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FORAGE AND BIOMASS DUAL-PURPOSE HARVEST SYSTEM USING NATIVE WARM-SEASON GRASSES

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To the Graduate Council:

I am submitting herewith a thesis written by David Weston McIntosh entitled "FORAGE AND BIOMASS DUAL-PURPOSE HARVEST SYSTEM USING NATIVE WARM-SEASON GRASSES." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

Gary E. Bates, Major Professor

We have read this thesis and recommend its acceptance:

Patrick Keyser, Fred Allen

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A Thesis Presented for the
Master of Science
Degree
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David Weston McIntosh
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ABSTRACT

There has been increasing interest in utilizing native warm-season grasses (NWSGs), especially switchgrass, as a biomass feedstock for cellulosic ethanol production. Millions of hectares of crop and pasture in the mid-South are forecast to potentially be planted with switchgrass for biomass feedstock production. This could have a substantial impact on the region's cattle industry, reducing forage production hectares. This study was conducted to determine the effect of early season harvest timing on forage and biomass of NWSGs designed for use in cellulosic ethanol production. The over-all hypothesis was to determine if an early forage harvest can be included in a dual-purpose system along with a fall biomass harvest for cellulosic ethanol production without significantly reducing fall biomass yields. The NWSGs used in this study were switchgrass (*Panicum virgatum* L.) monoculture (SG), SG/big bluestem (*Sorghastrum nutans* L.) /indiangrass (*Andropogon gerardii* V.) mixture (SGBBIG), and big bluestem/indiangrass mixture (BBIG). These NWSGs were harvested at fall dormancy for biomass only (FD), early boot plus FD (EBFD), and early seedhead plus FD (ESHFD). Therefore, the objectives of this study were to determine the effect (i) early-season harvest timing on fall biomass yield, (ii) early season forage harvests yield and quality, and (iii) species mixtures on biomass quality in a dual-purpose system. Results from this study should provide information about dual-purpose systems using NWSGs in monoculture and mixtures for both forage and biomass.

Keywords: biofuel, switchgrass, big bluestem, indiangrass, dual-purpose, hay production, ethanol, forage, native warm-season grasses, mid-South, fertilization, biomass, feedstock, forage quality, biomass quality, feedstock

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REVIEW OF RELEVANT LITERATURE

Chapter 1

The development of renewable energy sources has become an issue of increasing importance as it was recognized 25 years ago that bio-energy feedstocks would ultimately be important contributors to a national renewable energy supply (McLaughlin and Kszos, 2005). The potential of cellulosic ethanol production using native warm-season grasses (NWSGs) for biomass feedstock, especially switchgrass (SG), has grown since then (Schmer et al., 2010). If bio-refineries become more common-place across the country, they must have reliable biomass supplies, resulting in many hectares potentially being planted to biomass crops (Landis et al., 2008; Schmer et al., 2010). With estimates predicting that over 21 million hectares of SG will need to be produced annually, the importance of exploring bioenergy sources is becoming more evident (English et al., 2004). In the United States, bioenergy crops are estimated to exceed 22 million hectares by 2030, producing 60 billion gallons of ethanol and biodiesel (Sanderson and Adler, 2008; USDA Statistics Service, 2013). There are currently 412 million hectares of crop land, of which over 22 million hectares are forage crops that have potential to be planted to bioenergy crops (USDA Statistics Service, 2013). The demand for bioenergy crops could have a substantial impact on the livestock industry since the predictions for bioenergy crops is the same as the forage crop hectares already in production; therefore, alternative uses for NWSGs in biomass systems are being explored (Sanderson and Adler, 2008). Forage production hectares, already for grazing or hay, might be replaced with biomass fields with such a demand for biomass. This situation has been a concern in the Southern U.S. with economic estimations by English et al. (2006) that hay production hectares would suffer by decreasing while biomass hectares increased. However, this model predicted that current pastureland would expand in hectares that were not already in hay production, increase farm incomes, create job opportunities, and provide other economic benefits (English et al., 2006). Although the demand for biomass can almost be unpredictable, there will be a drastic change in current production systems.

Assessing the performance of a SG (*Panicum virgatum* L.) monoculture compared to NWSGs in mixture is important when evaluating systems for forage and biomass production. Previous studies for biomass production have focused on SG due to high yields, cost effectiveness, minimal environmental impacts, and cellulosic ethanol potential (Lynd et al., 1991; Sanderson et. al., 1996). Parrish and Fike (2005) reported that a single harvest of switchgrass from late fall or early winter resulted in the highest sustainable biomass. Switchgrass is known for its regional and ecotypical differences and constituents used in estimating ethanol yields (Bhandari et al., 2013). Switchgrass is typically converted into ethanol using the lignocellulosic process that involves fermentation of sugars (Sanderson et al., 2006). Biorefineries will require certain levels of each constituent and the prediction of ethanol yield changes with each process update and innovation. Most ethanol yield predictions have been based on cellulosic materials where fibers, lignin, and digestibility make up 95% of the information needed (Lorenz et al., 2009). Higher levels of lignin limit the conversion process by inhibiting sugar and fermentation recovery from biomass (Sanderson et al., 2006; Dien et al., 2006; Vogel and Jung, 2001). Current research into *in-vitro* true dry matter digestibility 48 hrs (IVTDMD48) estimates have shown them to be a leading constituent for estimating ethanol yield from switchgrass (Vogel et al., 2011). This indicates that digestibility is important in the sugar extraction process, where lower lignin levels can indicate higher cellulose availability needed to ferment into ethanol (Chang and Holtzapple, 2000; Lee, 2006). Also, mineral content can produce excess waste materials that can make the conversion of biomass into ethanol processes less efficient (Monti et al., 2008).

Other NWSGs have not been researched to the same extent as SG, however, these species have been discussed as a way to increase yield and quality attributes in both forage and biomass systems (Thomason et al., 2005; Tracy et al., 2010). Recent work on NWSGs in monoculture and mixtures reported that SG should be included in mixtures, as it resulted in higher biomass yield and lower cellulose levels compared to mixtures with big bluestem (*Sorghastrum nutans* L.) (BB) and indiangrass (*Andropogon gerardii* V.) (IG) (Hong et al., 2013). In that study, the addition of BB and IG resulted in more desirable biomass for ethanol production due to lower lignin levels than any monoculture or mixture, SG (Hong et al., 2013). Hong et al. (2013) also reported that in mixtures SG tended to decline with fewer plants surviving over time when included in a mixture with other species. However, NWSGs are known to produce biomass, under a single fall harvest, with high levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) and low levels of crude protein (CP) and ash (Mulkey et al., 2008). Levels of each biomass quality constituent will vary depending on the species and harvest system, whether a single fall harvest or a dual-purpose system.

These quality aspects are an important factor to consider when selecting NWSGs for forage production systems. Research has also shown that SG can be favorable for forage production with proper management and harvesting before maturity causes reduced forage quality (Mitchell et al., 2001). With the focus on forage production, desired forage quality can be met for all NWSGs if harvested at an early stage in production. These NWSGs could have CP levels of 150 g kg^{-1} if harvested at the EB stage (Griffin and Jung, 1983). As harvest is delayed, quality decreases dramatically, making it important to harvest based on plant phenology instead of yield (Waramit et al., 2012).

The consideration to utilize NWSGs in a dual-purpose system is to allow a portion of the yield to be diverted as an early forage harvest and then utilize the remaining growth as a biomass harvest for ethanol production. Previous research conducted on dual-purpose systems is leading the way to include mixtures over monocultures that have been the focus for several years. Recently, Mosali et al. (2013) published results indicating that SG can provide forage for stocker cattle into early spring and throughout the growing season while still removing a biomass harvest in the late fall. To remove two harvests each season has produced optimum yields in some systems, with the earlier harvest being high-quality forage and the later harvest for biofuel production (Sanderson et al., 1996). Other research concluded that a single spring harvest for forage followed by a final fall harvest for biomass would be the best approach for a dual-purpose system (Sanderson et al., 1999).

Parish and Fike (2005) found that a single harvest of switchgrass from late fall or early winter resulted in the highest sustainable biomass yields and good stand persistence from year to year compared to a dual-purpose system approach. In a two-harvest system in Mississippi, biomass yields for the one-harvest system were significantly higher than the two harvest system (Grabowskit et al., 2004). In this study, however, biomass harvest occurred prior to plant dormancy (Grabowski et al., 2004). Guretzky et al. (2011) evaluated SG for dual-purpose use at two locations in Oklahoma from 2008 to 2009 with the forage harvest occurred after boot stage and the biomass harvest occurring after frost. This study found forage quality to be poor when harvested any time after the reproductive stage had begun, and suggested that SG would need to be harvested in the early boot stage to have quality acceptable for livestock (Guretzky et al., 2011). This dual-purpose system evaluated by Guretzky et al. (2011) to determine if harvesting twice a year is possible if the first harvest is very early in the growing season and the biomass harvest is after the first killing frost; they concluded it was possible with proper management and increased inputs. Inputs such as fertilization and management will increase in order to produce high-quality forage and biomass in the same system (Brejda et al., 2000).

In systems using NWSGs, fertilizer inputs should adjusted for the type of production desired from these grasses. Typically native grasses have been listed as low input, but mainly for biomass production used in ethanol production. However, when reviewing recommendations for nitrogen (N) fertilization many differences in opinions and data surfaced. The only recommendations that are consistent were that fertilization with N is not recommended during the first year due to weed pressure; and that an early application of N as grass green-up will increase yield and nutrient quality (USDA, 2006; Thomason et al., 2005). Applications of up to N during the early growing season has been reported to increase yields and highest yields with a maximum 448 kg N ha^{-1} applied annually (Muir et al., 2001). In a multi-state and year study conducted it was found that split applications of N, in the two-harvest system, may have reduced N losses (Fike et al., 2006b). All N was applied in April for the one-harvest treatment, and for the two-harvest treatments N applications were equally split between April and the first harvest at 40 kg N ha-^{1} (Fike et al., 2006b). Expectations for switchgrass monocultures to require N fertilizer is known to be economically productive with so much material removed for biomass harvests (Heaton et al., 2004). Even though the research differs in N rates, it has been shown that N fertilization increased yields for different cutting systems (Madakadze et al., 1999). Reducing N loss is also important when NWSGs are used for hay production where it was found that split application of N in a two-harvest system may have reduced losses due to volatilization and run-off (Fike et al. 2006b).

All other fertilizer applications of phosphorus (P) and potassium (K) have been usually based on regular soil testing and recommendations (Thomason et al., 2005). These recommendations are generally reported as necessary only when soil test results show low levels, but this can be a problem if the NWSGs are for hay production and more material is removed during harvests (Fike et al., 2006b). This can be increased with N application, when other resources such as water, P, K, and calcium (Ca) are available, making nutrient content improve for forage and yields increase (Ocumpaugh et al., 2003; Epstein et al., 1996; Parrish et al., 2003; Muir et al., 2001). Soil nutrient uptake and cycling for a mixed species of NWSGs has not been studied, but several of the species have been analyzed separately. A study from Oklahoma looking at switchgrass response to harvest frequency and N application rates reported that there were no significant differences when N was applied at different rates to the control of no N applied; however, increased concentrations of P and K were noticed with increased yields (Thomason et al., 2005). As the species are combined more nutrients may be necessary depending on the needs for quality hay production. For biofuel feedstock production a single harvest for fall biomass is an option when a producer does not need another cash-crop that would remove less nutrients compared to a two harvest system (Guretzky et al., 2011). Their findings found that switchgrass increased in maintenance and fertility requirements when an early harvest was removed and then a biomass harvested (Guertzky et al., 2011). Where NWSGs can be grown in fields that are low in P levels making sure adequate levels is necessary to potentially increase yields by up to 17%, and the application of N can increase those yields even more up to over 45% in comparison to fields with no additional inputs (Kering et al., 2011).

Although most of the research in the forage and biomass dual-use concept has been conducted using SG, several other NWSGs have potential to be included in production systems. Selection of the lowland cultivar ‘Alamo’ switchgrass was due to the cultivar’s leafy, fast spring growth, long vegetative state, and high yielding attributes (Ball et al., 2007). This cultivar grows very well in the mid-South region where upland cultivars do not thrive to the same extent (Parrish and Fike, 2005). Selection of the cultivar was ‘Rumsey’ originating from the corn-belt area and is a later maturing variety having a mid-summer target harvest date for quality and yield. The cultivar of Indiangrass for this study was ‘OZ-70’ from the Ozark region that is highly adaptable to different climates and soil types.

The species BB and IG are considered to be high-quality forage species that mature later in the summer and are widely used for livestock forage in the mid-West (Ball et al., 2007; Mitchel et al., 2001; Mulkey et al., 2008). Compared to SG, BB and IG are generally the more palatable and nutritious species due to the leafiness of the forage during the early summer, and have the potential for ethanol production in the future (Ball et al., 2007; Mitchell et al., 2001; Redfearn and Nelson, 2003; Stubbendieck et al., 2002). Other research concluded that SG, BB, and IG could be ideal for sustainable forage production with proper management (Mulkey et al., 2008). Studies have demonstrated dual-purpose systems in which the species maintained yields even if the early harvest was taken early; however, quality of the forage harvest has not been a primary focus (McLaughlin et al., 1999; Sanderson et al., 1999). Including other species of NWSGs with SG may provide higher quality forage options or increased yields for both production systems in this model (Fike et al., 2006a; Posler et al., 1993; Sanderson et al., 2006). These same three NWSGs, when combined in a mixture, have not been studied to determine characteristics that each species could bring to a mixture harvested for forage followed by a biomass harvest in the fall.

With previous research and management recommendations this study should demonstrate that NWSGs in a field intended for biomass can produce acceptable forage yield and quality if a single harvest is made relatively early in the season. Determining the appropriate harvest timing will be specific to the NWSGs maturity at time of harvests and should be closely monitored during the growing season. The opportunity for producers to harvest both forage and biomass from NWSGs in the same field offers flexibility with potential to increase profits. The impact of harvest timings and specific NWSGs for both forage and biomass needs to be considered. This can contribute to current sustainable practices when producers want more flexibility in harvest management while satisfying multiple crop goals providing quality forage for livestock and a biomass crop (Mulkey et al., 2008).

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EARLY SEASON FORAGE HARVESTS COMPARING YIELD AND QUALITY OF NATIVE WARM-SEASON GRASSES DESIGNED FOR USE IN A BIOMASS SYSTEM

Chapter 2

ABSTRACT

Native warm-season grasses (NWSGs) have the potential to become a leading feedstock for ethanol production. Having long been considered a forage crop for livestock the biofuel movement allows these grasses to be considered a dual-purpose crop. A study was conducted to determine the effect of early season harvest timing on forage yield and quality of NWSGs designed for use in cellulosic ethanol production. The NWSGs used in this study were switchgrass (*Panicum virgatum* L.) monoculture (SG), SG/big bluestem (*Sorghastrum nutans* L.) /indiangrass (*Andropogon gerardii* V.) mixture (SGBBIG), and big bluestem/indiangrass mixture (BBIG). These NWSGs were harvested at fall dormancy for biomass only (FD), early boot (EB) plus FD, and early seedhead (ESH) plus FD. Harvesting at ESH produced more forage yield than mixtures harvested at EB. The SG and SGBBIG produced more yield compared to the BBIG for both early harvests. Forage harvested at EB had higher crude protein (CP) and higher estimated total digestible nutrient (TDN) levels compared to ESH, indicating a decrease in forage quality as plants matured. Fiber levels increased as the stage of maturity advanced from EB to ESH. The mixture of BBIG resulted in a higher quality forage compared to the SG and were similar to the

SGBBIG at both early harvests. The macro-nutrient removal of nitrogen (N), phosphorus (P), and potassium (K) by the forage harvests were significantly greater when harvested at ESH. An early season forage harvest, using NWSGs in a biomass field, has the potential to produce acceptable forage yield and quality, but plant phenology should be an important factor when determining harvest timings.

Abbreviations: ADF, acid detergent fiber; BB, big bluestem; CP, crude protein; DM, dry-matter basis; EB, early boot harvest; ESH, early seedhead harvest; FD, fall dormancy harvest; IG, indiangrass; NWSGs, native warm-season grasses; NIRS, near-infrared spectroscopy; NDF, neutral detergent fiber; N, nitrogen; P, phosphorus; K, potassium; PLS, pure live seed; SG, switchgrass.

LITERATURE REVIEW

The potential of cellulosic ethanol production has increased interest in using native warm-season grasses for biomass feedstock (Schmer et al., 2010). If bio-refineries become more common-place across the country, they must have reliable biomass supplies, resulting in many hectares potentially being taken to biomass crops (Landis et al., 2008; Schmer et al., 2010). With estimates to produce over 21 million hectares of SG annually, the importance of exploring bioenergy sources is becoming more evident (English et al., 2004). In the United States, bioenergy crops are estimated to exceed 22 million hectares by 2030, producing 60 billion gallons of ethanol and biodiesel (Sanderson and Adler, 2008; USDA Statistics Service, 2013). The demand for bioenergy crops could have a substantial impact on the livestock industry; therefore, alternative uses for NWSGs in biomass systems are being explored (Sanderson and Adler, 2008). There are currently 412 million hectares of crop land, of which over 22 million hectares are forage crops that have potential to be planted to bioenergy crops (USDA Statistics Service, 2013). The demand for bioenergy crops could have a substantial impact on the livestock industry since the predictions for bioenergy crops is the same as the forage crop hectares already in production; therefore, alternative uses for NWSGs in biomass systems are being explored (Sanderson and Adler, 2008). While the evaluation of SG has shown it is suitable for use as an energy feedstock for producing ethanol, other NWSGs have not been researched to the same extent (Thomason et al., 2005; Tracy et al., 2010).

Previous studies for biomass production have focused on SG monocultures due to high yields, cost effectiveness, minimal environmental impacts, and cellulosic ethanol potential (Lynd et al., 1991; Sanderson et. al., 1996). Parrish and Fike (2005) reported that a single harvest of switchgrass from late fall or early winter resulted in the highest sustainable biomass. Other NWSGs have not been researched to the same extent as SG, however, these species been discussed as a way to increase yield and quality attributes in both forage and biomass systems (Thomason et al., 2005; Tracy et al., 2010). Recent work on NWSGs in monocultures and mixtures reported that for biomass production, SG should be included in mixtures; the addition of BB and IG resulted in more desirable biomass for ethanol production (Hong et al., 2013). These NWSGs are known to produce biomass, under a single fall harvest, with high levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) and low levels of CP and ash (Mulkey et al., 2008). Research has also shown that SG can be favorable for forage production with proper management and harvesting before maturity causes reduced forage quality (Mitchell et al., 2001). With the focus on forage production, desired forage quality can be met for all NWSGs if harvested at an early stage in production. These NWSGs could have CP levels of 150 g kg^{-1} if harvested during the vegetative stages (Griffin and Jung, 1983). As harvest is delayed into the later stages of seed production quality decreases dramatically, making it important to harvest based on plant phenology instead of yield (Waramit et al., 2012).

The consideration to utilize NWSGs in a dual-purpose system is to allow a portion of the yield to be diverted as an early forage harvest and then utilize the remaining growth as a biomass harvest for ethanol production. Dual-purpose systems for grazing with a bioenergy harvest in the late fall have indicated that SG can extend the grazing season for stocker cattle into early spring and still remove a biomass harvest (Mosali et al., 2013). Removing one or two harvests each season has produced optimum yields in some systems, with the earlier harvest being high-quality forage and the later harvest for biofuel production (Sanderson et al., 1996). Other research concluded that a single spring harvest for forage followed by a final fall harvest for biomass would be the best approach for a dual-purpose system (Sanderson et al., 1999). In a dual-purpose system, inputs such as fertilization and management will increase in order to produce high-quality forage and biomass in the same system (Brejda et al., 2000). Soil nutrient uptake and cycling for a mixed species of NWSGs has not been studied, but a study from Oklahoma looking at switchgrass response to harvest frequency and time with different rates of N reported that concentrations of P and K increased with yields (Thomason et al., 2005). Previous research focused on forage and biomass systems reported that higher yielding perennial grasses removed more nutrients (Guretzky et al., 2011; Propheter and Staggenborg, 2010).

Although most of the research in the forage and biomass dual-use concept has been conducted using SG, several other NWSGs have potential in this model. The species BB and IG are considered to be high-quality leafy forage that matures later in the summer; and, is widely used for livestock forage in the mid-West (Ball et al., 2007; Mitchel et al., 2001; Mulkey et al., 2008). Compared to the SG, BB and IG are generally the more palatable and nutritious species due to the leafiness of the forage during the early summer and providing more consistent quality throughout the growing season (Ball et al., 2007; Mitchell et al., 2001; Redfearn and Nelson, 2003; Stubbendieck et al., 2002). Other research concluded that SG, BB, and IG could be ideal for sustainable forage production with proper management (Mulkey et al., 2008). Species selection for biomass crops have been based on previous research demonstrating dual-purpose systems in which the species maintained yields if the early harvest was taken early; however, quality of the forage harvest has not been a primary focus (McLaughlin et al., 1999; Sanderson et al., 1999). Including other species of NWSGs with SG may provide higher quality forage options or increased yields (Fike et al., 2006a; Posler et al., 1993; Sanderson et al., 2006). These three NWSGs, when combined in a mixture, have not been studied to determine characteristics that each species could bring to a mixture harvested for forage followed by a biomass harvest in the fall.

The opportunity for producers to harvest both forage and biomass from a field of NWSGs offers flexibility with potential to increase profits. The impact of specific species and harvest timings for both forage and biomass needs to be considered. This can contribute to current sustainable practices where producers want more flexibility in harvest management while satisfying multiple crop goals providing quality forage for livestock and a biomass crop (Mulkey et al., 2008). The objectives of this study were to determine the effect (i) early-season harvest timing, and (ii) species mixtures on forage yield and quality in a NWSGs biomass system.

MATERIALS AND METHODS

A 3 x 3 factorial experiment was conducted using NWSGs in monoculture and mixtures to evaluate the yield and quality of two different early-season forage harvest timings, and the effect of these early harvests on biomass. Harvest treatments were FD only, EB, and ESH. Species of NWSGs were: (1.) 100% SG, (2.) 65% BB and 35% IG (standard forage ratio), and (3.) 50% SG, 35% BB, and 15% IG (50:50 ratio of treatments 1 and 2). The seed were blended to the percentage specifications and seeding rate based on pure live seed (PLS). Seeding rate for SG was 7.85 kg ha^{-1} PLS; the three-way mixture SGBBIG was 7.85 kg SG ha^{-1} PLS; and, 8.97 kg BBIG ha^{-1} PLS, and the BBIG mixture was 8.97 kg BBIG ha^{-1} PLS. The varieties of NWSGs selected for this study were 'Alamo' SG, 'OZ-70' BB, and 'Rumsey' IG.

The experiment was conducted from 2010-2012 at three locations within the Appalachian and Interior Low Plateau regions of Tennessee. The first location was at the East Tennessee Research and Education Center (ETREC) in Knoxville, Tennessee (35° 54' 2", -83° 57' 36"; on an Etowah Silt Loam (fine-loamy, siliceous, semiactive, thermic Typic Paleudults) (NRCS, 2003). The second location was the Plateau Research and Education Center (PREC) in Crossville, Tennessee (36° 2' 38", -85° 9' 48"; on a Lily Loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludults) (NRCS, 2003). The third location was at the Highland Rim Research and Education Center (HHREC) near Springfield, Tennessee (36° 28' 22", 86° 49' 7"; on a Mountview Silt Loam (fine-silty, siliceous, semiactive, thermic Oxyaquic Paleudults) (NRCS, 2003).

Plots were established in 2008 at HRREC, and 2009 at ETREC and PREC. All sites were planted in early May. In the fall prior to establishment, an application of 2.24 kg ai/ha^{-1} glyphosate [*N*-(phosphonomethyl) glycine] was applied to the study area to eradicate existing vegetation. A second application was made two weeks prior to planting dates. Plots were established on a conventionally prepared seedbed using a no-till drill. Experimental plot size at ETREC was 1.83 x 7.62 m; for PREC and HRREC plots were 1.52 x 7.62 m. At establishment, BBIG plots were treated with an application of glyphosate (2.24 kg ai/ha^{-1}) and imazapic (0.11 kg ai/ha^{-1}) [2-[[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]]-5-methylnicotinic acid] to provide pre-emergence weed control. During the establishment year plots containing SG were mowed twice to reduce weed competition. During year two metsulfuron (14.0 g ai/ha-^{1}) [2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]-oxomethyl]sulfamoyl]benzoic acid methyl ester] was applied to all plots in late April for broadleaf weed control. In the third year, weed control was not necessary due to the stand density of the NWSGs.

Plots were fertilized with 100.88 kg N ha^{-1} annually. The FD harvest received the N at green-up in mid-April, while the dual-purpose treatments received half of the N at green-up and half after the early forage harvest. Lime, P, and K applications were made according to soil test recommendations.

Harvest and Data Collection

Harvest timings were based on the plant phenology of the SG monoculture for all treatments. Timing for the EB harvest was determined as prior to seedhead emerging from sheath. At the ESH harvest, a seadhead was present in the top portion of the elongated stem. The biomass only FD harvest was made after killing frost, usually early November.

Plots were harvested at a 15.24 cm residual height using a flail small-plot harvester with a 91.44 cm swath (Carter Mfg. Co., Inc. Brookston, IN; Swift Machine and Welding Ltd., Swift Current, SK). Harvested forage was weighed and a subsample was dried at 60°C in a forced air oven for 72 hours to determine moisture content and ultimately yield (Murray and Cowe, 2004). The dried subsamples were ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ) using a 2-mm screen, then re-ground with a UDY cyclone mill (UDY Corporation, Fort Collins, CO) through a 1-mm screen (Murray and Cowe, 2004). Samples were analyzed using a FOSS 6500 near-infrared spectrometer (NIRS) for CP, ADF, NDF, estimated TDN, relative forage quality (RFQ), N, P, and K (Foss NIRSystems, Inc., Laurel, MD). Equations for the forage nutritive analysis were standardized and checked for accuracy using equations developed by the NIRS Forage and Feed Consortium and are reported on a dry matter (DM) basis (Hillsboro, WI). Software used for NIRS analysis was WINSI II supplied by Infrasoft International LLC (State College, PA). Using these equations allowed the samples to be run against the Global H statistical test in the WINSI II program for accuracy (Murray and Cowe, 2004). All forage samples fit the equation with the ($H<3.0$) and were used to report results and identify outliers.

Data Analyses

Experimental design used in this study was a randomized complete block design (RCBD) with repeated measures. Independent variables were three species mixtures, three harvest treatments, with four replications at three locations. Dependent variables were treated as repeated measures. Models were analyzed with SAS V.9.3 software (SAS Institute, 2012). Random effects were included for year with replication nested in the location random effect. The null hypothesis was that yield and quality were not different across the NWSGs for each harvest treatment. Location and year differences were not reported as they were combined across all subsequent analyses to report main effects and interactions of harvests x species.

Normality of residuals and homogeneity of variances were assessed by the Shapiro-Wilk test ($W \geq 0.90$) and Levene's test ($P \leq 0.05$) using the PROC MIXED procedure producing ANOVA (SAS Institute, 2012). Data are shown by least significant difference (LSD) values at or below the five percent level ($P \leq 0.05$). Results from any treatment means being compared to differ by at least this amount were considered different and is reported accordingly.

RESULTS AND DISCUSSION

Forage Yield

Forage yield was affected by both harvest timing and forage species included in the mixture. As expected, the greatest forage yield came from the ESH harvest across all species and mixtures compared to EB ($P<0.0001$). Delaying forage harvest from EB to ESH resulted in 25-50% higher yields ($P<0.0001$). Averaged across species, EB yield averaged 6,406 kg DM ha^{-1} while ESH produced 10,078 kg DM ha^{-1} ($P<0.0001$). Within each forage harvest treatment, including SG in a mixture with BBIG resulted in increased yield (Figure 2.1; all tables and figures located in Appendix). When harvested at EB, SG yielded significantly more than SGBBIG, while the lowest yield was produced by BBIG (7,904, 6,660, and 4,655 kg DM ha^{-1}; respectively). The ESH harvest yields for both the SG and SGBBIG mixtures were higher than BBIG (12,285, 11,625, and 6,327 kg DM ha^{-1}; respectively), most likely due to earlier SG green-up and a longer vegetative growth stage. For both harvests, SG produced more forage than BBIG and the addition of SG to the BBIG mixture resulted in increased yields relative to BBIG ($P<0.0001$). The highest yields were produced by the SG monoculture, or by including SG in the species mixtures, causing SG and the SGBBIG mixtures to yield the most for both early forage harvests (Figure 2.1).

Forage Quality

Forage quality was affected by both harvest timing and forage species. Delaying harvest from EB to ESH reduced forage quality (Table 2.1). Levels of CP for all species were above 100 g kg^{-1} at EB, while ESH levels ranged from 87 to 93 g kg^{-1}. However, basic nutrient requirements for maintenance of a mature cow can be met by all NWSGs harvested at the EB and ESH stage since all levels of CP were above 70 g kg^{-1} (Hersom, 2010). These forages provided sufficient CP levels for maintenance of beef cattle with <1.5 kg average daily gain reported by the National Research Council (2000). Although there were no species x harvest interactions for CP levels, there were significant differences among main effects, with EB levels of CP 20 g kg^{-1} higher than ESH ($P<0.0001$). Delaying harvest from EB to ESH resulted in higher ADF levels (390 vs. 420 g kg^{-1}) averaged across forage species ($P<0.0001$). There was a trend for lower amounts of NDF at EB (640-690 g kg^{-1}) then increasing by the ESH stage to (670-730 g kg^{-1}) (Table 2.1). Estimated TDN levels of the two forage harvests were higher at EB compared to ESH (576 vs. 552 g kg^{-1}) ($P<0.0001$).

There were relatively consistent differences in forage quality constituents among species mixtures. Generally, treatments including SG had the lowest CP and estimated TDN, and higher ADF and NDF levels (Table 2.1). Within harvest treatments, species differed in CP levels, with BBIG having the highest level (Table 2.1). The monoculture and mixtures with SG decreased CP by an average of 6.2 g kg^{-1} ($P=0.0272$). At EB, SG had lower estimated TDN and higher NDF levels compared to mixtures containing BBIG (Table 2.1). For ESH, mixtures containing BBIG were consistently higher in CP and estimated TDN, and lower in ADF and NDF than the SG monoculture (Table 2.1).

Additionally, forage quality was compared using RFQ, which combines measures into a point system. This utilizes more quality constituents compared to the relative feed value (RFV).

The RFQ includes digestible fiber and should be more useful in predicting how an animal should perform on the NWSGs in mixtures with different harvest timings (Ball et al., 2001; Coleman and Moore, 2003). Species included in mixtures is an important factor that could increase the forage quality shown by RFQ. The NWSGs in this study met the “fair” rating at EB with a range between 95 and 100 total points; and, decreased significantly at the ESH harvest with the range in the “poor” level (85 to 90 total points) ($P<0.0001$). The forage harvested later at the ESH stage had lower RFQ for all mixtures with a decrease of almost 10 points compared to the EB harvest ($P=0.0115$). Using BBIG in the mixture resulted in a higher RFQ at EB (100) and (90) at ESH (Figure 2.2). As expected RFQ and yield showed an inverse relationship. With this analysis and previous research by Coleman and Moore (2003) animals fed EB harvested forage would be expected to be perform significantly better than those fed ESH forage.

Forage Nutrient Removal

Although the treatment effects on nutrient removal was not one of the original primary objectives of the study, the data presented an opportunity to generally compare N, P, and K removal differences. The greatest removal depended on the growth stage in all three NWSGs in mixtures (Table 2.2). The total N, P, and K concentration was highest at EB, but total removal was highest at ESH due to increased yield (Table 2.2). With BBIG, the nutrient removal was less than the SG monoculture and SGBBIG mixture (Table 2.2). Total nutrients removed by SG and the SGBBIG mixture was significantly above the applied levels for this study. The experimental plots in this study were not soil tested and fertilized with P and K separately; however, data indicate that increased N and K fertilization may be necessary to maintain yields. This work reports levels of K being used by the NWSGs to an extent not found in other studies where previous research reported levels for biomass production only (Brejda et al., 2000; Vogel, 2004). Removal of K, especially if SG was included in the mixture, was great enough that K deficiency could cause nutrient deficiency and reduced yield (Vicente-Chandler et al., 1962). In biomass production a single fall harvest is an option that would remove less nutrients compared to a dual-purpose system where Guertzky et al. (2011) reported that SG increased in maintenance and fertility requirements when an early harvest was removed. Current fertilizer recommendations are generally reported as necessary only when soil test results show low levels, but this can be a problem if the NWSGs are under a dual-purpose system for forage and biomass production (Fike et al., 2006b). As the NWSGs are combined into mixtures more nutrients may be necessary depending on the needs for quality forage production.

CONCLUSIONS

This study indicates that NWSGs in a field intended for biomass can produce acceptable forage yield and quality if a single harvest is made relatively early in the season. The addition of SG increased yield, while forage quality was highly dependent on the phenology at harvest. In this study, plots including SG produced the greatest yields, while BBIG plots produced the highest quality forage. The addition of the three-way mixture of SGBBIG provided higher yield, but similar forage quality to the BBIG mixture. Regardless of species chosen to include in a mixture, highest forage quality was produced when forage was harvested at EB, while greatest yield came at ESH. As yields increased at the ESH stage the quality of the forage declined. The difference in quality was improved when grasses like BB and IG were part of the mixtures. Additionally, macro-nutrient removal was significantly higher than expected and could not be compared to other studies that reported species individually or at later harvest stages. Further considerations for use of macro-nutrients in NWSGs should be considered carefully in high yielding production systems.

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IMPACTS OF AN EARLY SEASON FORAGE HARVEST ON NATIVE WARM-SEASON GRASSES IN A BIOMASS SYSTEM

Chapter 3

ABSTRACT

There has been increasing interest in utilizing native warm-season grasses (NWSGs), especially switchgrass, as a biomass feedstock for cellulosic ethanol production. Millions of hectares of crop and pasture in the mid-South are forecast to potentially be planted with switchgrass for biomass feedstock production. This could have a substantial impact on the region's cattle industry, reducing forage production hectares. Current recommendations for biomass production in our region include only a single fall harvest. A study was conducted to determine the effect of early season harvest timing on biomass yield and quality of NWSGs designed for use in cellulosic ethanol production. The NWSGs used in this study were switchgrass (*Panicum virgatum* L.) monoculture (SG), SG/big bluestem (*Sorghastrum nutans* L.) /indiangrass (*Andropogon gerardii* V.) mixture (SGBBIG), and big bluestem/indiangrass mixture (BBIG). These NWSGs were harvested at fall dormancy for biomass only (FD), early boot plus FD (EBFD), and early seedhead plus FD (ESHFD). Harvesting forage at early boot (EB) and early seedhead (ESH) decreased fall biomass yield compared to the single FD harvest. Results indicated the EB forage harvest caused less impact on fall biomass yield than did the ESH

harvest. Switchgrass and the mixture including switchgrass provided greatest fall biomass yield across all harvest treatments. The macro-nutrient removal of nitrogen (N), phosphorus (P), and potassium (K) was considerably higher when a forage harvest was made prior to a biomass harvest. According to this study, NWSGs in biomass fields can be harvested early for forage, but biomass yield will be reduced. A single biomass harvest is an option when a producer does not desire forage production. Based on N, P, and K removal estimates determined in this study, costs for inputs such as fertilization will increase in a dual-purpose system. These grasses can provide a viable option to produce hay from the same NWSGs in fields originally targeted for ethanol production. This research suggests that NWSGs, in a forage-biomass dual-purpose system, can be used to increase harvest options.

Abbreviations: ADF, acid detergent fiber; BB, big bluestem; DM, dry-matter basis; EB, early boot harvest; EBFD, early boot plus fall dormancy harvest; ESH, early seedhead harvest; ESHFD, early seedhead plus fall dormancy harvest; FD, fall dormancy harvest; IG, indiangrass; IVTDMD48, *in-vitro* true dry matter digestibility 48 hrs; NWSGs, native warm-season grasses; NIRS, near-infrared spectroscopy; NDF, neutral detergent fiber; N, nitrogen; P, phosphorus; K, potassium; PLS, pure live seed; SG, switchgrass

LITERATURE REVIEW

The development of renewable energy sources has become an issue of increasing importance as it was recognized 25 years ago that bio-energy feedstocks would ultimately be important contributors to a national renewable energy supply (McLaughlin and Kszos, 2005). The potential of cellulosic ethanol production using NWSGs for biomass feedstock, especially SG, has grown since then (Schmer et al., 2010). If bio-refineries become more common-place across the country, they must have reliable biomass supplies, resulting in many hectares potentially being planted to biomass crops (Landis et al., 2008; Schmer et al., 2010). With estimates predicting that over 21 million hectares of SG will need to be produced annually, the importance of exploring bioenergy sources is becoming more evident (English et al., 2004). In the United States, bioenergy crops are estimated to exceed 22 million hectares by 2030, producing 60 billion gallons of ethanol and biodiesel (Sanderson and Adler, 2008; USDA Statistics Service, 2013). There are currently 412 million hectares of crop land, of which over 22 million hectares are forage crops that have potential to be planted to bioenergy crops (USDA Statistics Service, 2013). The demand for bioenergy crops could have a substantial impact on the livestock industry since the predictions for bioenergy crops is the same as the forage crop hectares already in production; therefore, alternative uses for NWSGs in biomass systems are being explored (Sanderson and Adler, 2008). Forage production hectares, already for grazing or hay, would be replaced with biomass fields with such a demand for biomass. This situation has been a concern in the Southern U.S. with economic estimations by English et al. (2006) that hay production hectares would increase. However, this model predicted that current pastureland would expand in hectares, increase farm incomes, create job opportunities, and provide other economic benefits (English et al., 2006). Although the demand for biomass can almost be unpredictable, there will be a drastic change in current production systems.

Already, SG has shown it is suitable for use as an energy feedstock for ethanol production, other NWSGs have not been researched to the same extent (Thomason et al., 2005; Tracy et al., 2010). Assessing the performance of a SG monoculture compared to NWSGs in mixture is important when evaluating the production systems. Previous studies for biomass production have focused on SG due to high yields, cost effectiveness, minimal environmental impacts, and cellulosic ethanol potential (Lynd et al., 1991; Sanderson et. al., 1996). Parrish and Fike (2005) reported that a single harvest of switchgrass from late fall or early winter resulted in the highest sustainable biomass. Switchgrass is known for its regional and ecotypical differences and constituents used in estimating ethanol yields (Bhandari et al., 2013). Switchgrass, and other NWSGs, are typically converted into ethanol using the lignocellulosic process that involves conversion of sugars (Sanderson et al., 2006). Biorefineries will require certain levels of each constituent and the prediction of ethanol yield changes with each process update and innovation. Most ethanol yield predictions have been based on cellulosic materials where fibers, lignin, and digestibility make up 95% of the information needed (Lorenz et al., 2009). Higher levels of lignin limit the conversion process by inhibiting sugar and fermentation recovery from biomass (Sanderson et al., 2006; Dien et al., 2006; Vogel and Jung, 2001). Current research into IVTDMD48 (*in-vitro* true dry matter digestibility 48 hrs) estimates have shown them to be a leading constituent for estimating ethanol yield from switchgrass (Vogel et al., 2011). This indicates that digestibility is important in the sugar extraction process, where lower lignin levels can indicate higher cellulose availability needed to ferment into ethanol (Chang and Holtzapple, 2000; Lee, 2006). Also, mineral content can produce excess waste materials that can make the conversion of biomass into ethanol processes less efficient (Monti et al., 2008).

Other NWSGs have not been researched to the same extent as SG, however, these species been discussed as a way to increase yield and quality attributes in both forage and biomass systems (Thomason et al., 2005; Tracy et al., 2010). Recent work on NWSGs in monoculture and mixtures reported that SG should be included in mixtures, as it resulted in higher biomass yield and lower cellulose levels compared to mixtures with BB and IG (Hong et al., 2013). In that same study, the addition of BB and IG resulted in more desirable biomass for ethanol production resulting in lower lignin and fiber levels than any monoculture or mixture, SG (Hong et al., 2013). Hong et al. (2013) also reported that in mixtures that SG tended to decline over time when included in a mixture. However, NWSGs are known to produce biomass, under a single fall harvest, with high levels of neutral detergent fiber (NDF) and acid detergent fiber (ADF) and low levels of crude protein and ash (Mulkey et al., 2008). Levels of each biomass quality constituent will vary depending on the species and harvest system, whether a single fall harvest or a dual-purpose system. This is an important factor to consider when selecting NWSGs for production systems.

The consideration to utilize NWSGs in a dual-purpose system is to allow a portion of the yield to be diverted as an early forage harvest and then utilize the remaining growth as a biomass harvest for ethanol production. Previous research conducted on dual-purpose systems is leading the way to include mixtures over monocultures that have been the focus for several years. Recently, Mosali et al. (2013) published results indicating that SG can provide forage for stocker cattle into early spring and throughout the growing season while still removing a biomass harvest in the late fall. To remove two harvests each season has produced optimum yields in some systems, with the earlier harvest being high-quality forage and the later harvest for biofuel production (Sanderson et al., 1996). Other research concluded that a single spring harvest for forage followed by a final fall harvest for biomass would be the best approach for a dual-purpose system (Sanderson et al., 1999).

Parish and Fike (2005) found that a single harvest of switchgrass from late fall or early winter resulted in the highest sustainable biomass yields and good stand persistence from year to year compared to a dual-purpose system approach (Parrish and Fike, 2005). In a two-harvest system in Mississippi biomass yields for the one-harvest system were significantly higher than the two harvest system, however, in this study biomass harvest occurred prior to plant dormancy (Grabowski et al., 2004). Guretzky et al. (2011) evaluated SG for dual-purpose use at two locations in Oklahoma from 2008 to 2009 with the forage harvest occurred after boot stage and the biomass harvest occurred after frost. This study found forage quality to be poor when harvested any time after the reproduction stage had begun, and suggested that SG would need to be harvested in the early boot stage to have quality attributes that would make it an attractive forage source for livestock producers (Guretzky et al., 2011). This dual-purpose system evaluated by Guretzky et al. (2011) to determine if harvesting twice a year is possible if the first harvest is very early in the growing season and the biomass harvest is after the first killing frost; they concluded it was possible with proper management and increased inputs. Inputs such as fertilization and management will increase in order to produce high-quality forage and biomass in the same system (Brejda et al., 2000).

In these dual-purpose systems, soil nutrient uptake and cycling for a mixed species of NWSGs has not been studied, but a study from Oklahoma looking at switchgrass response to harvest frequency and time with different rates of N reported that concentrations of P and K increased with yields (Thomason et al., 2005). Previous research focused on biomass systems reported that higher yielding perennial grasses removed more nutrients (Propheter and Staggenborg, 2010). In typical biomass one-harvest systems, the harvest takes place after the first killing frost when macro-nutrient content declines, plants have lower levels of lignin and fiber, and cellulose content decreases (Sanderson and Wolf, 1995). This management practice is widely used in single harvest biomass systems and can be adapted to species mixtures in dual-purpose system for forage and biomass.

Although most of the research in the forage and biomass dual-use concept has been conducted using SG, several other NWSGs have potential in this model. The species BB and IG are considered to be high-quality forage species that mature later in the summer and are widely used for livestock forage in the mid-West (Ball et al., 2007; Mitchel et al., 2001; Mulkey et al., 2008). Compared to SG, BB and IG are generally the more palatable and nutritious species due to the leafiness of the forage during the early summer, and have the potential for ethanol production in the future (Ball et al., 2007; Mitchell et al., 2001; Redfearn and Nelson, 2003;

Stubbendieck et al., 2002). Other research concluded that SG, BB, and IG could be ideal for sustainable forage production with proper management (Mulkey et al., 2008). Studies have demonstrated dual-purpose systems in which the species maintained yields even if the early harvest was taken early; however, quality of the forage harvest has not been a primary focus (McLaughlin et al., 1999; Sanderson et al., 1999). Including other species of NWSGs with SG may provide higher quality forage options or increased yields for both production systems in this model (Fike et al., 2006a; Posler et al., 1993; Sanderson et al., 2006). These same three NWSGs, when combined in a mixture, have not been studied to determine characteristics that each species could bring to a mixture harvested for forage followed by a biomass harvest in the fall.

The opportunity for producers to harvest both forage and biomass from NWSGs in the same field offers flexibility with potential to increase profits. The impact of harvest timings and specific NWSGs for both forage and biomass needs to be considered. This can contribute to current sustainable practices when producers want more flexibility in harvest management while satisfying multiple crop goals providing quality forage for livestock and a biomass crop (Mulkey et al., 2008). Therefore, the objectives of this study were to determine the effect (i) early-season harvest timing on fall biomass yield, and (ii) species mixtures on biomass quality in a dual-purpose system.

MATERIALS AND METHODS

A 3 x 3 factorial experiment was conducted using NWSGs to evaluate the yield and quality of two different early-season forage harvest timings, and the effect of these early harvests on biomass. Harvest treatments were FD only, EBFD, and ESHFD. Species of NWSGs were: (1.) 100% SG, (2.) 65% BB and 35% IG (standard forage ratio), and (3.) 50% SG, 35% BB, and 15% IG (50:50 ratio of treatments 1 and 2). The seed were blended to the percentage specifications and seeding rate based on pure live seed (PLS). Seeding rate for SG was 7.85 kg ha^{-1} PLS; the three-way mixture SGBBIG was 7.85 kg SG ha^{-1} PLS; and, 8.97 kg BBIG ha^{-1} PLS, and the BBIG mixture was 8.97 kg BBIG ha^{-1} PLS. The NWSGs selected for this study were 'Alamo' SG, 'OZ-70' BB, and 'Rumsey' IG.

The experiment was conducted from 2010-2012 at three locations within the Appalachian and Interior Low Plateau regions of Tennessee. The first location was at the East Tennessee Research and Education Center (ETREC) in Knoxville, Tennessee (35° 54' 2", -83° 57' 36"; on an Etowah Silt Loam (fine-loamy, siliceous, semiactive, thermic Typic Paleudults) (NRCS, 2003). The second location was the Plateau Research and Education Center (PREC) in Crossville, Tennessee (36° 2' 38", -85° 9' 48"; on a Lily Loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludults) (NRCS, 2003). The third location was at the Highland Rim Research and Education Center (HHREC) near Springfield, Tennessee (36° 28' 22", 86° 49' 7"; on a Mountview Silt Loam (fine-silty, siliceous, semiactive, thermic Oxyaquic Paleudults) (NRCS, 2003).

Plots were established in 2008 at HRREC, and 2009 at ETREC and PREC. All sites were planted in early May. In the fall prior to establishment, an application of 2.24 kg ai/ha^{-1} glyphosate [*N*-(phosphonomethyl) glycine] was applied to the study area to eradicate existing vegetation. A second application was made two weeks prior to planting dates. Plots were established on a conventionally prepared seedbed using a no-till drill. Experimental plot size at ETREC was 1.83 x 7.62 m; for PREC and HRREC plots were 1.52 x 7.62 m. At establishment, BBIG plots were treated with an application of glyphosate (2.24 kg ai/ha^{-1}) and imazapic (0.11 kg ai/ha^{-1}) [2-[[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]]-5-methylnicotinic acid] to provide pre-emergence weed control. During the establishment year plots containing SG were mowed twice to reduce weed competition. During year two metsulfuron (14.0 g ai/ha-^{1}) [2-[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]-oxomethyl]sulfamoyl]benzoic acid methyl ester] was applied to all plots in late April for broadleaf weed control. In the third year, weed control was not necessary due to the stand density of the NWSGs.

Plots were fertilized with 100.88 kg N ha^{-1} annually. The FD harvest received the N at green-up in mid-April, while the dual-purpose treatments received half of the N at green-up and half after the early forage harvest. Lime, P, and K applications were made according to soil test recommendations.

Harvest and Data Collection

Early forage harvest timings were based on the plant phenology of the SG monoculture. The biomass only FD harvest was made after killing frost, usually early November. Plots were harvested at a 15.24 cm residual height using a flail small-plot harvester with a 91.44 cm swath

(Carter Mfg. Co., Inc. Brookston, IN; Swift Machine and Welding Ltd., Swift Current, SK). Harvested biomass was weighed and a subsample was dried at 60°C in a forced air oven for 72 hours to determine moisture content and ultimately yield (Murray and Cowe, 2004). The dried subsamples were ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ) using a 2-mm screen, then re-ground with a UDY cyclone mill (UDY Corporation, Fort Collins, CO) through a 1-mm screen (Murray and Cowe, 2004). Samples were analyzed using a FOSS 6500 near-infrared spectrometer (NIRS) for ADF, NDF, Lignin, ash, IVTDMD48, N, P, and K (Foss NIRSystems, Inc., Laurel, MD). Equations for the biomass nutritive analysis were standardized and checked for accuracy using equations developed by the NIRS Forage and Feed Consortium (Hillsboro, WI). Software used for NIRS analysis was WINSI II supplied by Infrasoft International LLC (State College, PA). Using these equations allowed the samples to be run against the Global H statistical test in the WINSI II program for accuracy (Murray and Cowe, 2004). All biomass samples fit the equation with the ($H<3.0$) and were used to report results and identify outliers.

Data Analyses

Experimental design used in this study was a randomized complete block design (RCBD) with repeated measures. Independent variables were three species mixtures, three harvest treatments, with four replications at three locations. Dependent variables were treated as repeated measures. Models were analyzed with SAS V.9.3 software (SAS Institute, 2012). Random effects were included for year with replication nested in the location random effect. The null hypothesis was that yield and quality were not different across the NWSGs for each harvest treatment. Location and year differences were not reported as they were combined across all subsequent analyses to report main effects and interactions of harvests x species.

Normality of residuals and homogeneity of variances were assessed by the Shapiro-Wilk test ($W \geq 0.90$) and Levene's test ($P \leq 0.05$) using the PROC MIXED procedure producing ANOVA (SAS Institute, 2012). Data are shown by least significant difference (LSD) values at or below the five percent level ($P \leq 0.05$). Results from any treatment means being compared to differ by at least this amount were considered different and is reported accordingly.

RESULTS AND DISCUSSION

Biomass Yield

In this study, the FD harvested SG monoculture provided control data to compare the effects of mixing species and taking an early forage harvest. At FD, the SG yielded the most compared to the mixed species treatments of SGBBIG and BBIG (Figure 3.1). Biomass yield for SG harvested at FD only were greater compared to SG harvested at EBFD or ESHFD (Figure 3.1). Taking an early season forage harvest decreased the biomass yield by 30-50% with significant differences found in the main effect treatments of both species and harvest ($P<0.0001$).

The three-way mixture of SGBBIG produced lower yields compared to the SG monoculture (Figure 3.1). The addition of BBIG in the mixture reduced biomass yields (16,572 vs 11,662 kg DM ha^{-1}; respectively). Taking an early season forage harvest resulted in a less dramatic reduction in yield in the three-way mixture with no differences for the EBFD and ESHFD treatments (Figure 3.1). However, for all harvest treatments the BBIG produced the lowest biomass yield of all the species treatments ($P<0.0001$). The BBIG mixture produced the lowest yields at FD, EBFD, and ESHFD in comparison to the other two species treatments. These differences indicated that BBIG harvested at FD and EBFD performed the same, only dropping in yield with the later forage harvest ESHFD (Figure 3.1). This was in agreement with other studies that have shown BBIG to produce lower yields compared to SG monocultures or SG in mixtures (Brejda et al., 2000; Vogel, 2004).

The greatest reduction in biomass yield, across all species and harvest treatments, resulted from taking the forage harvest at ESH ($P<0.0001$). However, the biomass yield from the SG monoculture and the SGBBIG mixture was statistically the same in the ESHFD treatment, but not with the FD harvest (Figure 3.1). Across all treatments there was a trend for reduced yields if early harvests for forage were taken, but the SG monoculture was affected the most (Figure 3.1).

Biomass Quality

The biomass material was at post-senescence when harvested, and levels of each constituent discussed will only be for that phonological stage of growth. Previous research concluded that biomass for ethanol production should have adequate levels of fiber, low mineral content, and higher digestibility levels (Vogel et al. 2013). Quality analysis of the FD harvested biomass evaluate constituents that are considered necessary for current ethanol production methods including ADF, NDF, ASH, Lignin, and IVTDMD48.

There were significant differences found between the species x harvest interaction for both ADF and NDF (Table 3.1). Taking an early harvest for hay production decreased the fiber content in the biomass harvest. Fall dormancy harvested biomass had the highest levels of ADF and NDF. The SG ADF level was highest at 530 g kg-1 harvested at FD. Mixtures containing BBIG had lower ADF and NDF levels when early harvests were taken for forage (Table 3.1). The ADF content of biomass for the FD, EBFD, and ESHFD harvest treatments on SG monoculture were 530.0, 503.1, and 481.6 g kg^{-1}; respectively. The mixture of SGBBIG had the lowest levels of ADF at the FD, EBFD, and ESHFD harvests (495.9, 490.1, and 477.5 g kg^{-1};

respectively). However, the BBIG mixture provided the most stable levels of ADF for all harvest treatments of FD, EBFD, and ESHFD (505.0, 507.1, and 491.4 g kg^{-1}; respectively). For NDF, the SG was affected the most by taking early forage harvests (Table 3.1). Across all harvest treatments, BBIG had the lowest NDF levels if included in mixtures compared to the SG monoculture (Table 3.1).

Although there were no species x harvest interactions for ash and lignin, there is potential to increase these levels by taking an early harvest (Table 3.1). Since ash represents the over-all mineral content in the biomass, possible differences could be due to the stage of the regrowth at the FD harvests across the three species treatments in this study. Increased in ash levels in the two species mixtures of SGBBIG and BBIG were found in the harvest treatments of EBFD and ESHFD (Table 3.1). For the FD harvest, all species treatments of SG, SGBBIG, and BBIG had ash content between 48.1 to 42 g kg^{-1} (Table 3.1).

Slight differences were found in the digestibility of the NWSGs across harvest treatments (Table 3.1). This made an interesting comparison of the SG monoculture to the mixtures with BBIG included where digestibility increased (Table 3.1). Only main effect significant differences for IVTDMD48 were detected among species treatments, however, no species x harvest interaction was found for the FD harvest alone ($P<0.0004$). The SG had lower digestibility than the mixtures. There was no difference in digestibility between SGBBIG and BBIG. Removing forage at EB or ESH did not affect digestibility of the fall biomass harvest. Species mixtures might be another way to alter biomass fiber digestibility without expensive and excessive time needed to breed better feedstock as recently reported by Vogel et al. (2013) using divergent breeding techniques. However, this statement would only be possible if mixtures of NWSGs can be applied to produce ethanol in the future. Research conducted by Hong et al. (2013) determined when NWSGs are in mixture that each bring certain constituent traits that can either damage or improve ethanol production (Sanderson and Adler, 2008). There could be potential to mix NWSGs to achieve these same goals with a more digestible biomass with consistent fiber, lignin, and ash levels if an early harvest is removed with a dual-purpose system.

Biomass Nutrient Removal

Although the treatment effects on nutrient removal was not one of the original primary objectives of the study, the data presented an opportunity to generally compare N, P, and K removal differences. The experimental plots in this study were not soil tested and fertilized with P and K separately; however, nutrient content of harvested material showed significant differences in removal rates ($P<0.0001$). The macro-nutrients removed for all species mixtures and harvest treatments reported only slight differences in biomass nutrient content, however, removal was affected by yield differences (Table 3.2). Treatments that contained BBIG produced lower yields and less total macro-nutrient removal (Table 3.2). Less N was removed in the biomass from the mixture treatments, primarily due to lower yields. For all harvest treatments of FD, EBFD, and ESHFD, SG removed similar amounts of N (74.6 to 73.1 kg ha^{-1}) (Table 2.2).

The removal of P and K levels were also lower with the mixtures (Table 3.2). If an early forage harvest was removed followed by a biomass harvest the addition of BBIG to a mixture provided lower K levels compared to the SG monoculture (Table 3.2). The three-way species mixture had higher K levels for the EBFD and ESHFD treatments, however, this increase was caused by the amount of biomass removed with the addition of SG to the BBIG mixture (Table 3.2). Although data presented consistent biomass macro-nutrient removal across species x harvest interactions, biomass yield should be considered the greatest factor in the differences reported by this study. Overall, the SG monoculture removed more nutrients by the harvest main effect and the BBIG mixture removed significantly less ($P<0.0001$).

Combined Nutrient Removal

In this dual-purpose system, early harvests of EB and ESH were followed by a FD harvest and the total of all macro-nutrients removed were totaled and compared (Table 3.3). The macro-nutrient removal of N, P, and K in the FD biomass showed lowest levels of removal for all the macro-nutrients ($P<0.0001$). As stated earlier, removal levels were primarily affected by yield, with BBIG having lowest removal levels for N, P, and K ($P<0.0001$).

Species treatments did show main effect differences where the SG monoculture removed the most macro-nutrients, however, no species x harvest interactions were detected except for levels of P where only slight differences were reported (Table 3.3). This work reports higher levels of K removal than similar studies for biomass production (Brejda et al., 2000; Vogel, 2004). Removal of K, especially if SG was included in the mixture, was great enough to cause nutrient deficiency and reduced yield (Vicente-Chandler et al., 1962). The total removal of N, P, and K was significantly higher, with the main effects of harvest, when a forage harvest was removed and then combined with a biomass harvest in the fall ($P<0.0001$). The dual-harvests removed about the same amount of macro-nutrients as the single FD harvested material (Table 3.3). These findings agree with previous research that showed that switchgrass increased in maintenance and fertility requirements when an early harvest was removed and then a biomass harvested (Guertzky et al., 2011). Kering et al. (2011) demonstrated that where NWSGs can be grown in fields that are low in P, adequate fertilization is necessary and can potentially increase yields by up to 17% (Kering et al., 2011). The application of N can increase those yields up to over 45% in comparison to fields with no additional inputs (Kering et al., 2011). According to this study, increased yields are related to the available soil macro-nutrients and levels should be monitored to determine if there is any deficiency and K levels (Table 3.3).

In biomass production a single fall harvest is an option that would remove less nutrients compared to a dual-purpose system where Guertzky et al. (2011) reported that SG increased in maintenance and fertility requirements when an early harvest was removed. Current fertilizer recommendations are generally reported as necessary only when soil test results show low levels, but this can be a problem if the NWSGs are under a dual-purpose system for forage and biomass production (Fike et al., 2006b).

CONCLUSIONS

Harvesting NWSGs for forage at early boot or early seed head stage decreased fall biomass yield compared to the single fall biomass harvest; however, results indicated the earlier forage harvest at EB had less impact than the ESH forage harvests. Switchgrass and the mixtures including switchgrass provided greatest fall biomass yield across all harvest treatments. However, the SG monoculture yield was only reduced by only a third, when forage was removed at EB, providing more potential to increase biomass yield in a dual-purpose system. With the addition of BBIG to mixtures fiber levels decreased and digestibility increased. There is potential to mix NWSGs in order to achieve a more digestible biomass required by the ethanol production process used in most biorefineries.

This suggests mid-South forage programs can use NWSGs in a forage and biomass dual-purpose system to increase harvest options and profitability. In a dual-purpose system inputs such as fertilization and harvesting will increase requiring more management in order to produce high-quality hay and biomass feedstock in the same system. The macro-nutrient use was considerably higher when an early harvest was removed for hay production and then combined with a biomass harvest in the fall. A single harvest for fall biomass is an option when a producer does not need another cash-crop. In this study, investigation of these grasses to provide an option to produce hay from NWSGs in fields originally targeted for ethanol production. The inclusion of NWSGs into a forage program in the mid-South can be important for many other reasons where cattle and crops are the major sources of income. Using NWSGs will make growing biomass for ethanol production more appealing by allowing a portion of the forages currently being grown to remain similar to the regions current way of farming. Future research should be conducted to focus on increasing the proper fertilization requirements for optimal hay and biomass production using NWSGs in mixtures; and, possibly change the application timing in order to improve the biomass feedstock quality for ethanol production if a hay harvest was removed. As the NWSGs are combined into mixtures more nutrients may be necessary depending on the needs for quality forage and biomass production. The total combined macro-nutrients removed by SG and the SGBBIG mixture was significantly above the applied levels for this study; and, the mixture of BBIG removed far less ($P<0.0001$). Further research for fertilization recommendations for dual-purpose systems using NWSGs for forage and biomass may be necessary.

With the slow transition into producing ethanol efficiently there the potential for a producer to have a hay crop along with the biomass harvest until biomass demands increase in the region. Ethanol production requirements can possibly be met by dual-purpose systems providing quality forage and biomass. However, the focus for future projects should be with goals of harvesting biomass with less fiber, minerals, and higher digestibility. Analysis of mixed NWSGs has not been researched to determine if the composition of combined species would create a more desirable biomass feedstock composed of traits needed to be more efficient in ethanol extraction.

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APPENDIX

FIGURES

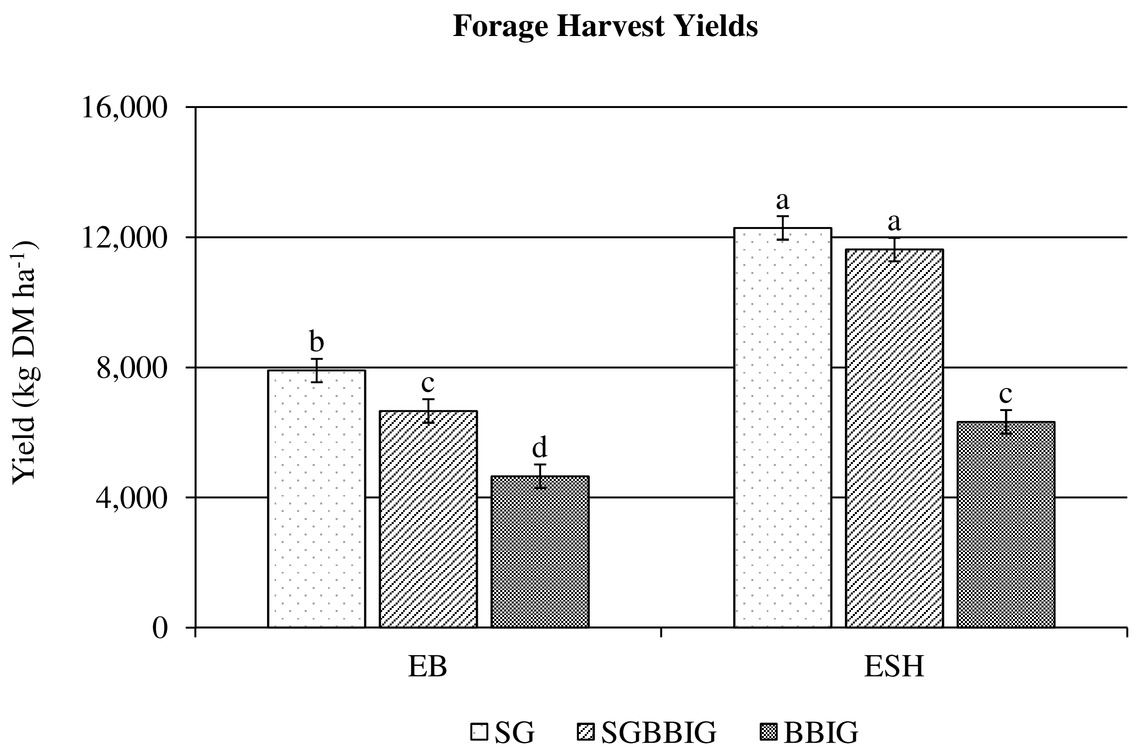

Figure 2.1. Forage harvest yield at two stages of maturity across all locations and years. Means not sharing a lowercase letter are significantly different (Fisher's Protected LSDα=0.05). Harvests (EB, Early Boot; ESH, Early Seedhead). Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass.

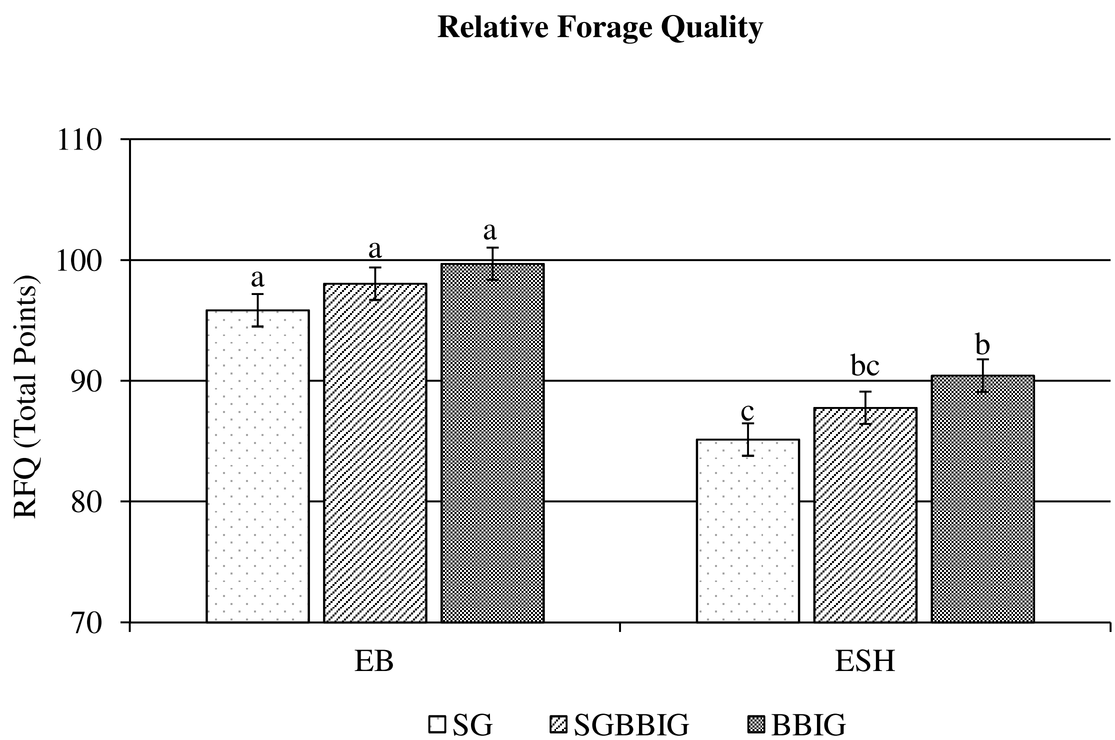

Figure 2.2. Forage harvest relative forage quality (RFQ) at two stages of maturity across all locations and years. Means not sharing a lowercase letter are significantly different (Fisher's Protected LSDα=0.05). Harvests (EB, Early Boot; ESH, Early Seedhead). Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).

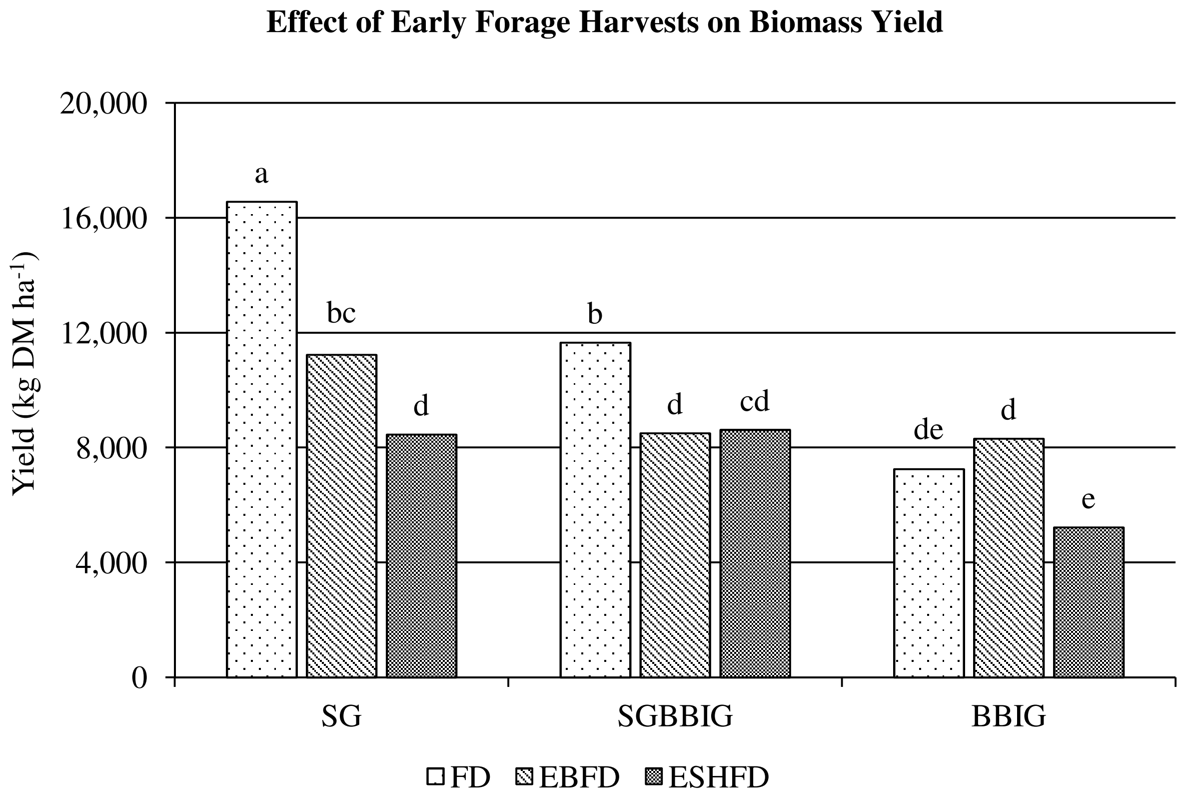

Figure 3.1. Effect of early forage harvests on biomass yield across all locations and years. Means not sharing a lowercase letter are significantly different (Fisher's Protected LSDα=0.05). Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass). Harvests (FD, Fall Dormancy; EBFD, Early Boot plus Fall Dormancy; ESHFD, Early Seedhead plus Fall Dormancy).

TABLES

Table 2.1. Forage quality at two stages of maturity across all locations and years on a DM basis.

Harvest[†]	Species[‡]	Forage Quality Constituents[¶] CP	ADF	NDF	TDN
		$g\ kg^{-1}$			
EB	SG	106.7b[§]	403.2b	685.0b	565.8c
	SGBBIG	106.8ab	387.2d	643.3d	584.0a
	BBIG	114.8a	391.5cd	648.1d	579.1ab
ESH	SG	86.8c	435.3a	729.6a	529.2d
	SGBBIG	88.4c	409.8b	684.8b	558.3c
	BBIG	93.0c	401.8bc	668.5c	567.3bc
	LSD	5.1	9.7	14.2	11.0

[†]Harvests (EB, Early Boot; ESH, Early Seed-Head).
[‡]Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).
[§]Means within a column not sharing a lowercase letter are significantly different (Fisher's Protected LSDα=0.05).
[¶]Forage Quality Constituents (CP, Crude Protein; ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; TDN, Total Digestible Nutrients).

Table 2.2. Macro-nutrients removed by forage harvests based on two stages of maturity across all locations and years.

Harvest[†]	Species[‡]	Content[¶] N	Content[¶] P	Content[¶] K	Removal N	Removal P	Removal K
		———g kg^{-1}———			———kg ha^{-1}———		
EB	SG	17.1b[§]	2.7a	19.1ab	131.5b	21.0b	152.9b
	SGBBIG	17.1b	2.6b	19.7a	111.4c	17.2c	133.2b
	BBIG	18.4a	2.5b	19.0b	84.8d	12.0d	87.5d
ESH	SG	13.9d	2.3c	16.2d	160.0a	28.0a	192.9a
	SGBBIG	14.1cd	2.2d	17.9c	155.9a	26.0a	205.8a
	BBIG	14.9c	2.2d	18.1c	92.1d	13.4d	110.6c
	LSD	0.8	0.1	0.7	15.1	5.6	22.6

[†]Harvests (EB, Early Boot; ESH, Early Seed-Head).
[‡]Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).
[§]Means within a column not sharing a lowercase letter are significantly different (Fisher's Protected LSDα=0.05).
[¶]Macro-Nutrient Content and Removal (N, Nitrogen; P, Phosphorus; K, Potassium).

Table 3.1. Biomass quality (DM basis) across all locations and years.

Harvest[†]	Species[‡]	Forage Quality Constituents[¶]				
		ADF	NDF	Lignin	Ash	IVTDMD48
		g kg^{-1}				
FD	SG	530.0a[§]	814.5a	63.6a	42.0c	317.7c
	SGBBIG	495.9cd	799.1bc	68.2a	47.7a	432.8a
	BBIG	505.0bc	809.7ab	61.2a	45.6abc	368.7abc
EBFD	SG	503.1bc	788.3c	66.9a	46.4ab	351.3bc
	SGBBIG	490.1de	789.5c	64.6a	48.5a	412.5ab
	BBIG	507.1b	811.1a	67.8a	43.5bc	381.4abc
ESHFD	SG	481.6ef	763.8e	64.2a	46.8ab	314.9c
	SGBBIG	477.5f	777.6d	65.2a	48.0a	410.7ab
	BBIG	491.4de	788.1cd	64.5a	48.1a	401.7ab
	LSD	17.5	10.7	7.0	4.1	78.1

[†]Harvests (FD, Fall Dormancy; EBFD, Early Boot plus Fall Dormancy; ESHFD, Early Seedhead plus Fall Dormancy).
[‡]Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).
[§]Means within a column not sharing a lowercase letter are significantly different (Fisher's Protected LSDα=0.05).
[¶]Biomass Quality Constituents (ADF, Acid Detergent Fiber; NDF, Neutral Detergent Fiber; Lignin; ash; IVTDMD48, *In-vitro* True Dry Matter Digestibility 48 hrs).

Table 3.2. Macro-nutrients removed by biomass harvest across all locations and years.

Harvest†	Species‡	Content¶			Removal		
		N	P	K	N	P	K
		g kg^{-1}			kg ha^{-1}		
FD	SG	5.6e§	2.1f	6.7e	74.6a	34.3a	100.0a
	SGBBIG	6.5cd	3.0cd	10.1c	60.8b	31.4ab	96.5a
	BBIG	6.8bcd	3.1c	10.7bc	47.1c	21.6cd	69.7b
EBFD	SG	7.4b	2.4e	7.3de	75.9a	26.2bc	74.9b
	SGBBIG	6.6bcd	3.2bc	11.0ab	52.5bc	26.6abc	82.7ab
	BBIG	6.3de	3.1bc	10.5bc	42.4cd	25.7bc	69.7b
ESHFD	SG	9.1a	2.9d	8.0d	73.1a	25.4bcd	64.2bc
	SGBBIG	7.4b	3.5a	11.6a	52.6bc	25.1abc	82.1ab
	BBIG	7.3bc	3.3b	10.9abc	34.9d	17.6d	46.4c
	LSD	0.8	0.2	0.8	11.6	7.9	20.3

†Harvests (FD, Fall Dormancy; EBFD, Early Boot plus Fall Dormancy; ESHFD, Early Seedhead plus Fall Dormancy).
‡Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).
§Means within a column not sharing a lowercase letter are significantly different (Fisher's Protected LSDα=0.05).
¶Macro-Nutrient Content and Removal (N, Nitrogen; P, Phosphorus; K, Potassium).

Table 3.3. Combined macro-nutrients removed by two early forage harvests plus a fall dormancy biomass harvest across all locations and years.

Harvest†	Species‡		Removal¶ N	Removal¶ P	Removal¶ K
			——— kg ha^{-1} ———		
FD	SG		74.6d§	37.1f	110.2e
	SGBBIG		60.8de	33.1f	104.0e
	BBIG		47.1e	22.6g	73.6f
EBFD	SG		204.2a	72.6bc	253.6bc
	SGBBIG		164.7b	66.3cd	243.2c
	BBIG		129.5c	53.5e	179.2d
ESHFD	SG		219.0a	80.0ab	275.8b
	SGBBIG		203.6a	82.6a	307.8a
	BBIG		148.0b	56.7de	206.5d
		LSD	17.6	9.4	20.3

†Harvests (FD, Fall Dormancy; EBFD, Early Boot plus Fall Dormancy; ESHFD, Early Seedhead plus Fall Dormancy).
‡Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).
§Means within a column not sharing a lowercase letter are significantly different (Fisher's Protected LSDα=0.05).
¶Macro-Nutrient Removal (N, Nitrogen; P, Phosphorus; K, Potassium).

SUPPLEMENTAL MATERIAL

Table 4.1. Experiment set-up information.

Information	Description	ETREC†	HRREC	PREC
Experiment Type		Small Plot	Small Plot	Small Plot
Experimental Layout		Randomized Complete Block Design (RCBD)	Randomized Complete Block Design (RCBD)	Randomized Complete Block Design (RCBD)
Treatments	*Factorial*	3 (Species) x 3 (Harvest)	3 (Species) x 3 (Harvest)	3 (Species) x 3 (Harvest)
Replications		4	4	4
Statistical Analysis	*ANOVA-Mixed Model*	SAS Version 9.3	SAS Version 9.3	SAS Version 9.3
Establishment Year		2009	2008	2009
Plot Size, m		1.83 x 7.62	1.52 x 7.62	1.52 x 7.62
Species Mixture, %	*SG‡* *SGBBIG* *BBIG*	100 50, 35, 15 65, 35	100 50, 35, 15 65, 35	100 50, 35, 15 65, 35
Harvest Treatments		EBFD§ ESHFD FD	EBFD ESHFD FD	EBFD ESHFD FD

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).
‡ Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).
§ Harvests (EB, Early Boot; ESH, Early Seed-Head; FD, Fall Dormancy).

Table 4.2. Experiment harvest details for all locations.

Information	Description	ETREC†	HRREC	PREC
Target Harvest Dates	*EB*‡	Early to mid-May	Early to mid-May	mid-May to early June
	ESH	Late June	Late June	Late June to Early July
	FD	Early November	Early November	Early November
Harvest Equipment Used		Small Plot Flail Harvester	Small Plot Flail Harvester	Small Plot Flail Harvester
Harvest Residual Height, cm		15.24	15.24	15.24

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).
‡ Harvest target dates based on plant phenology (EB, Early Boot; ESH, Early Seed-Head; FD, Fall Dormancy).
§ Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).

Table 4.3. Experiment locations.

Site Information	Description	ETREC†	HRREC	PREC
Site Coordinates	*Latitude, Longitude*	+35° 54' 2", -83° 57' 36"	+36° 28' 22", 86° 49' 7"	+36° 2' 38", -85° 9' 48"
	Altitude	886'	680'	1863'
Soil Type		Etowah Silt Loam	Mountview Silt Loam	Lily Loam
Soil Texture		Fine-Loamy	Fine-Silty	Fine-Loamy

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).

Table 4.4. Experiment preparation and fertilization for all locations.

Information	Description	ETREC†	HRREC	PREC
Planting Equipment Used		Small Plot no-Till Drill	Small Plot no-Till Drill	Small Plot no-Till Drill
Ground Preparation		Conventional Tilling and Cultipacking	Conventional Tilling and Cultipacking	Conventional Tilling and Cultipacking

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).
‡ Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).
§ Harvests (EB, Early Boot; ESH, Early Seed-Head; FD, Fall Dormancy).

Table 4.5. Soil test results for all locations.

Information	Description	ETREC† 2010	ETREC† 2011	ETREC† 2012	HRREC 2010	HRREC 2011	HRREC 2012	PREC 2010	PREC 2011	PREC 2012
Soil Test Results- Whole Plot Area‡	pH	6	7	6	6	6	6	6	6	6
	P, kg ha^{-1}	35	25	18	50	22	62	3	63	41
	K, kg ha^{-1}	176	133	99	106	84	157	99	269	138

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).
‡ Small plots were not individually soil tested.

Table 4.6. Experiment weed control details for all locations.

Technique Used	Description	ETREC†	HRREC	PREC
Weed Control Methods	Pre-emergence, SG and SGBBIG‡	Manual and Mowing	Manual and Mowing	Manual and Mowing
Weed Control Chemicals	*Pre-Planting*	glyphosate (2.24 kg ai/ha^{-1})	glyphosate (2.24 kg ai/ha^{-1})	glyphosate (2.24 kg ai/ha^{-1})
	Pre-Emergence, BBIG only	imazapic (0.11 kg ai/ha^{-1})	imazapic (0.11 kg ai/ha^{-1})	imazapic (0.11 kg ai/ha^{-1})
	Pre-Emergence, BBIG only	metsulfuron methyl (14.0 g ai/ha^{-1})	metsulfuron methyl (14.0 g ai/ha^{-1})	metsulfuron methyl (14.0 g ai/ha^{-1})

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).
‡ Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).

Table 4.7. Actual experiment harvest dates for all locations and years.

Location†	Harvest‡	Year 1	Year 2	Year 3
	EB	05-27-10	05-16-11	05-07-12
ETREC	*ESH*	06-29-10	06-21-11	05-29-12
	FD	10-31-10	10-26-11	10-31-12
	EB	05-28-10	05-24-11	05-10-12
HRREC	*ESH*	06-21-10	06-29-11	06-08-12
	FD	10-21-10	10-31-11	11-14-12
	EB	06-14-10	06-03-11	05-25-12
PREC	*ESH*	07-07-10	07-01-11	06-13-12
	FD	10-29-10	10-31-11	11-29-12

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).
‡ Harvest dates based on plant phenology (EB, Early Boot; ESH, Early Seed-Head; FD, Fall Dormancy).

Table 4.8. Mean species height at harvest.

		ETREC†			HREC			PREC		
Description	Species	EB‡	ESH	FD	EB	ESH	FD	EB	ESH	FD
Mean Species Height at Harvest, cm	*SG*§	108	99	156	94	90	158	94	83	137
	SGBBIG	92	86	157	94	85	143	86	77	116
	BBIG	80	72	182	82	68	139	57	62	86

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).
‡ Harvests (EB, Early Boot; ESH, Early Seed-Head; FD, Fall Dormancy).
§ Species (SG, Switchgrass; SGBBIG, Switchgrass/Big Bluestem/Indiangrass; BBIG, Big Bluestem/Indiangrass).

Table 4.9. Experiment Sample Preparation.

Information	ETREC†	HRREC	PREC
Sample Preparation Procedures	Forced Air Oven- 60°C for 72 hours	Forced Air Oven- 60°C for 72 hours	Forced Air Oven- 60°C for 72 hours
	DM yield calculated from swath, wet and dry weights	DM yield calculated from swath, wet and dry weights	DM yield calculated from swath, wet and dry weights
Sample Preparation Equipment	Willey Mill Grinder, 2 mm Screen	Willey Mill Grinder, 2 mm Screen	Willey Mill Grinder, 2 mm Screen
	Cyclone Grinder, 1 mm Screen	Cyclone Grinder, 1 mm Screen	Cyclone Grinder, 1 mm Screen
Sample Analysis Equipment	FOSS 6500 NIR Spectrometer	FOSS 6500 NIR Spectrometer	FOSS 6500 NIR Spectrometer
	WINSI II Software	WINSI II Software	WINSI II Software
	NIRS Consortium Equations	NIRS Consortium Equations	NIRS Consortium Equations

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).

Table 4.10. Fertilization protocol for all locations.

Information	Description	ETREC†	HRREC	PREC
Fertilization Protocol (P, K, pH)		Establishment and maintenance recommendations from the Soil, Plant and Pest Center in Nashville, TN	Establishment and maintenance recommendations from the Soil, Plant and Pest Center in Nashville, TN	Establishment and maintenance recommendations from the Soil, Plant and Pest Center in Nashville, TN
Fertilization (N) for Experiment	*EBFD*‡	50.44 kg N/ha^{-1} green-up, and 50.44 kg N/ha^{-1} after first harvest	50.44 kg N/ha^{-1} green-up, and 50.44 kg N/ha^{-1} after first harvest	50.44 kg N/ha^{-1} green-up, and 50.44 kg N/ha^{-1} after first harvest
	ESHFD	50.44 kg N/ha^{-1} green-up, and 50.44 kg N/ha^{-1} after first harvest	50.44 kg N/ha^{-1} green-up, and 50.44 kg N/ha^{-1} after first harvest	50.44 kg N/ha^{-1} green-up, and 50.44 kg N/ha^{-1} after first harvest
	FD	100.88 kg ha^{-1}at green-up	100.88 kg ha^{-1}at green-up	100.88 kg ha^{-1}at green-up

† Locations (ETREC, East Tennessee Research and Education Center, Knoxville, TN; HRREC, Highland Rim Research and Education Center, Springfield, TN; PREC, Plateau Research and Education Center, Knoxville, TN).
‡ Harvests (EB, Early Boot; ESH, Early Seed-Head; FD, Fall Dormancy).

VITA

David Weston McIntosh is from both Chuckey and Gatlinburg, Tennessee where he grew up on a farm growing tobacco, raising cattle, riding horses, working in his grandfather's hardware store, and enjoying the mountains. After completing high school, he attended Tennessee Technological University for his first two years of undergraduate studies. In 2000 he completed his Bachelor of Arts degree at the University of Tennessee in Knoxville with a concentration of Technical Writing and Communications. After graduating, David spent several years traveling the country, living in Santa Fe, New Mexico and Boulder, Colorado. In that time he developed his first career passion of being a chef working for bakeries, restaurants, and on the private excursion train, "American Orient Express". After traveling across Canada, Mexico, and the United States several times he moved to Florida where he worked in the accounting industry for several years. In 2007, David returned home to Tennessee where he started working for Dr. Gary Bates in the University of Tennessee Forage Research Program. In past 6 years, he has worked as a student worker, graduate student, research associate, and is currently the coordinator for the UT Beef and Forage Center. Upon completion of his Master of Science Degree, David will be enjoying his new home, son Lucas, and spending time with his wife Katherine.