DEVELOPMENT, QUALITY, GROWTH, AND YIELD OF TWO DIVERSE SWITCHGRASS CULTIVARS RECEIVING NITROGEN FERTILIZER IN INDIANA

by

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A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Agronomy

West Lafayette, Indiana

December 2018

THE PURDUE UNIVERSITY GRADUATE SCHOOL
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In dedication to my grandparents, John and Jo Stefancik.

ACKNOWLEDGMENTS

I would like to acknowledge my advisor, Keith Johnson, for all his support as I went through the journey to complete my Master's Degree. Our travels across Indiana to various Purdue Extension functions allowed for great discussions on forages and life. I would also like to thank my committee members, Ron Lemenager and Jeff Volenec, for supporting me as I completed classes and research work. Thank you to all the student workers, especially Hillary Booher, Amelia Laub, and Amanda Duncan, who helped me accomplish this research; without their help this paper would not be possible. You made the research more fun as we traveled across Indiana collecting switchgrass and making memories. I'd like to express gratitude for Mike Casler and Joe Halinar allowing me to visit the Dairy Forage Research Center in Madison, WI, and for helping me as I analyzed my plethora of samples with your NIRS machine. Additionally, Jason Adams at the Indiana Corn and Soybean Innovation Center, for assistance as we utilized his facility for sample processing and storage. Finally, thank you to Corey Gerber and Braden Carpenter with the Purdue Crop Research and Diagnostic Training Center for providing me a graduate assistantship in exchange for working with forage demonstration plots. The experience and knowledge I gained is invaluable.

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ABSTRACT

Author: Stefancik, Brooke A.. MS
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Degree Received: December 2018
Title: Development, Quality, Growth, and Yield of Two Diverse Switchgrass Cultivars Receiving Nitrogen Fertilizer in Indiana
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Switchgrass (*Panicum virgatum* L.) is an important warm-season perennial grass in livestock systems and has been extensively researched as an herbaceous energy crop. Objectives of this series of studies were to compare morphological development, compositional quality, crop growth, and yield of a recently developed biofuel cultivar ‘Liberty’ to an improved forage cultivar ‘Shawnee’ in multiple Indiana environments. Pure stands of each cultivar were sampled in the field at Trafalgar and Roann, Indiana in 2016. In 2017, samples were collected at Trafalgar, Roann, and Lafayette, Indiana. Samples were collected weekly during the early season and every other week in the late season with development determined by use of the Mean Stage Count (MSC) and Mean Stage Weight (MSW) system.

In the morphological development study, MSC and MSW were linearly related to both GDD and DOY for both years. ‘Liberty’ growth lagged behind ‘Shawnee’ throughout the whole growing season by approximately seven days. Prediction equations for MSC and MSW were developed based on accumulated GDD and DOY for Trafalgar and Roann in 2017. The prediction equations for MSC as predicted by GDD explained from 84 to 93 percent of the variation in MSC across locations for ‘Shawnee’ and between 90 to 94 percent of the variation for ‘Liberty’. For MSW, ‘Shawnee’ and ‘Liberty’ prediction equations explained from 84 to 93 percent and 90 to 95 percent of the variation as predicted by GDD across locations, respectively.

In the compositional quality study, samples from every other sampling date were ground and analyzed using near-infrared reflectance spectroscopy (NIRS). Increasing nitrogen fertilizer caused a higher nitrogen concentration at a given MSC. The 0 kg N ha^{-1} fertilizer rate dropped below 10 mg g^{-1} nitrogen by MSC 2.2, whereas the 134 kg N ha^{-1} fertilizer rate had greater than 10 mg g^{-1} until MSC 2.7. 'Liberty' had increased Neutral Detergent Fiber (NDF) concentration as compared to 'Shawnee'. For whole-plant samples, 'Liberty' averaged 727 mg g^{-1} NDF as compared to 'Shawnee' which averaged 718 mg g^{-1}. 'Liberty' had 18 mg g^{-1} higher acid detergent fiber (ADF), on average, as compared to 'Shawnee'. Acid Detergent Lignin (ADL) was not different among nitrogen fertilizer treatments. Stem-plus-sheath material accounted for a higher percentage of NDF, ADF, and ADL, in whole-plants as MSC increased, as compared to leaf blades. 'Shawnee' had higher IVDMD as compared to 'Liberty' and the biggest differences occurred around MSC 2.9. At MSC 2.9, 'Shawnee' whole-plant IVDMD was 448 mg g^{-1} and 'Liberty' whole-plant IVDMD was 430 mg g^{-1}. Whole-plant ash concentration decreased as MSC increased.

For the study that evaluated crop growth and yield, differences in grams m^{-2}, mass $tiller^{-1}$, and tiller number per unit area were analyzed in response to growing degree days (GDD) and day of year (DOY). Number of tillers had a negative linear response to GDD and DOY for both years, whereas, mass $tiller^{-1}$ had a positive linear response to GDD and DOY for both years. Grams m^{-2} responded quadratically to GDD and DOY. Generally, 'Liberty' had 20 percent higher mass $tiller^{-1}$ and lower number of tillers per m^{-2} at the end of the season as compared to 'Shawnee.' Addition of nitrogen fertilizer generally increased mass $tiller^{-1}$ and grams m^{-2}. Roann, the northern most site, also had highest tiller numbers at the beginning of the season and decreased faster than at the central Indiana sites. ‘Liberty’ yielded 8.8 percent higher than ‘Shawnee’ across locations, nitrogen rates, and sampling years. Addition of nitrogen fertilizer did not conclusively increase yield. Grams m^{-2}, mass $tiller^{-1}$, and tillers per sample area helped explain some yield differences. For example, ‘Liberty’ had increased yield as compared to ‘Shawnee’, and ‘Liberty’ also had higher mass $tiller^{-1}$ with no differences in tiller number between cultivars. While additions of nitrogen fertilizer increased grams per tiller, yield was not significantly increased with added nitrogen fertilizer. Therefore, these measures should not stand alone as a predictor of yield differences between cultivars. Switchgrass is a bunchgrass and has inherent difference in numbers of plant and tillers per plant within a plot, which may not be truly represented by one crop growth parameter alone.

This study confirms that switchgrass has great potential as a forage and biofuel crop in Indiana with low nitrogen fertilizer requirements and high yield. Understanding how switchgrass morphological development, compositional quality, growth, and yield responds in Indiana environments across locations, years, and nitrogen rates will help guide the future switchgrass management decisions of producers and researchers.

CHAPTER 1. INTRODUCTION

As a result of the 1970's oil shortage, the United States' general public became more sensitive to energy use and the country's dependency on foreign fuel supplies (Hohenstein et al., 1994). The U.S. Energy Information Administration projected global energy consumption would increase by 28 percent between 2015 and 2040 (IEO, 2017). Worldwide coal use is estimated to remain stable, while petroleum and other liquids will decrease by just two percent. In areas of the world where coal use is expected to decline, energy will be created from alternative sources including natural gas, renewable fuels, and nuclear power. In addition, growing global population is expected to reach ten billion by 2050, which leads to a 50 percent increase needed in food production (FAO 2017). In order to sustainably meet increasing energy demands and to feed a growing population, innovation in novel fuel sources must be researched.

There have been widespread and differing research interests into renewable and sustainable energy sources coming from herbaceous biomass. While initial interest in renewable fuels was based on woody crops, a program focused on herbaceous energy crops (HEC) research was started in 1984. The HEC program requested submissions of proposals to screen herbaceous crops for their ability to produce high biomass yield and have the composition to be converted into a renewable fuel source. Screening trials began in 1985 at Auburn, Cornell, Purdue, and Virginia Tech universities. At the conclusion of these screening trials, switchgrass (*Panicum virgatum* L.) was selected as the single "model" crop species in 1991 (Wright et al., 2010).

1.1 Selection of Switchgrass as a Bioenergy Crop

Switchgrass is a perennial, C_4 grass commonly found across North America, and was a dominant species in the native prairie. Switchgrass was commonly found from Central America to Southern Canada and as far west as Arizona and Nevada (Hitchcock et al., 1935). Selecting switchgrass as a model HEC occurred for many reasons. It can be established from seed and has high dry matter yields, genetic variability, and positive environmental attributes (Wright 1992). Initial trials at Purdue University showed yields from 10 to 15.2 Mg ha^{-1} (Cherney et al., 1990). Switchgrass has environmental versatility as it was native to North America and found across the country in varying environments (Wright et al., 1992). Thus, it has adapted different ecotypes which have individual characteristics such as drought resistance, high yield, or winterhardiness. This provided a wide genetic selection for futuristic breeding improvement efforts. In addition, it could be grown on marginal lands with limited fertilizer and pesticide inputs (Wright et al., 1992). Due to its ability to grow on marginal lands, switchgrass grown as a HEC will not need to compete with crops grown on prime farmland for food production.

After the initial screening trials, breeding efforts were started to develop a switchgrass cultivar that combined the best attributes of the varying ecotypes. Switchgrass populations can be grouped into two main ecotype groups, “lowland” and “upland” (Porter et al., 1966). Lowland ecotypes are generally more sensitive to moisture stress, have taller and coarser stems, and larger panicles than upland ecotypes (Porter et al., 1966). Ploidy levels vary within switchgrass ecotypes. All lowland ecotypes have been found to be tetraploid, while upland ecotypes can be tetraploid, hexaploid, or octoploid (Hopkins et al., 1996). As a result of breeding efforts that took place at the University of Nebraska’s Agricultural Research and Development Center, ‘Liberty’ was developed as an improved variety through a paired plant crossing with an upland parent

'Summer' and a lowland parent 'Kanlow'. 'Liberty' was found to have up to 40 percent greater yields and could maintain stand persistence (Vogel et al., 2014).

1.2 History of Switchgrass as a Forage

While it has become a model HEC species, switchgrass was first used by wildlife for grazing and shelter, then by humans as a forage for grazing livestock. Early research focused on increasing the forage value and yield of switchgrass (Anderson et al., 2000). Consequently, 'Shawnee' switchgrass was developed from the selection of high in vitro dry matter digestibility (IVDMD) characteristics from the cultivar 'Cave-in-Rock'. Shawnee was an important contribution to using switchgrass as a forage due to its increased forage quality, as measure by IVDMD, and its increased yield (Vogel, 1996). Current research for utilizing switchgrass as a forage includes incorporating legumes into the stand to determine yield response, and evaluating the belowground metabolism in rhizomes (Jakubowski et al., 2017; Palmer et al., 2017).

The economics of systems that combine both grazing and harvesting for bioenergy have been recently modeled (Biermaker et al., 2017). Producers' perceived economic decisions are heavily based on the prices paid for bioenergy crops or cattle. Biermaker et al (2017) concluded that if farmers were offered prices for biomass from \$55 to \$82 Mg^{-1}, a combination of grazing and bioenergy harvest would be economically ideal; however, if prices were to go above \$110 Mg^{-1}, harvesting only for bioenergy would be chosen. Conversely, if prices were \$0 Mg^{-1}, farmers would choose to graze only.

1.3 Morphological Development

Moore et al. (1991) developed a system to quantify morphological growth stages of perennial forage grasses. This system defines five major stages to grass development: germination, vegetative, elongation, reproductive, and seed ripening. Each of these stages have substages that pertain to development that occurs within each stage. The germination, reproductive, and seed ripening stages each have five substages to quantify growth, while vegetative and elongation stages have no set number of substages in order to account for differences in number of leaves or nodes usually accumulated by different species. Utilizing a numerical index to quantify growth allows statistical analysis to be conducted; additionally, it allows for growth to be predicted by a model. Once growth is documented, numerical indices can be applied by utilizing equations to calculate either Mean Stage Count (MSC) or Mean Stage Weight (MSW). Mean Stage Count is based on the mean growth of a sample based on the number of tillers in each stage and in the whole sample; whereas, MSW is the mean growth of a sample based on the dry weight of the tillers in each stage and the dry weight of the whole sample (Moore et al., 1991). MSC and MSW can range from 0.0 (dry seed) to 4.9 (endosperm is dry).

Development of equations to predict MSC and MSW in switchgrass has been accomplished in Nebraska (Moore et al., 1997). Switchgrass development can be predicted by a linear model based on Growing Degree Days (GDD) or Day of Year (DOY). Models that utilized GDD and DOY explained 94 percent and 98 percent of the variation in development, respectively, and had low root mean square error (RMSE) values. GDD prediction for switchgrass utilizes a base temperature of 10 °C, while DOY predictions is based on the number of days passed since January 1. Breaking dormancy and vegetative development are closely related to growing degree days, while reproductive development is more closely related to DOY, which suggests a photoperiod effect (Sanderson et al., 1995). 'Alamo' switchgrass exhibited inflorescence emergence and reproductive development around the same day each year independent of rainfall or temperature (Sanderson et al., 1995).

In addition to prediction of MSC and MSW, Mitchell et al. (2001) developed equations to predict forage quality based on GDD, DOY, MSC, or MSW. Prediction of forage quality can be useful to guide harvest management decision, and can be accomplished by understanding the relationship of plant maturity to quality characteristics (Mitchell et al., 2001; Kalu et al., 1983; Moore et al., 1995). In vitro digestible dry matter (IVDDM) and crude protein (CP) were best predicted by GDD equations, with 86 percent of the variation accounted for in those models. Crude protein was reported to be well predicted by DOY, MSC, and MSW as well, indicating that it follows a predictable pattern during a growing season. In addition, NDF concentration were best predicted by MSC and MSW, which accounted for 82 and 83 percent of the variability, respectively. Overall, the authors concluded that MSC and MSW are affected by many environmental factors; therefore, it may be best to use caution in relying solely on a predicted MSC and MSW to infer forage quality (Mitchell et al., 2001).

In addition to MSC and MSW, research tracking tiller numbers over a season can give insight to canopy changes because of the differences in environmental events that year. Canopy architecture of a grass sward has been reported as a function of tiller morphology depending on the developmental stage of the tillers within the sward (Nelson et al., 1995). A grass sward's canopy can directly relate to a plant's physiological response, light interception, and consequently, the yield realized from the stand (Nelson et al., 1994). Most of the dry matter yield of a grass comes from reproductive tillers, while a small portion comes from the vegetative tillers. As a result, increased yield is realized from grasses that have an increased number of reproductive tillers (Kalmbacher et al., 1983). It has been reported that 65 percent of the total aboveground biomass is found in the stem, leaf sheath, and inflorescence (Lemus et al., 2002). Mitchell et al. (1997) evaluated tiller demographics over a season and reported that tiller number m^{-2} decreased as stands matured. While information for wide geographical areas is available for switchgrass production as either forage or biomass, more local information will be key to developing a successful HEC production system (Aurangzaib et al., 2015).

1.4 Evaluating Switchgrass Quality with Compositional Analysis

1.4.1 Conversion of Switchgrass Biomass to Energy

Ethanol can be produced from switchgrass via two different routes established for lignocellulosic crops: biochemical and thermochemical conversion (Demirbas et al., 2007). The first goal is similar in each process as cellular components must be broken down into intermediates which will can eventually be converted to ethanol (Mu et al., 2010).

1.4.2 Biochemical Conversion

The first step in biochemical conversion of biomass is a pretreatment step that breaks down the plants into three components: cellulose, hemicellulose, and lignin. These components are then broken down into simple sugars via hydrolysis. Lignin cannot be broken down during hydrolysis, but it can be extracted and combusted to generate heat and electricity to fuel the conversion process (Mu et al., 2010). Some research also suggests that lignin may be gasified prior to fermentation (Datar et al., 2004). The simple sugars from hydrolysis are then fermented for several days and finally distilled to ethanol. Hydrolysis and fermentation can occur concurrently in a process called saccharification and fermentation (SSF; Takagi et al., 1977). This process utilizes enzymes instead of chemicals and can decrease the cost of equipment because only one reactor is needed and it does not need to be resistant to strong acids (Wright et al., 1988).

According to an interactive map from Ethanol Producer Magazine (2018), there is only one ethanol biorefinery in the United States that is currently able to utilize switchgrass. This facility is the "ICM Inc. Pilot Integrated Cellulosic Biorefinery" located in St. Joseph, MO. This refinery utilizes a variation of biochemical conversion which includes a pre-treatment, enzymatic hydrolysis, and co-fermentation to create fuel ethanol and other by-products (US DOE, 2012). Several pre-treatment options were studied by Smullen et al. (2017), and it was concluded that methanol resulted in the highest conversion yields following SSF. It was reported that SSF was a necessary addition due to an increased conversion rate and reduced inhibitor formation (Smullen et al., 2017).

1.4.3 Thermochemical Conversion

In thermochemical conversion there are two main methods to convert biomass to ethanol, gasification and pyrolysis (Mu et al., 2010). Pyrolysis is a thermochemical conversion that occurs in the absence of oxygen (Hornung, 2014). Fast pyrolysis is the most widely suggested method for switchgrass conversion and it occurs at temperatures from 400°C to 500°C (Mante, 2011). This process yields char, condensable gasses, and non-condensable gasses including H_2, CO, and CO_2 (Boateng et al., 2006). Some of the resulting non-condensable gases can be captured as syngas, which is a mixture of primarily hydrogen and carbon monoxide. Syngas can be converted to ethanol or synthetic natural gas by utilizing catalysts. Condensable gases are captured as pyrolytic oils and can be used as a heating oil or further processed into hydrocarbon fuels (Boateng et al., 2006). Gasification is a similar process to pyrolysis, but differs in the temperature used during the reaction (Mante, 2011). Gasification occuring between 800°C and 1000°C and results in higher amounts of non-condensable gas products which can be utilized as a synthetic natural gas; whereas, utilizing a higher temperature of 1200°C to 1400 °C results in a higher amount of syngas production (Boateng et al., 2006, Mante, 2011). A life cycle assessment by Mu et al. (2010) concluded that environmentally and technically, thermochemical conversion of biomass may be preferred over biochemical conversion due to its decreased fresh water consumption and its lesser use of chemical additives.

1.4.4 Effects of Minerals on Thermochemical Conversion

Thermochemical conversion may be more sensitive to levels of certain elements as compared to biochemical conversion. Total biomass yield, therefore, is a primary goal with thermochemical conversion, but quality of the biomass is also an important consideration. Excess of some elements can cause corrosion or fouling of the boiler and other components at the power plant, which decreases efficiency and increases labor (Miles et al., 1996). Obernberger et al. (2006) released guidelines for recommended allowance of certain undesirable elements for thermochemical conversion of switchgrass. On a dry basis, nitrogen (N) should be less than 0.6 mg kg^{-1}, chloride (Cl) less than 0.1 mg kg^{-1}, and sulfur (S) less than 0.1 mg kg^{-1}. N can lead to NO_x emission, Cl can lead to corrosion, and S can lead to SO_x emission. Additional attention should be given to elements such as P, K, Fe, Ca, Mg, and Na due to their ability to lower the overall melting temperature, which would lead to slagging and a decrease in efficiency and longevity of the conversion system (Pronobis et al., 2005). Profitability of a HEC system will depend on producing a high yielding crop with high concentrations of cellulose and hemicellulose, but with low levels of water, N, and ash (McKendry et al., 2002). Delaying harvest of switchgrass until after senescence occurs can help decrease undesirable mineral elements, due to the natural leaching of these materials from the plant residue, but it can also cause a reduction in total biomass yield (Christian et al., 2002; McLaughlin et al., 1996; Casler et al., 2003).

1.4.5 Thermal Conversion

A final method for utilization of switchgrass to produce energy is a thermal process where biomass is directly co-fired with coal. When compared to crop residues like wheat straw or corn stover, switchgrass is more ideal for direct combustion due to its lower ash and higher energy content (Mani et al., 2004). A few pilot co-fire tests have occured (Amos et al., 2002; Southern Research Institute, 2001). It is estimated that up to 20 percent switchgrass could be added with coal during the co-firing process, but it must be combined and pulverized with coal prior to combustion (Southern Research Institute, 2001). If switchgrass was not pulverized prior to use, as little as five percent would block the flow of the coal bunkers. During switchgrass-coal co-fire tests, it was reported that no unusual fouling or slagging could be linked to the switchgrass at the conclusion of the trial (Amos et al., 2002; Southern Research Institute, 2001).

1.4.6 Forage Quality

For a producer wanting to utilize switchgrass primarily as a forage, nutritive value of the forage will be of high importance. Nutritive value of the forage directly relates to the animal's performance. The most widely used parameters in evaluating forage quality for research purposes include: In vitro dry matter digestibility (IVDMD), acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein (CP; Kering et al., 2013). In the case of cattle, a producer is primarily concerned with supplying energy, protein, water, vitamins, and minerals (Lemenager, 2011). Generally, vitamins and minerals can be met with a good commercial supplement, and fresh water should be accessible at all times. Common parameters given by a forage test, such as ADF, NDF, and CP, can help producers decide on how or if they need to supplement their animal's diet with higher quality forages or concentrates (Lemenager, 2011). An animal's dry matter intake (DMI) can be predicted using NDF, while digestibility can be predicted using ADF. ADF can be utilized to estimate net energy of maintenance (NE_m), lactation (NE_l), or growth (NE_g; Buckmaster, 2011). In practice, these values are most often from sampled hay or silage samples and less often from pasture. Continuing with cattle as an example, minimum quality forage for maintenance should contain 80-100 g kg^{-1} CP, 430 - 450 g kg^{-1} ADF, and 610 -650 g kg^{-1} NDF (Forage Field Guide).

Switchgrass has been previously researched as a hay and pasture grass (Sanderson et al., 2010; Biermacher et al., 2017; Guretzky et al., 2011). Biermacher et al. (2017) evaluated the potential use of switchgrass as a pasture or as a pasture/bioenergy harvest rotation for stocker cattle in south-central Oklahoma, where producers generally have a gap in forage production between late April and late June. Systems that graze and then harvest for biomass can result in up to 35 percent less total biomass yield. The crude protein of the pasture ranged from 150 g kg^{-1} before grazing started in mid-April to 52 g kg^{-1} at physiological maturity. ADF concentration increased from 320 to 430 g kg^{-1} and NDF increased from 590 to 770 g kg^{-1} (Mosali et al., 2013). In south-central Oklahoma, switchgrass as a pasture has potential to adequately meet stocker calves requirements. Calves gained 0.83 kg day^{-1} hd^{-1} on the lowest stocking density to 1.05 kg day^{-1} hd^{-1} on the highest stocking density (Mosali et al., 2013). While it may seem counter-intuitive that the lowest stocking rate had the lower rate of gain, one of the explanations is that the cattle were unable to graze the switchgrass sward efficiently, so more tillers were able to mature. Thus, the increased maturity caused a decrease in the feed value of that sward. At a higher stocking density rate, the cattle were able to graze the switchgrass more efficiently and keep it at a vegetative state (Mosali et al., 2013).

Sanderson et al. (2010) compared upland cultivars in an intake and digestion trial for sheep in Pennsylvania. The cultivars were evaluated using a single-cut system where harvest occured in late June. The tillers were in the late vegetative to early boot stage. Nutritive value of the hays did not differ among cultivars. CP ranged from 92-97 g kg^{-1}, ADF from 364-380 g kg^{-1}, and NDF from 692-697 g kg^{-1}. A two-cut system was also analyzed for its impact on forage quality. First-cut switchgrass hays (June 16) had ranges from 137-144 g kg^{-1} for CP, 342-372 g kg^{-1} for ADF, and 704-734 g kg^{-1} for NDF. Second cut hays (August 8) had ranges from 115 to 131 g kg^{-1} for CP, 367 to 383 g kg^{-1} for ADF, and 717 to 735 g kg^{-1} for NDF. A three-cut system was reported to give 11 to 24 percent higher dry matter yield, higher CP, and lower NDF than the two-cut system (Sanderson, 2008). Overall, switchgrass as a hay can be utilized in different cutting schedules and has potential to adequately meet the nutritional requirements of sheep (Umberger et al., 2009).

There has been little research outlining the use of switchgrass as a silage, but Huntington (2007) compared morning versus afternoon harvesting of switchgrass and gamagrass as baleage. Afternoon harvest was taken on August 5, and morning harvest was taken August 6. Afternoon harvest of switchgrass resulted in greater DM and in vitro true dry matter disappearance (IVTDMD), and less CP, NDF, and ADF. From morning to evening, CP decreased from 98 to 95 g kg^{-1}, ADF decreased from 425 to 411 g kg^{-1}, and NDF decreased from 718 to 706 g kg^{-1}.

Nutritional quality of the baleage from this study was lower than studies that evaluated use as a pasture and hay above, which is likely due to the differences in harvest dates. Steers were reported to have gained, on average, 0.40 kg/d. Switchgrass as a baleage increases the non-protein nitrogen(NPN) load on the animal, so an animal would need more readily fermentable dietary energy to support conversion of the NPN to microbial protein (Huntington et al., 2007).

1.5 Seasonal Crop Growth

From the early 1900's, various measures of crop growth have been introduced and used in research (Blackman, 1919; Radford, 1967). One early study focused on the growth of annual plants and how their growth rate may be compared to that of a continuous interest formula used in economics (Blackman, 1919). Radford (1967) explained the different growth rate variables and their mathematical strengths and weaknesses, so that future research may proceed effectively.

Of particular interest to switchgrass growth is the overall 'Crop Growth Rate' (CGR), which is defined as the change in plant weight divided by change in time (Radford, 1967). By utilizing calculus, a mean crop growth rate can be found for a grass sward over time. The mean crop growth rate can be expressed as an equation that relates to a line or smooth curve which represents the average growth change over time (Radford, 1967). Net Assimilation Rate (NAR), Relative Growth Rate (RGR), and Leaf Area Ratio (LAR) are additional measures that can be used to evaluate plant growth over time. NAR is defined as 'the increase of plant material per unit of assimilatory material per unit of time" (Radford, 1967; Blackman, 1919). Relative Growth Rate is defined as the increase of plant material per unit of material present per unit of time’ (Radford, 1967; Blackman, 1919). Additionally, LAR can be defined as ‘the ratio of the assimilatory material per unit of plant material present’ (Radford, 1967; Blackman, 1919).

While many studies have analyzed growth rates for a variety of crop species, there is not a multitude of published research describing the growth rates of switchgrass in field environments at differing N rates and between two diverse cultivars (Giannoulis 2016; Kephart 1971; Perry, 1975; Na, 2015). One study evaluated different cultivars of switchgrass for their seedling NAR, RGR, and LAR to determine differences in seedling growth rates among the cultivars to determine which characteristics may help a seedling compete post-emergence with surrounding plants (Perry, 1975). In this study, switchgrass was planted and studied over ten weeks in a growth chamber. While no significant differences were found between NAR and RGR, there were cultivar differences in LAR, which would suggest that the cultivar with the higher LAR would be more competitive post-emergence in the field (Perry, 1975). In Iowa, a study compared the LAR, CGR, and dry weights of leaf and stem under different irradiance treatments (Kephart, 1971). Increased LAR was found to occur in low irradiance, but the weight per leaf decreased, meaning that while the plants had more leaves the mass per leaf decreased. Switchgrass had the highest crop growth rate of the species studied, and it increased linearly with increasing levels of irradiance. Additionally, unlike the tall fescue, reed canarygrass, deertongue grass, and big bluestem studied, switchgrass was found to respond to irradiance levels by changing the weight of leaf and stem components (Kephart, 1971).

Switchgrass growth has also been studied internationally (Giannoulis, 2016; Jeke, 2016). A study that occurred in Greece evaluated switchgrass in a field experiment for its changes to dry matter partitioning of leaf, stem, or flower over two years under both irrigation and no irrigation (Giannoulis, 2016). It was concluded that in the third year of growth the dry matter partitioned towards leaf stem, or flower remained consistent at a level of seventy percent, twenty percent, and ten percent, respectively. Additionally, leaf area was significantly higher for those plants receiving irrigation (Giannoulis, 2016). In Canada, aboveground and belowground biomass growth rates were studied in a controlled environment growth room after being treated with biosolids (Jeke, 2016). It was determined that a three-parameter logistic model was most accurate in describing the growth of the above and belowground biomass. Additionally, a longer lag in biomass accumulation was seen for the belowground portion. Belowground biomass reached peak growth rates 19 days later than the aboveground biomass peak growth rates.

1.6 Nitrogen Fertilization and Management Factors Impact on Yield

Establishment of a stand can take between two and three years to reach maximum yields; however, under adequate management, a stand can last longer than ten years (Perlack et al., 2011). Stand success is impacted by selecting an appropriate cultivar, soil fertility, climate conditions, and harvest management (Casler et al., 2004; Casler et al., 2005). In cultivar selection, it is important to select a cultivar that is adapted to the climate and latitude of the selected field. Especially in areas of the country that experience more harsh winter weather, perennial grasses must reach adequate dormancy before freezing temperatures occur. Some lowland ecotypes may not be adjusted to the photoperiod differences in the northern latitude; thus, they may not reach dormancy or have adequate amounts of metabolites stored before a freeze (Casler et al., 2004; Casler et al., 2005).

Harvest management is critical to maintaining stands of switchgrass for both forage and biomass systems. While forage-livestock systems harvest to increase nutritive value, biomass harvests are generally targeted towards highest economical yield (Mitchell et al., 2010). Switchgrass can be harvested for biomass with existing forage equipment and can be baled using traditional hay-making practices. When managing for maximum yield in a biomass production system, switchgrass harvested during the late reproductive phase is ideal (Mitchell et al., 2010). In the Midwest, switchgrass reaches maximum yields in mid-August at the full panicle emergence to post-anthesis stage of development, and yields decreased up to 20 percent when harvested after a killing freeze (Vogel, 2002a). While harvesting switchgrass during late summer to early autumn may maximize yield, it could lead to a decline in stand longevity (Casler et al., 2003; Mitchell et al., 2010). In addition, delayed harvest could increase the amount of ash found in the feedstock (Casler et al., 2003).

Vogel et al. (2002a) evaluated yield and harvest management for switchgrass in the Midwest. Maximum first cut yields were found when plants have fully emerged peduncles to post anthesis, which usually occurs in the beginning of August. This harvest timing could be advantageous because it will occur before fall grain harvest for Midwest farmers. Harvesting after a killing freeze may result in lower yields, but could allow the plant to translocate N back to its crown and roots. Thus, N fertilization could be lower in subsequent years under a delayed harvest routine than when switchgrass is cut earlier in the season (Vogel et al., 2002a).

Switchgrass yield has been reported to range from as low as 1 Mg ha^{-1} to as high as 40 Mg ha^{-1} (Wullschleger et al., 2010). Across differing soils and environment, switchgrass generally yields from 10 to 14 Mg ha^{-1}. There have been considerable differences in yield between upland and lowland ecotypes, but also between cultivars within each ecotype. Generally, ‘Alamo’ and

'Kanlow' cultivars are the most reported and highest yielding lowland ecotypes, while 'Cave-in-Rock' has the most observations and higher yields among the upland cultivars (Wullschleger et al., 2010). Biomass yield generally increases with temperature up to a limit, before decreasing. While temperature during the growing season is important, switchgrass may be more sensitive to spring temperatures especially in environments where the growing season is shorter and experiences cooler average temperatures (e.g. Canada and North Dakota; Wullschleger et al., 2010; Berdahl et al., 2005; Madakadze et al., 1998a). Madakadze et al. (1998b) reported that plants broke winter dormancy up to 35 days earlier when a warmer spring occurred. Earlier spring growth and warmer springs may allow for extra growing days, and thus increases in biomass yield. Finally, average temperatures experienced during winter can affect stand persistance, especially with lowland cultivars, due to the variability in winter dormancy and survival (Vogel et al., 2002b).

In addition to selecting the correct cultivar, soil fertility is important to switchgrass production. N application is not recommended in the first year to decrease weed competition and cost in the seeding year. N management can be difficult for grasses due to the flux of N that can be expected from the soil each year depending on moisture and temperature (Brejda et al., 2000). Due to these naturally occurring N fluctuations, switchgrass response to N fertilization can vary greatly between sites and years. Brejda et al. (2000) reported that across many studies and environments, N response in switchgrass has resulted from 0 to 7.0 Mg ha^{-1} increases in yield when compared to a 0 N control. During forage production, haying results in higher N removal rates than grazing, meaning switchgrass stands managed as a hay would require higher N fertilizer (Brejda et al., 2000). Localized N recommendations should be based on average growing days and annual precipitation, but independent of location, and it is recommended that N be applied after initial green up to reduce stimulation of cool-season grass competition and to prevent N losses (Bredja et al., 2000). Vogel, et al. (2002a) recommended applying 10 to 12 kg N ha^{-1} for each Mg of biomass production expected. Wullschleger et al. (2010) compiled research from multiple studies completed in the United States and reported that for lowland cultivars a N response could be seen up to 100 kg ha^{-1}, but in many cases the 0 kg ha^{-1} rate performed just as well. Upland ecotypes responded similarly with increasing yield up to around 100 kg N ha^{-1} and then began to decline at higher levels (Wullschleger et al., 2010). While most crops require phosphorus and potassium fertilizer, it has been documented that switchgrass is able to successfully grow in P and K limited soils (Bredja et al., 2000; Mitchell et al., 2010; Woodson et al., 2011).

1.7 Objectives of Research

Previous research has shown promise for prediction of switchgrass growth by utilizing GDD and DOY methods (Moore et al., 1997; Sanderson et al., 1995). In addition, switchgrass compositional quality has shown potential for prediction by use of GDD. This would give producers a guide line to harvest so that quality of the feedstock for either forage or biomass may be optimized (Mitchell et al., 2001). Furthermore, multiple studies have shown varied responses to N fertilizer (Bredja et al., 2000; Wullschleger et al., 2010). Most of the previously mentioned studies have taken place in the Great Plains. Due to the regional climate differences in the United States, future use of prediction equations by producers would perform best when regionally developed equations are utilized (Mitchell et al., 2001). Additionally, the newly released variety ‘Liberty’, which was bred for increased biomass production and winterhardiness, has not had growth and development extensively compared to other cultivars in an Indiana environment.

Our objectives were to compare the differences in growth of ‘Liberty’ and ‘Shawnee’ at different locations within Indiana and how their growth and compositional quality may respond to N fertilizer, GDD, and DOY. It is hypothesized that ‘Liberty’ will remain vegetative longer than ‘Shawnee’, and that ‘Liberty’ will have improved quality characteristics as a biofuel and lesser value as a forage as compared to ‘Shawnee’. Furthermore, it is hypothesized that both cultivars increase yield as N fertilizer application increased for both cultivars. Overall by utilizing our data, prediction equations for growth and quality in an Indiana climate can be made for future use by forage and biomass producers to make management decisions for their crop.

CHAPTER 2. DEVELOPMENTAL MORPHOLOGY OF TWO DIVERSE SWITCHGRASS CULTIVARS RECEIVING DIFFERENT RATES OF NITROGEN FERTILIZER IN INDIANA

2.1 Abstract

Switchgrass (*Panicum virgatum* L.) is an important warm-season perennial grass in livestock systems and has been extensively researched as an herbaceous energy crop. The objectives of this study were to compare the morphological development of a recently developed biofuel cultivar ‘Liberty’ to an improved forage cultivar ‘Shawnee’ in multiple Indiana environments, and to predict morphological development in response to growing degree days (GDD) and day of year (DOY). Switchgrass growth varies across environments. Thus, to accurately predict morphology, locally developed prediction equations are needed. Pure stands of each cultivar were sampled in the field at Trafalgar and Roann, Indiana in 2016. Samples were collected weekly during the early season and every other week in the late season with growth determined by use of the Mean Stage Count (MSC) and Mean Stage Weight (MSW) system. In 2017, samples were collected at Trafalgar, Roann, and Lafayette, Indiana. Prediction equations for MSC and MSW were developed based on accumulated GDD and DOY for Trafalgar and Roann in 2017. The equations were validated using 2016 data at Trafalgar and Roann, and 2017 data at Lafayette. MSC and MSW were linearly related to both GDD and DOY for both years. ‘Liberty’ growth lagged behind ‘Shawnee’ throughout the whole growing season by approximately seven days. Additionally, the northern location, Roann, accumulated less GDD, but morphological development progressed more quickly than central Indiana locations, Trafalgar and Lafayette. The validation equations for MSC as predicted by GDD explained from 84 to 93 percent of the variation in MSC across locations for ‘Shawnee’ and between 90 to 94 percent of the variation

for ‘Liberty’. For MSW, ‘Shawnee’ and ‘Liberty’ validation equations explained from 84 to 93 percent and 90 to 95 percent of the variation as predicted by GDD across locations, respectively. Morphological development in Indiana can be accurately predicted using either GDD or DOY equations, and neither GDD or DOY was more accurate than the other.

2.2 Introduction

Switchgrass morphology has been studied to understand how a grass sward matures over a season, and how that may relate to yield and forage quality composition (Mitchell et al., 2001; Kalu et al., 1983; Moore et al., 1995; Nelson et al., 1994). Recent breeding efforts between an upland and lowland cultivar resulted in the new cultivar, ‘Liberty’, which has increased yield and persistence (Vogel et al., 2014). The morphological development system, as described by Moore et al. (1991), can be used to evaluate growth differences between ‘Liberty’ and the forage cultivar ‘Shawnee’. These growth differences may explain the previously documented yield increase for ‘Liberty’ over ‘Shawnee’. While a previous study has developed prediction equations for switchgrass morphology, the study was in Nebraska where the environmental factors of GDD and rainfall are different than in Indiana (Mitchell et al., 1997). In 1999, Sanderson et al. tested the equations developed in Nebraska in Texas, and concluded that due to differences in environments, prediction equations specific to the local environment were important to creating accurate predictions. Thus, for widespread future use to be realistic, locally developed equations will be critical to accurately predict switchgrass morphology. Overall, by utilizing the morphological development measures, MSC and MSW, plant maturity can be modeled in response to either temperature or daylength by comparing morphology to GDD or DOY, respectively. By creating local prediction equations, future switchgrass growth modeling can be more reliable due to more prediction equations from different environments available in the literature. These models can also be used by producers making management decisions based on the maturity of their crop.

Our objective was to compare and predict differences in morphological development of ‘Liberty’ and ‘Shawnee’ at different locations within Indiana and how they may respond to N fertilizer, GDD, and DOY. Our hypotheses were that ‘Liberty’ morphological development would be slower as compared to ‘Shawnee’ across DOY and GDD measures, growth and development would advance more quickly at higher N rates, and that the Roann location would have a lower morphology index on a given DOY as compared to Trafalgar.

2.3 Materials and Methods

To determine and predict morphological development of ‘Shawnee’ and ‘Liberty’, field studies were conducted in 2016 and 2017. In 2012, plots of ‘Liberty’ and ‘Shawnee’ were seeded near Roann, IN (40° 54’ 02.6” N, 85° 57’ 48.4” W) on a Martinsville sandy loam soil (fine-loamy, mixed, active, mesic Typic Hapludalfs). In 2013, plots of ‘Liberty’ and ‘Shawnee’ cultivars were seeded at the Indiana FFA Leadership Center located near Trafalgar, IN (39° 22’ 25.6” N, 86° 07’ 21.6” W) on a Fincastle silt loam soil (fine-silty, mixed, superactive, mesic Aeric Epiaqualfs). Cultivars were planted at a rate of nine kg of pure live seed hectare^{-1}, and no fertilizer was added in the seeding year. The experimental design was a randomized complete block design, where whole plots were cultivar and split plots were N rate. Whole plot dimensions were 42 by 24 meters and 30 by 24 meters at Roann and Trafalgar, respectively. There were two replications at each location. N, in the form of urea (46-0-0), was applied annually in mid-May at three differing rates: 0, 67, and 134 kg ha^{-1}. In the seeding year, quinclorac (3, 7-dichloro-8-quinolinecarboxylic acid) herbicide was applied at a rate of 435 g active ingredient (a.i.) ha^{-1}, and Atrazine 4L (6-Chloro-N-ethyl-N′-(1-methylethyl)-1,3,5-triazine-2,4-diamine) was applied at a rate of 970 g (a.i.) ha^{-1}. In 2016 at Roann, potassium fertilizer, in the form of potassium chloride (0-0-60), was applied at a rate of 48 kg K ha^{-1}. Soil tests taken in the spring of 2016 indicated low potassium levels at Roann, but not at Trafalgar. Samples were harvested weekly (Appendix Table 1) from mid-May until mid-August, and then every other week until the conclusion of seed ripening. Samples were harvested by clipping tillers at the ground level from two randomly placed 0.09 m^2 quadrats in each split plot for each replication. After clipping, tillers were stored on ice during transport and in a cooler at 1.7 °C before processing. After processing, all samples were weighed and then dried in an oven at 60°C. Dry weight measurements were taken for all samples.

In 2016, each sample from each split plot was evenly divided into thirds, with one - third being processed for morphological development. The other two-thirds were used for studies reported in Chapter 3 and 4. In 2017, samples were only taken from the 67 kg N ha^{-1} plots because no differences in morphological development were found among N rates in 2016. Additionally, 2017 samples were split in half, with one-half being used for morphological development and one-half for other measurement parameters.

In 2017, a third location, the Throckmorton Purdue Agricultural Center located near Lafayette, IN (40° 17’ 43.4” N, 86° 53’ 41.9” W) was sampled for validation of prediction equations with the same sampling method as the previous locations. The Lafayette location was seeded on a Toronto-Millbrook complex soil (Fine, mixed, superactive, mesic Udollic Epiaqualfs) and an Octagon silt loam (Fine-loamy, mixed, active, mesic Mollic Oxyaquic Hapludalfs) for a cultivar trial in 2014. Border plots (1.2 m X 4.6 m) of ‘Shawnee’ and ‘Liberty’ were sown in four replications with ‘Shawnee’ planted on the eastern border and ‘Liberty’ on the western border. Herbicide was applied after planting, but before switchgrass emergence. Plots were sprayed with a tank mixture of 420 g a.i. ha^{-1} quinclorac (3, 7-dichloro-8-quinolinecarboxylic acid), 970 g a.i. ha^{-1} atrazine (6-Chloro-N-ethyl-N′-(1-methylethyl)-1,3,5-triazine-2,4-diamine), and 1.8 liters per hectare of methylated seed oil (MSO) adjuvant. No fertilizer was added in the seeding year, but N fertilizer, in the form of urea (46-0-0) was applied at a rate of 112 kg N ha^{-1} annually in mid-May starting in 2015. Sampling at Lafayette was done using the same method as described for Roann and Trafalgar, but only one $0.09m^2$ sample was taken per replication. Thus, for 2017 the total number of samples were the same for all sites for each harvest date, but Lafayette had four replications with one sample each, as compared to the other locations which had two replications and 2 subsamples from each replicate.

Morphological development was scored according to the system developed by Moore et al. (1991). Accumulated Growing Degree Days were calculated using a base temperature of 10°C from January 1 and were downloaded from the cli-MATE database hosted by the Midwest Regional Climate Center (https://mrcc.illinois.edu/CLIMATE/). Day of year was calculated from January 1. The weather stations were located 13 km and 17 km from the plots at Trafalgar and Roann, respectively. The Lafayette weather station was located on site, but not directly adjacent to the plots.

To calculate MSC and MSW, understanding normal morphological development is necessary to calculate indices for vegetation and elongation stages. Switchgrass plants can accumulate different numbers of leaves or nodes in different environments before progressing to the next stage (Moore et al., 1991; Table 2.1). Reproductive and seed development stages occur the same across all environments, so the index stages for these do not vary based on local variation. For all three Indiana locations, vegetative development occurred until the fourth leaf collar emerged. Thereafter, elongation began until the eighth node was palpable, and then the plant started reproductive development. To calculate MSC for vegetative and elongation stages N=4 and N=8 were used, respectively. MSC was calculated by summing the product of each stage index times the number of tillers within the stage, then dividing by the total number of tillers (Moore et al., 1991). MSW was calculated by summing the product of each stage index times the dry weight of tillers within the stage, then dividing by the total dry weight of tillers (Moore et al., 1991).

Statistical analysis was completed using mixed models procedure in SAS 9.4 using the restricted maximum likelihood method. Fixed categorical variables were location, cultivar, N rate (2016), and each replication within location was considered random. GDD and DOY were continuous variables. All two- and three-way interactions between the fixed, random, and continuous variables were included in the model. The dependent variable was either MSC or MSW. Variables were considered significant when $P < 0.05$.

Using the Bayesian Information Criterion (BIC), based on published work by Gideon Schwarz in 1978, it was determined whether to use a polynomial or linear model. The BIC assesses the model fit by comparing the total fit to the model to the total number of variables in the model, and penalizes for increased variable number (Schwarz, 1978). This is unlike the R-squared criteria, which usually increases with each additional variable added (Schwarz, 1978). Polynomial relationships were considered for each model, where the continuous variables acted as the slope predictor. The polynomial or linear model was selected by which model produced the lowest BIC when the polynomial term and all its associated 2- and 3-way interactions were included. Calibration equations (Appendix Table 2) were calculated for all data in 2016 and 2017.

Table 2.1 The numerical indices and corresponding descriptions for staging the growth and development of perennial grasses as described by Moore et al. (1991).

Stage	Index	Description
		Vegetative - leaf development
Ve or V0	1.0	Emergence of first leaf
V1	(1/N) + .9	First leaf collared
V2	(2/N) + .9	Second leaf collared
Vn	(n/N) + .9	N^{th} leaf collared
		Elongation - stem elongation
E0	2.0	Onset of stem elongation
E1	(1/N) + .9	First node palpable/visible
E2	(2/N) + .9	Second node palpable/visible
En	(n/N) + .9	N^{th} node palpable/visible
		Reprodutive - floral development
R0	3	Boot Stage
R1	3.1	Inflorescence emergence/first spikelet visible
R2	3.3	Spikelets fully emerged/peduncle not emerged
R3	3.5	Inflorescence emerged/peduncle fully elongated
R4	3.7	Anther emergence/anthesis
R5	3.9	Post-anthesis/fertilization
		Seed development and ripening
S0	4	Caryopsis visible
S1	4.1	Milk
S2	4.3	Soft Dough
S3	4.5	Hard Dough
S4	4.7	Endosperm hard/physiological maturity
S5	4.9	Endosperm dry/seed ripe

Where n equals the event number (number of leaves or nodes) and N equals the number of events within the primary stage (total number of leaves or nodes developed). General formula is P + (n/N) - 0.1; where P equals primary stage number(1 or 2 for vegetative and elongation, respectively) and n equals the event number. When N > 9, the formula P + 0.9 (n/N) should be used.

To develop prediction equations in Indiana for switchgrass morphology, the morphological data from Trafalgar and Roann in 2017 were analyzed using the mixed models program in SAS. Validation equations for MSC and MSW based on GDD and DOY were created from the estimates given by SAS. The 2017 validation equations were used to predict morphological development in 2016 at Trafalgar and Roann, as well as Lafayette in 2017. The 2017 equations, which were based on the 67 kg N ha^{-1} treatments, were used to predict all N rates, since no significant differences due to N rate were found during the 2016 sampling season. Since Lafayette had accumulated approximately the same GDD as Trafalgar at the end of the 2017 season, the validation equations created for Trafalgar were used for prediction at Lafayette. The predicted values were then compared to the actual measured values. The differences between the predicted value and average value were used to estimate the Root Mean Square Error (RMSE) and Coefficient of Determination (R^2).

2.4 Results and Discussion

2.4.1 Environment

According to the National Oceanic and Atmospheric Administration, Indiana had “much above average” temperatures and “above average” precipitation for 2016 and 2017 (https://www.ncdc.noaa.gov/sotc/national). In 2016, fused anthers occurred at the Roann location, thus providing unreliable morphological development after anthesis. At Trafalgar, seed development was inhibited, likely due to storm events occurring around anthesis, so a majority of tillers did not produce seed. Therefore, morphological data after August 8, 2016 and August 23, 2016 at Roann and Trafalgar, respectively, were not used for the statistical analysis for morphology based on GDD or DOY.

2.4.2 Morphology

In both 2016 and 2017 and at all locations, MSC and MSW were linearly correlated to both GDD and DOY ($P < 0.0001$; Appendix Table 2), which is consistent with previous studies of switchgrass morphology (Moore et al., 1997). In 2016 and 2017 for both MSC and MSW, there were significant differences between locations (data not shown). This is most likely due to the differences in environment between the three locations. Roann is in north central Indiana while Trafalgar is in central Indiana and Lafayette is in west central Indiana. Differences in temperature most likely cause the differences in morphological development. For example, in 2016, Trafalgar had accumulated 318 GDD by June 1 whereas, Roann had only accumulated 262 GDD. By October 1, Trafalgar had accumulated a total of 1853 GDD, whereas Roann had accumulated 1721 GDD. Other switchgrass morphology studies have shown differences in response to location, but generally they are comparing a greater difference in distance between locations as compared to this study (Boe et al., 2005; Sanderson et al., 1995). In 2016, N rate did not significantly impact MSC or MSW at either location when predicted by either GDD or DOY (data not shown). This contrasts with previous research conducted in Iowa, where switchgrass morphological development progressed faster at increasing N rates (Waramit et al., 2014).

In both years, there were two significant two-way interactions, one between GDD and location (2017 shown in Figure 2.1; 2016 described in Appendix Table 2), and another between DOY and location (Appendix Table 2). These interactions were expressed by Roann having lower intercept values and higher slope values as compared to Trafalgar and Lafayette. There was no cultivar by location interaction, therefore cultivars were averaged in Figure 2.1 to show the different response of morphological development among the three locations. Switchgrass grown at Roann breaks dormancy later than Trafalgar and Lafayette, but also advances through growth stages at a faster rate. In 2017, this accelerated morphological development allowed Roann switchgrass to complete the reproductive and seed development stages in less time than switchgrass at Trafalgar. There were no other significant interactions between location, cultivar, N rate, GDD, or DOY for either year. A previous study (Boe et al., 2005) reported location by cultivar interactions, but the locations were a greater distance apart than in this study. One experiment was in Arlington, Wisconsin, while the other was in Brookings, South Dakota.

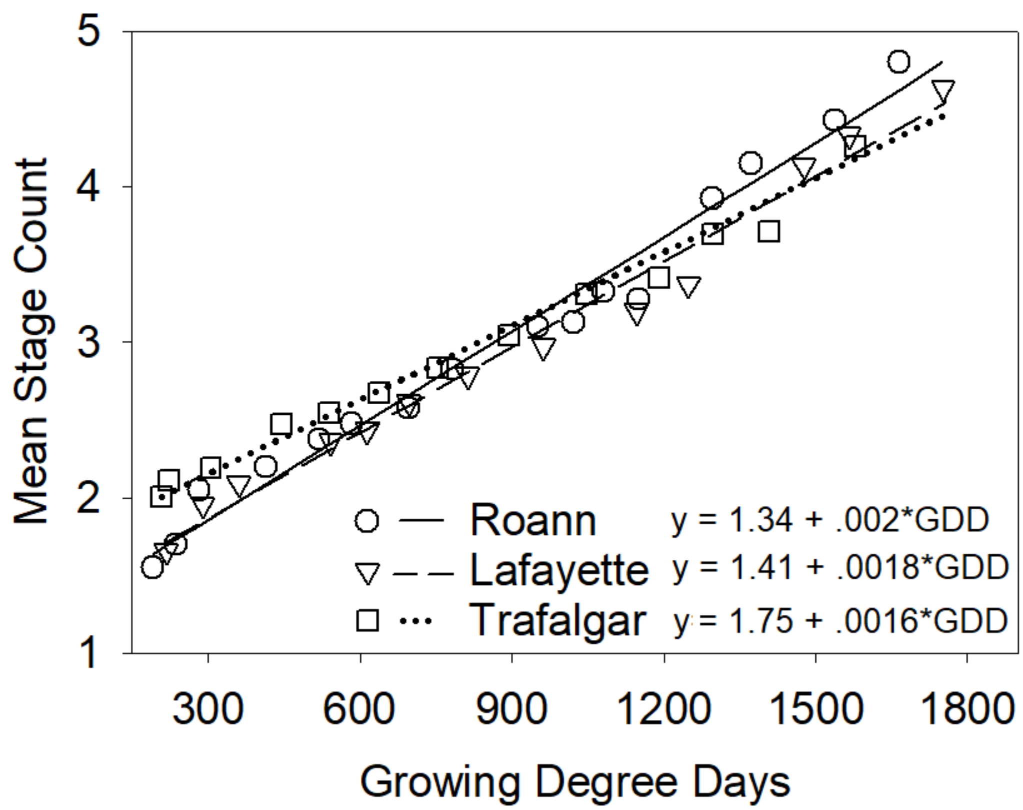

Figure 2.1 Response of Mean Stage Count to Growing Degree Days for three Indiana locations in 2017. Each point represents the collected mean data and each line represents the predicted equation.

In 2016, ‘Liberty’ morphological development lagged behind ‘Shawnee’. The difference was approaching significance for MSC (P =0.059) and MSW (p =0.087) when predicted by GDD, but not significant when predicted by DOY (See Appendix Table 1 for 2016 equations). In 2017, the differences in morphological development were significantly different between ‘Liberty’ and ‘Shawnee’ for both MSC (p =0.03) and MSW (p =0.02) when predicted by GDD (Figure 2.2), but not significantly different when predicted by DOY. This difference was expressed as a lower intercept value for the ‘Liberty’ prediction equation as compared to the ‘Shawnee’ equation. Overall, ‘Liberty’ was less mature at a given GDD than ‘Shawnee’, but the difference was not always significant. Recently published research compared differences in morphology between upland and lowland ecotypes (Aurangzaib et al., 2018). They found that upland cultivars always had a higher MSC than the lowland cultivars. Our study agrees with these results when ‘Liberty’ and ‘Shawnee’ are compared on a GDD basis; however, for both years, morphology was not different when compared on a DOY basis.

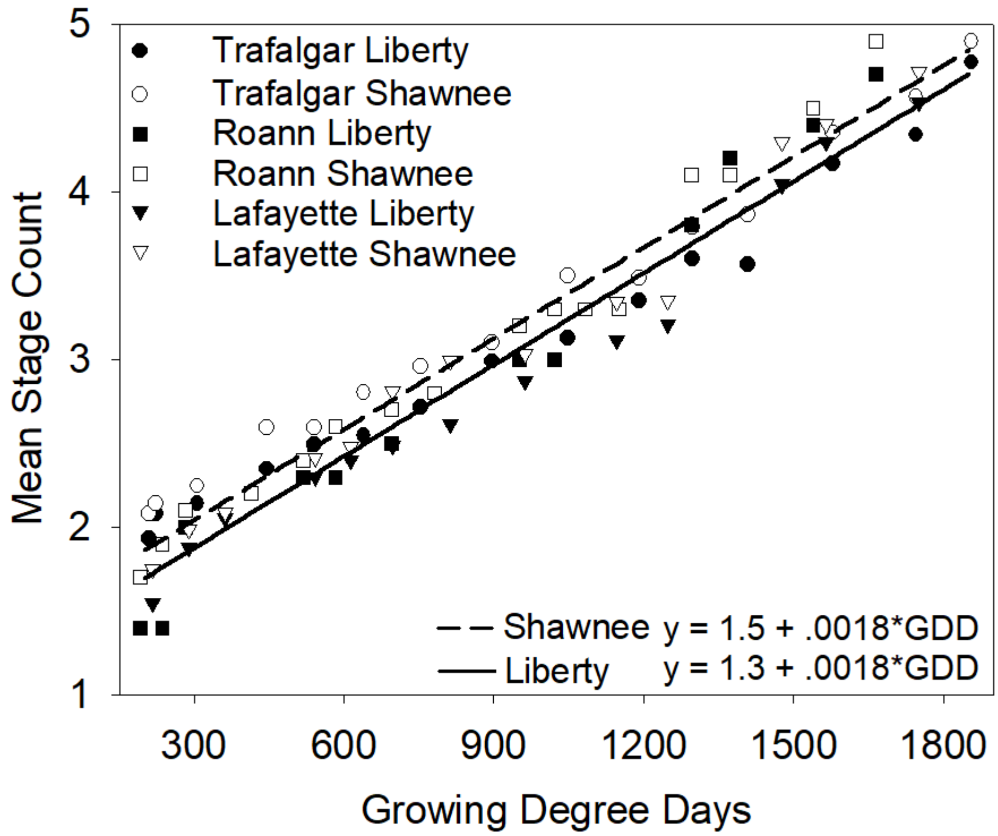

Figure 2.2 Response of Mean Stage Count to Growing Degree Days of 'Shawnee' and 'Liberty' switchgrass in 2017. Each point represents the collected mean data and each line represents the predicted equation.

Additionally, MSC did not provide a significantly different mean stage on a given GDD or DOY as compared to MSW (Figure 2.3). As previously reported in the literature, MSC equally accounts for each tiller in a certain stage in the mean, so it can give a lower result as MSW, which accounts for the dry matter associated with each stage in its mean (Moore et al., 1997). Unlike switchgrass, some species of warm-season grasses, such as indiangrass or big bluestem, have a large portion of tillers that may never advance to reproductive stages (Mitchell et al., 1997). Therefore, those species are more likely to show differences in MSC and MSW as the growing season progress. For switchgrass, utilization of MSC is a reliable predictor of morphology throughout the season and utilizing mean stage weight did not give a significantly different mean stage. Producers and researchers in Indiana studying switchgrass can use MSC for future morphological development research, as it is time efficient and easier than calculating MSW.

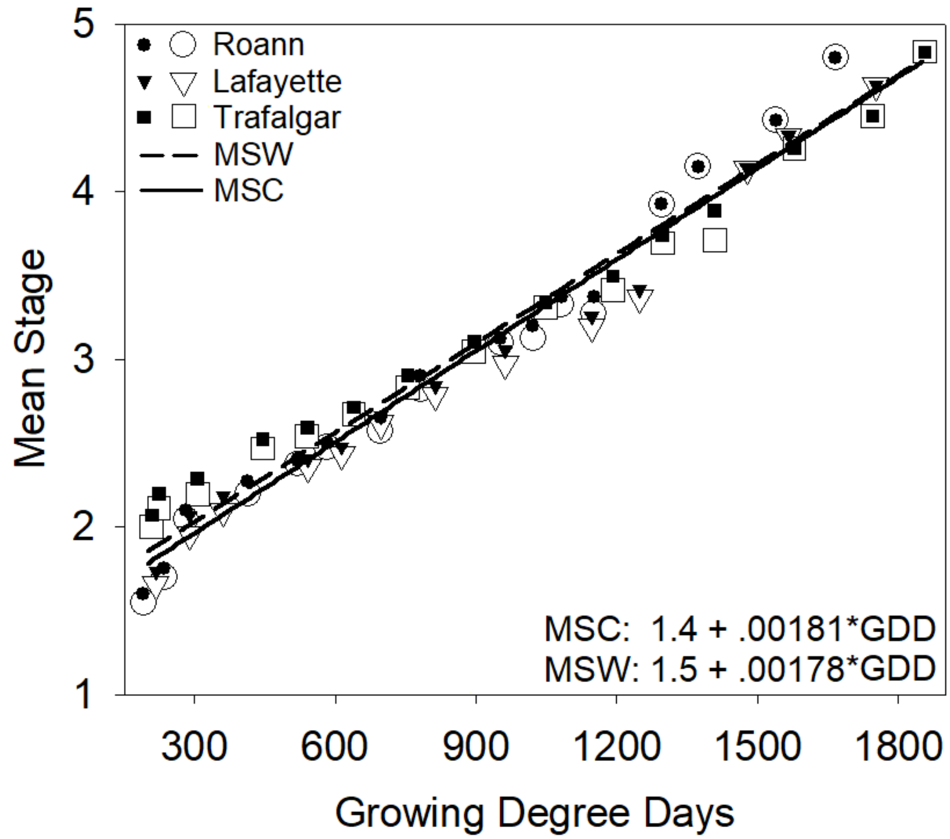

Figure 2.3 Difference between Mean Stage Count (MSC) and Mean Stage Weight (MSW) as predicted by Growing Degree Days in 2017. Each point represents the collected average data and each line represents the predicted equations.

2.4.3 Predicting Morphology

The resulting linear calibration equations that described MSC and MSW in 2017 were compared to measured MSC and MSW values to create validation equations. Root Mean Square Error (RMSE) values were calculated to give an additional measure of fit other than the BIC score (Table 2.2). Validation equations for predicting MSC and MSW by GDD and DOY were linearly related, with no added predictive value from including quadratic terms (Table 2.2). Developed validation equations and the R^2 and root mean square error (RMSE) values that correspond to the predictive value of using 2017 calibration equations to predict 2016 growth are listed in Table 2.2. A value of 1 for R^2 and 0 for RMSE would indicate a perfect fit.

Validation equations for MSC as predicted by GDD explained from 84 to 93 percent of the variation in MSC across locations for ‘Shawnee’ and between 90 to 94 percent of the variation for ‘Liberty’. For MSW as predicted by GDD, the validation equations explained from 84 to 93 percent of the variation in MSW across locations for ‘Shawnee’ and between 90 to 95 percent of the variation for ‘Liberty’. ‘Liberty’ R^2 values were higher than ‘Shawnee’, which suggests its morphological development may be more consistent across locations and years as compared to ‘Shawnee’ (Table 2.2). Mitchell et al. (1997) developed prediction equations in Nebraska, which accounted for 94 to 95 percent of the variation in MSC for GDD, and between 92 and 93percent of the variation in MSW for GDD.

For MSC as predicted by DOY, validation equations explained from 79 to 97 percent and 88 to 97 percent of the variation in MSC across locations for ‘Shawnee’ and ‘Liberty’, respectively. Validation equations for MSW as predicted by DOY explained from 84 to 96 percent of the variation in MSC across locations for ‘Shawnee’ and 90 to 97 percent of the variation for ‘Liberty’. DOY equations developed by Mitchell et al. (1997) explained 96 percent of the variation for predicting both MSC and MSW. While our RMSE and R^2 values in this experiment are lower than those found in Nebraska, an acceptable response variation with fewer sampling years was attained. Additionally, this Indiana experiment was based on two different cultivars and three different N rates, which were not previously studied in switchgrass prediction morphology.

Table 2.1 Validation equations for predicting morphological development as determined by Mean Stage Count (MSC) and Mean Stage Weight (MSW) with Growing Degree Days (GDD) or Day of Year (DOY) across three Indiana locations.†

		MSC Predicted by GDD				MSW Predicted by GDD				MSC Predicted by DOY				MSW Predicted by DOY			
Location	Cultivar	b_o‡	b_1§	RMSE ⁋	R^2 #	b_o	b_1	RMSE	R^2	b_o	b_1	RMSE	R^2	b_o	b_1	RMSE	R^2
Trafalgar	Shawnee	1.75	0.0016	0.25	0.84	1.83	0.0016	0.21	0.87	-0.76	0.02	0.28	0.79	-0.64	0.02	0.24	0.84
	Liberty	1.60	0.0016	0.19	0.90	1.70	0.0015	0.18	0.91	-0.84	0.02	0.21	0.88	-0.68	0.019	0.19	0.9
Roann	Shawnee	1.34	0.002	0.24	0.85	1.42	0.002	0.22	0.87	-1.19	0.02	0.23	0.85	-1.05	0.021	0.2	0.9
	Liberty	1.17	0.0021	0.17	0.91	1.23	0.002	0.19	0.9	-1.44	0.02	0.17	0.91	-1.35	0.021	0.17	0.91
Lafayette	Shawnee	1.75	0.0016	0.24	0.93	1.83	0.0016	0.24	0.93	-0.76	0.02	0.17	0.97	-0.64	0.02	0.17	0.96
	Liberty	1.60	0.0016	0.22	0.94	1.70	0.0015	0.2	0.95	-0.84	0.02	0.15	0.97	-0.68	0.019	0.14	0.97

† Validation equations were created using 2017 regression equations to predict MSC and MSW in 2016.

‡ b_o: Y intercept

§ b_l: Linear slope coefficient

⁋ RMSE: Root Mean Square Error

R^2: Coefficient of Determination

2.5 Conclusions

This study increased knowledge of switchgrass morphological development in Indiana, and examined differences exhibited between ‘Liberty’ and ‘Shawnee’, two diverse switchgrass cultivars. Both growing degree days (GDD) and day of year (DOY) were acceptable predictors of switchgrass morphology. Previous researchers have suggested that GDD may offer a better predictive ability over a wider range of locations. Our study did not show that GDD or DOY was a better predictor; however, our prediction equations were made and validated at the same or very similar locations. Additional research studying the predictive power of both GDD and DOY equations created in this study at more diverse locations throughout the Midwest, may determine that GDD prediction equations are more robust. N rate did not significantly affect the developmental morphology of ‘Liberty’ or ‘Shawnee’. For both cultivars, location had the largest impact on developmental progression. Differences in location are often driven by temperature and daylength, which can be represented by the predictive terms GDD and DOY. Additionally, the recently released cultivar ‘Liberty’ exhibited slower morphological growth than ‘Shawnee’ by approximately seven to ten days. Delayed development has been previously reported to allow increased biomass accumulation and less occurrence of detrimental foliar disease. Differences between MSC and MSW at each sampling date was minimal. Thus, MSC may be a more practical system for use by producers because MSW would require samples to be dried and weighed which is not as time efficient or as practical. Morphological prediction equations reported in this study will be helpful in developing future switchgrass cultivars for forage and bioenergy purposes, as they contribute to the understanding of switchgrass growth in varying locations and among different N rates.

CHAPTER 3. CHANGES IN COMPOSITION AND QUALITY OF MORPHOLOGICAL COMPONENTS FOR TWO DIVERSE SWITCHGRASS CULTIVARS RECEIVING THREE NITROGEN RATES AT TWO INDIANA LOCATIONS

3.1 Abstract

Switchgrass (*Panicum virgatum* L.) is an important warm-season perennial grass with the ability to be utilized as a feedstuff for livestock and as an herbaceous energy crop. The objectives of this study were to compare the compositional quality of a newly developed biofuel cultivar ‘Liberty’ to an improved forage cultivar ‘Shawnee’ in two Indiana environments receiving three N rates. Compositional development was reported in response to increasing maturity as measured by Mean Stage Count. Difference among the leaf blade, stem-plus-sheath, and whole-plant tissues were evaluated. Pure stands of each cultivar were destructively sampled in the field at Trafalgar and Roann, Indiana in 2016. Samples were collected weekly during the early season and every other week in the late season and Mean Stage Count (MSC) was determined. Samples from every other sampling date were ground and analyzed using near-infrared reflectance spectroscopy (NIRS). Increasing N (N) fertilizer caused a higher N concentration at a given MSC. The 0 kg N ha^{-1} fertilizer rate dropped below 10 mg g^{-1} N by MSC 2.2, whereas the 134 kg N ha^{-1} fertilizer rate had greater than 10 mg g^{-1} until MSC 2.7. Leaf blades had the highest N concentrations as the season progressed. ‘Liberty’ had increased neutral detergent fiber (NDF) concentration as compared to ‘Shawnee’. For whole-plant samples, ‘Liberty’ averaged 727 mg g^{-1} NDF as compared to ‘Shawnee’ which averaged 718 mg g^{-1}. ‘Liberty’ had 18 mg g^{-1} higher acid detergent fiber (ADF), on average, as compared to ‘Shawnee’. Acid detergent lignin (ADL) was not different among N fertilizer treatments. Stem-plus-sheath material accounted for a higher percentage of NDF, ADF, and ADL, in whole-plants as MSC increased, when compared

to leaf blades. ‘Shawnee’ had higher IVDMD as compared to ‘Liberty’ and the biggest differences occurred around MSC 2.9. At MSC 2.9, ‘Shawnee’ whole-plant IVDMD was 448 mg g^{-1} and ‘Liberty’ whole-plant IVDMD was 430 mg g^{-1}. Ash concentration decreased as MSC increased. Switchgrass has potential use in Indiana for grazing ruminant animals when cool-season grasses decrease in productivity due to increasing temperatures when grazed or cut for hay before MSC 2.5. High hemicellulose and cellulose and low N concentrations suggests Indiana as a good environment for production of switchgrass for biofuel purposes.

3.2 Introduction

When growing switchgrass for either forage or biomass purposes, composition of the crop is vital to the success of the operation. Switchgrass has the potential to be used as both a forage and biofuel crop in one season (Richner et al., 2014). Previously studied systems for dual use management systems include: grazing at boot stage then biomass harvest after frost, biomass harvest before anthesis then grazing regrowth in late summer, and biomass harvest after anthesis then grazing regrowth in late summer (Richner et al., 2014). Additionally, switchgrass could be used only for forage or biomass in a season. When used for grazing, livestock preferentially graze leaves. When assessing nutritive value of switchgrass as a grazed forage, analysis of the leaf blade may give the best insight into the potential performance by the grazing animal. Whole-plant compositional analysis describes the forage value of switchgrass baled for hay or for biofuel. Analyzing switchgrass leaf blade, stem-plus-sheath, and whole-plant composition through the growing season gives insight into proper management and ideal harvest times when used as a feedstuff for livestock or as a biofuel. ‘Shawnee’ is a cultivar selected for increased IVDMD from the variety ‘Cave-in-Rock’. ‘Liberty’ is a cultivar selectively bred for increased biomass yield and winterhardiness by crossing the varieties ‘Kanlow’ and ‘Summer’. The objectives of this study were to analyze how quality in whole-plant samples change as maturity increases, how leaf blades and stem-plus-sheath influence the change in whole-plant quality, and how N fertilizer treatments at different locations may impact quality as plants mature.

3.3 Materials and Methods

To determine and predict composition of ‘Shawnee’ and ‘Liberty’, field studies were conducted during 2016 and 2017. Plot establishment protocol was the same as described in Chapter 2. Each sample from each split plot was evenly divided into thirds, with one - third being processed into either leaf blades or stem plus leaf sheath. One-third of the sample was left as a whole-plant sample for analysis. The final third was used for morphological staging, as discussed in Chapter 2. After processing, all samples were dried in an oven at 60°C.

After drying, samples from every other sampling date, beginning with the first sampling date, were ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ) using a 6-mm screen. During the initial 6-mm grind, the entire sample was ground and thoroughly mixed before selecting a subsample, which was stored in a plastic vial. Each subsample was ground using a UDY cyclone mill (Udy Corp., Fort Collins, CO) with a 1-mm screen. After fine grinding, samples were stored at room temperature before being evaluated using near-infrared reflectance spectroscopy (NIRS). Each sample was thoroughly mixed again before analysis. NIRS was completed at the Dairy Forage Research Center in Madison, Wisconsin with a Foss Model 6500 (Foss-NIRSystems Inc., Silver Spring, MD, USA). The spectra of this machine was standardized to match a master machine, which is managed by the NIRS Forage and Feed Testing Consortium (NIRSC, Hillsborough, WI). Before samples were loaded and processed through the NIRS machine, it was allowed approximately two hours to warm up and a known test sample was analyzed to ensure the machine was operating within normal parameters. NIRS prediction equations were developed and summary statistics for predicted parameters can be found in Vogel et al. (2010).

Data were analyzed for statistical significance using the mixed models procedure in SAS 9.4. Fixed categorical variables were location, cultivar, N rate, plant part (leaf blades, stem-plus-sheath, and whole-plant) and each replication within location was considered random. For this study, MSC was selected as the continuous variable to compare compositional differences equally across locations and cultivars. Utilization of MSC allowed plant samples to be compared at a similar maturity, which has been previously reported as a major contributor to forage quality (Anderson et al., 1989). All two- and three-way interactions between the fixed, random, and continuous variables were included in the model. The dependent variable was the compositional parameter (i.e. N, ADF, etc). Variables were considered significant when $P < 0.05$. Variables were centered before analysis of variance was completed, in order to decrease correlations between squared and cubic terms. Using the estimate function in SAS, the significant model, linear, quadratic, or cubic, was found for each dependent variable. The full model table of equations can be found in Appendix Table 3.

3.4 Results and Discussion

3.4.1 Nitrogen Concentration

N concentration was significantly different across locations, cultivars, N rates, and plant parts. Switchgrass grown at Roann had 1.5 mg g^{-1} higher N concentration than plants grown at Trafalgar (data not shown; P <.001). ‘Liberty’ had 1 mg g^{-1} lower concentration of N as compared to ‘Shawnee’ over the season ($P < 0.0001$; data not shown). A previous study comparing upland and lowland cultivars found that lowland cultivars had lower N concentration as compared to upland cultivars (Aurangzaib et al., 2016). Increased N fertilizer applications increased average N concentration ($P < 0.001$). There was a two-way interaction between MSC and N rate ($P < .001$). Switchgrass whole-plant tissue receiving 0 kg N ha^{-1} had lower N concentration as compared to the 67 and 134 kg N ha^{-1} treatment as MSC increased (Figure 3.1; $P < 0.001$). Waramit et al. (2010), also found increased N concentrations with higher N fertilization rates. They found a N concentration of 20 to 30 mg g^{-1} for the first harvest date which quickly decreased during the first third of the growing season. After the initial fast decline, the N concentration decrease slowed when it reached between 10 and 15 mg g^{-1}, and dropped as low as 5 mg g^{-1} at the end of the season. In our study, at the first sampling dates, N concentration was around 25 mg g^{-1}. N concentration quickly decreased to around 10 mg N g^{-1} between MSC of 2.25 to 2.50 which is early to mid-elongation. Loss of N slowed after that point. At the end of the season, the plants had 3 to 5 mg N g^{-1} on a dry matter basis.

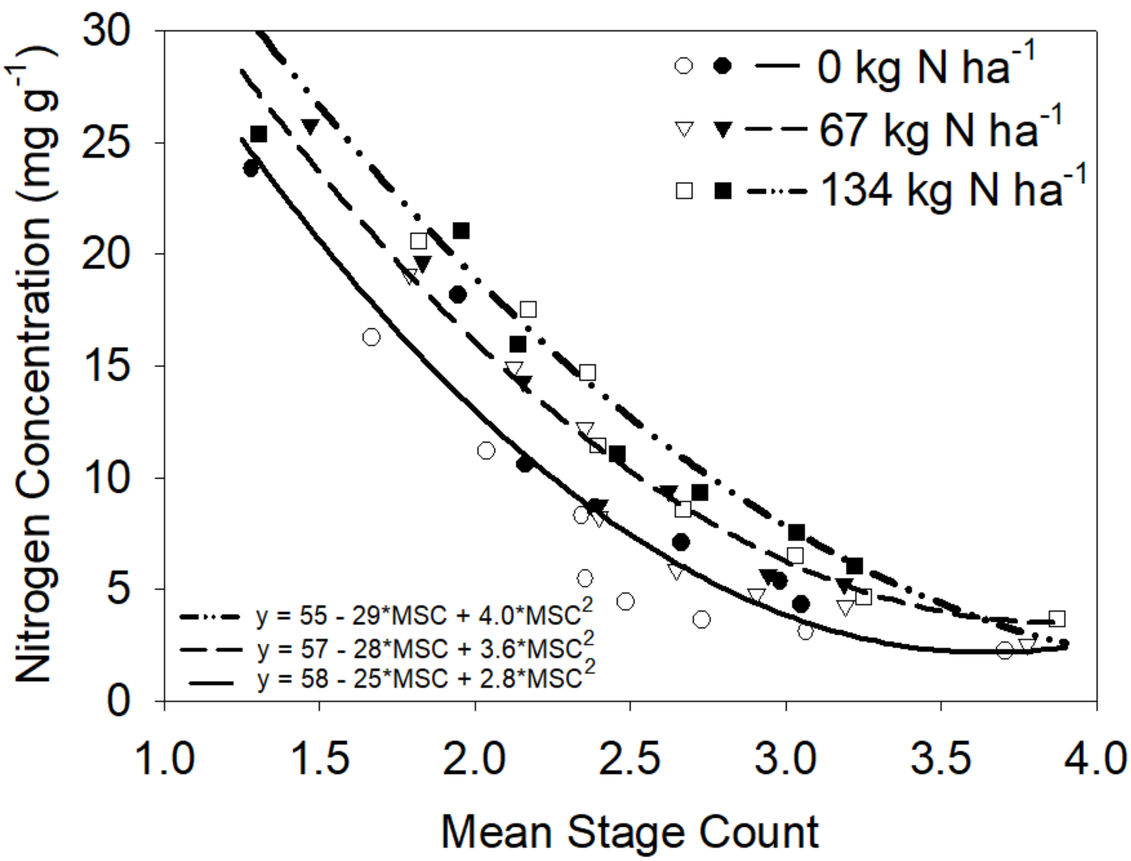

Figure 3.1 Effect of three nitrogen fertilizer rates on the dry matter nitrogen concentration of switchgrass as maturity increases, as measured by Mean Stage Count. Open and closed symbols represent Trafalgar and Roann data, respectively.

There was a two-way interaction among MSC and leaf blade, stem-plus-sheath, or whole-plant. This interaction was due to the difference in how fast N concentration declined among leaf blades, stem-plus-sheath, and whole-plant samples. Leaf blade had a slower decline in N concentrations, expressed as a less negative slope, as compared to the stem plus leaf sheath or whole-plant samples as MSC increased ($P < 0.0001$). The stem-plus-sheath portion had lower N concentration than whole-plant samples as maturity increased (Figure 3.2; $P < 0.0001$). These findings are consistent with previous research completed by Twidwell et al. (1988) in Indiana, where switchgrass leaf blades had higher N concentration when compared to stem or sheath samples.

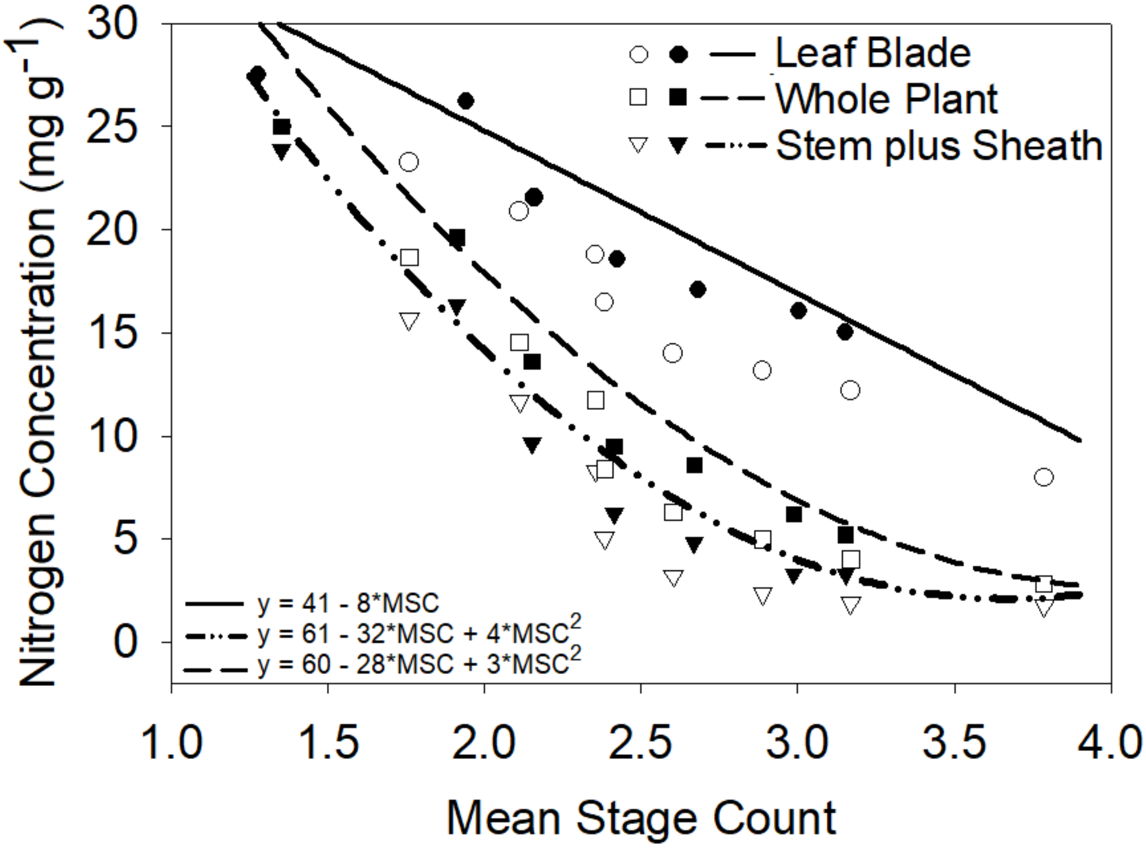

Figure 3.2 Response of dry matter nitrogen concentration to increasing maturity, as measured by Mean Stage Count, for leaf blades, stem-plus-sheath, and whole-plant of ‘Shawnee’ and ‘Liberty’ switchgrass. Open and closed symbols represent Trafalgar and Roann data, respectively.

3.4.2 Neutral Detergent Fiber

The NDF was significantly different across cultivars, N rates, and plant parts. There was no significant difference between Roann and Trafalgar. ‘Liberty’ had increased NDF concentration as compared to ‘Shawnee’ (Figure 3.3; P < 0.0001). For whole-plant samples, ‘Liberty’ averaged 727 mg g^{-1} NDF as compared to ‘Shawnee’ which averaged 718 mg g^{-1}. Aurangzaib et al. (2016) compared cellulose, hemicellulose, and lignin separately, as compared to the additive term, NDF. Aurangzaib et al. (2016) found that the lowland cultivar ‘Kanlow’ had higher cellulose and hemicellulose as compared to the upland cultivar ‘Cave-in-Rock’.

Additions of 134 kg N ha^{-1} resulted in 8.8 mg g^{-1} higher NDF as compared to the 0 kg N ha^{-1} rate ($P < 0.01$). There was no significant difference between 0 and 67 ($P = 0.2$) or 67 and 134 kg N ha^{-1} ($P = 0.06$) rates (data not shown). A study completed in Iowa found that lignin and cellulose increased in response to each addition of N fertilizer, but no increases in hemicellulose were found with additions of N fertilizer (Waramit et al., 2011). Guretzky et al. (2011), found that higher N fertilizer rates had either no change or decreased NDF on switchgrass harvested after seed set or after a frost event.

There were significant differences in NDF among leaf blades, stem-plus-sheath, and whole-plants ($P < 0.0001$). There was a two-way interaction between MSC and plant part ($P < 0.0001$; Figure 3.3). Leaf blades accumulated less NDF as compared to the whole-plant and stem plus leaf samples as maturity increased. As switchgrass matured, stem-plus-sheath had higher accumulation of NDF as compared to whole-plants ($P < 0.0001$). Twidwell et al. (1988) found that leaf blade NDF did not increase for a 14 day sampling period; however, stem and sheath NDF showed significant increases in NDF over the sampling period. Concentrations reported in the Twidwell et al. (1988) study were similar to this study.

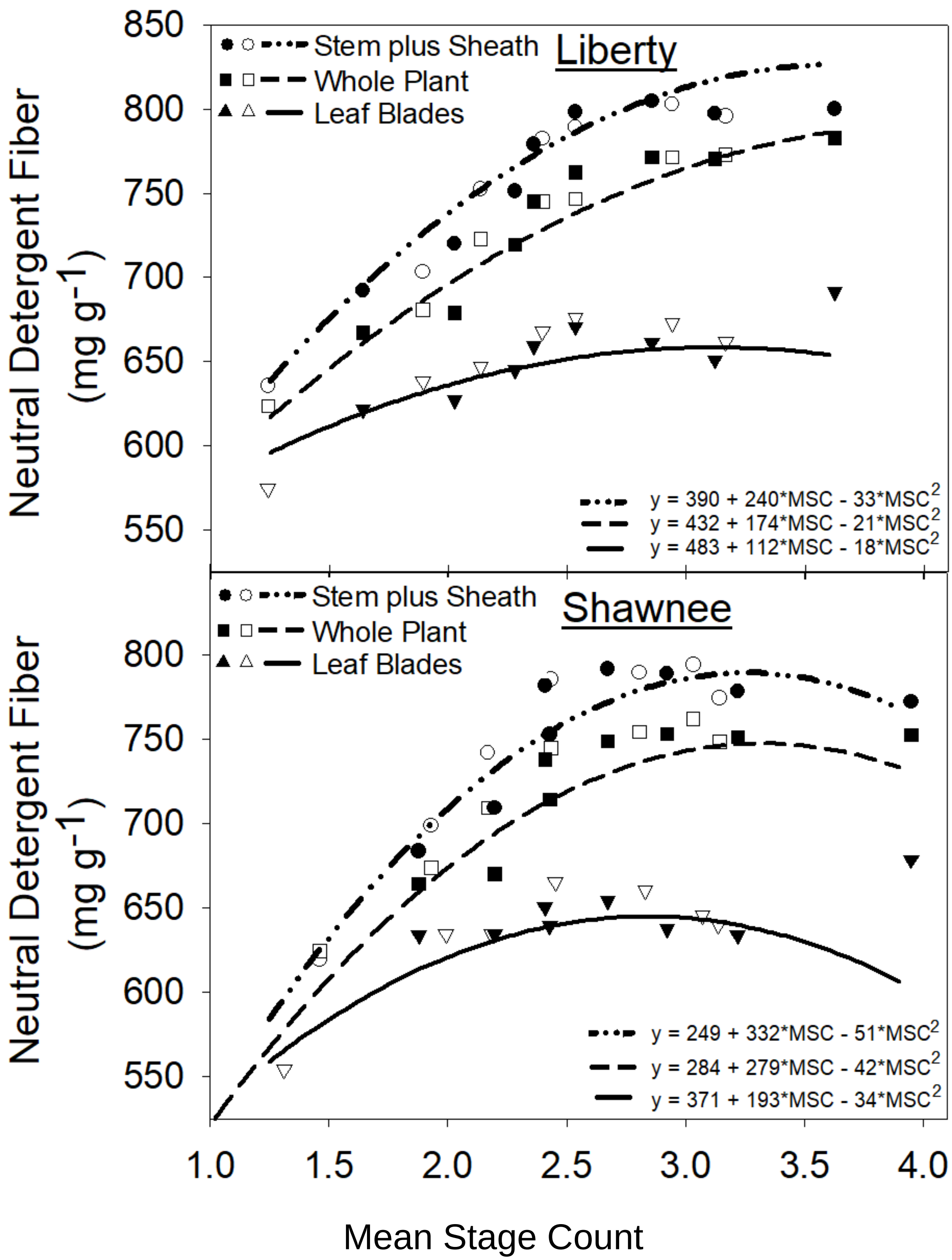

Figure 3.3 Response of dry matter Neutral Detergent Fiber (NDF) concentration to increasing maturity, as measured by Mean Stage Count, for leaf blades, stem-plus-sheath, and whole-plant of 'Shawnee' and 'Liberty' switchgrass. Open and closed symbols represent Trafalgar and Roann, respectively.

3.4.3 Acid Detergent Fiber

ADF was significantly different across cultivars and plant parts. There were no significant main effect differences between locations or among N rates. There was a significant difference among N rates for NDF concentrations. This infers that increasing N rates increases the hemicellulose, but not the cellulose or lignin fractions. Waramit et al. (2011) and Guretzky et al. (2011), found that increasing N fertilizer levels increased ADF in ‘Cave in Rock’ and ‘Alamo’ switchgrass, respectively. Not all additions of N fertilizer, in the Guretzky et al. (2011) study, resulted in increased ADF, but the additions of N fertilizer increased incrementally by 45 kg N ha^{-1} up to a maximum of 225 kg N ha^{-1}.

ADF concentration at Roann and Trafalgar are consistent with ADF concentrations reported in several studies (Mosali et al., 2013; Adler et al., 2006; Waramit et al., 2011; Sanderson et al., 2010; Kering et al., 2010). ‘Liberty’ whole-plant samples averaged 375 mg ADF g^{-1} as compared to ‘Shawnee’, which averaged 357 mg g^{-1}. Aurangzaib et al. (2016), found similar results in their study where ‘Kanlow’ had higher ADF as compared to ‘Cave in Rock’. ‘Kanlow’ is the lowland parent of ‘Liberty’, and ‘Shawnee’ is a selection from ‘Cave in Rock’. Thus, it is not surprising ‘Liberty’ has higher ADF concentration as compared to ‘Shawnee’ ($P < 0.0001$; Figure 3.4).

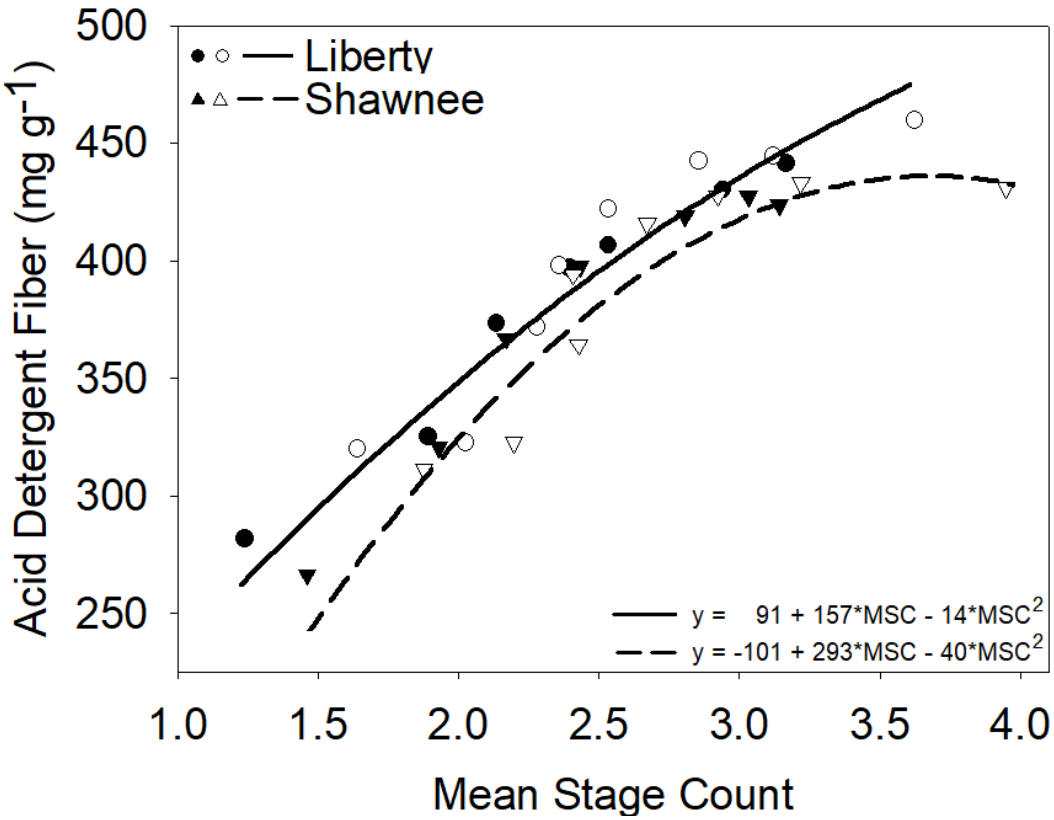

Figure 3.4. 'Liberty' and 'Shawnee' dry matter Acid Detergent Fiber concentration with increasing maturity, as measured by Mean Stage Count. Open and closed symbols represent Trafalgar and Roann data, respectively.

Leaf blades had lower ADF concentration as compared to stem-plus-sheath or whole-plant samples. There was a two-way interaction between MSC and plant part (Figure 3.5; $P < 0.0001$). Mean leaf blade ADF concentration was 271 mg g^{-1} at the first sampling date; whereas, stem-plus-sheath and whole-plant samples averaged 307 and 295 mg ADF g^{-1}, respectively. By MSC 3.0, mean leaf blade ADF concentration increased to 320 mg g^{-1}, and stem-plus-sheath and whole-plant samples averaged 461 and 435 mg g^{-1}, respectively. Furthermore, leaf blades accumulated less ADF as compared to stem-plus-sheath and whole-plant samples as MSC increased, with the biggest differences occurring after MSC 2.0 (Figure 3.5; $P < 0.0001$). Previous research has also indicated that leaf blades had lower ADF as compared to stem or sheath tissues (Twidwell et al., 1988). Twidwell et al. (1988) leaf blades, stem and sheath had similar ADF concentration. They found no difference among ADF concentration for leaves as the plants matured, however sampling only occurred over a four-week period once the flag leaf became visible.

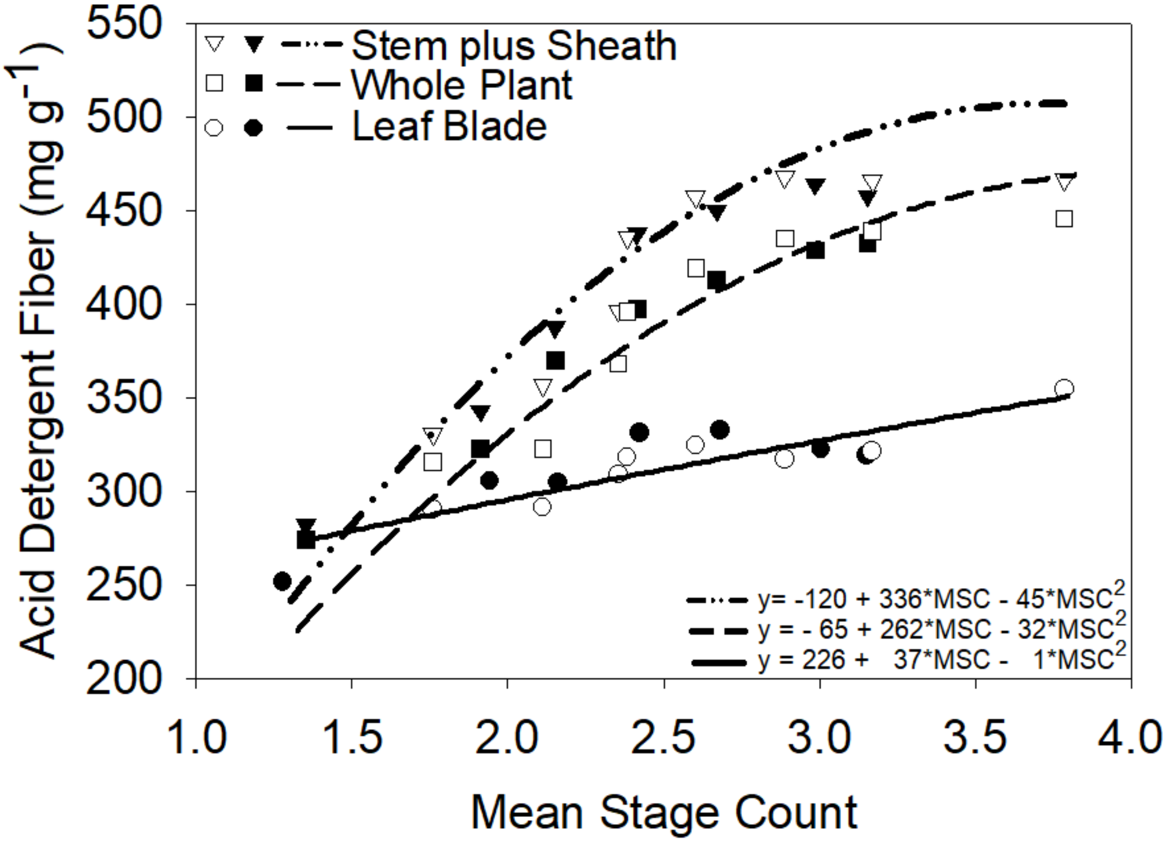

Figure 3.5. Change in dry matter Acid Detergent Fiber for leaf blades, stem-plus-sheath and whole-plant with increasing maturity, as measured by Mean Stage Count. Open and closed symbols represent Trafalgar and Roann data, respectively.

3.4.4 Acid Detergent Lignin

The ADL was significantly different across locations, cultivars, and plant parts. The ADL increased as the season progressed in response to MSC. Previous studies also found that lignin increased as plants matured (Adler et al., 2006; Aurangzaib et al., 2016; Mitchell et al., 2001). There was a two-way interaction between MSC and location. ADL concentrations were higher for switchgrass grown at Roann as compared to Trafalgar before MSC 2.5. After MSC 2.5, plants at Roann began accumulating higher levels of ADL than plants grown at Trafalgar ($P < 0.0001$). ‘Liberty’ whole-plants averaged 49 mg ADL g^{-1} and ‘Shawnee’ whole-plants averaged 48 mg ADL g^{-1} on a dry matter basis for the season, and the difference was significant ($P < 0.01$; data not shown). A previous study found that ‘Kanlow’ had 2.2 mg g^{-1} lower lignin concentration as compared to ‘Cave-in-Rock’ (Aurangzaib et al., 2016).

Leaf blades had lower ADL concentrations as compared to stem-plus-sheath and whole-plant ($P < 0.0001$). Whole-plant samples also had less ADL as compared to stem-plus-sheath ($P < 0.0001$). Leaf blades averaged 37 mg ADL g^{-1}, as compared to 56 and 52 mg ADL g^{-1} for the stem-plus-sheath and whole-plant samples, respectively. There was a two-way interaction between MSC and plant part (Figure 3.6). Stem-plus-sheath and whole-plant samples accumulated higher ADL as MSC increased ($P < 0.0001$). Leaf blade ADL remained relatively constant. Twidwell et al. (1988) found that leaf blades, stems, and sheaths increased in lignin as time progressed. Leaf blades had less ADL as compared to stem and sheath components in their study. Fourteen days after the flag leaf was visible, which would be around MSC 3.0, the measured ADL in leaf blades was 33 mg g^{-1}, whereas stems and sheaths were 98.7 and 57.9 mg ADL g^{-1}, respectively (Twidwell et al., 1988). There was no difference in ADL for increasing N rates. A previous study in Iowa found that increasing N fertilizer rates increased lignin content in switchgrass, and the differences in lignin, among N rates, were more prominent at the end of the season as compared to the beginning of the season (Waramit et al., 2011).

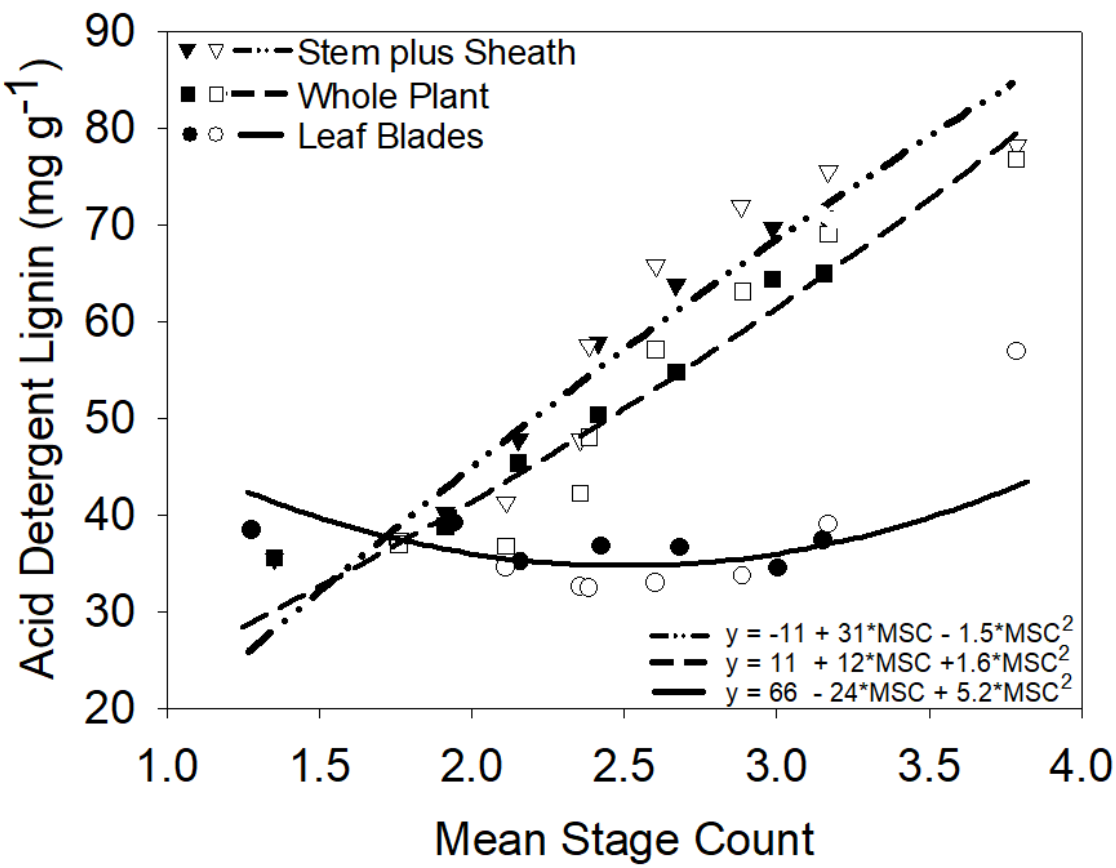

Figure 3.6. Differences in Acid Detergent Lignin with increasing maturity, as measured by Mean Stage Count, among leaf blades, stem-plus-sheath, and whole-plant switchgrass tissues. Open and closed symbols represent Trafalgar and Roann data, respectively.

3.4.5 In-vitro Dry Matter Digestibility

Dry matter IVDMD was significantly different across cultivars, N rates, and plant parts. The IVDMD decreased as switchgrass matured. The decline of IVDMD has been previously documented in literature (Twidwell et al., 1988; Mitchell et al., 2001). ‘Liberty’ had lower IVDMD concentration as compared to ‘Shawnee’ (*P* < 0.0001; Figure 3.7). ‘Liberty’ and ‘Shawnee’ whole-plant dry matter IVDMD averaged 552 mg IVDMD g^{-1} and 582 mg IVDMD g^{-1}, respectively. For the whole-plants on the first sampling date, ‘Liberty’ averaged 732 mg IVDMD g^{-1} and ‘Shawnee’ averaged 739 mg IVDMD g^{-1}; however, when the plants reached

MSC 2.9 ‘Liberty’ dry matter IVDMD had decreased to 430 mg IVDMD g^{-1}, while ‘Shawnee had 448 mg IVDMD g^{-1}. Additions of 67 and 134 kg N ha^{-1} increased IVDMD by 16 and 25 mg g^{-1}, respectively (P < 0.01). The difference between the 67 and 134 kg N ha^{-1} rate was approaching significant (p = 0.08). A study in Oklahoma found that IVDMD increased as N rate increased for the first cutting, but regrowth IVDMD was not different (Kering et al., 2012). Guretzky et al. (2011), however, found that increasing N rates did not impact IVDMD in their study, also located in Oklahoma.

There was a two-way interaction between MSC and plant part (P < 0.0001). Leaf blades had higher IVDMD than the stem-plus-sheath or whole-plant (Figure 3.7). Leaf blades averaged 653 mg IVDMD g^{-1}, as compared to 505 and 543 g mg^{-1} IVDMD for the stem-plus-sheath and whole-plant, respectively. Additionally, leaf blades had higher IVDMD as compared to the stem-plus-sheath or whole-plants tissues, as MSC increased (P < 0.0001). These results are consistent with previous research where leaf blades had the highest IVDMD followed by the leaf sheath, then stem tissues (Twidwell et al., 1988).

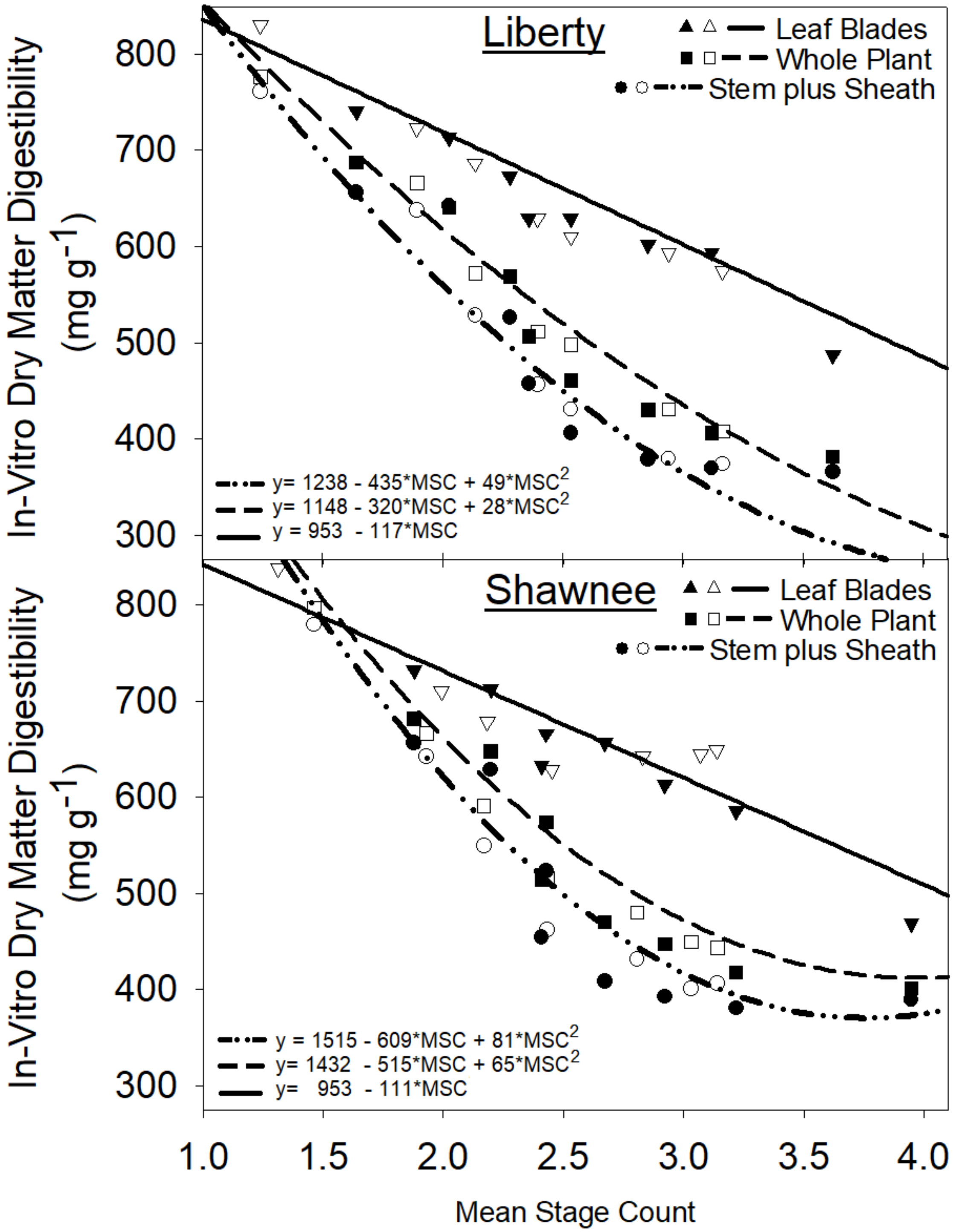

Figure 3.7. Dry mater in-vitro dry matter digestibility with increasing maturity, as measured by Mean Stage Count, for leaf blades, stem-plus-sheath, and whole-plant for 'Shawnee' and 'Liberty' switchgrass. Open and closed symbols represent Trafalgar and Roann data, respectively.

3.4.6 Ash

Mean ash concentrations were consistent with previous research which examined ash either over a season or at an autumn harvest date (Aurangzaib et al., 2016, Lemus et al., 2002, Waramit et al., 2011). Aurangzaib et al. (2016) reported that ash decreased as the season progressed, however there were no differences among varieties at a single harvest date. Similarly, in our study, cultivar did not impact ash concentrations. In addition, there was no difference in ash with increasing N fertilizer. Waramit et al. (2011) also found that increasing N rates did not impact ash concentrations in switchgrass or big bluestem (*Andropogon gerardii* Vitman). Ash was significantly different across plant parts (data not shown; $P < .01$). There was a two-way interaction between MSC and plant part (Figure 3.8). Leaf blades had significantly less ash as compared to stem-plus-sheath and whole-plant before MSC 2.3 ($P < .001$). After MSC 2.3, leaf blades had significantly higher ash as compared to stem-plus-sheath and whole-plant tissues ($P < .0001$). Hu et al. (2010) also found increased ash content in leaves as compared to nodes and internodes for 'Kanlow' and 'Alamo' switchgrass grown in Georgia.

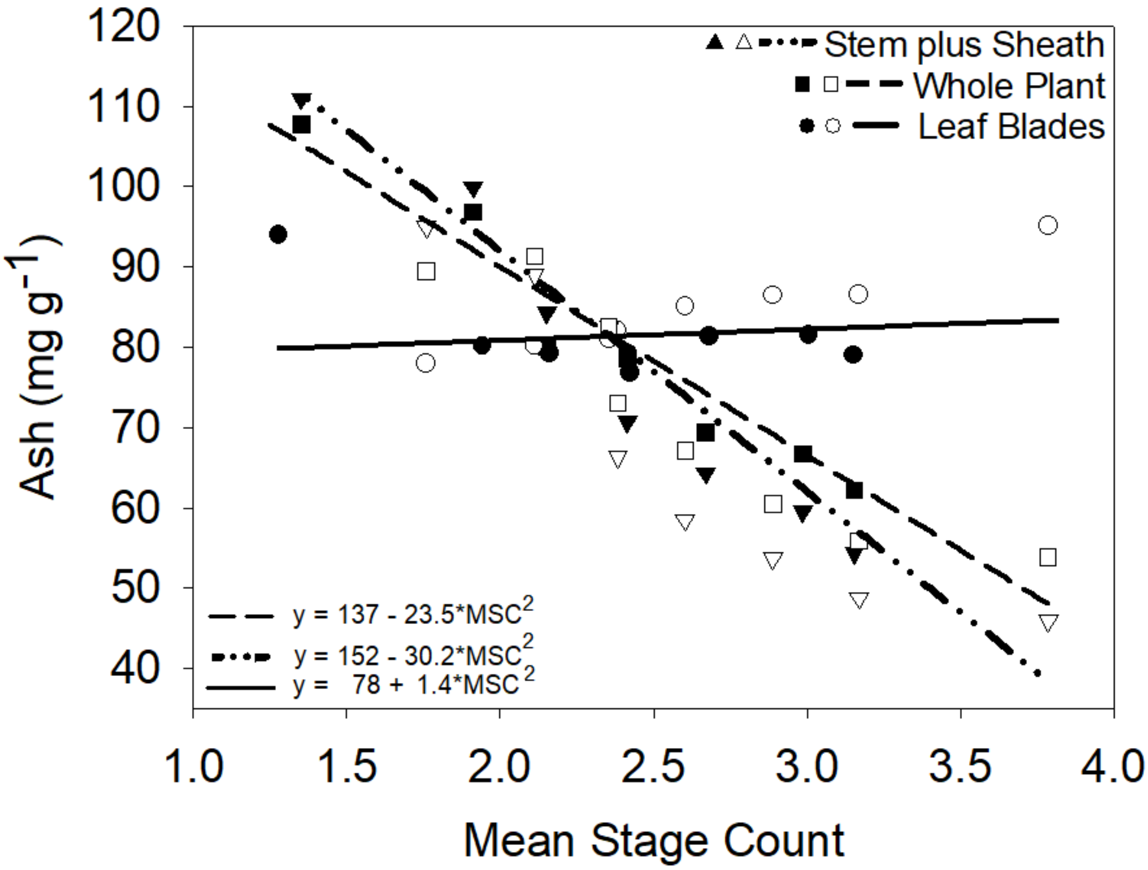

Figure 3.8. Response of dry matter ash to increasing maturity, as measured by Mean Stage Count, for leaf blades, stem-plus-sheath, and whole-plant of ‘Shawnee’ and ‘Liberty’ switchgrass. Open and closed symbols represent Trafalgar and Roann data, respectively.

3.4.7 Applied Discussion of Switchgrass for Ruminant Animals

Producers in Indiana and across the Midwest may consider adding switchgrass into their production system, as it can be used for grazing, hay, or harvested as a biofuel crop. Switchgrass can be added to a cool-season dominated rotational grazing program by seeding switchgrass in a designated paddock. This helps increase forage availability for livestock when cool-season grasses are beginning to become less productive during the hotter temperature of the summer months (Hintz et al., 2004). As a cattle feed, producers would be most interested in N and fiber concentration (Lemenager, 2011). Switchgrass can meet protein requirements of early to mid-gestation beef cattle, replacement heifers, and stocker calves (Lemenager, 2011; Mosali et al., 2013). While it is recommended to graze cool-season grasses when they reach 18 to 25 centimeters in height, it has been recommended to graze switchgrass when it reaches 45 to 60 centimeters in height, and to stop grazing at 20 to 30 centimeters (Bates et al., 2008). Switchgrass has elevated meristematic tissue, which is sensitive to over grazing. Mosali et al. (2013) reported a study in Oklahoma where grazing stocker calves were allowed access to switchgrass when it was 36 centimeters in growth and were removed when switchgrass reached 7.5 cm. Due to the quick growth of switchgrass, cattle needed to be removed from the paddock before they could graze plants to 7.5 or 20 centimeters because the forage nutritive value was too low to support the cattle's nutritional requirements need (Mosali et al., 2013).

Beef cattle in mid to late gestation require a diet containing 13 to 16 mg g^{-1} N (National Research Council, 2000). Our study indicated that switchgrass whole-plant tissue meets these protein requirements until it reaches a MSC between 2.25 to 2.5, which would be mid-elongation. Addition of N fertilizer can help maintain higher crude protein content as maturity increases. Our study showed that additions of 134 kg N ha^{-1} kept N concentrations above 10 mg g^{-1} until MSC 2.7, whereas the 0 kg N ha^{-1} study dropped below 10 mg g^{-1} by MSC 2.2. Grazing switchgrass would allow for livestock to preferentially eat the highest quality herbage. Switchgrass leaf blades maintained N content above 15 mg g^{-1} until MSC 3.0, which is boot stage. Switchgrass used for grazing would have a wider timeframe to be utilized to meet livestock needs, as compared to mowing it for hay, due to the selective grazing of leaf blades.

It has been previously noted that horses and lambs consuming switchgrass could develop photosensitivity, which is due to glycosidic steroidal saponins found in the plant (Lee et al., 2001; Puoli et al., 1992). Therefore, switchgrass should be used with caution if considered as a forage for horses and lambs. While most research on switchgrass as an animal feedstock has focused on cattle, Sanderson and Burns (2010) studied different hay harvest systems for switchgrass and the effect on forage digestibility in mature sheep wethers. Switchgrass received 100 kg N ha^{-1} and was harvested in either a two- or three- cut system. Sheep preferred eating switchgrass from the first cutting, which had greater NDF and hemicellulose digestibilities, as compared to the second cutting. A series of eight studies reported by Fisher et al. (2005) found no difference in preference for cattle when consuming switchgrass mowed for hay in the morning or afternoon. In the same studies, sheep and goats showed preference for switchgrass hay mowed in the afternoon for some of the studies, but not all. Sheep, goats, and cattle preferred hays that had higher crude protein, as a result of applying N fertilizer, even though the soluble carbohydrate levels were reduced (Fisher et al., 2005).

The major drawback to producers grazing or feeding switchgrass is the fiber content of the plant. High quality forage is considered to have NDF concentration less than 45 percent, moderate quality forage NDF is between 45 and 60 percent and low quality forage would be samples with greater than 60 percent NDF (Purdue University Forage Field Guide). NDF concentration at the first harvest were 60 and 65 percent for leaf blade and whole-plant tissues. By MSC of 2.5, leaf blades and whole-plant tissues had 65 and 75 percent NDF. This means that the dry matter intake of the cattle is reduced due to the high concentration of fiber, since mastication and rumination will take longer. Physical fill is also affected by increased fiber, as cattle will stop grazing before they can consume adequate nutrition for their needs. ‘Shawnee’ switchgrass may be a better choice for the cattle producer, as it had higher IVDMD and lower ADF as compared to ‘Liberty’. Higher IVDMD and lower ADF suggests that even with similar NDF, ‘Shawnee’ would be more digestible and provide greater energy for the consuming animal. . Sanderson and Burns (2010), however, found no difference between ‘Shawnee’ and ‘Cave-in-Rock’ dry matter digestibility in vivo when sheep consumed the hay in a feeding trial. Future research comparing in vivo dry matter digestibility of ‘Liberty’ and ‘Shawnee’ would be beneficial.

Previous research has indicated potential for utilizing switchgrass as a forage and biofuel crop in one season (Biermacher et al., 2017). Options include grazing in late May to early June and harvesting regrowth as a biofuel crop, or harvesting as a biofuel crop in late July and grazing regrowth once it reaches 46 to 61 centimeters (Biermacher et al., 2017). Producers should consider cost and profit for each system for each year to make the management decision on whether to use switchgrass for both grazing and biofuel, or for one purpose. It is important to make sure to leave at least ten centimeters of stubble for the plant to survive winter whether grazing or harvesting for biomass (Mitchell et al., 2010).

3.4.8 Final Harvest Composition

Whole-plant samples were harvested at the end of September at Roann and Trafalgar. The samples were statistically analyzed separately from previous data due to the absence of an accurate MSC to compare location and cultivars due to occurrence of fused anthers and smut. Mean values are listed in Table 3.1. When harvesting switchgrass for biofuel, the whole-plant will be cut and baled. Therefore, only whole-plant composition was analyzed for the final sampling dates.

N concentration was significantly different between locations and N rates. N concentration was 1.1 mg g^{-1} higher for plants grown at Roann, as compared to Trafalgar (P < 0.001). There were no differences between ‘Liberty’ and ‘Shawnee’ (P = 0.1). Additions of N fertilizer at a rate of 67 and 134 kg N ha^{-1} increased N concentrations by 0.9 and 1.3 mg g^{-1} as compared to the 0 kg N ha^{-1} treatment (P < 0.01). There was no difference between the 67 and 134 kg N ha^{-1} fertilizer rates (P = 0.1).

The dry matter NDF was significantly different between locations and cultivars. The NDF was 12 mg g^{-1} higher for switchgrass grown at Roann as compared to Trafalgar (P < 0.05). ‘Liberty’ had 30 mg NDF g^{-1} higher as compared to ‘Shawnee’ (P < 0.0001). ‘Liberty’ averaged 799 mg NDF g^{-1} at the final sampling date.

The ADF was significantly different between locations, cultivars, and N rates. Switchgrass grown at Roann had 14 mg ADF g^{-1} higher as compared to Trafalgar (P < 0.01). ‘Liberty’ switchgrass had 24 mg ADF g^{-1} higher as compared to ‘Shawnee’ (P < 0.0001). There was a N rate by location interaction (P < 0.05). Plants grown at Roann receiving 0 kg N ha^{-1} had 30 mg ADF g^{-1} higher as compared to switchgrass grown at Trafalgar receiving 0 kg N ha^{-1}. There were no differences for ADF among the 0, 67, and 134 kg N ha^{-1} fertilizer treatments at Roann. At Trafalgar, however, switchgrass receiving 67 and 134 kg N ha^{-1} had 22 and 27 mg ADF g^{-1} higher as compared to the 0 kg N ha^{-1} treatment (P < 0.05). There was no difference between the 67 and 134 kg N ha^{-1} treatments.

The ADL was 3.4 mg g^{-1} higher for switchgrass grown at Roann as compared to Trafalgar, and the difference was approaching significance (P =0.06). There was a significant interaction between location and N rate. Switchgrass at Roann receiving 0 kg N ha^{-1} had 7.6 mg ADL g^{-1} higher as compared to plants at Trafalgar receiving 0 kg N ha^{-1} (P < 0.05). There were no significant differences between location, cultivar, or N rate for ash concentration for the final harvest date.

Table 3.1 Compositional Quality of 'Liberty' and 'Shawnee' switchgrass whole-plant tissue at two Indiana locations receiving three N rates in 2016. Roann was sampled September 27 and Trafalgar was sampled September 21.

Location	Cultivar	Nitrogen Rate (kg N ha^{-1})	Nitrogen (mg g^{-1})	NDF† (mg g^{-1})	ADF‡ (mg g^{-1})	ADL§ (mg g^{-1})	Ash (mg g^{-1})
Roann	Liberty	0	2.7	816	486	86	57
Roann	Liberty	67	3.5	804	485	86	55
Roann	Liberty	134	4.0	799	478	87	55
Roann	Shawnee	0	2.7	777	452	83	56
Roann	Shawnee	67	3.8	767	453	83	55
Roann	Shawnee	134	3.6	775	467	84	53
Trafalgar	Liberty	0	1.5	782	450	77	53
Trafalgar	Liberty	67	2.3	797	475	81	54
Trafalgar	Liberty	134	2.6	794	478	85	54
Trafalgar	Shawnee	0	1.6	759	428	77	57
Trafalgar	Shawnee	67	2.6	763	446	83	51
Trafalgar	Shawnee	134	3.4	770	458	85	50
Standard Error (p < .05)			0.4	11	11	2	16

† Neutral Detergent Fiber
‡ Acid Detergent Fiber
§ Acid Detergent Lignin

3.4.9 Discussion: switchgrass composition post senescence for biofuel

It has been previously recommended that switchgrass for biofuel should contain high levels of cellulose and hemicellulose, and low levels of lignin, N, and ash (McKendry et al., 2002). Obernberger et al. (2006) has recommended that switchgrass for thermochemical conversion, where the energy products are ethanol and syngas, contain less than 6 mg N g^{-1} on dry matter basis. N concentrations were lower for both ‘Liberty’ and ‘Shawnee’ than the maximum recommended for biofuel conversion at all N fertilizer rates applied (Table 3.1). Both cultivars had increased N concentrations with additions of fertilizer, but there was no difference between the 67 and 134 kg N ha^{-1} treatments. Guretzky et al. (2010) found increasing N concentrations for each increase in N fertilizer for switchgrass harvested after seedset. Switchgrass N concentrations late in the season are similar or lower to those previously reported (Adler et al., 2006; Guretzky et al., 2010; Hoagland et al., 2013; De Koff et al., 2015).

Both cultivars had higher hemicellulose plus cellulose concentration than what has been previously reported for switchgrass (Lindsey et al., 2013, Waramit et al., 2011). ‘Liberty’ and ‘Shawnee’ averaged 799 and 769 mg g^{-1} NDF, which is the sum of hemicellulose, cellulose, and lignin. ADL concentration for ‘Liberty’ and ‘Shawnee’ averaged 84 and 83 mg g^{-1}, respectively. By subtracting ADL from NDF, we can determine dry matter concentration of hemicellulose plus cellulose. Therefore, ‘Liberty’ and ‘Shawnee’ averaged 715 and 686 mg g^{-1} of hemicellulose plus cellulose, respectively.

Similar to previous research, delaying harvest timing into late September helped to decrease N and ash content of the whole-plant, and also increases the hemicellulose and cellulose concentrations (Christian et al., 2002; McLaughlin et al., 1996; Casler et al., 2003). While delayed harvesting can help increase stand longevity, it has been reported that delayed harvesting also reduces total yield for the season (Casler et al., 2003). Producers should consider this tradeoff and decide whether obtaining a higher yield by harvesting earlier and risking stand persistence or having decreased yield from a post-senescence harvest and ensuring increased stand persistence may be better for their production system.

For biochemical conversion of switchgrass, there is not as much concern about the levels of N and other minerals because they are not being burned, as in thermochemical conversion, which releases toxic by-products (Pronobis et al., 2005). However, low levels of lignin are more ideal for biochemical conversion, as lignin is unable to be broken down efficiently by the enzymes and chemicals used during pre-treatment and fermentation (Mu et al., 2010). While there have been no suggested levels of lignin for ideal conversion to the author's knowledge, 'Liberty' and 'Shawnee' both had less than 87 mg ADL g^{-1} in this study. This lignin concentration value is lower than previously reported in switchgrass (Hu et al., 2010; Lindsey et al., 2013; Adler et al., 2006).

3.5 Conclusions

This study increased knowledge of the composition and quality two diverse switchgrass cultivars when grown in two different Indiana environments and receiving three N rates. Increasing N fertilizer caused increases in N concentrations at a given MSC. Leaf blades maintained higher N concentrations as the season progressed when compared to stem-plus-sheath and whole-plant samples. 'Liberty' had increased NDF and ADF as compared to 'Shawnee', as MSC increased. ADL was not different for the two cultivars or three N rates. Stem-plus-sheath accounted for a higher percentage of the increase in NDF, ADF, and ADL in whole-plants, as compared to leaf blades. 'Shawnee' exhibited higher IVDMD as compared to 'Liberty' throughout the season. Ash concentrations decreased as MSC increased.

At the final composition harvest date in late September, switchgrass that received N fertilizer in early May still had increased N concentration as compared to switchgrass that received no N fertilizer. ‘Liberty’ maintained higher NDF and ADF concentration in the final harvest and the whole-plant concentrations were similar to whole-plant concentrations found at MSC 3.5. Switchgrass grown at Roann had higher N, NDF, ADF, and ADL concentrations as compared to switchgrass grown at Trafalgar. Ash concentration was similar to whole-plant concentrations at MSC 3.5. The final composition harvest showed that switchgrass grown in Indiana has high concentration of hemicellulose and lignin and low concentration of ash and N, which is ideal for conversion of switchgrass to biofuel products.

3.6 Final Thoughts

Switchgrass has great potential as a biofuel and forage crop in Indiana. Producers interested in incorporating switchgrass into their pasture rotations should consider ‘Shawnee’ as it delivers a nutritional profile that is more suitable to ruminant animals as compared to ‘Liberty’. Additionally, grazing switchgrass can offer a longer utilization timeframe in the spring for the first growth to be utilized for animal feed as compared to mowing it for hay, as fiber content in the stems increases quickly as it matures. Grazing would allow animals to selectively graze leaves which maintain a higher nutritional profile with increasing maturity as compared to the stem-plus-sheath. Additions of N fertilizer will increase N concentration of the plant and allow it to be higher protein for a longer timeframe as compared to plants that receive no N fertilizer.

Producers considering adding switchgrass for wildlife or environmental benefits, who may want the possibility of cutting once a year to be used as a biomass crop, may want to consider utilizing ‘Liberty’ due to its increased concentrations of cellulose and hemicellulose for biomass purposes when receiving no N fertilizer. ‘Liberty’ also had less lodging issues as compared to ‘Shawnee’. Indiana would benefit from future research exploring use of switchgrass in pasture rotations or as a dual-use hay and biofuel crop.

Breeding efforts to create ‘Liberty’, a cultivar with improved properties for utilization as a biofuel feedstock, were successful as there was increased fiber concentration and decreased N concentrations when compared to ‘Shawnee’. ‘Liberty’ also had increased hemicellulose and cellulose concentration as compared to previous studies. Future pilot studies utilizing switchgrass for thermochemical or biochemical conversion in an applied setting could be used to spark local interest and investment, develop a biomass supply chain, and provide infrastructure to meet future biofuel standards. Producers may consider growing switchgrass in buffer strips within their row cropping system (Schultz et al., 1995). Utilizing ‘Liberty’ for buffer strips would have added value with the prospect of being sold as a biofuel crop or harvested as hay for ruminants in years when a feed shortage occurs.

CHAPTER 4. SEASONAL CROP GROWTH AND FINAL YIELD OF TWO DIVERSE SWITCHGRASS CULTIVARS AT THREE INDIANA LOCATIONS

4.1 Abstract

Switchgrass (*Panicum virgatum* L.) is an important warm-season perennial grass in livestock systems and has been extensively researched as an herbaceous energy crop. To fully examine the potential for utilizing switchgrass as a widespread biofuel crop, studies of yield and growth across states and environments are important. Objectives of these field studies were to compare the crop growth and yield of a recently developed biofuel cultivar, 'Liberty', to an improved forage cultivar, 'Shawnee', receiving three different N rates in multiple Indiana environments. Differences in grams m^{-2}, mass $tiller^{-1}$, and tiller number per unit area were analyzed in response to growing degree days (GDD) and day of year (DOY). Pure stands of each cultivar were sampled in field studies at Trafalgar and Roann, Indiana in 2016 and 2017. A third location, Lafayette, IN, was also sampled in 2017. Samples were collected weekly early in the season and every other week late in the season to analyze accumulation of dry matter per m^{-2}, dry matter per tiller, and total number of tillers per sample area. Number of tillers and mass $tiller^{-1}$ responded linearly to both GDD and DOY for both years. Grams m^{-2} responded quadratically to both GDD and DOY. 'Liberty' had 20 percent higher mass $tiller^{-1}$ at the end of the season as compared to 'Shawnee.' Addition of N fertilizer generally increased mass $tiller^{-1}$ and yield m^{-2}. Roann, the northern most site, also had highest tiller numbers at the beginning of the season and these decreased faster than at the central Indiana sites. 'Liberty' yielded 8.8 percent higher than 'Shawnee' across locations, N rates, and sampling years. Addition of N fertilizer did not conclusively increase forage yield. Grams m^{-2}, mass $tiller^{-1}$, and tillers per sample area helped

explain some yield differences. For example, ‘Liberty’ had increased yield as compared to ‘Shawnee’, and ‘Liberty’ also had higher mass tiller^{-1} with no differences in tiller number as compared to ‘Shawnee’.

4.2 Introduction

While forage composition for both animal and biofuel purposes is important, it is also essential that the crop be high yielding. In addition to measuring total yield for the desired purpose as feed for ruminants or as a biofuel, understanding how the crop grows over time can help make management decisions and direct future breeding improvement efforts. While the concept of studying crop growth has been discussed since the early 1900’s, relatively little research of switchgrass growth, measured by dry matter accumulation, throughout the season has been published. This is most likely due to the amount of labor needed to collect adequate samples. However, Boe et al. 2007, have studied how mass tiller^{-1} and tiller number related to final yield measurements. This study is similar in design, but analyzes the mass tiller^{-1} and tiller number changes over an entire growing season. By understanding how switchgrass accumulates dry matter over the season, insight can be gained as to how genetic and environmental factors impact yield.

4.3 Materials and Methods

4.3.1 Crop Growth

To determine crop growth of ‘Shawnee’ and ‘Liberty’ switchgrass, field studies were completed during 2016 and 2017. Plot establishment and sample collection occurred as outlined in the Materials and Methods section of Chapter 2. For this study, each sample was evenly split into thirds, with one - third being processed as whole-plant samples. The other two-thirds were used for studies reported in Chapters 2 and 3. After processing, all samples were dried in a forced air oven at 60°C. Dry weights for each third were summed to determine the total dry weight per sample area (0.09 m^{-2}). Dry weights were converted to grams per m^{-2} and used to measure dry matter accumulation over the sampling period for the 2016 and 2017 seasons. Total tiller number in the 0.09 m^{-2} sample area was also recorded before drying and used to determine mass $tiller^{-1}$.

Data was evaluated using the mixed models procedure in SAS, as described in Chapter 2. Fixed categorical variables were location, cultivar, N rate (2016), and each sample replication was treated as an independent random variable within each location. In 2017, only the 67 kg N ha^{-1} treatment at Roann and Trafalgar, and the 112 kg N ha^{-1} treatment at Lafayette were sampled as N rate did not affect morphological development in 2016. Thus, N rate was not a fixed effect in models for 2017 data. The GDD and DOY were continuous variables. All two- and three-way interactions among fixed, random, and continuous variables were included in the model. The dependent variable was either grams m^{-2}, mass $tiller^{-1}$, or tiller number per sample area. Variables were considered significant when $P < 0.05$. Data based on GDD are discussed in the results section. This allows for better comparisons across locations, as it compares growth across similar GDD. Equations for crop growth and DOY are reported in Appendix Table 4.

4.3.2 Yield

After senescence in 2014 to 2016, a biomass harvest was collected from a 1.2 m X 7.6 m area with a Wintersteiger Cibus S cut at a height of 15 cm. In 2017, a biomass measurement sample was taken using a sickle bar walk behind mower (Garden Way Incorporated, Model Number: 34063) from a 1.2 X 6 m area. At Roann, final harvests were taken on December 2, 2014, November 19, 2015, November 15, 2016, and November 8, 2017. At Trafalgar, final harvests were taken on October 24, 2014, November 23, 2015, November 16, 2016, and November 3, 2017.

To determine total biomass from the harvested area, the total sample was weighed by the Wintersteiger in 2014 to 2016, and with a tripod scale in 2017. To determine dry matter yield, a subsample was collected randomly from the total sample and dried in a forced air oven at 60°C. Percent dry matter from each subsample was used to calculate dry matter yield of harvested switchgrass.

In the SAS model, the fixed categorical variables were location, cultivar, N rate, and year. Each sample replication was treated as an independent random variable within each location. All two- and three-way interactions were included in the model. The dependent variable was dry matter yield measured in kg ha^{-1}. Variables were considered significant when $P < 0.05$. Data for years 2014 to 2016 were compared to each other, but data from 2017 was analyzed separately due to the difference in harvest method.

4.4 Results

4.4.1 Crop Growth measured in grams m^{-2}

Total dry weights were measured for each sampling date listed in Appendix Table 1. In 2016 and 2017, grams m^{-2} of 'Shawnee' and 'Liberty' receiving three N rates were best related to GDD in a quadratic model (Table 4.1). In 2016, there was a significant interaction between GDD and N rate (P =0.02). As GDD increased, the zero kg ha^{-1} N treatments accumulated significantly less dry matter as compared to the 67 and 134 kg ha^{-1} N treatments (Figure 4.1). This difference is accounted for by a decreased slope for the 0 kg N ha^{-1} predicted equation (Table 4.1). There was no significant difference between 67 and 134 kg ha^{-1} N treatments. Location and cultivar were not significantly different in 2016 for grams m^{-2}. While there was no significant difference between cultivars or a significant cultivar by N rate by GDD interaction, ‘Liberty’ and ‘Shawnee’ are graphed separately in Figure 4.1 to compare the trends of each cultivar. ‘Liberty’ tended to increase more with applications of nitrogen fertilizer as compared to ‘Shawnee’. ‘Shawnee’ trends showed higher grams m^{-2} when receiving 67 kg N ha^{-1} as compared to 134 kg N ha^{-1}, whereas ‘Liberty’ receiving 67 and 134 kg N ha^{-1} were more similar.

Table 4.1 Accumulation of grams m^{-2} predicted by Growing Degree Days (GDD) for ‘Shawnee’ and ‘Liberty’ switchgrass in two sampling years in Indiana receiving varying nitrogen rates.

				Grams m^{-2} predicted by GDD		
Year	Location	Cultivar	Nitrogen Rate (kg ha^{-1})	b_o †	b_l ‡	b_q §
2016	Trafalgar	Shawnee	0	-225	5.19	-0.0016
			67	-1357	10.43	-0.0041
			134	-1089	10.95	-0.0047
2016	Trafalgar	Liberty	0	-532	6.21	-0.0024
			67	-761	9.18	-0.0036
			134	-827	9.34	-0.0030
2016	Roann	Shawnee	0	-965	8.33	-0.0033
			67	-854	8.77	-0.0030
			134	-989	10.19	-0.0041
2016	Roann	Liberty	0	-1331	11.76	-0.0051
			67	-990	11.54	-0.0035
			134	-1490	10.98	-0.0035
2017	Trafalgar	Shawnee	67	-993	10.20	-0.0042
		Liberty	67	-1496	11.22	-0.0043
2017	Roann	Shawnee	67	-1194	8.49	-0.0033
		Liberty	67	-1398	9.48	-0.0037
2017	Lafayette	Shawnee	112	-499	6.65	-0.0028
		Liberty	112	-372	4.66	-0.0012

†Y intercept

‡Linear Slope Coefficient

§Quadratic Slope Coefficient

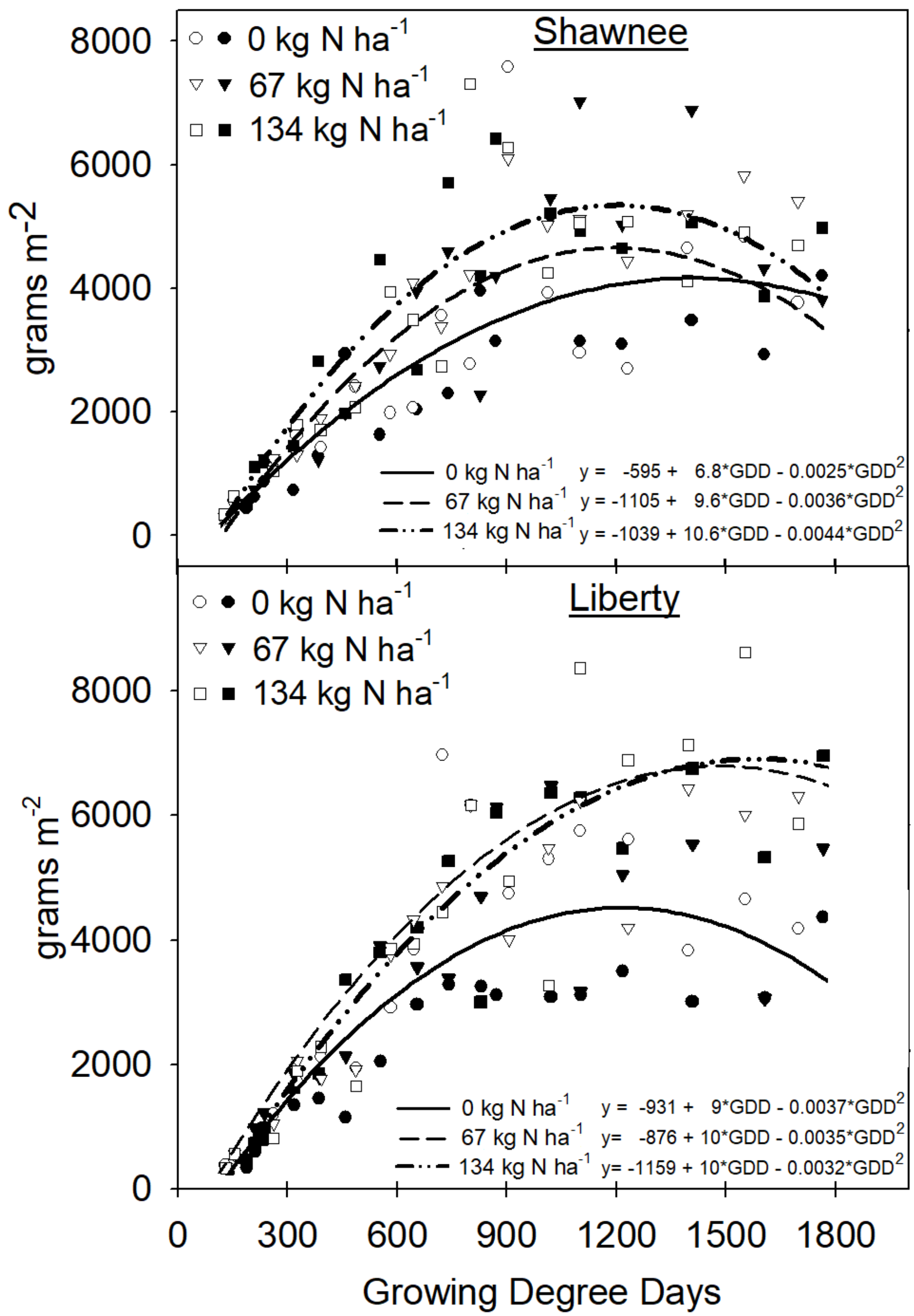

Figure 4.1 Dry matter accumulation of ‘Liberty’ and ‘Shawnee’ switchgrass in grams m^{-2} as predicted by growing degree days (GDD) for 2016. Each point represents the mean data and each line represents the predicted equation. Open and closed symbols represent Trafalgar and Roann, respectively.

In 2017, there was a significant interaction between GDD and location (Figure 4.2). As GDD accumulated, the rate of growth was fastest at Trafalgar, followed by Roann. Lafayette has the slowest rate of growth. The differences in accumulated grams m^{-2} were primarily influenced by increased slope values and not intercept values (Table 4.1). While there was no significant difference between cultivars, Figure 4.2 illustrates grams m^{-2} trends for ‘Liberty’ and ‘Shawnee’ as GDD increase. For both ‘Liberty’ and ‘Shawnee’, Trafalgar had the highest grams m^{-2} through the growing season. ‘Liberty’ at Roann tended to have a faster decline in grams m^{-2} after GDD of 1200, as compared to ‘Shawnee’ at Roann. ‘Liberty’ at Lafayette continued to increase until the final harvest, as compared to ‘Shawnee’ at Lafayette, which decreased after GDD of 1100.

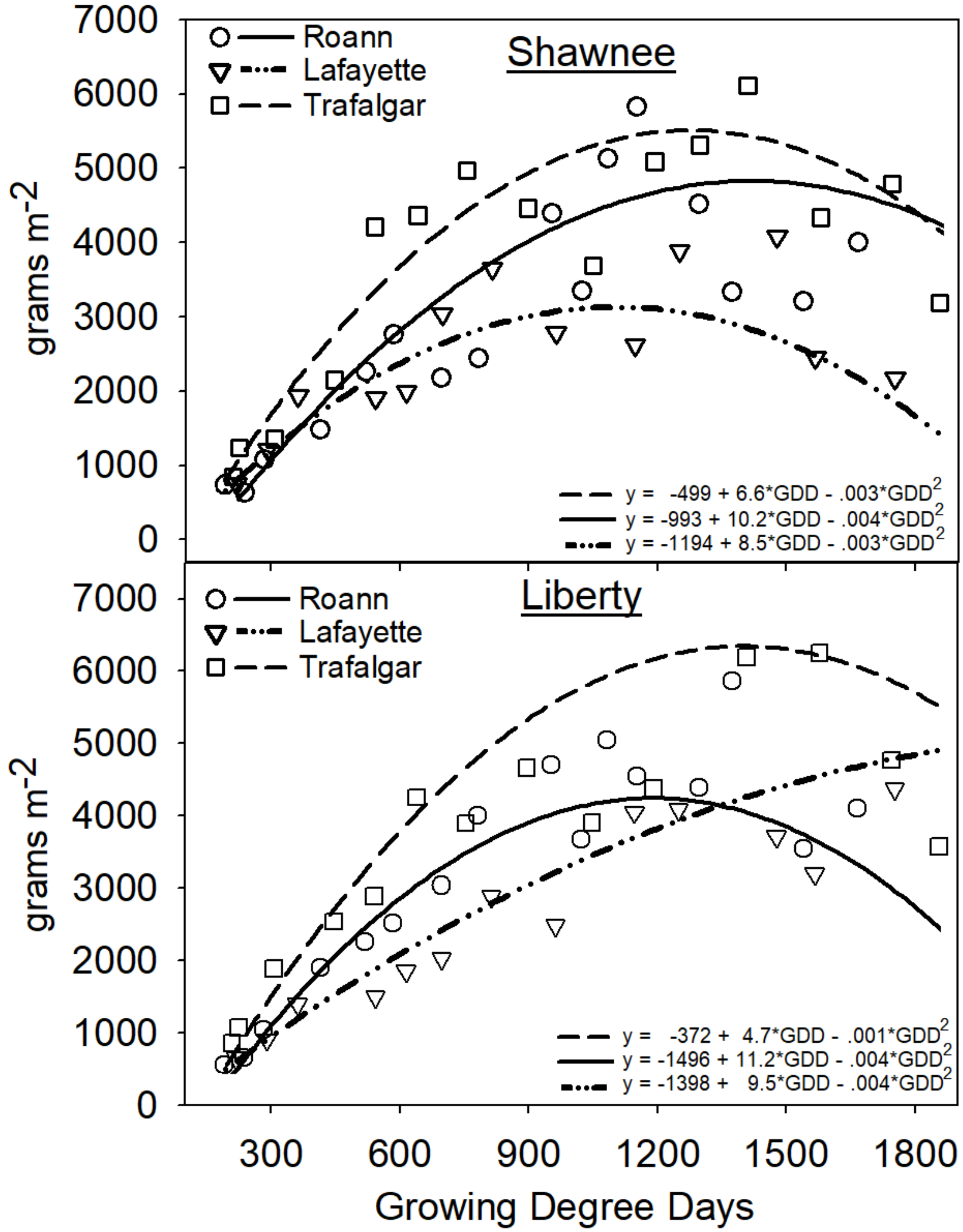

Figure 4.2 Dry matter accumulation in grams m^{-2} for ‘Liberty’ and ‘Shawnee’ switchgrass cultivars as predicted by Growing Degree Days in 2017. Each point represents the mean data and each line represents the predicted equation.

4.4.2 Crop growth measured in mass tiller^{-1}

In 2016 and 2017, mass tiller^{-1} was significantly related to GDD (P <.0001). Accumulated mass tiller^{-1} was best related to GDD in a linear model (Table 4.2). In 2016 and 2017, there was a significant two-way interaction between GDD and cultivar (P < 0.01). As GDD accumulated over the growing season, 'Liberty' had significantly higher weight per tiller as compared to 'Shawnee' (P < 0.01; Figure 4.3). The higher grams per tiller is expressed as an increased slope for 'Liberty' as compared to 'Shawnee'. While 'Shawnee' had initially higher dry weight means, 'Liberty' had higher dry weights beginning mid-July, or approximately 850 GDD, and continued until the final harvest. At the first sampling date in 2016, 'Shawnee' averaged 0.33 g tiller^{-1}, whereas 'Liberty' averaged 0.30 g tiller^{-1}. However, by the final sample date, 'Shawnee' averaged 5.8 g tiller^{-1}, whereas 'Liberty' averaged 7.2 g tiller^{-1}. In 2016, there was a significant interaction between GDD and N rate (P < 0.0001). The zero N treatment accumulated significantly less (P < 0.001) mass tiller^{-1} as GDD increased as compared to the 67 and 134 kg ha^{-1} N treatments as indicated by the lower slope as GDD increased (Figure4. 3). There was no statistical difference between the 67 and 134 kg N ha^{-1} treatments in mass tiller^{-1}. While there was no significant interaction between GDD, cultivar, and N rate, 'Liberty' and 'Shawnee' are graphed separately in Figure 4.3 to illustrate that 'Liberty' tended to have an increased mass tiller^{-1} response with additions of 134 kg N ha^{-1} as compared to 'Shawnee'.

Table 4.2 Accumulation of mass tiller^{-1} predicted by Growing Degree Days for 'Liberty' and 'Shawnee' switchgrass cultivars in two sampling years in Indiana receiving varying nitrogen rates.

				Mass tiller^{-1} predicted by GDD	
Year	Location	Cultivar	Nitrogen Rate (kg ha^{-1})	b_o †	b_l ‡
2016	Trafalgar	Shawnee	0	0.483	0.0027
			67	0.534	0.0044
			134	0.295	0.0048
2016	Trafalgar	Liberty	0	0.018	0.0039
			67	0.263	0.0050
			134	0.056	0.0063
2016	Roann	Shawnee	0	0.186	0.0035
			67	-0.295	0.0054
			134	0.153	0.0052
2016	Roann	Liberty	0	0.628	0.0042
			67	-0.180	0.0054
			134	-0.412	0.0061
2017	Trafalgar	Shawnee	67	1.224	0.0035
		Liberty	67	0.959	0.0051
2017	Roann	Shawnee	67	-0.188	0.0043
		Liberty	67	-0.115	0.0051
2017	Lafayette	Shawnee	112	0.143	0.0039
		Liberty	112	-0.764	0.0057

†Y intercept

‡Linear Slope Coefficient

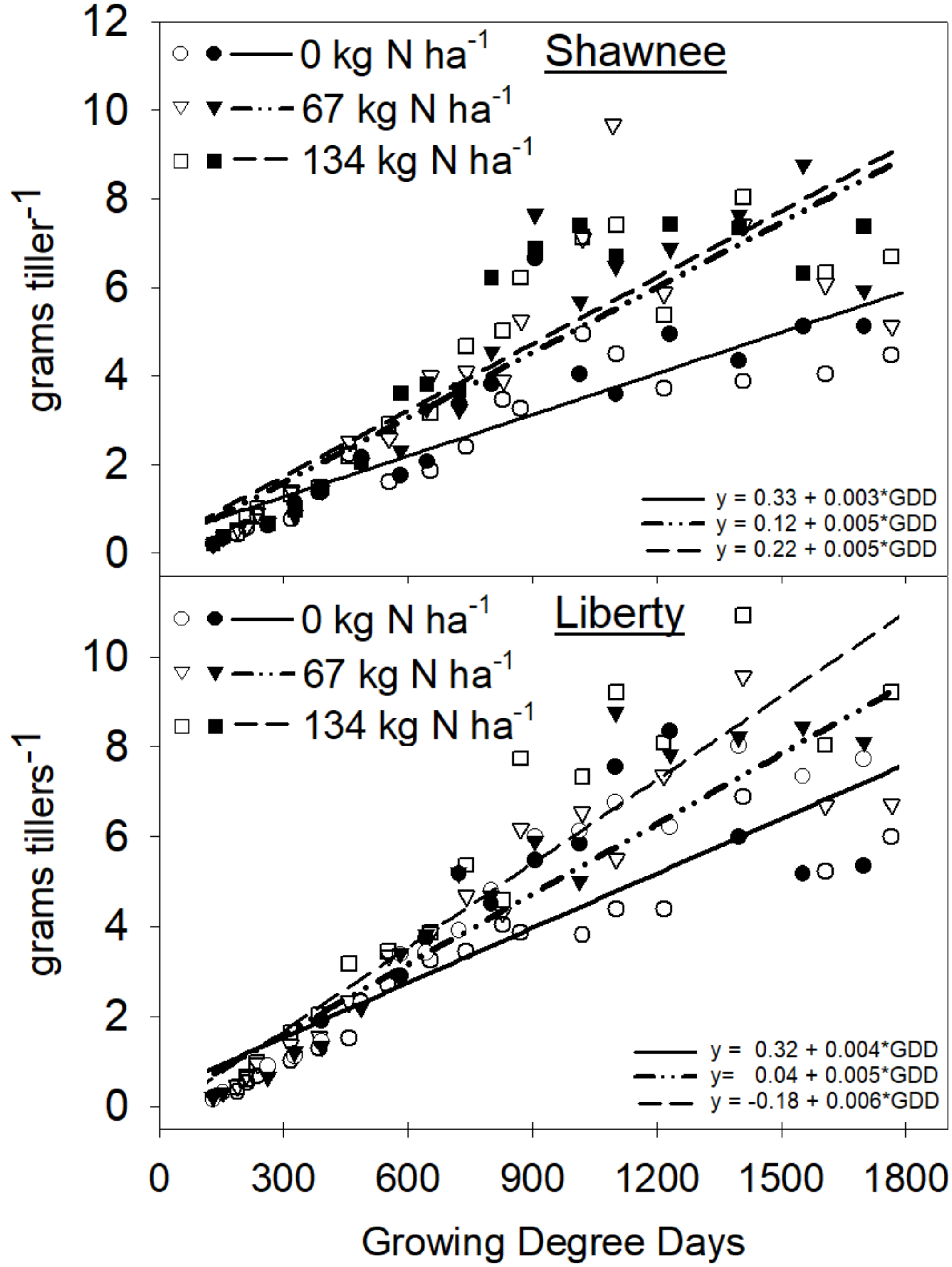

Figure 4.3 Dry matter accumulation in mass tiller^{-1} as predicted by Growing Degree Days (GDD) of 'Shawnee' and 'Liberty' switchgrass in 2016. Each point represents the mean data and each line represents the predicted equation. Open and closed symbols represent Trafalgar and Roann data, respectively.

4.4.3 Tiller Number

In 2016, there were significant differences in number of tillers per 0.09 m^2 between locations ($P < 0.05$). Significant two-way interactions were found between GDD and location and GDD and N rate ($P < 0.05$). Tiller number at Roann had higher intercept values, indicating greater tiller number in the beginning of the season as compared to Trafalgar (Table 4.3). Roann also had more negative slope values, meaning that the number of tillers per sample area decreased more quickly at Roann than at Trafalgar. Tiller numbers also decreased slower for plots receiving 0 and 67 kg N ha^{-1} through the growing season, as compared to the 134 kg N ha^{-1} plots. There was a three way interaction between cultivar, N rate, and GDD ($P < 0.05$). 'Liberty' receiving 67 kg N ha^{-1} had more negative slopes as compared to the 0 and 134 kg N ha^{-1} rates (Table 4.3), so tiller number decreased the fastest for 'Liberty' receiving 67 kg N ha^{-1} as GDD accumulated. For 'Shawnee', each addition of N fertilizer caused a more negative slope, meaning tiller number decreased fastest for the 134 kg N ha^{-1} rate, as compared to the 0 and 67 kg N ha^{-1} rate.

Table 4.3 Regression equations describing how tiller number per 0.09 m^{-2} changes as Growing Degree Days increase for ‘Shawnee’ and ‘Liberty’ switchgrass grown at two Indiana locations when receiving three nitrogen rates in 2016.

Location	Cultivar	Nitrogen Rate (kg N ha^{-1})	b_o †	b_1 ‡
Roann	Liberty	0	135.7	-0.049
		67	167.4	-0.075
		134	144.8	-0.052
Roann	Shawnee	0	129.5	-0.040
		67	151.8	-0.063
		134	149.6	-0.069
Trafalgar	Liberty	0	104.6	-0.031
		67	116.7	-0.039
		134	102.7	-0.030
Trafalgar	Shawnee	0	102.8	-0.016
		67	101.5	-0.023
		134	124.7	-0.041

†Y intercept

‡Linear Slope Coefficient

Tiller number decreased linearly as GDD increased for both sampling years, but there were no significant differences between cultivars. In 2017, there was a GDD by location interaction (Figure 4.4). Roann and Lafayette had the highest tiller numbers at the first sampling date, but by the end of the season Trafalgar had the highest tiller number. Roann and Lafayette had more negative slope values as compared to Trafalgar, which accounts for the change in rank of tiller number that occurred between 900 and 1200 GDD.

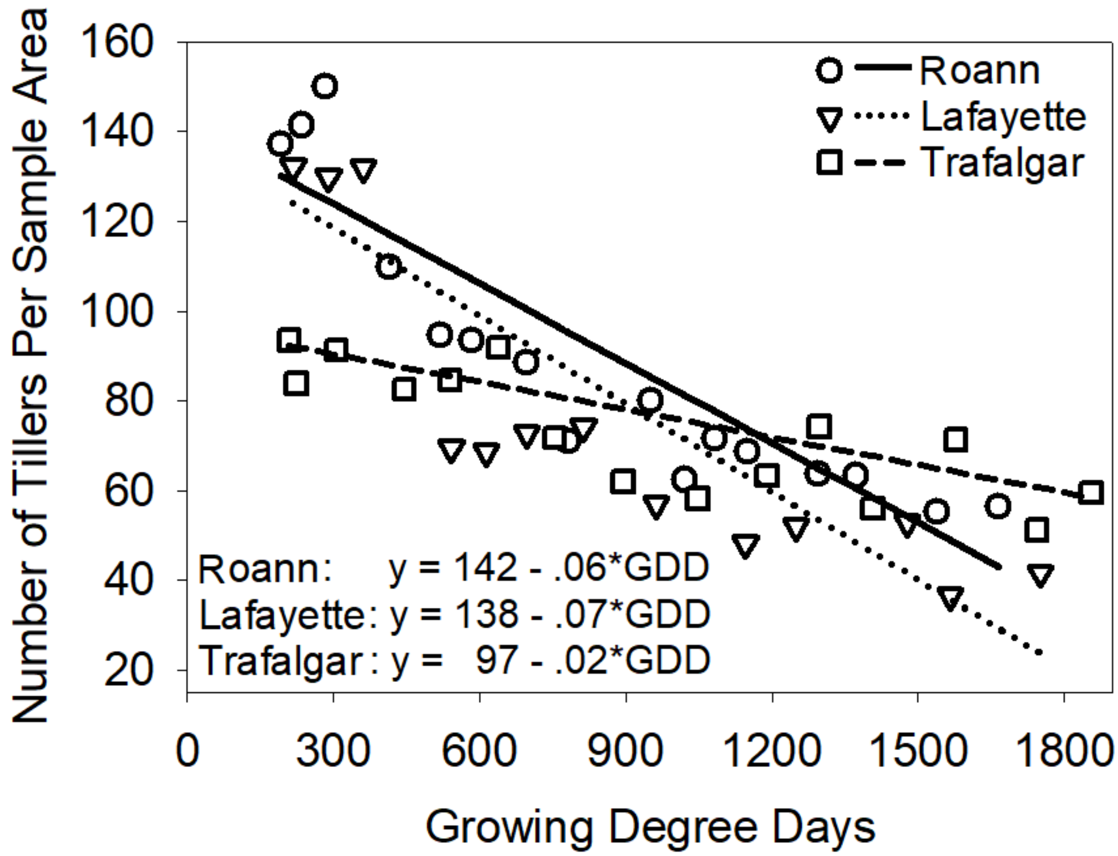

Figure 4.4 Tiller number per 0.09 m^{-2} for the 2017 growing season as measured across Growing Degree Days for three Indiana locations.

4.4.4 Yield

Final yield measurements were taken in 2014 through 2017 at Trafalgar and Roann. Yield for years 2014 through 2016 were analyzed together, while 2017 was analyzed separately due to differences of harvest method.

There were no significant differences in yield between locations. Within N rates, the difference between 0 and 67 kg N ha^{-1} was approaching significance (P = 0.078). However, the yield of the 0 and 134 kg N ha^{-1} and 67 and 134 kg N $^{-1}$ were similar (P > 0.10). Application of 67 kg N ha^{-1}

increased yield from 7323 to 8414 kg ha^{-1}, but adding a total of 134 kg N ha^{-1} resulted in a yield of 8219 kg ha^{-1}. Visual scoring of plots was completed on a scale of one to nine with nine meaning plants are standing upright with no lodging. Switchgrass receiving 67 and 134 kg N ha^{-1} had a mean lodging score of six, whereas switchgrass receiving 0 kg N ha^{-1} had a mean lodging score of 8 (data not shown). This creates a problem when trying to harvest switchgrass for biomass, as plants that are lodged are harder to mow and leave more biomass in the field after harvest than non-lodged crops. In addition, 'Liberty' had a mean lodging score of 7 and 'Shawnee' had a mean lodging score of 6 (data not shown).

Yield differed significantly across years ($P < 0.01$; Table 4.4). Yield was significantly higher in 2015 as compared to 2014 ($P < 0.01$) and 2016 ($P < 0.01$). In 2015, mean yield was 2581 and 2775 kg ha^{-1} higher than in 2014 or 2016, respectively ($P <0.01$). 'Liberty' had higher yield in 2015 and 2016 than 'Shawnee'. In 2015, 'Liberty' yield was 10,492 kg ha^{-1}, while 'Shawnee' averaged 9,048 kg ha^{-1}. Our research confirms that 'Liberty' was 8.8 percent higher yielding in multiple Indiana environments and over four years as compared to 'Shawnee'.

In 2017, there were no significant differences between locations, cultivars, N rates, or the interactions among the variables. On May 7 and 8 of 2017, there were two nights after the switchgrass plants had broken dormancy, where temperatures dropped below 0°C at Roann. Visual inspection of the field noted that one replication of 'Liberty' at Roann was more affected by this freeze, due to lower lying topography, as compared to other plots. While the switchgrass seemed to recover well from the injury, this event could be negatively impacting the realized yield and growth of switchgrass at Roann in 2017, especially 'Liberty'. 'Liberty' mean yield was

8167 and 11965 kg ha^{-1} at Roann and Trafalgar, respectively. Mean yield at Roann and Trafalgar for ‘Shawnee’ kg ha^{-1} was 8939 and 10269, respectively.

Table 4.4 Dry Matter Yield (kg ha^{-1}) of 'Shawnee' and 'Liberty' receiving three nitrogen rates at two Indiana locations over four years.

Location	Cultivar	Nitrogen Rate (kg N ha^{-1})	Dry Matter Yield (kg ha^{-1})				
			2014	2015	2016	2017	Mean
Roann	Liberty	0	5928	8976	8290	8051	7811
		67	7463	11301	7815	8245	8706
		134	6944	11550	6670	8206	8342
Roann	Shawnee	0	6062	6714	6123	8002	6725
		67	8407	9119	6338	8560	8106
		134	8923	8788	6159	10256	8532
Trafalgar	Liberty	0	6410	9990	7955	10244	8650
		67	7168	10093	7176	12182	9155
		134	7646	11045	8446	13468	10151
Trafalgar	Shawnee	0	7312	8198	5923	8278	7428
		67	7473	11518	7101	12154	9561
		134	6540	9954	5959	10374	8206
Standard error (P < 0.05)			887			770	

4.5 Discussion

4.5.1 Relating Crop Growth and Biomass Yield

Previous studies with switchgrass have reported that differences in biomass production between cultivars can be related back to weight tiller^{-1} and tillers m^{-2} (Boe et al., 2005; Boe 2007). Weight tiller^{-1} accounts for more variability in biomass production that tillers m^{-2} (Boe et al., 2005; Boe 2007). Other measures, such as weight per reproductive tiller and weight per phytomer, have been suggested as having strong influence on biomass yield (Boe et al., 2008; Boe et al., 2005). In Miscanthus (Miscanthus X giganteus), a strong linear relationship occurred between biomass yield and total number of tillers, weight tiller^{-1}, and number of reproductive tillers (Lee et al., 2017). Sanderson and Reed (2000) found that switchgrass tiller weight was related to availability of water and N, whereas, tiller number was related to aboveground competition among plants. In this study, 'Liberty' yielded 8.8 percent higher biomass yields, on average, than 'Shawnee'. 'Liberty' also had higher weight tiller^{-1} than 'Shawnee' with no difference occurring between the cultivars for the total number of tillers per 0.09 m^{-2}. Wullschleger et al., 2010, also found that lowland ecotypes exhibited significantly higher yield (P < 0.001) than upland ecotypes. Since 'Liberty' is a cross between and upland and lowland ecotype, it would reason that it should have increased biomass yield when grown in a similar environment as 'Shawnee'.

In 2016, higher biomass yield (P < 0.10) occurred with the addition of 67 kg N ha^{-1}. Greater mass tiller^{-1} was found for the 67 kg N ha^{-1} treatment as compared to the 0 kg N ha^{-1} rate. However, nitrogen rates did not conclusively increase yield in all years. Previous research has reported great variability in switchgrass response to N fertilizer application (Wullschleger et al., 2010; Brejda et al., 2000). A comparative analysis of many research trials by Wullschleger et al. (2010) found that upland cultivars increased in yield with as much as 100 kg N ha^{-1}, and decreased yield was found at higher N treatments. The increase in tiller weight seems to be a large contributor to greater biomass yield in switchgrass with the tradeoff that number of tillers will decrease as the season progresses. Due to the nature of switchgrass as a bunchgrass, number of tillers per area varies widely within one plot. Where one area with a vigorous plant may exhibit high tiller numbers, the adjoining area could be bare, with no plant growing. For future research efforts examining accumulated grams m^{-2} or tillers m^{-2}, it would be ideal for larger and more sampling areas to be taken from within the field to accurately assess the stand.

4.6 Conclusions

By examining crop growth measures like tiller number and dry weight per tiller, factors that may affect final yield measurements can be analyzed. Our study found higher mass $tiller^{-1}$ for 'Liberty' as compared to 'Shawnee', and additions of 67 kg N ha^{-1} increased mass $tiller^{-1}$ as the season progressed. There was no difference in tiller number per sample area between 'Liberty' and 'Shawnee'. From our studies, switchgrass grown in Indiana did not benefit from high N fertilizer applications, and plants receiving no N fertilizer yielded as high as the plants receiving moderate N fertilizer levels for the final harvest. Switchgrass receiving N fertilizer was often lodged by the final harvest, which creates difficulties during harvest. Switchgrass breeding efforts to produce a higher yielding biomass crop were successful with the creation of 'Liberty'. 'Liberty' was 8.8 percent higher yielding in Indiana and lodged less at the final harvest when compared to 'Shawnee'. While some crop growth measures showed higher mass per tiller or higher tiller numbers m^{-2}, these results didn't always directly correlate to the differences found in final yield. Thus, utilizing various crop growth parameters, such as mass $tiller^{-1}$ and tiller number per area, with final yield can help reach a more comprehensive understanding of the factors that may or may not influence cultivar yield differences.

CONCLUSIONS

These studies increased knowledge of switchgrass morphological development, compositional quality, growth, and yield in Indiana, and examined differences exhibited between 'Liberty' and 'Shawnee', two diverse switchgrass cultivars, when receiving varying N rates across Indiana. Objectives of this series of studies were to compare morphological development, compositional quality, crop growth, and yield of a recently developed biofuel cultivar 'Liberty' to an improved forage cultivar 'Shawnee' in multiple Indiana environments. Both growing degree days (GDD) and day of year (DOY) were acceptable predictors of switchgrass morphological development. Previous researchers have suggested that GDD may offer a better predictive ability over a wider range of locations. This study did not show that GDD or DOY was a better predictor; however, prediction equations were made and validated at the same or very similar locations. Additional research studying the predictive power of both GDD and DOY equations created in this study at more diverse locations throughout the Midwest, may determine that GDD prediction equations are more robust. N rate did not significantly affect the developmental morphology of 'Liberty' or 'Shawnee'. For both cultivars, location had the largest impact on developmental progression. Differences in location are often driven by temperature and daylength, which can be represented by the predictive terms GDD and DOY. Additionally, the recently released cultivar 'Liberty' exhibited slower morphological development than 'Shawnee' by approximately seven to ten days. Differences between MSC and MSW at each sampling date were minimal. Thus, MSC may be a more practical system for use by producers because MSW would require samples to be dried and weighed which is not as time efficient.

Regarding compositional quality, N fertilizer applications increased N concentrations at a given MSC. Leaf blades maintained higher N concentrations as the season progressed when compared to stem-plus-sheath and whole-plant samples. ‘Liberty’ had increased NDF and ADF as compared to ‘Shawnee’, as MSC increased. ADL was not different for the two cultivars or three N rates. Stem-plus-sheath accounted for a higher percentage of the increase in NDF, ADF, and ADL in whole-plants, as compared to leaf blades. ‘Shawnee’ exhibited higher IVDMD as compared to ‘Liberty’ throughout the season. Whole-plant ash concentrations decreased as MSC increased. Producers interested in incorporating switchgrass into their pasture rotations should consider ‘Shawnee’ as it delivers a nutritional profile that is more suitable to ruminant animals as compared to ‘Liberty’.

At the final composition harvest date in late September, switchgrass that received N fertilizer in early May still had increased N concentration as compared to switchgrass that received no N fertilizer. ‘Liberty’ had higher NDF and ADF than ‘Shawnee’ at the final harvest and the whole-plant concentrations were similar to whole-plant concentrations found at MSC 3.5. Switchgrass grown at Roann had higher N, NDF, ADF, and ADL concentrations as compared to switchgrass grown at Trafalgar. Final harvest ash concentration was similar the whole-plant concentrations found at MSC 3.5. The final composition harvest showed that switchgrass, especially ‘Liberty’, grown in Indiana has high concentration of hemicellulose and lignin and low concentration of ash and N, which is ideal for conversion of switchgrass to biofuel products.

The study of crop growth and yield examined measures such as tiller number and dry weight per tiller to see if those factors can explain differences found in final yield. While some crop growth measures showed higher grams per tiller or higher tiller numbers m^{-2}, these results didn't always directly correlate to the differences found in final yield. Switchgrass grown in Indiana did not benefit from high N fertilizer applications, and plants receiving no N fertilizer yielded as high as the plants receiving moderate N fertilizer levels. Switchgrass receiving N fertilizer was often lodged by the final harvest, which creates difficulties during harvest. Switchgrass breeding efforts to produce a higher yielding biomass crop were successful with the creation of 'Liberty'. 'Liberty' was 8.8 percent higher yielding in Indiana, and was less lodged at each final harvest when compared to 'Shawnee'. Utilizing various crop growth parameters, such as mass $tiller^{-1}$ and tiller number per area, with final yield can help reach a more comprehensive understanding of the factors that may or may not influence cultivar yield difference.

APPENDIX

Table A.2. Sampling Dates and the respective Growing Degree Day (GDD) or Day of Year (DOY) for 2016 and 2017 across three Indiana locations.

Location	Date	DOY	GDD	Location	Date	DOY	GDD	Location	Date	DOY	GDD
Roann	5/16/2016	136	130	Trafalgar	5/11/2016	131	189	Lafayette	5/15/2017	134	217
	5/23/2016	143	154		5/18/2016	138	211		5/22/2017	141	290
	6/1/2016	152	262		5/24/2016	144	237		5/31/2017	150	361
	6/8/2016	159	327		5/31/2016	151	318		6/14/2017	164	542
	6/14/2016	165	392		6/6/2016	157	386		6/19/2017	169	614
	6/21/2016	172	487		6/13/2016	164	459		6/27/2017	177	697
	6/29/2016	180	582		6/20/2016	171	554		7/6/2017	186	813
	7/6/2016	187	645		6/27/2016	178	655		7/17/2017	197	962
	7/12/2016	193	723		7/6/2016	187	741		7/31/2017	211	1146
	7/18/2016	199	801		7/13/2016	194	829		8/10/2017	221	1249
	7/25/2016	206	906		7/19/2016	200	872		8/30/2017	241	1477
	8/2/2016	214	1014		7/26/2016	207	1021		9/12/2017	254	1566
	8/8/2016	220	1101		8/1/2016	213	1102		9/26/2017	268	1752
	8/17/2016	229	1232		8/9/2016	221	1217				
	8/30/2016	242	1397		8/23/2016	235	1408				
	9/13/2016	256	1552		9/7/2016	250	1606				
	9/26/2016	269	1699		9/21/2016	264	1766				
	5/17/2017	136	191		5/15/2017	134	209				
	5/24/2017	143	236		5/22/2017	141	224				
	5/30/2017	149	282		5/30/2017	149	306				
	6/12/2017	162	414		6/12/2017	162	446				
	6/20/2017	170	519		6/19/2017	169	540				
	6/27/2017	177	583		6/26/2017	176	639				
	7/6/2017	186	696		7/7/2017	187	754				
	7/13/2017	193	782		7/18/2017	198	896				
	7/26/2017	206	952		7/28/2017	208	1048				
	8/1/2017	212	1022		8/7/2017	218	1191				
	8/8/2017	219	1082		8/14/2017	225	1297				
	8/15/2017	226	1151		8/23/2017	234	1408				
	8/29/2017	240	1296		9/5/2017	247	1578				
	9/11/2017	253	1373		9/19/2017	261	1745				
	9/25/2017	267	1539		10/2/2017	274	1856				
	10/11/2017	283	1666								

Table A.2. Calibration equations describing morphological growth of two switchgrass cultivars receiving various nitrogen fertilizer rates at three Indiana locations during 2016 and 2017.

				MSC Predicted by GDD			MSW Predicted by GDD			MSC Predicted by DOY			MSW Predicted by DOY		
Year	Location	Cultivar	Nitrogen Rate (kg N ha^{-1})	b_o†	b_1‡	RMSE§	b_o	b_1	RMSE	b_o	b_1	RMSE	b_o	b_1	RMSE
2016	Trafalgar	Shawnee	0	1.68	0.0013	0.20	1.80	0.0013	0.21	-0.29	0.016	0.17	-0.21	0.016	0.15
			67	1.69	0.0015	0.15	1.78	0.0015	0.16	-0.49	0.019	0.23	-0.39	0.018	0.16
			134	1.74	0.0014	0.17	1.83	0.0014	0.16	-0.39	0.017	0.16	-0.28	0.017	0.17
2016	Trafalgar	Liberty	0	1.52	0.0015	0.17	1.67	0.0014	0.17	-0.70	0.018	0.14	-0.48	0.017	0.13
			67	1.56	0.0015	0.17	1.73	0.0014	0.16	-0.72	0.018	0.13	-0.44	0.017	0.12
			134	1.64	0.0014	0.18	1.76	0.0013	0.17	-0.46	0.017	0.13	-0.27	0.016	0.13
2016	Roann	Shawnee	0	1.46	0.0016	0.24	1.55	0.0016	0.22	-1.10	0.019	0.16	-1.10	0.020	0.17
			67	1.40	0.0017	0.22	1.49	0.0018	0.20	-1.42	0.021	0.13	-1.39	0.022	0.16
			134	1.45	0.0017	0.23	1.54	0.0017	0.21	-1.32	0.021	0.15	-1.29	0.021	0.16
2016	Roann	Liberty	0	1.26	0.0018	0.21	1.37	0.0018	0.17	-1.56	0.022	0.11	-1.49	0.022	0.16
			67	1.30	0.0018	0.21	1.38	0.0018	0.17	-1.46	0.021	0.12	-1.44	0.022	0.16
			134	1.32	0.0017	0.19	1.38	0.0018	0.18	-1.40	0.021	0.14	-1.40	0.021	0.15
2017	Trafalgar	Shawnee	67	1.75	0.0016	0.11	1.83	0.0016	0.09	-0.76	0.020	0.11	-0.64	0.020	0.09
		Liberty	67	1.60	0.0016	0.13	1.70	0.0015	0.20	-0.84	0.020	0.23	-0.68	0.019	0.21
2017	Roann	Shawnee	67	1.34	0.0020	0.15	1.42	0.0019	0.14	-1.19	0.021	0.12	-1.05	0.021	0.11
		Liberty	67	1.17	0.0021	0.15	1.23	0.0020	0.14	-1.44	0.022	0.12	-1.35	0.022	0.11
2017	TPAC	Liberty	112	1.23	0.0018	0.14	1.33	0.0018	0.14	-1.30	0.022	0.11	-1.11	0.021	0.11
		Shawnee	112	1.411	0.0018	0.12	1.51	0.0018	0.11	-1.083	0.021	0.1	-0.916	0.0208	0.09

†Y intercept

‡Linear Slope Coefficient

§Quadratic Slope Coefficient

Table A.3. Regression Equations describing the full model response of Nitrogen concentration to Mean Stage Count (MSC) for two cultivars, three nitrogen fertilizer rates, and two Indiana locations.

				Nitrogen (mg g^{-1})			
Location	**Cultivar**	**Nitrogen Rate (kg ha^{-1})**	**Plant Part**	**bo †**	**b1 ‡**	**bq §**	**bc ⁋**
Roann	Liberty	0	Leaf	-6.3	65.4	-38.1	6.1
			Stem	4.5	51.0	-36.3	6.3
			Whole Plant	-7.3	67.4	-41.8	6.9
		67	Leaf	-7.0	66.2	-36.4	5.6
			Stem	20.4	29.5	-26.2	4.8
			Whole Plant	10.1	43.1	-30.2	5.1
		134	Leaf	-11.4	63.7	-31.4	4.4
			Stem	15.6	30.5	-23.7	4.0
			Whole Plant	4.6	44.8	-27.7	4.3
	Shawnee	0	Leaf	-15.5	73.4	-40.4	6.4
			Stem	0.3	51.7	-35.0	6.0
			Whole Plant	-12.5	69.4	-41.0	6.6
		67	Leaf	-34.6	97.3	-47.6	7.0
			Stem	-2.4	53.2	-33.8	5.6
			Whole Plant	-13.5	68.1	-38.4	6.0
		134	Leaf	-17.8	71.7	-33.9	4.7
			Stem	13.9	31.1	-22.6	3.7
			Whole Plant	2.3	46.6	-27.2	4.1
Trafalgar	Liberty	0	Leaf	40.6	-17.4	2.3	NS#
			Stem	54.4	-34.6	5.6	NS
			Whole Plant	54.5	-32.1	4.9	NS
		67	Leaf	45.7	-14.7	1.2	NS
			Stem	62.4	-36.2	5.3	NS
			Whole Plant	61.4	-32.4	4.5	NS
		134	Leaf	-4.7	43.6	-19.0	2
			Stem	10.3	26.0	-17.6	3
			Whole Plant	6.2	33.2	-19.5	3
	Shawnee	0	Leaf	101.4	-84.3	26.6	-3
			Stem	106.7	-90.5	25.7	-2
			Whole Plant	100.5	-79.8	21.8	-2
		67	Leaf	55.4	-20.6	2.1	NS
			Stem	69.4	-39.2	5.6	NS
			Whole Plant	69.6	-36.4	4.9	NS
		134	Leaf	41.5	-8.1	NS	NS
			Stem	70.2	-37.7	5.2	NS
			Whole Plant	69.7	-33.7	4.3	NS

†Y intercept ‡Linear Slope Coefficient §Quadratic Slope Coefficient ⁋ Cubic Slope Coefficient # Not Significant

Table A.4. Regression Equations describing the full model response of Acid Detergent Fiber (ADF) to Mean Stage Count (MSC) for two cultivars, three nitrogen fertilizer rates, and two Indiana locations.

				Acid Detergent Fiber (mg g^{-1})			
Location	**Cultivar**	**Nitrogen Rate (kg ha^{-1})**	**Plant Part**	**bo †**	**b1‡**	**bq §**	**bc ⁋**
Roann	Liberty	0	Leaf	101.8	152.6	-24.7	NS#
			Stem	542.8	-552.4	351.0	-58.4
			Whole Plant	488.0	-443.3	277.1	-45.0
		67	Leaf	84.3	182.4	-32.9	NS
			Stem	554.0	-556.1	345.8	-56.5
			Whole Plant	440.0	-353.5	228.9	-37.2
		134	Leaf	81.2	176.4	-30.9	NS
			Stem	655.5	-692.1	395.2	-61.6
			Whole Plant	493.5	-425.5	253.7	-39.3
	Shawnee	0	Leaf	90.1	162.7	-28.8	NS
			Stem	644.1	-677.3	394.5	-63.2
			Whole Plant	530.2	-494.2	291.5	-46.2
		67	Leaf	18.6	238.5	-46.4	NS
			Stem	563.1	-590.5	367.2	-60.6
			Whole Plant	391.9	-314.0	221.2	-37.6
		134	Leaf	-43.5	276.9	-52.3	NS
			Stem	558.7	-608.5	371.4	-59.7
			Whole Plant	334.7	-268.0	200.8	-33.9
Trafalgar	Liberty	0	Leaf	244.8	30.2	NS	NS
			Stem	280.7	-95.8	117.3	-21.3
			Whole Plant	616.3	-510.9	266.5	-38.3
		67	Leaf	234.2	35.0	NS	NS
			Stem	-120.9	346.4	-49.6	NS
			Whole Plant	324.1	-182.8	139.3	-21.6
		134	Leaf	240.4	32.2	NS	NS
			Stem	425.6	-305.9	196.6	-29.5
			Whole Plant	654.1	-563.5	278.3	-37.8
	Shawnee	0	Leaf	240.7	24.7	NS	NS
			Stem	-147.4	365.0	-54.4	NS
			Whole Plant	226.0	-92.3	109.7	-18.8
		67	Leaf	-436.4	751.2	-250.5	27.9
			Stem	-262.7	435.4	-64.6	NS
			Whole Plant	-201.1	368.3	-52.7	NS
		134	Leaf	-252.7	541.2	-173.1	18.7
			Stem	-235.6	402.3	-56.7	NS
			Whole Plant	-179.4	338.2	-45.8	NS

†Y intercept ‡Linear Slope Coefficient §Quadratic Slope Coefficient ⁋ Cubic Slope Coefficient # Not Significant

Table A.5. Regression Equations describing the full model response of Neutral Detergent Fiber (NDF) to Mean Stage Count (MSC) for two cultivars, three nitrogen fertilizer rates, and two Indiana locations.

				Neutral Detergent Fiber (mg g^{-1})			
Location	**Cultivar**	**Nitrogen Rate (kg ha^{-1})**	**Plant Part**	**bo†**	**b1‡**	**bq §**	**bc ⁋**
Roann	Liberty	0	Leaf	325.6	245.6	-42.7	NS #
			Stem	660.7	-232.4	217.4	-41.4
			Whole Plant	636.9	-185.9	174.8	-32.5
		67	Leaf	347.3	240.5	-44.0	NS
			Stem	784.2	-374.4	266.9	-46.6
			Whole Plant	696.7	-229.7	179.7	-31.6
		134	Leaf	364.4	213.5	-38.0	NS
			Stem	787.8	-365.9	248.8	-41.6
			Whole Plant	690.5	-207.1	157.3	-26.4
	Shawnee	0	Leaf	298.4	261.5	-47.2	NS
			Stem	754.9	-372.3	275.1	-49.1
			Whole Plant	671.8	-247.8	200.4	-36.1
		67	Leaf	260.8	311.4	-60.6	NS
			Stem	763.2	-386.8	285.0	-51.0
			Whole Plant	617.8	-164.2	165.8	-32.0
		134	Leaf	243.3	309.8	-58.6	NS
			Stem	719.5	-340.3	257.0	-45.1
			Whole Plant	559.9	-103.6	133.5	-25.8
Trafalgar	Liberty	0	Leaf	574.1	34.2	NS	NS
			Stem	312.6	323.5	-52.9	NS
			Whole Plant	350.3	261.1	-39.5	NS
		67	Leaf	196.0	458.5	-155.0	18.1
			Stem	292.7	313.4	-47.5	NS
			Whole Plant	342.1	244.6	-33.6	NS
		134	Leaf	579.1	25.9	NS	NS
			Stem	372.6	245.2	-34.4	NS
			Whole Plant	731.6	-205.0	131.0	-19.5
	Shawnee	0	Leaf	9.3	645.2	-221.4	25.9
			Stem	277.3	320.1	-49.7	NS
			Whole Plant	300.2	270.4	-39.3	NS
		67	Leaf	-378.5	1057.1	-361.6	40.8
			Stem	-220.1	834.3	-224.2	19.3
			Whole Plant	240.1	308.9	-45.6	NS
		134	Leaf	-169.8	821.7	NS	30.5
			Stem	247.3	322.2	-47.3	NS
			Whole Plant	279.0	270.1	-38.0	NS

†Y intercept ‡Linear Slope Coefficient §Quadratic Slope Coefficient ⁋ Cubic Slope Coefficient # Not Significant

Table A.6. Regression Equations describing the full model response of Acid Detergent Lignin (ADL) to Mean Stage Count (MSC) for two cultivars, three nitrogen fertilizer rates, and two Indiana locations.

				Acid Detergent Lignin (mg g^{-1})			
Location	**Cultivar**	**Nitrogen Rate (kg ha^{-1})**	**Plant Part**	**bo†**	**b1‡**	**bq §**	**bc ⁋**
Roann	Liberty	0	Leaf	34.2	0.05	NS	NS #
			Stem	158.4	-215.5	113.4	-17.1
			Whole Plant	113.2	-137.2	71.0	-10.3
		67	Leaf	-4.7	72.3	-36.5	5.7
			Stem	134.6	-167.4	87.3	-12.9
			Whole Plant	14.7	15.4	NS	NS
		134	Leaf	-18.0	82.7	-38.7	5.7
			Stem	159.9	-198.9	99.2	-14.3
			Whole Plant	91.4	-89.6	44.9	-6.1
	Shawnee	0	Leaf	37.7	-0.9	NS	NS
			Stem	191.5	-249.6	122.3	-17.5
			Whole Plant	137.6	-161.0	76.8	-10.5
		67	Leaf	43.9	-2.5	NS	NS
			Stem	129.2	-160.7	82.8	-12.0
			Whole Plant	14.5	14.9	NS	NS
		134	Leaf	41.7	-1.5	NS	NS
			Stem	153.0	-192.6	95.9	-13.7
			Whole Plant	76.3	-73.0	38.5	-5.3
Trafalgar	Liberty	0	Leaf	106.2	-66.3	14.3	NS
			Stem	109.5	-139.4	75.5	-10.9
			Whole Plant	177.4	-210.3	95.7	-12.5
		67	Leaf	111.1	-66.6	13.9	NS
			Stem	122.9	-144.9	71.8	-9.7
			Whole Plant	179.4	-196.8	83.5	-10.2
		134	Leaf	38.0	29.2	-25.5	5.2
			Stem	126.0	-139.7	66.3	-8.6
			Whole Plant	171.0	-179.6	74.5	-8.8
	Shawnee	0	Leaf	72.5	-37.8	8.5	NS
			Stem	148.0	-182.5	88.5	-11.9
			Whole Plant	206.5	-243.2	105.5	-13.3
		67	Leaf	67.8	-30.6	6.8	NS
			Stem	123.5	-147.2	71.4	-9.4
			Whole Plant	172.1	-188.9	80.0	-9.7
		134	Leaf	15.2	9.3	NS	NS
			Stem	125.4	-142.5	67.1	-8.6
			Whole Plant	161.3	-172.1	72.2	-8.6

†Y intercept ‡Linear Slope Coefficient §Quadratic Slope Coefficient ⁋ Cubic Slope Coefficient # Not Significant

Table A.7. Regression Equations describing the full model response of In-vitro Dry Matter Digestibility (IVDMD) to Mean Stage Count (MSC) for two cultivars, three nitrogen fertilizer rates, and two Indiana locations.

				In-vitro Dry Matter Digestibility ($mg\ g^{-1}$)			
Location	**Cultivar**	**Nitrogen Rate ($kg\ ha^{-1}$)**	**Plant Part**	**bo†**	**b1‡**	**bq §**	**bc ⁋**
Roann	Liberty	0	Leaf	1245.1	-382.9	52.0	NS #
			Stem	291.4	1100.5	-722.5	121.5
			Whole Plant	350.7	979.3	-629.1	103.3
		67	Leaf	1189.4	-346.7	47.6	NS
			Stem	295.7	1004.2	-640.0	104.4
			Whole Plant	498.9	666.9	-450.7	73.3
		134	Leaf	956.6	-119.2	NS	NS
			Stem	362.3	852.1	-538.5	85.2
			Whole Plant	547.9	545.0	-367.4	57.3
	Shawnee	0	Leaf	1256.8	-410.5	62.9	NS
			Stem	213.2	1172.0	-730.6	120.3
			Whole Plant	361.5	931.3	-591.1	96.7
		67	Leaf	1253.9	-424.1	70.6	NS
			Stem	340.5	949.1	-612.6	101.4
			Whole Plant	627.1	492.4	-377.2	65.0
		134	Leaf	1295.6	-425.2	67.8	NS
			Stem	524.6	705.2	-486.8	79.8
			Whole Plant	803.9	278.7	-269.5	46.5
Trafalgar	Liberty	0	Leaf	911.9	-120.3	NS	NS
			Stem	804.5	168.7	-240.2	44.6
			Whole Plant	473.0	581.6	-381.2	59.2
		67	Leaf	963.7	-130.6	NS	NS
			Stem	1553.3	-687.2	98.1	NS
			Whole Plant	1409.7	-534.0	68.2	NS
		134	Leaf	948.9	-115.7	NS	NS
			Stem	536.9	489.6	-330.8	50.2
			Whole Plant	337.1	716.7	-394.0	55.1
	Shawnee	0	Leaf	993.0	-161.0	6.0	NS
			Stem	1643.9	-753.1	110.3	NS
			Whole Plant	1544.0	-635.5	87.2	NS
		67	Leaf	2053.8	-1310.7	416.1	-46.7
			Stem	1740.7	-782.3	112.0	NS
			Whole Plant	1610.6	-650.0	87.4	NS
		134	Leaf	1613.2	-825.7	NS	-27.8
			Stem	1660.4	-705.4	95.8	NS
			Whole Plant	1558.1	-591.6	75.4	NS

†Y intercept ‡Linear Slope Coefficient §Quadratic Slope Coefficient ⁋ Cubic Slope Coefficient # Not Significant

Table A.8. Regression Equations describing the full model response of Ash to Mean Stage Count (MSC) for two cultivars, three nitrogen fertilizer rates, and two Indiana locations.

				Ash ($mg\ g^{-1}$)			
Location	**Cultivar**	**Nitrogen Rate ($kg\ ha^{-1}$)**	**Plant Part**	**bo†**	**b1‡**	**bq§**	**bc⁋**
Roann	Liberty	0	Leaf	105.3	-9.4	NS #	NS
			Stem	-14.0	235.9	-134.5	21.2
			Whole Plant	22.2	166.9	-96.6	15.1
		67	Leaf	237.0	-193.9	77.2	-10.1
			Stem	53.7	128.6	-81.0	12.8
			Whole Plant	140.1	-26.0	NS	NS
		134	Leaf	293.9	-274.0	114.5	-15.6
			Stem	154.6	-31.9	NS	NS
			Whole Plant	141.5	-25.4	NS	NS
	Shawnee	0	Leaf	90.0	-4.1	NS	NS
			Stem	-112.8	354.8	-180.1	26.8
			Whole Plant	-32.2	223.7	-116.5	17.4
		67	Leaf	87.9	-3.1	NS	NS
			Stem	-74.8	307.0	-157.3	23.2
			Whole Plant	31.7	136.5	-76.9	11.7
		134	Leaf	291.5	-251.8	96.9	-12.1
			Stem	12.1	190.4	-103.5	15.1
			Whole Plant	147.6	-26.6	NS	NS
Trafalgar	Liberty	0	Leaf	77.5	3.2	NS	NS
			Stem	49.8	116.7	-73.3	11.4
			Whole Plant	-69.5	249.8	-119.3	16.6
		67	Leaf	69.7	5.9	NS	NS
			Stem	-4.3	169.2	-83.7	11.2
			Whole Plant	-98.7	263.0	-112.9	14.3
		134	Leaf	64.7	7.5	NS	NS
			Stem	-44.3	209.5	-95.5	12.2
			Whole Plant	-119.3	279.6	-115.7	14.2
	Shawnee	0	Leaf	60.9	9.6	NS	NS
			Stem	248.0	-114.5	16.2	NS
			Whole Plant	49.7	94.5	-54.0	7.7
		67	Leaf	52.9	11.3	NS	NS
			Stem	35.8	135.6	-74.8	10.4
			Whole Plant	-15.6	167.3	-78.3	10.2
		134	Leaf	200.7	-147.2	NS	-6.0
			Stem	55.5	112.9	-64.3	8.8
			Whole Plant	24.2	120.9	-58.8	7.5

†Y intercept ‡Linear Slope Coefficient §Quadratic Slope Coefficient ⁋ Cubic Slope Coefficient # Not Significant

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