkansas and Louisiana the flowering time for upland and lowland ecotypes is around 150th and 175th DOY respectively for Arkansas and 130th and 155th DOY respectively for Louisiana.

4. Discussion

We developed a new model that accounts for both temperature and photoperiod effects on switchgrass phenology. The model has a great potential and offers a mechanistic approach to estimate switchgrass life cycle phenology. Our model can predict switchgrass flowering time at different latitude and different ecotype. The model prediction (see Fig. 4.5) is in agreement with what is known for switchgrass adaptability in different geographic locations in U.S. (Mitchell et al. 1998, Vogel et al. 2002; Casler et al. 2004). This information regarding flowering time is an important aspect for perennial grasses being used for biomass production. Moreover, this is the first study that also attempted to estimate the critical photoperiod value for switchgrass (Fig. 4.4). Though this information is fundamental and can inform future breeders programs, still, there is no comprehensive data in the literature (Aurangzaib 2014, unpublished). An important reason for this gap in the literature is not the lack of photoperiodic response in switchgrass but the difficulty in performing planting date experiments in perennial species or sophisticated greenhouse experiments (Van Esbroeck et al. 2003; Yin et al. 2008). Generally, to estimate photoperiod effects in field crops, researchers perform multi-season and multi-location experiments (Carbery et al. 1992), and then through statistical optimization estimate the parameters. Thus, currently the only available option for switchgrass to estimate its critical photoperiod and difference between ecotypes is by using multi-site experiments. In this work we followed the methodology similar to Archontoulis et al. (2014), Yang et al. (2014) and Sarath et al. (2015).

For evaluation of the performance of this model, it will be important to consider the uncertainties associated with the database used. The fact that the calibrated model is not quite accurate in extrapolating the phenological predictions for other geographic zones is due to the inadequate data to estimate photoperiod sensitivity parameters for other locations. Sarath et al. (2014) reported similar limitation while calibrating phenology sub-model for APSIM-Oryza. Therefore, this calibrated model may require more experiments for estimation of photoperiod sensitivity parameters in controlled environment or wide geographic zones (Awan et al. 2014; Olsen et al. 2012).

Most of the data in literature is available about switchgrass flowering time, but data related to regrowth, end of juvenile and flower initiation is very rare. Especially, data for end of juvenile stage is generally not recorded, which is mostly associated with the changes in leaf morphology and anatomy (Sylvester et al. 2001). This transition might be abrupt or gradually buildup. However, this model still has the ability to predict these aforementioned stages and also allow the incorporation of additional developmental stages if they exist.

In this study we generalized and propose the use of generic parameters by ecotype. This simplification lost some prediction power (Fig. 4.1) but on the other hand substantially increased the potential applicability of our model. For example the model can be used to explore the adaptability of switchgrass ecotypes to new environments which is the first step in any decision making process. These parameters can serve also as starting value to develop new parameters for switchgrass varieties. It also allows researchers to explore and understand temperature × photoperiod interactions in switchgrass cultivars. It also facilitates them to optimize with minor modifications in the following parameters in R code: T_o, T_{max}, T_{min}, P_{crit}, P_{sen}, a_1, a_2, and a_3 (table 4.1).

This model demonstrates potential for calibrating multiple parameter input phenological model that takes into consideration temperature × photoperiod interaction in crop life cycle from regrowth and flowering information. Though the prediction ability of the model is not yet at a very satisfactory level (RMSE of 8.8 days), we have developed a framework that can be applied to a larger database in the future for higher quality models to estimate better critical photoperiod in switchgrass and to further strengthen the model performance. Such a model is not only useful for agronomists and policy makers to make informed decisions about the adaptability of switchgrass but also very useful for modelers. This is because quantification of phenology is the starting point and the most important step in the development of simulation models. Also phenology captures much of the genotypic variation among different cultivars. This model can also serve as a template towards development of new crop models and optimization of phenology parameters for other short day grass species as well.

Tables

Table 4.1. List of the parameters and acronyms used in this study, with their definitions, values and units.

Acronym	Definition	Value	Unit
DAE	Days after emergence	-	d
VE_JUV	Emergence to end of Juvenile phase	F(t)	d
JUV_EL	End of juvenile to elongation (also floral initiation)	F(t) and F(P)	d
EL_FL	End of elongation phase to flowering	F(t) and F(P)	d
T_o	optimum temperature for maximum growth	30	^oC
T_{base}	Base temperature below which there is no growth	10	^oC
T_{max}	Maximum temperature above which there is no growth	40	^oC
P	photoperiod (length of the daylight)	0-24	Hours
PM	Physiological maturity		d
P_{crit}	Day length above which the development rate is sensitive to photoperiod	12.75	Hours
P_{sen}	photoperiod sensitive coefficient	0.2	1/hours
a_1	Optimum days to complete VE-JUV	5	d
a_2	Optimum days to complete JUV_EL	10	d
a_3	Optimum days to complete EL_FL	25	d

Here F(T) demonstrates that the duration of this phase is a function of temperature, and F(P) demonstrates that the duration of a phase is a function of photoperiod

Table 4.2. Literature datasets used in this analysis

Data Set	Location	Latitude (ºN)	Years	Cultivar	Phenological data					Reference
1	Ames, IA	42.11	2012-13	5	EM	VE	EL	FL	PM	Exp. Data
2	Mead, NE	41.11	1990-91	1	EM	VE	EL	FL	PM	Mitchell et al. 1997
3	Mead, NE	41.11	1992-93	1	EM	VE	EL	FL	na	Mitchell et al. 1998
4	Mandan, ND	46.48	2000-02	8	na	na	na	FL	na	Berdahl et al. 2005
5	Mead NE	41.11	1994-95	1	EM	na	na	FL	na	Vogel et al. 2002
	Ames, IA	42.011								
6	Ames, IA	42.011	2006-07	1	EM	VE	EL	FL	na	Waramit et al. 2013
7	Temple, TX	31.53	1995-96	1	EM	VE	EL	FL	na	Kiniry et al. 1999
8	Frederick, OK	34.23	2008-09	1	na	na	na	FL	na	Guretzky et al. 2010
	Burnsville, OK	33.89								
9	Montreal , QC	45.28	1993-95	9	na	na	na	FL	na	Madakadzi et al. 1998
10	Columbus, OH	40.01	2010	16	na	na	na	FL	na	Stottlemyer, 2012

EM: emergence; VE: vegetative; EL: elongation; FL: flowering; PM: physiological maturity

Table 4.3. Phenological parameter estimates for five cultivars (from experimental data Ames, IA) from the automated calibration method

Cultivar	p_{crit} hour	p_{sen} 1/hour	a_1	a_2 Optimum days (d)	a_3
"--- using 2012-2013 data from Iowa ---"					
Cave-in-Rock	13.1	0.2	4	16.2	25.2
Trailblazer	13.1	0.2	4.9	15.4	24.7
Blackwell	13.1	0.2	4.8	16.3	25.8
Kanlow	12.75	0.2	4.9	29.1	23.9
Alamo	12.75	0.2	5.4	31.3	21.7
"---averaged over ecotype---"					
Upland	13.1	0.2	4.6	16.0	25.2
Lowland	12.75	0.2	5.2	30.2	22.8
"---averaged over ecotype, new round of optim. for p_{crit}---"					
Upland	14.4	0.2	4.6	16.0	25.2
Lowland	13.5	0.2	5.2	30.2	22.8
"---averaged over ecotype, new round of optim. for p_{crit} and a_2---"					
Upland	13.6	0.2	4.6	15.60	25.2
Lowland	12.6	0.2	5.2	21.50	22.8

Figures

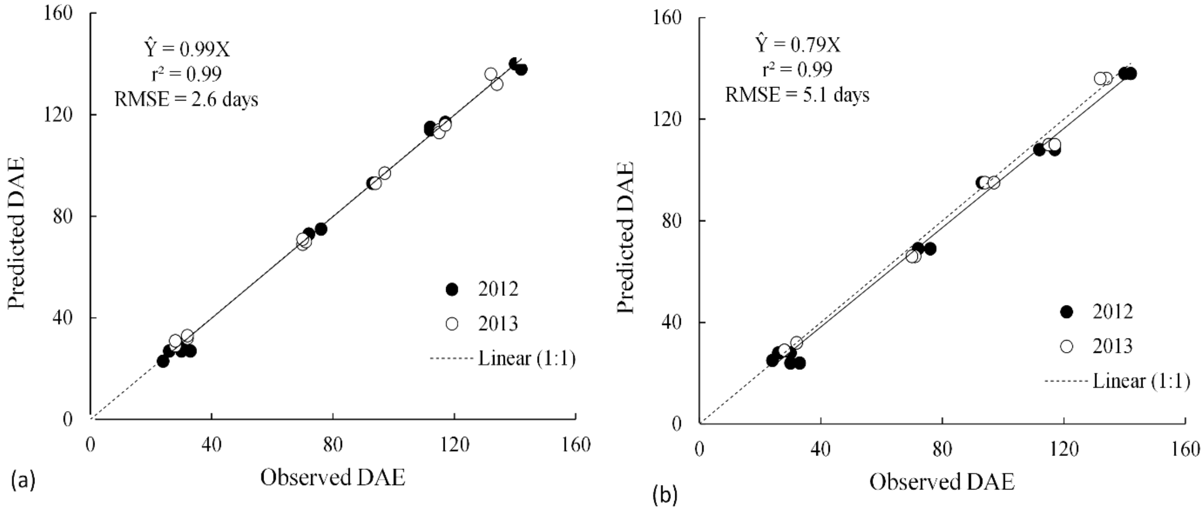


Figure 4.1. Developmental stages observed vs predicted DAE (days after emergence) for switchgrass cultivars for year 2012 and 2013 in Ames, IA. Parameters used for Fig. 4.1a are the estimated parameters for individual cultivar used for calibration, and parameters used for Fig. 4.1b are the estimated average parameters for upland and lowland ecotypes.

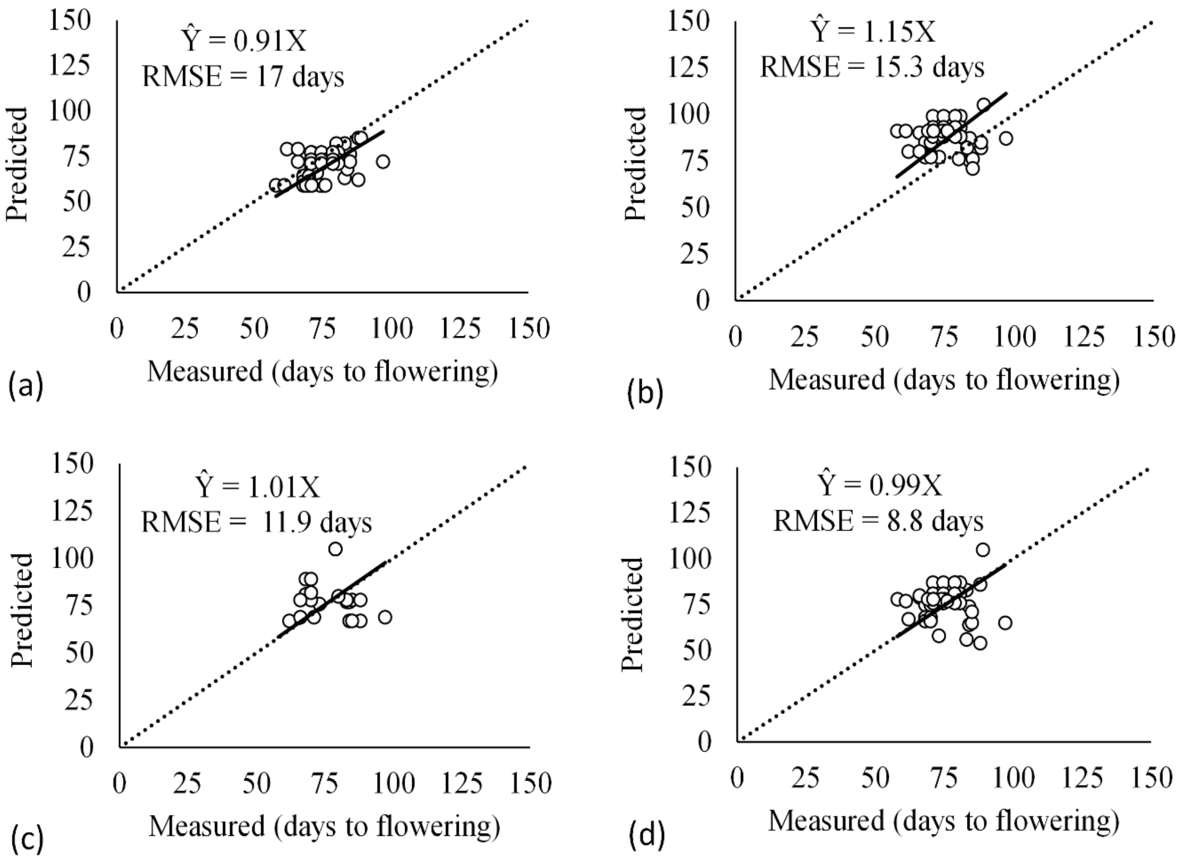


Figure 4.2. Flowering data observed vs predicted DAE (days after emergence) for literature data used in this study. Parameters used are average of the estimated parameters for upland and lowland ecotypes from each analysis.

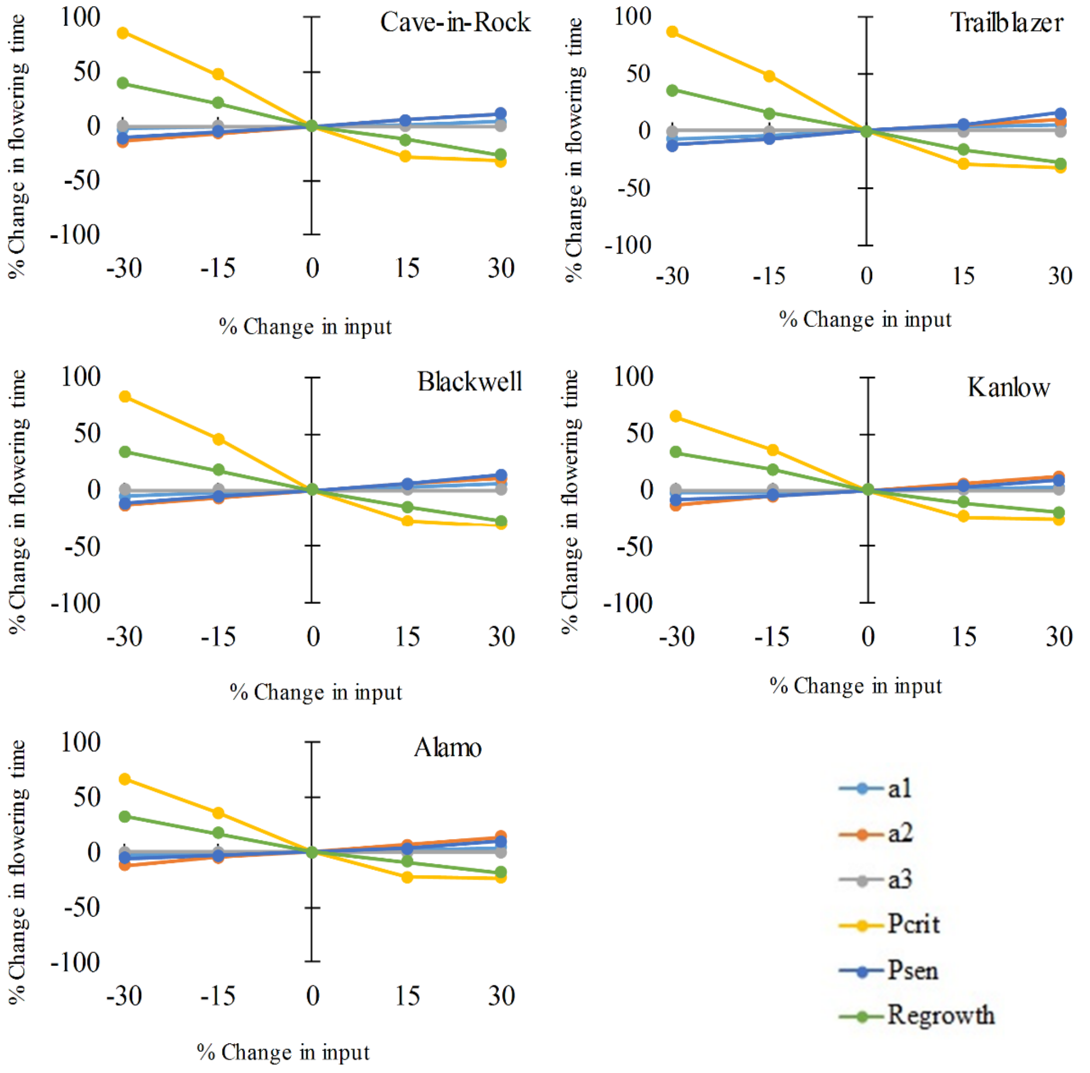
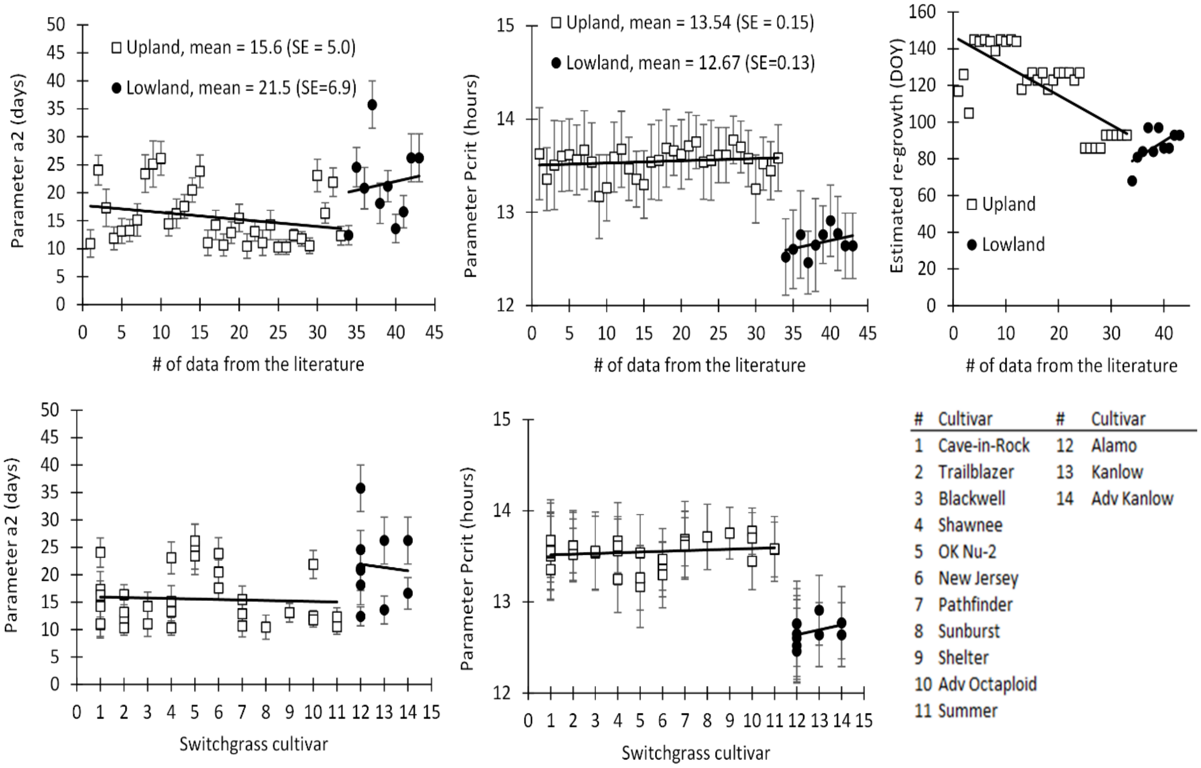


Figure 4.3. Sensitivity analysis performed for input parameters (a_1, a_2, a_3 P_{crit}, P_{sen} and regrowth) one at a time by ± 15 % and ± 30 % to study their sensitivity to flowering time for 2012 Ames, IA weather data.



#	Cultivar	#	Cultivar
1	Cave-in-Rock	12	Alamo
2	Trailblazer	13	Kanlow
3	Blackwell	14	Adv Kanlow
4	Shawnee		
5	OK Nu-2		
6	New Jersey		
7	Pathfinder		
8	Sunburst		
9	Shelter		
10	Adv Octaploid		
11	Summer		

Figure 4.4. Parameter estimates from the optimization program for a_2, p_{crit} and date of regrowth from the literature database.

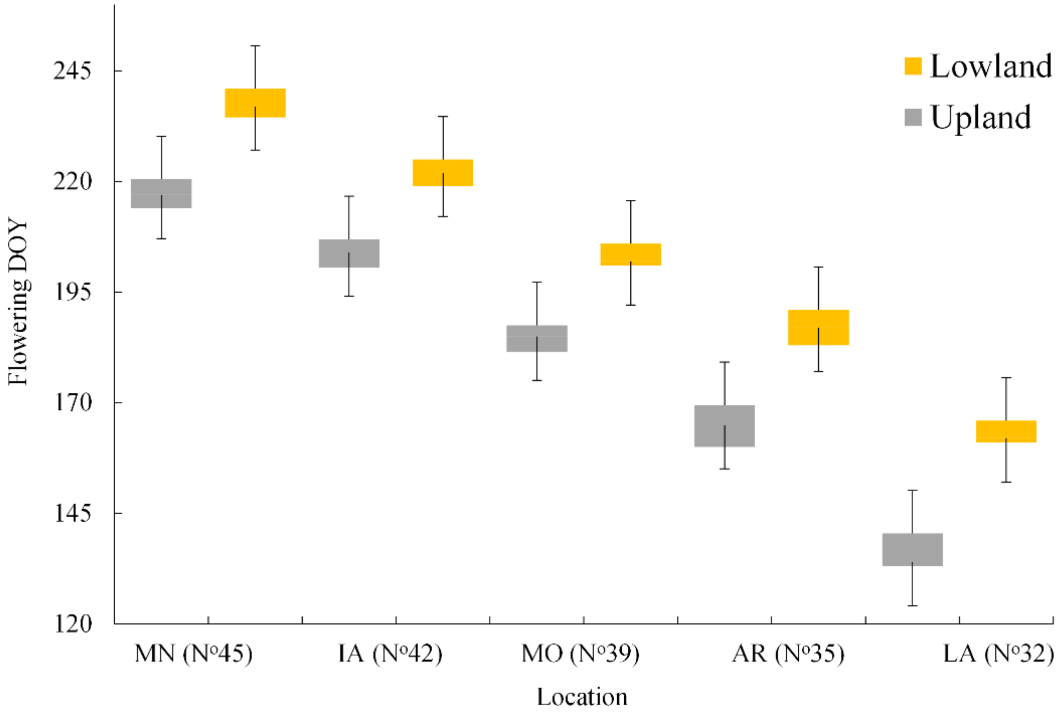


Figure 4.5. Switchgrass adaptability test- Predicted flowering time DOY (day of year) for upland and lowland switchgrass ecotypes in five US states (Minnesota (MN), Iowa (IA), Missouri (MO), Arkansas (AR) and Louisiana (LA)). The length of each box indicate the variability in model predictions created by replicating the simulation for 30 years (1980-2010).

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CHAPTER 5

GENERAL CONCLUSIONS

The sustainable biomass production with enhanced feedstock quality are important aspects of a sustainable bioenergy production (Schmer et al. 2008; Heaton et al. 2008; Tilman et al. 2011; Waramit et al. 2011; Valentine et al. 2012), and switchgrass is considered an important candidate crop to serve this purpose (Tilman et al. 2011, Waramit et al. 2011). Switchgrass being a C_4 grass is more efficient than C_3 grasses with respect to their water use efficiency, N use efficiency and biomass production per unit of land.

Sustainable and successful development of bioenergy industry strongly depends upon quantity and quality of the bioenergy feedstock produced. Therefore, the two most important aspects of an ideal bioenergy crop will the greater biomass yield production and quality of the feedstock produced. Switchgrass has already demonstrated great potential for being an ideal bioenergy feedstock in Central and North America (Bransby et al. 1999; Douglas et al. 2004; Cassida et al. 2005; Bouton J., 2007). The wide natural geographic distribution of switchgrass cultivars have resulted into two ecotypes i.e., upland and lowland cultivars (McLaughlin et al. 2005). These ecotypes have been observed to have genotypic, phenotypic and physiological differences (Stroup et al. 2003; Casler et al. 2004; Das et al. 2004; Gunter et al. 1996; Yang et al. 2009; Cortese et al. 2010). In geographic regions where these ecotypes overlap clear morphological differences have been detected between the ecotypes like phenological traits (Casler, 2012; Aspinwall et al. 2013). These phenological traits also have a significant role in biomass accumulation.

Results of two years of growth and developmental study of switchgrass ecotypes confirmed significant variation between switchgrass ecotypes for their biomass yields and phenology. We observed that MSC, LAI and biomass yields were different between switchgrass ecotypes. Upland ecotypes generally had higher MSC and LAI at a given day of year than the lowland ecotypes. Ecotypic differences were also observed among switchgrass cultivars for their harvest timing, but delaying harvest to later maturity always ensured greater biomass production in all cultivars. Upland ecotypes usually matured about 3 weeks earlier than the lowland ecotypes in both years.

Lowland ecotypes i.e., Kanlow and Alamo had low MSC than the upland ecotypes but they produced the highest dry matter yields in both years. However, greatest dry matter yields in all switchgrass cultivars were observed post flowering. Having determinate growth habit, switchgrass vegetative growth/biomass accumulation is mostly terminated as the inflorescence starts developing. This suggests that greater dry matter yield production by the lowland ecotypes might be attributed to their delayed flowering. Thus, one delayed harvest to the most advance maturity stage or the fall harvest after the killing frost will ensure greater dry matter yield production. Moreover, this delayed harvest will also ensure stand persistence, because earlier harvests might hinder the metabolite translocation process to the below ground plant parts, which is an important phenomenon for perenniality (Beale and Long 1997; Madakadze et al. 1999; Wilson et al. 2013).

Results from this study also demonstrate MSC quadratic equation relationship with LAI and DMY. Overall the models predicted well, but there was also a slight under prediction in both years after the mid-season of crop growth and development, which might be due to faster LAI and biomass accumulation (Kalu and Fick, 1981; Moore et al., 19991; Mitchell et al., 2001). We also observed year to year variation in the MSC quadratic equations which suggest that these relationships can vary from one year to another depending upon the climatic conditions.

As mentioned earlier that the successful development of bioenergy industry also depend upon quality of the bioenergy feedstock produced. Generally, for an ideal bioenergy feedstock higher concentration of fiber (lignin, cellulose and hemicellulose) and reduced levels of nitrogen and ash are desirable (Ablevor et al. 1992; Sanderson et al. 2007). Concentrations of these important feedstock components can vary significantly due to geographic location, genetic factors, plant maturity and agronomic practices (Vogel et al. 2002; Adler et al. 2006; Royal Society, 2008). Therefore, based on the desired feedstock quality traits such as cellulose and hemicellulose for biochemical conversion and lignin for thermochemical conversion we should optimize the production management techniques particularly the harvest management practices and selection of cultivar.

Results from this study demonstrated a significant variation among switchgrass ecotypes for the important quality traits. Harvest time was also observed to greatly influence not only the dry matter yields but the feedstock quality as well by increasing or decreasing the lignin, cellulose, hemicellulose, N and ash concentrations over the growing season. Notably, the delayed harvests to later maturity stages always ensured higher lignocellulosic concentration and lower N and ash concentration. Similar findings are also reported by the previous researchers (Jung and Vogel, 1992; Madakadze et al. 1999; Mitchell et al. 2001; Mulkey et al. 2006; Waramit et al. 2011). Moreover, the results also demonstrate that ecotypic differences also exist among switchgrass cultivars for their fiber contents. Lowland ecotypes generally produce higher concentrations of cellulose and hemicellulose than the upland ecotypes. Higher concentrations of cellulose and hemicellulose are highly desirable for the biochemical conversion systems. Higher lignin concentrations on the other hand, were found in Cave-in-Rock which is an upland ecotype. Similar findings have been previously reported that the upland ecotypes produce higher lignin contents (Lemus et al. 2002; Bhandari et al. 2014). Higher lignin concentrations are desirable for thermochemical conversion systems (Hayn et al. 1993). We also observed that N and ash concentrations in both ecotypes decrease with advanced maturity. This curvilinear decrease in N and concentrations with respect to delayed harvests might be the result of nutrients translocation to the below ground plant parts (Beale and Long 1997; Madakadze et al. 1999; Sanderson et al. 1999; Wilson et al. 2013). Carbon to N ratio on the other hand continuously increased in all cultivars over the growing season. Generally, lowland cultivars had higher C-N ratio as compared to their counterpart upland cultivars. This increase in C-N ratio over the growing season might be the result of decrease in N contents and increase in carbohydrate levels as the plants mature and prepare for the winter dormancy.

Based on results presented in this study and elsewhere, we conclude that ecotypic differences do exist between switchgrass cultivars for their fiber, nitrogen and ash concentrations. Lowland ecotypes usually have greater cellulose, hemicellulose and lower nitrogen and ash contents; whereas, upland ecotypes produce higher lignin, nitrogen and ash concentrations. Furthermore, delayed biomass harvest to later maturity stages or until fall will ensure greater fiber and lower nitrogen and ash concentrations.

Morphological development of switchgrass is similar to other perennial grasses and follows the general developmental pattern described by Moore et al. (1991), but when both ecotypes are grown at the same latitude significant differences are observed in terms of phenology and morphology (Casler et al. 2012; Aspinwall et al. 2013). Therefore, the final goal of this research was to develop switchgrass phenological model that incorporates environmental factors such as photoperiod and thermal effects on switchgrass development.

The developed model demonstrate a great potential and propose a mechanist approach to estimate switchgrass life cycle phenology. This model also has the ability to predict switchgrass flowering time for different latitudes as well as ecotypes. The model prediction regarding switchgrass flowering time in different latitudes is similar to what is known about switchgrass adaptability in different geographic regions in U.S. (Mitchell et al. 1998, Vogel et al. 2002; Casler et al. 2004). Furthermore, to our knowledge this is the first study that also attempted to estimate the critical photoperiod of switchgrass cultivars. However, this is just an initial step towards development of more robust switchgrass phenology model. Though the information provided by this model is fundamental, but still it can give an insight to the researchers for future breeding programs. One of the limitation faced while developing this model was the lack of literature related to switchgrass phenology and photoperiod effect. This inadequate data reduced our efficiency for the calibration of switchgrass photoperiod sensitivity parameters for other locations.

However, despite the fact that there are some limitations, the model demonstrates its capability for calibrating multi input phenology model that accounts for temperature × photoperiod interactions from regrowth and flowering information of switchgrass growth cycle. The developed model framework has the ability to be used in future for larger datasets for the development of more advance and better quality models. It can also serve as a template for the development of new crop models and for the optimization of phenology parameters for other short day grasses.

In summary, this study was designed to understand the feedstock compositional and morphological differences between upland and lowland ecotypes and their harvest management. Drawing from all the results presented in the manuscripts it can be concluded that ecotypic differences are present in switchgrass cultivars for their composition, biomass yield, morphological traits such as phenology and LAI and their harvest timing. The results also offer support for planting lowland varieties in Iowa to gain higher biomass yields. Although, their delayed maturity might also decrease their viability in northern regions in the long term. Temperatures were fairly typical during the course of this study and we didn’t observe stand losses. Therefore, we will also suggest that lowland ecotypes should be further tested for their cold tolerance before making any recommendation regarding the use of lowland ecotypes grown commercially for biomass production in Iowa.

Moreover, the results of MSC relationship to LAI and DMY study can also be beneficial in development of new and improvement of existing switchgrass morphological models for LAI and DMY prediction. The switchgrass phenology model can also be a helpful tool for improvement and development of calibration protocols for other models and it can also serve as a template for the development of phenology models for other perennial grasses.

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