ABSTRACT

RIVERA CHACON, RAUL. Defoliation Frequency and Timing Effects on Productivity and Tissue Composition of Two Switchgrass Cultivars. (Under the direction of Dr. Miguel S. Castillo).

Environmental concerns due to climate change and government mandates have provided impetus for bioenergy production in the USA, particularly from native grasses than can be grown in marginal lands. Switchgrass (*Panicum virgatum* L.) is native from the North American prairies and can serve as a feedstock source for bioconversion. Two switchgrass cultivars, 'BoMaster' and 'Performer', were released by the USDA-NCSU because of their potential for bioenergy and forage, respectively, compared to standard cultivars grown in the southeastern region. Information on defoliation management, including delayed harvest following a freeze event, is limited for these cultivars. This information is critical to evaluate whether a longer biomass supply window from the field to the biorefinery is feasible, for example during the months of November to February in North Carolina, and regionally. The objectives of this study were to determine the effect of harvest frequency (HF) and harvest timing (HT) defoliation strategies on feedstock characteristics and nutritive value. Feedstock characteristics included biomass yield, dry matter (DM) concentration, tissue ash concentration, and nutrient (N, P, K) removal. Nutritive value estimates included crude protein (CP), *in vitro* true digestibility (IVTD), neutral detergent fiber (NDF), and acid detergent fiber (ADF). The experiment was conducted at the Central Crop Research Station, Clayton, NC, for two years (2016-2017 and 2017-2018). Treatments were the factorial combination (3 x 2) of end-of-season HT [before frost (BF), after frost (AF), and late winter (LW)] and HF [full-season growth (1X) and two clippings per season (2X-June+2X regrowth)]. The experimental design was a split plot-design with the main-plot factor arranged in a completely randomized block design replicated three times. The main-plot factor was HF and the sub-plot factor was HT. Biomass yield ranged from ~10 to 16 Mg ha^{-1} for 'BoMaster' and ~6 to ~15 Mg ha^{-1} for 'Performer'. Biomass yield was greater for 2X across all HT for ‘Performer’ and ‘BoMaster’ with the exception of BF for ‘BoMaster’ where 1X vs. 2X were not different. Dry matter concentration ranged from ~445 to ~916 g kg^{-1} and ash concentration from ~33 to ~17 g kg^{-1}; DM concentration was greater for LW and lower for BF harvest and ash concentration followed an inverse trend. Removal of N, P, and K was greater for 2X vs. 1X and it was greater for BF compared to LW for both cultivars. Nutrient removal ranged from ~33 to 137 kg ha^{-1} for N, ~3 to 29 kg ha^{-1} for P, and from ~42 to 213 kg ha^{-1} for K. Tissue CP concentration for both cultivars ranged from ~33 to 82 g kg^{-1} and it was greater for the 2X-June and 2X regrowth-BF clippings during the frost-free period and lower for 1X-LW. Tissue IVTD followed a similar pattern than CP and ranged from ~244 to700 g kg^{-1}. Both NDF and ADF followed an inverse trend compared to CP and IVTD. Fiber components ranged from 745 to 864 g kg^{-1} for NDF and from 431 to 582 g kg^{-1} for ADF. Greater biomass yield was achieved with 2X clipping and this defoliation management resulted in consistent biomass yield after frost events and delayed harvest with no penalty on yield compared to lower values for 1X. Lower ash, greater DM concentration, and lower nutrient removal are desirable for bioenergy feedstock, were achieved when harvesting occurred at AF and LW; however, at the expense of lower biomass yield. Forage mass from the 2X defoliation treatment is marginally suitable to meet the nutrient requirements of a mature dry beef cow. Consequently, herbage from the other treatments is suitable for feedstock due to lower CP and IVTD, and greater fiber components important for bioenergy.

Defoliation Frequency and Timing Effects on Productivity and Tissue Composition of Two Switchgrass Cultivars.

by
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DEDICATION

This work is dedicated to my parents Urbelinda and Ysaul for their constant support and encouragement during the years as an undergraduate and graduate student, and to my fiancée Khaterine, who has been always by my side pushing me to move forward.

BIOGRAPHY

Raul was born in Tacna, Perú. Since he was a child, he was interested and amazed by the day to day activities in his family dairy farm. After finishing high school, he moved to Lima to study Animal Science at Universidad Nacional Agraria La Molina. He graduated with his B.S. degree in 2013 and soon after he started working as a research assistant at La Molina with responsibilities to conduct field research work with dairy producers. In 2016, he arrived to Raleigh to start his MS program in Crop Science under the direction of Dr. Miguel S. Castillo.

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CHAPTER 1: Defoliation Management Effects Following a Freeze Event of Two Switchgrass Cultivars

ABSTRACT

Switchgrass (*Panicum virgatum* L.) is a potential dedicated bioenergy feedstock. Defoliation strategies following a freeze event may represent a viable strategy to extend the period of switchgrass supply directly from the field to biorefineries. The objectives of this study were to determine the effect of harvest frequency (HF) and harvest timing (HT) on biomass yield, ash and dry matter (DM) concentration, and nutrient (N, P, K) removal for switchgrass cultivars ‘BoMaster’ and ‘Performer’. Treatments were the factorial combination of two HF [full-season growth (1X) and two clippings per season (2X = 2X-June+2X regrowth)] and three HT [(BF, before frost; AF, after frost; and LW, late winter)]. Clipping 2X, retained greater biomass yield across HT compared to 1X for both cultivars; except for BF harvest for ‘BoMaster’ where 1X vs. 2X were not different. Biomass yield ranged from ~10 to 16 Mg ha^{-1} for ‘BoMaster’ and ~6 to ~15 Mg ha^{-1} for ‘Performer’. Greater biomass yield with 2X resulted in greater nutrient removal. Delayed harvest, such as in LW, resulted in lower nutrient removal. Nutrient removal ranged from ~33 to 137 kg ha^{-1} for N, from ~3 to 29 kg ha^{-1} for P, and from ~42 to 213 kg ha^{-1} for K. Greater DM concentration, lower ash concentration, and lower nutrient removal were consistently achieved by harvesting LW. Our results highlight the impact of defoliation strategies on feedstock responses and the trade-off between high biomass yield and tissue composition, which are both factors of interest for the biofuel industry.

INTRODUCTION

Finite fossil fuel reserves, government mandates enforcing the use of renewable energy, and controversy associated with the use of land dedicated to animal-agriculture vs. bioenergy crops, have turned attention to the use of grasses as potential feedstock for bioenergy production. Converting plants into biofuels, industrial products, and human-used products, has been termed the biorefinery concept (Sanderson et al., 2006). Switchgrass is a C_4 grass native to the American prairies (Vogel, 2004) with potential to be used as forage and bioenergy feedstock. Positive attributes that make switchgrass a candidate bioenergy feedstock include: high biomass production (McLaughlin and Ksazos, 2005; Parrish and Fike, 2005), greater N-use efficiency because of the C_4 photosynthetic pathway (i.e. greater biomass yield per unit of N intake; Brown, 1978; Friesen and Catani, 2017), adaptation to marginal lands that are less suitable for row crop production (Jung et al., 1988), drought tolerance (Barney et al., 2009; Liu et al., 2015), and potential to support environmental conservation goals such as increasing diversity of grassland birds (Roth et al., 2005).

Switchgrass production systems with multiple clippings per year as a management practice, or grazing during the first portion of the season, have potential to serve as versatile systems, allowing land managers to utilize the earlier herbage growth for forage (either clipping or grazing; Burns et al., 1984; Mosali et al., 2013) and later regrowth to be clipped for bioenergy (Sanderson et al., 1999). Clipping defoliation management trials have traditionally focused on defoliation schedules before first-frost occurrence at the end of the growing season. However, delaying end-of-season harvest to after-frost periods, such as during the winter and spring months (e.g. December to February in North Carolina), may be an effective strategy to provide constant supply of biomass from the field to the bio-processing plants and, consequently, to help reduce the need of on-site storage capacity. Reduction of storage capacity at the processing sites results in considerable cost savings for the whole biomass logistics function (Rentizelas et al., 2009).

Leaf losses due to delayed harvest, in addition to lodging which complicates field pick up of the biomass, results in lower yields (Adler et al., 2006); however, the amount of leaf loss may be dependent on the age of the plant tissue at harvest time (e.g. season-long mature plants vs. half-season regrowth) and the environmental conditions. Sanderson et al. (1999) reported that total yields decreased approximately 16% when the final autumn harvest was delayed to November compared to September in Texas for switchgrass cultivar ‘Alamo’. In Pennsylvania, Adler et al. (2006) reported that switchgrass yields decreased almost 40% in winters with above-average snowfall when harvest was delayed over winter until spring for cultivars ‘Cave-In-Rock’, ‘Shawnee’, and ‘Trailblazer’. Tissue ash and N concentration of switchgrass decrease as switchgrass matures (Sanderson and Wolf, 1995) and delayed harvest resulted in lower ash and N concentrations for reed canarygrass (*Phalarisarundinacea*L.) (Burvall, 1997) and *Miscanthus* sp. (Lewandosky et al, 2003); the before-mentioned factors resulting in greater biofuel quality of the harvested feedstock.

Information is needed regionally, and in North Carolina specifically, to determine the impact of delayed harvest following a freeze event on harvestable biomass. This information is critical to evaluate whether a longer biomass supply window directly from the field to the bio-refineries may be possible. The objectives of the experiment were to determine the effect of combining harvest frequency and harvest timing defoliation strategies on biomass yield, nutrient (N, P, K) removal, and tissue ash and dry matter (DM) concentration of switchgrass cultivars ‘BoMaster’ and ‘Performer’. Switchgrass cultivars ‘BoMaster’ and ‘Performer’ were released because of greater potential for biomass production and improved nutritive value, respectively, compared to cultivars ‘Alamo’ and ‘Cave-in-Rock’ (Burns et al., 2008a, 2008b).

MATERIALS AND METHODS

Experimental Site, Plot Management, and Weather

The experiment was conducted for two growing seasons (2016-2017 and 2017-2018) at the Central Crop Research Station, Clayton, NC (35°40’ N, 78°29’W). Two mature stands (>8 yr) of switchgrass cultivars ‘BoMaster’ and ‘Performer’ were used for this experiment. Prior to initiation, the plots of both cultivars where managed with maintenance fertilization and a single-clipping event at the end of the growing season, followed by residue-burning in February of each year. The accumulated biomass from the 2015 growing season was clipped and removed from the plots in late September 2015 in preparation for this experiment.

The soil series was classified as Wedowee sandy loam (fine, kaolinitic, thermic Typic Kanhapludults). Soil samples collected on February 2016 indicated soil pH of 6.0 and Mehlich-3 extractable P and K concentrations (mg kg^{-1}) of 205 (very high) and 195 (high), respectively. Soil samples were tested by routine procedures at the NCDA&CS (Hardy et al., 2014). Nutrient analyses on Mehlich-3 soil extracts were performed using inductively coupled plasma (ICP) and soil pH was determined on a 1:1 soil:water volume ratio (Mehlich, 1984). Fertilizer amendments were broadcast-applied by hand in a single application on mid-April for both years. In 2016, N was applied at a rate of 134 kg ha^{-1} using a granular formulation of pre-mixed urea-ammonium sulfate blend for both cultivars. In 2017, ‘BoMaster’ was fertilized at a rate of 101 kg ha^{-1} using a granular formulation of di-ammonium phosphate-ammonium nitrate blend that was prepared on site (final concentration of 322 g N kg^{-1}, 460 g P kg^{-1}); and ‘Performer’ was fertilized at a rate of 176 kg N ha^{-1} using a granular formulation of ammonium sulfate-ammonium nitrate blend (final concentration of 314 g N kg^{-1}). Although N fertilization rates and sources varied among years, the applied N rates were in the higher range, or above, for reported switchgrass response to N fertilization rates in the region (Brejda, 2000; Obour et al., 2017).

Average daily maximum and minimum temperatures per month, monthly rainfall, and 30-yr average monthly rainfall are presented in Fig. 1.1. Dates of last frost at the beginning of the growing season were 10 April 2016 and 23 April 2017. Dates of first frost at the end of the growing season were 11 November 2016 and 4 November 2017. Total rainfall was 1591, 1327, and 1253 mm in 2016, 2017, and the 30-yr average, respectively.

Treatments and Experimental Design

Treatments were the factorial combination (3 x 2) of end-of-season harvest timing (HT) and harvest frequency (HF). The three HT levels were: before frost (BF), after frost (AF), and late winter (LW). Harvest dates for BF were 6 and 5 October in 2016 and 2017, respectively; for AF, 17 November in both years; and for LW, 7 and 6 February in 2016 and 2017, respectively. The two HF levels were: full-season growth (1X) and two clippings per season (2X). For the 2X treatment, the first harvest event occurred by mid-June (2X-June; 22 and 15 June in 2016 and 2017, respectively), which is approximately half-way through the active growing season for switchgrass in North Carolina, and the second harvest event occurred at the end of the growing season based on HT (2X regrowth-BF, 2X regrowth-AF, 2X regrowth-LW). The HF treatments were selected because of their potential to result in higher biomass yields (i.e. 1X) and to provide a flexible forage/biomass system (i.e. 2X system with first harvest potentially used for forage and the second for biomass). The HT treatments were selected to evaluate the impact of frost and delayed harvest of mature (i.e. 1X-BF, -AF, and -LW) vs. younger (i.e. 2X regrowth-BF, -AF, -LW) plant tissue on switchgrass responses.

The experimental design was a split plot-design with the main-plot factor arranged in complete randomized block design replicated three times. Main-plot factor was HF and sub-plot factor was HT. Treatments in 2017 were imposed on the same corresponding experimental units than in 2016. Treatment were imposed to each of two separate stands for the switchgrass cultivars, one for ‘BoMaster’ and one for ‘Performer’; consequently, each cultivar constituted an experiment and the data from each cultivar was analyzed separately with inferences limited to treatment effects for each cultivar. Experimental unit size was 4.9-m wide by 4.9-m long with 2.4-m wide alleys between plots.

Response Variables

Biomass Yield

Biomass yield was estimated by clipping a 7.5-m^2 area (2.5-m wide by 3-m long) to 15-cm stubble height using hedge trimmers. The harvested material was weighed fresh in the field and a sub-sample (approximately 1000 g fresh weight) was used to determine dry matter (DM) concentration and dry biomass yield. After biomass samples were collected, the remained of the plot was clipped to 15-cm stubble height and the residues were removed from the field. After LW harvest, which was the last harvest of the season, all plots were completely cleared by clipping the standing biomass to the target stubble height and the clipped material was removed out of the field.

Tissue Dry Matter and Ash Concentrations

Concentration of DM was determined by drying the harvested tissue at 60ºC until constant weight using a forced-air drying oven. Ash concentration was determined using a muffle furnace for loss on ignition determination (Thiex et al., 2012). The impact of freeze event and delayed harvest on DM and ash concentrations were evaluated using the mature (i.e. 1X-BF, -AF, and -LW) vs. younger tissue (i.e. 2X regrowth-BF, -AF, -LW) treatments.

Removal of N, P, and K

Dried subsamples were ground with a Thomas Willey mill model (Thomas Scientific, NJ, USA) to pass 1-mm screen in preparation for laboratory analysis and were sent to the NCDA&CS laboratory for nutrient analysis. N was determined by gas chromatography with an elemental analyzer (NA1500; CE Elantech Instruments; Lakewood, NJ) (AOAC 1990; Campbell 1992) and P and K concentrations were determined by inductively-couple-plasma-optical emission spectrometry (ICP-OES) (Spectro Arcos EOP, Spectro Analytical, Mahwah, NJ) (Donohue and Aho 1992; adapted USEPA 2001). Nutrient removal for N, P, and K were calculated by multiplying tissue nutrient concentrations by the corresponding biomass yield at each harvest, and values from each of the two harvests (i.e. 2X-June and 2X-BF, -AF, and -LW) were added to provide an estimate of nutrient removal for 2X treatment.

Statistical Analysis

Analyses of variance were performed using the PROC GLIMMIX procedure of SAS (SAS Institute, Cary, NC). The analyses were conducted by cultivar for the reasons explained earlier. Treatment, year, and their interaction were considered fixed effects. Year was considered as repeated measured with the variance component modeled using compound symmetry as the covariance structure based on smaller AIC values. Block was considered random effect. When an interaction effect was significant the SLICE statement of SAS was used for an F-test for each frequency and to compare timings. Mean separation was based on the LINES option of LSMEANS using SAS. Treatment differences were declared significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Biomass Yield

For cultivar ‘BoMaster’, there was a HF by HT interaction (Table 1.1). The interaction effect occurred because biomass yield was not different between 1X and 2X (approximately 14.5 Mg ha^{-1}) for BF; however, delaying end-of-season harvest resulted in lower biomass yield for 1X by approximately 22% for AF harvest and 30% for LW harvest compared to BF (Table 1.1). Two-clippings (2X) per year as a management system for cultivar ‘BoMaster’ is a viable option to delay end-of-season harvest to November and February with no penalty on biomass yield in North Carolina. If biomass is to be harvested before occurrence of a freeze event, then either 1X or 2X clipping management result in similar biomass yields.

For cultivar ‘Performer’, there was a three-way interaction of HF by HT by year; therefore, the data were analyzed by year. In 2016, there was a HF by HT interaction that occurred because biomass yield remained constant among HT for 2X clipping; however, biomass yield for 1X was lower and not different between AF and LW compared to BF (Table 1.2). In 2017, there were main effects of HF and HT. Biomass yield from 2X was approximately 17% greater than 1X and there was a trend (P= 0.07 for HT) for greater biomass yield for BF, intermediate for AF, and lowest for LW (Table 1.2). A two-clipping per year management system for ‘Performer’, as in the 2X treatment, resulted in greater biomass yield across all treatment combinations in this study.

Wide variation in switchgrass responses due to the environment, harvest management, and genotype have been reported previously (Fike et al., 2006; Lemus et al., 2002). In terms of clipping frequency, there are several reports indicating either similar or slightly greater yields for the two-clipping vs. one-clipping per year system. Fike et al. (2006) reported 38% greater biomass yield for a two-clipping system vs. one-clipping per year for cultivar ‘Cave-in-Rock’ grown across several states in the transition region in USA. The same trend was reported for cv. ‘Alamo’ by Guretzky et al. (2010) in Oklahoma and by Burns et al. (2010) in North Carolina working with switchgrass cultivars ‘Alamo’ and ‘Cave-in-Rock’. However, there have been limited reports for cultivars ‘BoMaster’ and ‘Performer’ especially with delayed end-of-season harvest following a freeze event. In general, delaying harvest timing at the end of the growing season results in lower biomass yield caused by plant tissue losses, mainly due to leaves losses, but also due to difficulty to pick up stems by the harvesting equipment (Adler et al., 2006); however, the extent of the reduction is site-specific. Our results indicate that a two-clippings per year management system, with first clipping occurring by mid-June, allows to successfully delay field harvest of the biomass feedstock until February in North Carolina with no penalty losses of biomass yield for both cultivars ‘BoMaster’ and ‘Performer’. If the goal is to harvest before frost, then the two-clipping per year system resulted in approximately 26% greater biomass yield for cv. ‘Performer’ and either clipping once or twice per year resulted in similar biomass yield for cv. ‘BoMaster’,

Tissue DM and Ash Concentrations

For cultivar ‘BoMaster’, there was a HT effect on DM concentration. Concentration of DM increased by almost two-fold from 453 g kg^{-1} at BF compared to LW, and it was intermediate for AF (Table 1.3). For ash concentration there were main effects of HT and HF. Ash concentration was lower for 1X vs. 2X regrowth and it decreased almost two-fold as HT was delayed from BF to LW (Table 1.3).

For ‘Performer’ switchgrass, there was a three-way interaction effect of HF by HT by year for DM concentration; consequently, data were analyzed by year. In 2016, there was a HF by HT interaction effect that occurred because DM concentration was not different between 1X and 2X regrowth for LW; however, DM concentration was greater for 1X compared to 2X regrowth that

BF and AF (Table 1.4). In 2017, there was a HT effect. Concentration of DM was almost two-fold greater for LW compared to BF and it was intermediate for AF (Table 1.4). For ash concentration there were main effects of HT and HF. Ash concentration was greater for 2X regrowth compared with 1X and it decreased when end-of-season harvest was delayed from BF to LW (Table 1.5).

Dry matter and ash concentration values observed in our study coincide with previous reports in the literature for cultivars ‘Alamo’, ‘Cave-in-Rock’ and ‘Sunburst’ (Ashworth et al., 2017; Lindsay et al., 2018, Casler and Boe, 2003; Tubeileh et al., 2014). In general, DM concentration increased as plants matured from vegetative to reproductive and as harvest was delayed from BF to LW; nevertheless, ash concentration followed an inverse pattern. Higher DM concentrations reduce the cost of artificial drying and risk of spoilage when the biomass is stored or transported (Lewandowski and Kicherer, 1997). High ash concentration reduces energy conversion efficiency (Agblevor et al., 1994; Jenkins et al., 1998). Our results indicate that driest biomass, with at least 870 g kg^{-1} DM concentration concomitant with lowest ash concentrations are achieved when end-of-season harvest occurred at LW for both cultivars, irrespective of HF treatments.

Nitrogen Removal

For ‘BoMaster’, there was a HF effect. Removal of N, averaged across HT, was about three-fold greater for 2X compared with 1X (Table 1.6). For cultivar ‘Performer’, there were HF and HT main effects. Removal of N for ‘Performer’, averaged across HT, was about four-fold greater for 2X compared to 1X and it decreased by about 20 kg ha^{-1} when end-of-season harvest was delayed from BF to AF and LW (Table 1.7). Lower N removed in the harvested tissue is desirable to increase efficiency of bioconversion processes such as pyrolysis (Wilson et al., 2013). Our results indicate that harvesting at AF or LW results in lower N removal in the harvested tissue.

In addition, lower N removal in the harvested tissue, if recycled back to the soil through litter, could potentially reduce N fertilization inputs.

Approximately 80% of the total N is removed by the 2X-June clipping for ‘BoMaster’ and 82% for ‘Performer’. These results agree with the 78% value previously reported by Reynolds et al. (2000) for six switchgrass cultivars grown for five years in Tennessee. Similar N removal values compared to those in our study have been reported in the literature for ‘Alamo’ cultivar (Lemus et al., 2009; Lindsay et al., 2018, Ashworth et al., 2017). Lower N removal by the 1X treatment may be a function of N translocated to storage organs, such as the rhizomes, as it was reported by Beaty et al. (1978). Therefore, lower N removal for delayed harvest frequency, such as AF and LW, is attributed to a combination of lower biomass yields and N translocation to storage organs in this study.

Phosphorus Removal

For ‘BoMaster’, P removal ranged from ~4 to 25 kg ha^{-1} equivalent to 1 and 7% removal rate, respectively of available P in the soil. There was a HF by HT interaction effect on P removal for ‘BoMaster’ that occurred because P removal was similar among HT for 2X clipping but it was lowest for 1X-LW compared to 1X-BF and 1X-AF (Table 1.6). Removal of P for ‘Performer’ switchgrass ranged from ~3 to 29 kg ha^{-1} equivalent to 0.25 and 4% removal rate, respectively of available P in the soil and there was a three-way interaction effect of HF by HT by year. In 2016, there was a HF by HT interaction effect and P removal for ‘Performer’ was lowest for 1X-LW; however, P removal was not different among HT for 2X. In 2017, there were HF and HT effects for ‘Performer’, P removal was greater for 2X vs. 1X. Averaged across HF, P removal was lower for LW compared to BF and AF harvests (Table 1.8).

In general, P removal followed a similar trend than N removal, that is, lower nutrient removal was observed for 1X vs. 2X clippings and also lower P removal values were observed as end-of-season harvest was delayed from BF to LW. Similar P removal values to those found in our study have been reported in the literature when switchgrass is harvested during the frost-free period (Ashworth et al. 2017; Seepaul et al. 2014). Silveira et al. (2013) reported greater P removal values, at about 56 kg ha^{-1}, for ‘Alamo’ switchgrass. Greater P removal values reported by Silveira et al. (2013) in Florida were most likely due to the more intense clipping frequency utilized (clipped every 6 wk from May until November) and because switchgrass was grown on a manure-impacted soil with approximately 232 mg kg^{-1} Mehlich 1-P concentration in the Ap (0 - 15 cm) horizon; this may open a different approach if the producer is interested in a particular nutrient removal like P. Concentration and P removal is reduced with plant maturity as demonstrated by Black, 1968 with native grasses.

Potassium Removal

For ‘BoMaster’, there was a HF effect. Removal of K was about four-fold greater for 2X compared to 1X (Table 1.6) and equivalent to 88 and 22%, respectively of available K in the soil removal rate. For ‘Performer’ switchgrass, there were main effects of HF and HT. Removal of K for ‘Performer’ switchgrass was also greater by about four-fold for 2X vs. 1X (with 89 and 20% respectively of available K in the soil) and it was greatest when harvest occurred BF and AF and lowest for LW (Table 1.7). Removal of K values ranged from ~42 to ~213 kg ha^{-1} for both cultivars.

Ashworth et al. (2017) reported K removal of 136 kg ha^{-1} for an early-July clipping vs. 22 kg ha^{-1} for a February clipping for cv. ‘Alamo’ in Arkansas under a single harvest management, following the same trend compared with both cultivars in our study. In an experiment working with cv. ‘Alamo’ Kering et al. (2013) reported more than 70% reduction of K removal from the first clipping (vegetative) compared to the one after frost similar to the value found in this study (89%). Lower K removal is explained due to the K leaching out of the plant tissue at LW with plants standing longer periods in the field with delayed harvest (1X) (Koelling and Kucera, 1965; Christian et al., 2002; Parrish and Fike, 2005; McLaughlin et al., 1996). Also, Yang et al. (2009) indicated that a single harvest in late fall or early winter would maintain more soil nutrients compared with a two-clipping management with several cultivars in Oklahoma. In addition, Himken et al. (1997) reported an increase of K content in rhizomes because of translocation of nutrients from aboveground to belowground plant structures at the end of the growing season in Miscanthus. Finally, biomass normally has higher alkaline concentration (K) which may represent a challenge for pyrolysis process in biorefineries (Trendewicz et al. 2015).

CONCLUSIONS

Frost events and late winter harvest don’t reduce biomass yield if the clipping management is 2X harvest. This is an important finding in terms of providing a ready-to-use biomass supply from the field to the biorefineries in North Carolina. Greater DM concentration, lower ash concentration, and lower nutrient (N, P, and K) removal is consistently achieved with LW harvest but at the trade-off of lower biomass yield for bioenergy production. The 2X HF represents an opportunity for producers to extend the window of time for providing biomass to the biorefineries with the consideration of higher nutrients removed for 2X-BF. Biomass harvested after senescence with lower alkaline concentration of K and reduced N removal has greater quality for bioenergy (Parrish et al. 2008) and is possible with delayed harvest, such as after a frost event, or with 1X-clipping harvest.

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TABLES

Table 1.1. Biomass yield (SE = 0.4) of ‘BoMaster’ switchgrass as a function of harvest frequency (HF; 1X vs. 2X) and harvest timing (HT; BF = before frost, AF = after frost, and LW = late winter).

Harvest Time	Harvest Frequency		P-value[†]	Mean
	1X	2X		
	--- Mg ha^{-1} ---			Mg ha^{-1}
BF	14.4 a[‡]	14.7	0.63	-
AF	11.2 b	15.9	< 0.01	-
LW	10.1 b	16.0	< 0.01	-
Mean	-	-		

[†] P-value for the effect of harvest frequency within harvest time treatment.

[‡] Means within columns by harvest frequency followed by different letters are different at $P \leq 0.05$.

Table 1.2. Biomass yield (SE = 0.6) of ‘Performer’ switchgrass as a function of harvest frequency (HF; 1X vs. 2X) and harvest timing (HT; BF = before frost, AF = after frost, and LW = late winter).

	2016				2017			
Harvest Time	Harvest Frequency				Harvest Frequency			
	1X	2X	*P*-value[†]	Mean	1X	2X	*P*-value	Mean
	--- Mg ha^{-1} ---			Mg ha^{-1}	--- Mg ha^{-1} ---			Mg ha^{-1}
BF	11.7a[‡]	14.8	< 0.01	-	10.4	11.4	-	10.9 a
AF	7.7 b	15.4	0.02	-	8.5	10.5	-	9.5 ab
LW	6.1 b	13.9	< 0.01	-	8.0	9.5	-	8.8 b
Mean	-	-			9.0	10.5	0.04	

[†] *P*-value for the effect of harvest frequency.

[‡] Values within columns followed by different letters are different at $P \leq 0.05$

Table 1.3. Dry matter (SE = 10.5) and ash concentration (SE = 0.8) for 'BoMaster' switchgrass as a function of harvest frequency (HF; 1X vs. 2X regrowth) and harvest timing (HT; BF = before frost, AF = after frost, and LW = late winter).

	Harvest Frequency			
	1X	2X regrowth		
Harvest Time	Dry matter concentration		*P*-value[†]	Mean
	--- g kg^{-1} ---			g kg^{-1}
BF	466	440	-	453 c[‡]
AF	517	515	-	516 b
LW	890	895	-	892 a
Mean	-	-		
	Ash concentration			
	--- g kg^{-1} ---			g kg^{-1}
BF	29	36	-	33 a
AF	25	31	-	28 b
LW	15	20	-	17 c
Mean	23	29	< 0.01	

[†] *P*-value for the effect of harvest frequency.

[‡] Means within columns by harvest frequency followed by different letters are different at $P \leq 0.05$

Table 1.4. Dry matter concentration (SE = 10.5) for ‘Performer’ switchgrass as a function of harvest frequency (HF; 1X vs. 2X regrowth) and harvest timing (HT; BF = before frost, AF = after frost, and LW = late winter).

Harvest Time	2016				2017			
	Harvest Frequency				Harvest Frequency			
	1X	2X regrowth	*P*-value[†]	Mean	1X	2X regrowth	*P*-value	Mean
	--- g kg^{-1}---			g kg^{-1}	--- g kg^{-1}---			g kg^{-1}
BF	509 c[‡]	474 c	< 0.01	-	457	432	-	445 c
AF	609 b	569 b	< 0.01	-	527	548	-	537 b
LW	911 a	916 a	0.67	-	868	879	-	874 a
Mean	-	-			-	-		

[†] *P*-value for the effect of harvest frequency.

[‡] Values within columns followed by different letters are different at $P \leq 0.05$

Table 1.5. Ash concentration (SE = 0.7) for ‘Performer’ switchgrass as a function of harvest frequency (HF; 1X vs. 2X regrowth) and harvest timing (HT; BF = before frost, AF = after frost, and LW = late winter).

Harvest Time	Harvest Frequency			
	1X	2X regrowth	*P*-value[†]	Mean
	--- g kg^{-1} ---			g kg^{-1}
BF	29	36	-	33 a[‡]
AF	25	33	-	29 b
LW	15	21	-	18 c
Mean	23	30	< 0.01	

[†] *P*-value for the effect of harvest frequency within harvest time treatment.

[‡] Means within columns by harvest frequency followed by different letters are different at $P \leq 0.05$.

Table 1.6. Nitrogen (N), phosphorus (P) and potassium (K) removal (SE = 5.4, 1.5, and 13.5 for N, P, and K, respectively) of 'BoMaster' switchgrass as a function of harvest frequency (HF; 1X vs. 2X) and harvest timing (HT; BF = before frost, AF = after frost, and LW = late winter).

	Harvest Frequency			
	1X	2X		
Harvest Time	N removal		*P*-value[†]	Mean
	--- kg ha^{-1} ---			kg ha^{-1}
BF	63	137	-	-
AF	36	138	-	-
LW	29	135	-	-
Mean	43	137	< 0.01	
	P removal			
	--- kg ha^{-1} ---			kg ha^{-1}
BF	15 a[‡]	25	< 0.01	-
AF	7 b	25	< 0.01	-
LW	4 b	21	< 0.01	-
Mean	-	-		
	K removal			
	--- kg ha^{-1} ---			kg ha^{-1}
BF	98	199	-	-
AF	55	231	-	-
LW	9	208	-	-
Mean	54	213	< 0.01	

[†] *P*-value for the effect of harvest frequency within harvest time treatment.

[‡] Means within columns by harvest frequency followed by different letters are different at $P \leq 0.05$.

Table 1.7. Nitrogen removal (N; SE = 4.8) and potassium removal (K; SE = 9.9) of 'Performer' switchgrass as a function of harvest frequency (HF; 1X vs. 2X) and harvest timing (HT; BF = before frost, AF = after frost, and LW = late winter).

	Harvest Frequency			
	1X	2X		
Harvest Time	N removal		P-value†	Mean
	--- kg ha^{-1} ---			kg ha^{-1}
BF	48	130	-	89 a‡
AF	27	111	-	69 b
LW	22	117	-	70 b
Mean	33	119	< 0.01	
	K removal			
	--- kg ha^{-1} ---			kg ha^{-1}
BF	76	187	-	132 a
AF	44	203	-	124 a
LW	5	173	-	89 b
Mean	42	188	< 0.01	

† P-value for the effect of harvest timing within a given year.

‡ Means within columns by harvest frequency followed by different letters are different at $P \leq 0.05$.

Table 1.8. Phosphorus removal (P; SE = 1.0) of 'Performer' switchgrass as a function of harvest frequency (HF; 1X vs. 2X) and harvest timing (HT; BF = before frost, AF = after frost, and LW = late winter).

Harvest Time	2016				2017			
	Harvest Frequency				Harvest Frequency			
	1X	2X	*P*-value[†]	Mean	1X	2X	*P*-value	Mean
	--- kg ha^{-1} ---			kg ha^{-1}	--- kg ha^{-1} ---			kg ha^{-1}
BF	15 a[‡]	28	< 0.01	-	12	19	-	16 a
AF	10 a	29	< 0.01	-	8	16	-	12 b
LW	3 b	25	< 0.01	-	3	15	-	9 c
Mean	-	-			8	17	< 0.01	

[†] *P*-value for the effect of harvest timing within a given year.

[‡] Values within columns followed by different letters are different at $P \leq 0.05$.

FIGURES

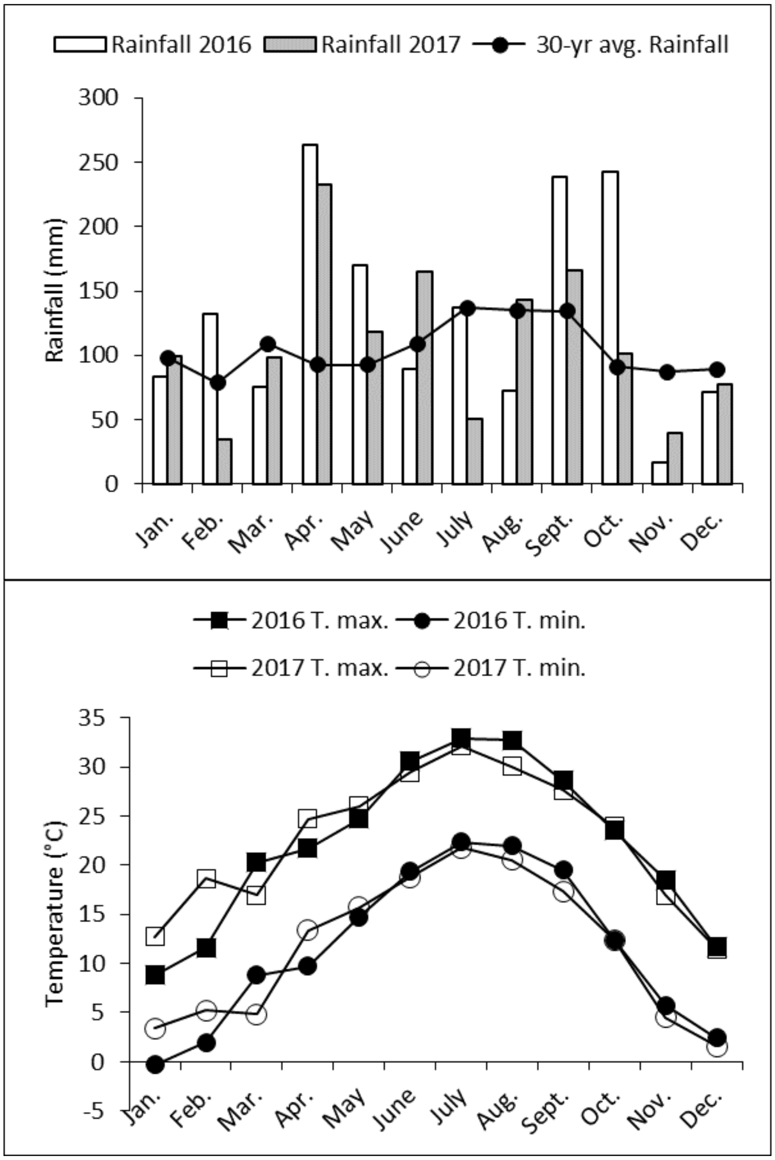

Figure 1.1. Monthly rainfall for 2016, 2017, and 30-yr average, and average temperatures (max. and min. for 2016 and 2017) at the Central Crops Research Station, Clayton, NC.

CHAPTER 2: Nutritive Value of Two Switchgrass Cultivars Harvested Following a Freeze Event

ABSTRACT

Information on the impact of defoliation management and harvest time at the end of the growing season is needed to determine the feasibility to support dual-purpose forage and feedstock switchgrass production systems. The objectives of the experiment were to determine 1) the impact of time of clipping during the frost-free period and 2) the interaction effect of harvest frequency (HF) and end-of-season harvest time (HT) on crude protein (CP), *in vitro* true digestibility (IVTD), and fiber composition [neutral- (NDF) and acid- (ADF)-detergent fibers)] for switchgrass cultivars 'BoMaster' and 'Performer'. Treatments were the factorial combination of two HF [full-season growth (1X) and two clippings per season (2X)] and three HT [(before frost (BF), after frost (AF), and late winter (LW)]. For both cultivars, CP ranged from 33 to ~82 g kg^{-1} being lower for 1X harvested at LW and greater when harvested half-way during the frost-free period. Tissue IVTD followed a similar pattern than CP and it ranged from ~244 to 700 g kg^{-1}. Both fiber components followed an inverse trend compared to CP and IVTD and ranged from ~745 to 864 g kg^{-1} for NDF and from ~431 to ~582g kg^{-1} for ADF. Herbage from the 2X defoliation treatment meets the nutrient requirement of a mature dry beef cow. Biomass from the rest of treatments is better suited as feedstock, especially when harvested following a freezing event, because of lower tissue CP and IVTD and greater NDF and ADF concentrations.

INTRODUCTION

Warm-season C_4 plants represent the main forage resource for ruminant production not only in the USA, but also in tropical and subtropical areas of the world (Burns et al., 2004). Switchgrass is a C_4 perennial warm-season grass, native to the Great Plains and broadly adapted throughout the USA transition region (Hitchcock and Chase, 1950), with potential to be used for wildlife habitat, erosion control, forage production, and as a bioenergy feedstock (Keshwani and Cheng, 2009; Moore et al., 2004, Sanderson and Wolf, 1995). Due to its perennial growth characteristic, farmers can take advantage of switchgrass' regrowth potential and minimize the risk of forage shortages due to delayed plantings that can occur when working with annual forage systems (Vogel, 2004). In North Carolina specifically, switchgrass can be utilized as early as mid-April or early May, overlapping with cool-season forages, and before other perennial warm-season grasses start to regrow (Burns et al., 1984).

Plant breeding efforts have resulted in the development and release of switchgrass cultivars with improved nutritive value, e.g., cultivars 'Trailblazer' (Vogel et al., 1991), 'Shawnee' (Vogel et al., 1996), and 'Performer' (Burns et al., 2008a), and also as dedicated biomass cultivars, e.g. 'Colony' (Burns et al., 2010) and 'BoMaster' (Burns et al., 2008b). Defoliation management schedules can impact productivity and quality of the harvested herbage (Bekewe et al., 2018; Chaparro and Sollenberger, 1997; Sanderson, 2008) and may interact with cultivars (Ashworth et al., 2013; Sanderson and Burns, 2010). Ashworth et al. (2013) reported reduced grass stands for lowland cultivars 'Alamo' and 'Kanlow' when clipping at 20-cm stubble height but not for the upland-type 'Cave-in-Rock'. Sanderson and Burns (2010) reported that differences among switchgrass cultivars in nutritive value and in vivo fiber and dry matter digestion mostly were due to differences in plant maturity and leaf to stem ratio.

Similar to other warm-season grasses, the nutritive value of switchgrass is initially moderate to high but declines rapidly as the plant matures (Burns et al., 1997; Griffin and Jung, 1983; Perry and Baltensperger, 1979). Crude protein of switchgrass cv. 'Alamo' decreased from 81 to 72 and 31 g kg^{-1} when cut at vegetative stage, boot stage, and one month after frost, respectively (Kering et al., 2013). Working with switchgrass cultivars 'Cave-in-Rock', 'Shawnee' and 'Trailblazer', Sanderson (2008) reported greater CP concentration from herbage harvested out of a 3- vs. 2-clippings per year management system. In Pennsylvania, Sanderson and Burns (2010) harvested 'Pathfinder' and 'Shawnee' switchgrass twice a year (mid-June and early-August) and reported greater crude protein for the mid-June clipping. In addition, clipping management during the frost-free period and time of harvest at the end of the growing season can also impact productivity and nutritive value of the harvested herbage. Working with cv. 'Alamo', Sanderson et al. (1999) reported lower CP when end-of-season harvest was delayed from October to November. Kering et al. (2013) reported that IVTD decreased from about 635 to 415 g kg^{-1} from a June harvest to harvesting after frost.

An early first clipping done at mid-season while switchgrass is at the vegetative stage, followed by forage regrowth clipped at the end of the growing season may be an alternative to support dual-purpose systems for production of forage for livestock and feedstock for bioenergy purposes when utilizing switchgrass cultivars 'BoMaster' and 'Performer'. Information on the impact of defoliation management on nutritive value is limited for cultivars 'Performer' and 'BoMaster' and particularly as a function of following a freeze event and delayed harvest at the end of the growing season. Cultivar 'BoMaster' was released because of its potential as a biomass crop and cultivar 'Performer' was released because of its improved nutritive value compared to other switchgrass cultivars grown in the transition region (Burns et al., 2008a, b). The objectives of the experiment were to determine 1) the impact of time of clipping during the frost-free period and 2) the interaction effect of harvest frequency and delayed harvest following a freeze event on switchgrass nutritive value estimates.

MATERIALS AND METHODS

Experimental Site, Plot Management, and Weather

The experiment was conducted for two seasons (2016-2017 and 2017-2018) at the Central Crop Research Station, Clayton, NC (35°40’ N, 78°29’W). Two mature stands (>8 yr) of switchgrass cultivars ‘BoMaster’ and ‘Performer’ were used for this experiment. Prior to initiation of this experiment the plots of both cultivars were managed with maintenance fertilization and a single-clipping event at the end of the growing season, followed by residue-burning in February of each year.

The soil series was classified as Wedowee sandy loam (fine, kaolinitic, thermic Typic Kanhapludults). Soil samples collected in February 2016 indicated soil pH of 6.0 and Mehlich-3 extractable P, K, Ca, and Mg concentrations of 205, 195, 733, and 218 g kg^{-1}, respectively, for the ‘BoMaster’ site, and pH of 6.2 and Mehlich-3 extractable P, K, Ca, and Mg concentrations of 622, 182, 1610, and 312 g kg^{-1} for the ‘Performer’ site. A new set of soil samples was also collected in February 2017.

Fertilization was applied following the North Carolina Department of Agriculture and Consumer Services (NCDA&CS; Hardy et al., 2014) recommendations for growing switchgrass and fertilizer amendments were broadcast-applied in a single application on mid-April for both years. In 2016, N was applied at a rate of 134 kg ha^{-1} using a granular formulation of pre-mixed urea-ammonium sulfate blend for both cultivars. In 2017, ‘BoMaster’ was fertilized at a N rate of 101 kg ha^{-1} using a granular formulation of di-ammonium phosphate-ammonium nitrate blend that was prepared on site (final concentration of 322 g N kg^{-1}, 460 g P kg^{-1}); and 'Performer' was fertilized at a rate of 176 kg N ha^{-1} using a granular formulation of ammonium sulfate-ammonium nitrate blend (final concentration of 314 g N kg^{-1}). Although N fertilization rates and sources varied among years, the applied N rates were in the higher range, or above, for reported switchgrass' response to N fertilization rates in the region (Brejda, 2000; Obour et al., 2017).

Minimum and maximum average monthly temperatures ranged from -0.3 to 32.9°C for January and July, respectively in 2016 and from 1.6 to 32.2°C for December and June in 2017. Total rainfall was 1591, 1327, and 1253 mm in 2016, 2017, and the 30-yr average, respectively. Dates of last frosts at the beginning of the growing season were 10 April 2016 and 23 April in 2017. Dates of first frosts at the end of the growing season were 11 November 2016 and 4 November in 2017.

Treatments and Experimental Design

Treatments were the factorial combination (2 x 3) of harvest frequency (HF) and end-of-season harvest time (HT). The two HF levels were full-season growth (1X) and two clippings per season (2X). The three HT levels were before frost (BF), after frost (AF), and late winter (LW). For the 2X treatment factor, the first harvest event occurred by mid-June (2X-June; on 22 and 15 June in 2016 and 2017, respectively), which is half-way the active growing season for switchgrass in North Carolina, and the second harvest occurred at the end of the growing season based on HT (2X regrowth-BF, -AF, and -LW). Harvest dates at the end of the growing season for BF were 6 and 5 October in 2016 and 2017, respectively; for AF, 17 November in both years; and for LW, 7 and 6 February in 2017 and 2018, respectively. The HT treatments were selected to test the impact of a freeze event and delayed harvest on nutritive value of mature (i.e. 1X) vs. younger (i.e. 2X regrowth) plant tissue on switchgrass responses.

The experimental design was a split plot-design with the main-plot factor arranged in a complete randomized block design replicated three times. Main-plot factor was HF and sub-plot factor was HT. Treatments were imposed to each of the two separate stands of switchgrass cultivars, one for ‘BoMaster’ and one for ‘Performer’; consequently, each cultivar constituted an experiment and the data from each cultivar were analyzed separate with inferences limited to treatment effects for each cultivar. Experimental unit size was 4.9-m wide by 4.9-m long with 2.4-m wide alleys between plots.

Response Variables

Nutritive value measurements included crude protein (CP), *in vitro* true digestibility (IVTD), and fiber composition [neutral detergent fiber (NDF), and acid detergent fiber (ADF)]. Concentrations of CP, IVTD, NDF, and ADF were determined using near-infrared spectroscopy. Calibration equations were developed correlating NIR spectra to wet chemistry estimates of CP, IVTD, NDF, and ADF. Samples were scanned using a 5000 NIRS equipment (Foss North America, Inc., Eden Prairie, MN) and reflectance was determined in 2 nm wavelength-increments (from 1100 to 2500 nm). Wet chemistry analyses for CP, IVTD, NDF, and ADF were performed by Dairy One Laboratory (Ithaca, NY) (Dairy One, 2015). In summary for wet chemistry analyses, CP was calculated by multiplying the concentration of total N (determined by dry combustion) by 6.25; IVTD was determined through a 48-h *in vitro* digestion procedure, and NDF and ADF were determined using an Ankom fiber analyzer.

Forty-two samples, which correspond to 39% of the total number of samples in the dataset, were randomly selected for wet chemistry analysis and were used for model selection, calibration, and validation using a NIR pipeline in R. Mathematical pretreatments applied to the spectra were Savitzky-Golay smoothed spectra (using five points; SG-5) for CP, standard normal variate (SNV) for IVTD, and SNV followed by SG-7 for NDF and ADF. Partial least square equations were then developed and used to predict the remaining of samples. Cross-validation was performed using ‘leave-one-out’. Number of factors in the model, coefficients of determination of calibration (R2C) and cross-validation (R2CV), and standard errors of calibration (SEC) and cross-validation (SECV) are presented in (Table 2.1).

Statistical Analysis

Analyses of variance were performed using the PROC GLIMMIX procedure of SAS (SAS Institute, Cary, NC). The analyses were conducted by cultivar for the reasons explained earlier. Two separate analyses of variance were set up. The first analysis was set up to compare the 2X-June vs. 2X regrowth treatments and the second analysis was set up to test the interaction effect of HF by HT. Treatments, year, and their interaction were considered fixed effects. Year was considered as repeated measured with the variance component modeled using compound symmetry as the covariance structure based on smaller AIC values. Block was considered random effect. When an interaction effect was significant the SLICE statement of SAS was used to set up an F-test for each harvest frequency and to compare harvest timings. The LINES option of LSMEANS using SAS was used for mean separation. Treatment differences were declared significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Crude Protein

For ‘BoMaster’, there was a trend ($P = 0.07$) toward greater CP concentration for 2X-June compared to 2X regrowth-BF (Table 2.2). There was, however, a year effect ($P = 0.01$) and CP concentration averaged across treatments was greater in 2016 (77.8 g kg^{-1}) compared to 2017 (67.4 g kg^{-1}). Lower CP concentration in 2017 is attributed to the lower N fertilization rate in 2017 compared to 2016. There was a HF by HT interaction effect that occurred because CP concentration for 2X regrowth-BF was greater than 2X regrowth-AF and -LW while there was no difference between HT for 1X (Table 2.3). Our results indicate that 2X clipping results in greater CP concentration compared to 1X and this effect remained consistent even after a freeze event and delayed harvest up until the last harvest date in February in this study.

For cultivar ‘Performer’, concentration of CP was greater for 2X-June (82.4 g kg^{-1}) compared to 2X regrowth-BF (71.8 g kg^{-1}). There was no interaction effect of HT by HF, but only main effects of HF and HT. Concentration of CP was greater for younger tissue (i.e. 2X regrowth vs. 1X) and greatest when harvested BF (Table 2.4). Our results indicate that CP concentration was greater for the first clipping of the season once switchgrass was clipped in June when switchgrass plants were predominantly at vegetative stage. Delaying harvest at the end of the growing season resulted in lower CP concentration following a freeze event and CP concentration was not different whether the forage was clipped AF or LW.

Similar CP concentration ranges to those found in our study for both cultivars have been reported for cultivars ‘Alamo’, ‘Cave-in-Rock’, ‘Shawnee’, and ‘Trailblazer’ (Mosali et al., 2013; Moore et al., 2004; Sanderson, 2008). Slightly higher CP values, up to 124 g kg^{-1}, were reported by Hedtcke et al. (2014) working with ‘Cave-in-Rock’ cultivar in Wisconsin and Michigan. Burns et al. (1997) reported two-fold decrease in CP concentration from early June to August harvest when working with cv. ‘Kanlow’ in North Carolina; a similar trend occurred in our study and it demonstrates the impact of harvest timing on CP concentration concomitantly with plant maturation and shift from vegetative to reproductive stages. Based on NRC (1984) tables, the CP levels of the 2X-June and 2X regrowth-BF would be enough to meet the daily CP requirement of a mature dry beef cow while the rest of treatments would not meet the requirement.

Digestibility

For ‘BoMaster’, there was a year by treatment interaction effect when comparing 2X-June vs. 2X regrowth-BF. The interaction effect occurred because digestibility for 2X-June was lower in 2016 compared to 2017; however, there was no difference between years for the 2X-BF (Table 2.2). Averaged across years, digestibility was greater for 2X-June (Table 2.2). There was no HF by HT interaction effect but there were HF and HT main effects. Averaged across HT, IVTD was greater for 2X regrowth vs. 1X; and averaged across HF, IVTD was greatest for BF, intermediate for AF, and lowest for LW (Table 2.3).

For ‘Performer’, digestibility for 2X-June was greater (700 g kg^{-1}) compared to 2X regrowth-BF (476 g kg^{-1}). There was a three-way interaction effect of HF by HT by year ($P = 0.01$) and therefore the data were analyzed by year (Table 2.5). In 2016, there was a HF by HT interaction effect ($P = 0.03$) that occurred because IVTD concentration for 2X regrowth was greater than 1X when harvest occurred at BF; however, it was not different between AF and LW. In 2017, there were main effects of HF and HT ($P < 0.0001$). Tissue IVTD concentration was lower for 1X compared to 2X regrowth and IVTD decreased as harvest timing was delayed from BF to LW (Table 2.5).

The wide range in digestibility values for herbage harvested in the frost-free (i.e. 2X-June and 2X regrowth-BF) in our study are within the range reported by several other studies and cultivars (Richner et al. 2014, Mosali et al. 2013, Kering et al. 2013, Burns et al. 2010). Lower digestibility in more mature stands and following a freeze event is explained because of a greater maturity stage at harvesting time (reproductive stage), with more lignified cell walls (Buxton, 1996), decreased leaf to stem ratio (Kirch et al. 2007; Griffin and Watson, 1982; Twidwell et al. 1988) and translocation of carbohydrates to the roots and crown (Parrish and Wolf, 1992, 1993).

Fiber Composition

For ‘BoMaster’, NDF concentration was lower for 2X-June (750 g kg^{-1}) compared to 2X regrowth-BF (764 g kg^{-1}) and there was no difference in ADF concentration (average of 483 g kg^{-1}). There was no interaction effect of HF by HT for NDF and ADF concentrations and both response variables followed the same response pattern with main effects of HF and HT. Tissue NDF and ADF concentrations were greatest for 1X and for LW harvest (Table 2.3).

For ‘Performer’ switchgrass, NDF concentration was lower for 2X-June (745 g kg^{-1}) compared to 2X regrowth-BF (763 g kg^{-1}); concentration of ADF was not different between 2X-June and 2X regrowth-BF (462 g kg^{-1}). There was a HF by HT interaction effect for NDF concentration that occurred because NDF concentration for 2X regrowth harvested at AF was intermediate between lower for BF and higher for LW; however, for 1X the NDF concentration was not different between AF and LW and lowest for BF (Table 2.4). For ADF concentration there were HF and HT main effects only (Table 2.4). Averaged across HT, ADF concentration was greater for 1X vs. 2X regrowth by about six percentage points, and averaged across HF, ADF concentration was lowest for BF, intermediate for AF, and greatest for LW.

Greater NDF and ADF concentration with plant maturity has been reported previously in the literature for warm-season grasses (Van Man and Wiktorsson, 2003). Similar values in ADF and NDF for 2X-June and 2X regrowth-BF than in our study were reported for lowland cultivars ‘Alamo’, ‘BoMaster’, and ‘Performer’ (Mosali et al. 2013; Burns et al. 2010, Sanderson et al. 1999; Anderson and Matches, 1983). In a grazing experiment with switchgrass cultivar ‘Cave-in-Rock’, Moore et al. (2004) reported lower NDF concentration in mid-summer compared with the values obtained for ‘BoMaster’ and ‘Performer’ in our study. Delayed harvest during the season increase fiber components sharply as demonstrated by previous work (Burns et al. 1997; Guretzky et al. 2011; Kering et al. 2013) with switchgrass cultivars ‘Kanlow’ and ‘Alamo’. In general, greater NDF concentration for longer regrowth intervals occurs as a phenological response to hot temperatures and shorter days (Buxton, 1996), but also associated with a growth of reproductive structures and greater proportion of structural carbohydrates (Twidwell et al., 1988; Wilson and Hatfield, 1997; Buxton and Redfearn, 1997). Jung and Vogel (1992) reported that NDF and ADF increase for both leaves and stems as result of physiological maturation with switchgrass. The NDF concentration increase is also related with digestibility decreasing as demonstrated by Burns and Fisher (2011) when delayed the clipping until September compared with June and this is due to the reduction of leaves and increase of stem as a proportion of the forage mass.

CONCLUSIONS

For both cultivars, CP ranged from 33 to 82 g kg^{-1} being lower for 1X harvested at LW and greater for the 2X-June and 2X-BF harvests. Tissue IVTD followed a similar pattern than CP and it ranged from ~244 to 700g kg^{-1}. Both fiber components followed an inverse trend compared to CP and IVTD and ranged from 745 to 864 g kg^{-1} for NDF and from 484 to 571 g kg^{-1} for ADF. Herbage from the 2X defoliation treatment is suitable to meet the nutrient requirement of a mature dry beef cow. However, greater nutritive value herbage can be obtained with more frequent defoliation regimes (clipping or grazing) when switchgrass plants are in the vegetative state and by utilizing it before the jointing stage (Anderson and Matches, 1983; Burns and Fisher, 2013). Herbage from the 1X clipping is better suited as feedstock specially when harvested following a freezing event, and also for 2X if harvested after frost, because of lower tissue CP and IVTD concentrations and greater NDF and ADF concentrations.

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TABLES

Table 2.1. Near-infrared reflectance spectroscopy calibration and validation statistics for crude protein (CP), *in vitro* true digestibility (IVTD), neutral detergent fiber (NDF), and acid detergent fiber (ADF).

Constituent	Mathematical treatment (# factors)	R^2_C†	R^2_{CV}‡	SE_C§	SE_{CV}¶
				--- g kg^{-1} ---	
CP	SG-5 (9)	0.99	0.98	2	6
IVTD	SNV (9)	0.99	0.98	17	20
NDF	SNV-SG7 (10)	0.98	0.91	7	15
ADF	SNV-SG7 (5)	0.88	0.85	25	29

† R^2_C = coefficient of determination for calibration

‡ R^2_{CV}= coefficient of determination for cross-validation

§ SE_C= standard error of calibration

¶ SE_{CV}= standard error of cross-validation

Table 2.2. Clipping treatment by year interaction effect on crude protein (CP; $P = 0.07$; n = 3; SE = 4), and *in vitro* true digestibility (IVTD; $P = 0.03$; n = 3; SE = 13) for 'BoMaster' switchgrass.

Year	Treatment		
	2X-June	2X regrowth-BF	
	CP		*P*-value[†]
	--- g kg^{-1} ---		
2016	90 a[‡]	65	0.02
2017	74 b	61	0.11
Mean	82	63	0.07
	IVTD		
	--- g kg^{-1} ---		
2016	620 b	452	< 0.01
2017	694 a	450	< 0.01
Mean	657	451	< 0.01

[†] *P*-value for the effect of harvest treatment within a given year.

[‡] Means within columns by harvest treatment followed by different letters are different at $P \leq 0.05$.

Table 2.3. Crude protein (CP; SE = 2), *in vitro* true digestibility (IVTD; SE = 5), neutral detergent fiber (NDF; SE = 3), and acid detergent fiber (ADF; SE = 4) of ‘BoMaster’ switchgrass as a function of harvest frequency (HF; 1X vs. 2X regrowth) by harvest time (HT; BF = before frost, AF = after frost, and LW = late winter).

Harvest Time	Harvest Frequency			
	1X	2X regrowth		
	CP		*P*-value[†]	Mean
	---- $g\ kg^{-1}$ ---			$g\ kg^{-1}$
BF	39	63 a[‡]	< 0.01	-
AF	32	45 b	< 0.01	-
LW	33	47 b	< 0.01	-
Mean	-	-		
	IVTD			
	--- $g\ kg^{-1}$ ---			$g\ kg^{-1}$
BF	374	451	-	412 a
AF	267	323	-	295 b
LW	215	282	-	249 c
Mean	285	352	< 0.01	
	NDF			
	--- $g\ kg^{-1}$ ---			$g\ kg^{-1}$
BF	804	764	-	784 b
AF	867	835	-	851 a
LW	866	846	-	856 a
Mean	846	815	< 0.01	
	ADF			
	--- $g\ kg^{-1}$ ---			$g\ kg^{-1}$
BF	530	468	-	499 c
AF	578	520	-	549 b
LW	604	560	-	582 a
Mean	571	516	< 0.01	

[†] *P*-value for the effect of harvest treatment within a given year.

[‡] Means within columns by harvest treatment followed by different letters are different at $P \leq 0.05$.

Table 2.4. Crude protein (CP; SE = 2), neutral detergent fiber (NDF; SE = 6), and acid detergent fiber (ADF; SE = 4) of 'Performer' switchgrass as a function of harvest frequency (HF; 1X vs. 2X regrowth) by harvest time (HT; BF = before frost, AF = after frost, and LW = late winter).

	Harvest Frequency			
	1X	2X regrowth		
Harvest Time	CP		*P*-value[†]	Mean
	--- g kg^{-1} ---			g kg^{-1}
BF	43	72	-	57 a[‡]
AF	35	51	-	43 b
LW	38	57	-	48 b
Mean	39	60	< 0.01	
	NDF			
	--- g kg^{-1} ---			g kg^{-1}
BF	819 b	763 c	< 0.01	-
AF	850 a	819 b	< 0.01	-
LW	864 a	843 a	0.04	-
Mean	-	-	-	
	ADF			
	--- g kg^{-1} ---			g kg^{-1}
BF	513	441	-	477 c
AF	553	495	-	524 b
LW	589	529	-	559 a
Mean	552	488	< 0.01	

[†] *P*-value for the effect of harvest treatment within a given year.

[‡] Means within columns by harvest treatment followed by different letters are different at $P \leq 0.05$.

Table 2.5. *In vitro* true digestibility (IVTD; SE = 12), of ‘Performer’ switchgrass as a function of harvest frequency (HF; 1X vs. 2X regrowth) by harvest time (HT; BF = before frost, AF = after frost, and LW = late winter).

Harvest Time	2016 Harvest Frequency 1X	2016 Harvest Frequency 2X	2016 P-value[†]	2016 Mean	2017 Harvest Frequency 1X	2017 Harvest Frequency 2X	2017 P-value[†]	2017 Mean
	--- g kg^{-1} ---			g kg^{-1}	--- g kg^{-1} ---			g kg^{-1}
BF	389 a[‡]	462 a	0.01	-	416	490	-	453 a
AF	340 ab	394 b	0.05	-	274	313	-	294 b
LW	304 b	273 c	0.24	-	215	273	-	244 c
Mean	-	-			302	359	< 0.01	

[†] P-value for the effect of harvest treatment within a given year.

[‡] Values within columns followed by different letters are different at $P \leq 0.05$.