DUAL USE SWITCHGRASS:

MANAGING SWITCHGRASS FOR BIOMASS PRODUCTION AND SUMMER GRAZING

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

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DUAL USE SWITCHGRASS

Managing Switchgrass for Biomass Production and Summer Grazing

Jordan Richner

Robert Kallenbach, Advisor

ABSTRACT

Two studies were conducted on established switchgrass plots at Columbia and Mount Vernon, Missouri. The Columbia site was located on Mexico silt loam, and the Mount Vernon site on Viraton silt loam. The first study examined the feasibility of harvesting switchgrass for biomass and summer forage within a season. Yields and forage quality were compared between four harvest management treatments: a single post-frost harvest for biomass, a forage harvest at boot stage followed by a post-frost biomass harvest, biomass harvest at post-anthesis with summer regrowth harvested as forage, and biomass harvest at pre-anthesis with summer regrowth harvested as forage. Summer regrowth was minimal at Mount Vernon due to a fragipan and shallower rooting depth at that site. Regrowth had greater lignin content and as a result, was less digestible. The second study attempted to determine the effect of switchgrass maturity on efficiency of conversion to glucose through enzymatic hydrolysis. These maturity stages included boot stage, pre-anthesis, post-anthesis, full seed, and post-frost. Lignin was not shown to negatively affect efficiency of enzymatic hydrolysis. Rather, hemicellulose was shown to negatively impact conversion efficiency, possibly because acid pretreatment was incomplete and thus some hemicellulose remained in the digested material.

GENERAL INTRODUCTION

The dependence on fossil fuel exhibited by today's society and the possible environmental effects of its continued use has led to an interest in developing new sources of energy (Parrish and Fike, 2005). Among these alternative energy sources is biomass (Demirbas, 2007). Although plant sources cannot completely replace fossil fuels, they may reduce our dependence on finite fossil fuels (Schubert, 2006) by providing a viable, renewable source of energy (Demirbas, 2007). One species that has gained attention as a possible biomass crop is switchgrass (*Panicum virgatum L.*) (Heaton et al., 2004).

Switchgrass is also commonly used as a summer forage crop in the Midwest (McLaughlin and Walsh, 1998), especially on operations where rotational stocking is practiced. A dual harvest system may allow producers to take advantage of the biomass market when and if such a market is developed. This could potentially provide an additional source of income for producers if biomass production and rotational stocking can be integrated. This study examines some harvesting schemes which may allow such an integration.

LITERATURE REVIEW

Switchgrass (*Panicum virgatum L.*) is a warm-season grass native to North America. It has been available as forage to ruminant wildlife species in the Great Plains for centuries, and is well adapted to grazing and occasional fire. Naturally, it was utilized as a forage when settlers arrived with domesticated livestock species. Switchgrass gained little attention until the 1970's, when it began to be studied as a monoculture forage and improved varieties were developed. Today, the most common use of switchgrass is summer grazing, but it can also be utilized as hay, silage, erosion control, phytoremediation, and wildlife habitat (Parrish and Fike, 2005).

In 1992, the Department of Energy's Biofuels Feedstock Development Program (BFDP) chose switchgrass as a model species for biofuel feedstock. After extensive study, switchgrass had shown efficient use of water and nutrients, environmental soundness, and suitable productivity across many climatic and soil conditions, including marginal land (McLaughlin and Kszos, 2005). Since then, switchgrass has been one of the major species considered as a source of biofuel.

Switchgrass

Switchgrass exhibits significant variability in morphology. The species can be generally categorized into upland and lowland forms. Upland populations are associated with drier soils in higher landform positions, while lowland populations tend to be found in more moist soils. Lowland types are characterized by having thicker stems and coarser leaves, a more bluish-green color, and are taller with larger panicles. Upland populations

have longer roots and more active rhizomes. Lowland cultivars tend to be tetraploid, while upland types can be tetraploid, hexaploid, or octoploid (Porter, 1966).

Since development of improved switchgrass varieties has taken place only over the past few decades, commercially available cultivars still exhibit a wide range of genetic variability (Casler et al., 2004). This fact is partly due to the self-incompatible and open pollinating nature of the switchgrass plant (Martinez-Reyna and Vogel, 2002).

Along with the wide genetic variation of switchgrass, is adaptability across a wide range of environmental conditions. Natural switchgrass populations have been observed across much of North America east of the Rocky Mountains extending from Texas to as far north as Southern Canada. Lowland varieties are adapted to lower latitudes while upland types are prevalent at higher latitudes (Casler et al., 2007). Switchgrass is also known to have a large tolerance to acidic soils (Hopkins and Taliaferro, 1997) and forms symbiotic relationships with mycorrhizal fungi, which increase productivity, improve stress tolerance, and enhance nutrient uptake, especially phosphorus (Parrish and Fike, 2005).

Being a C_4 plant, switchgrass has an optimum growing temperature of 30 to 35° C (Hsu et al., 1985) and grows during the summer when growth of cool-season grasses has slowed (Moore et al, 2004). Spring growth of switchgrass tillers is initiated when a temperature adequate for growth is reached. This temperature is dependent upon cultivar (Casler et al., 2007).

Reproductive development is initiated by photoperiod and vegetative growth ceases when the reproductive stage begins. Switchgrass is classified as a short-day plant, and the day length at which flowering begins is dependent upon cultivar and the latitude

to which the cultivar is adapted. Genotypes with later flowering dates have a longer growing period, which generally allows these cultivars to produce more biomass. As a result, southern varieties, if planted in a more northern location, remain vegetative longer and can produce greater yields. Northern varieties planted further south mature early, and tend to have lesser yields (Casler et al., 2007).

Plant Cell Walls

When switchgrass is harvested for biomass, it is the components of the plant's cell walls that are utilized in production of biofuel (Keshwani and Cheng, 2009). The main components that make up plant cell walls are cellulose, hemicellulose, and lignin. Cellulose, the most abundant, is a polymer consisting of a long, straight chain of glucose molecules linked by β(1-4) glycosidic bonds. Hemicellulose is a branched, amorphous polymer (Jeoh, 1998) composed primarily of pentose sugars that surrounds and reinforces bundles of cellulose in the cell wall. Lignin is a water insoluble polymer (Demirbas, 2007) of cross-linked phenolic compounds, which acts to further reinforce the cell wall (Sarath et al., 2007). Collectively, these fibers are called lignocellulose (Sun and Cheng, 2002).

In the cell wall, bundles of cellulose polymers are linked by hydrogen bonds, both intramolecularly between glucose units in the same polymer and intermolecularly between adjacent polymers, to form microfibrils. These hydrogen bonds enhance the rigidity and insolubility of the cellulose fiber. The hemicellulose is also bound to the cellulose by hydrogen bonds but is more readily broken down into its component sugars. Lignin fills in some of the spaces between cellulose and hemicellulose like glue, limiting

accessibility to the cellulose and hemicellulose and increasing rigidity of the cell wall (Jeoh, 1998).

Initially, plant cells deposit cellulose, pectins, and xylans to form the primary cell wall. After cell elongation ceases, secondary cell wall growth begins. The secondary cell wall, composed primarily of cellulose and xylan, develops inside the primary cell wall. It is in this phase when lignin deposition occurs. Incorporation of lignin begins in the outermost and oldest part of the cell wall and continues inward through the primary cell wall to the secondary cell wall. Lignification then, tends to be greatest in the primary cell wall and least in the youngest, innermost portion of the secondary cell wall (Jung and Allen, 1995).

Because lignin and fiber increase as the plant matures and digestibility concomitantly decreases, plants still in the vegetative stage of growth are more desirable to ruminant animals. However, as forages mature, yield increases. Producers strive to harvest forage when the stand has produced sufficient yield but has not become too indigestible (Burns et al., 1997).

Thickness of the secondary cell wall and lignin concentration vary in different cell types. The epidermis, xylem, and sclerenchyma, with thicker cell walls and more lignin, are among the least digestible. Phloem and collenchyma are thinner walled and more easily broken down, while mesophyll and parenchyma tissues are the most easily degraded. Of course, stems contain a larger proportion of structural components than leaves and are more resilient to degradation (Moore and Jung, 2001).

Plants grown under greater light intensity have a greater rate of photosynthesis and show increased levels of soluble carbohydrates and therefore are more digestible.

Warmer temperatures enhance production of structural components (Van Soest et al., 1978) and lignification, reducing forage quality (Moore and Jung, 2001).

Environmental stresses can retard plant growth and delay maturity, maintaining forage quality. Water deficit plants tend to be more digestible than plants with sufficient water (Van Soest et al., 1978). Shaded or nutrient deficient plants may also exhibit delayed maturity (Moore and Jung, 2001). Obviously, these factors reduce yield as well as maintain quality (Van Soest, 1978).

Cellulosic Ethanol

Due to concerns raised in recent years regarding the supply, cost, and environmental impact of fossil fuels, many researchers are seeking to utilize the cellulose in biomass to produce alternative fuels (Schmer et al, 2008). Biomass, a term that refers to all living matter, is one of the Earth's most abundant resources and is renewable (Demirbas, 2007). Energy from biomass can be utilized through direct combustion or through conversion to liquid fuels (Adler et al., 2006). The most common biofuel in the U.S. is ethanol (Schmer et al., 2008).

Ethanol (C_2H_5OH) is alcohol, the same as in alcoholic beverages, but can also be used pure or mixed with gasoline as fuel for internal combustion engines. Ethanol for fuel is commonly produced through yeast fermentation of sugars from starchy plant products, of which corn is the primary example in the US. However, use of grain as a source of fuel ethanol limits the supply of grain for food and feed (Keswani and Cheng, 2009). Brazil utilizes sugar supplied from sugar cane to produce a substantial amount of fuel ethanol (Somerville, et al., 2010) The lignocellulose that makes up a significant

portion of all plant material is another source of fermentable sugar (Demirbas, 2007). If the cellulose in plant cell walls can be broken down into its component glucose molecules, it can be used as a source of fermentable sugar for ethanol production (Sun and Cheng, 2002).

One of the processes used to produce cellulosic ethanol is enzymatic hydrolysis, also called saccharification. This method uses cellulase enzymes to break down the cellulose into glucose. The difficulty with producing ethanol from cellulosic sources is that hemicellulose and lignin prevent access to the cellulose by the enzymes. Because of this, a pre-treatment step is necessary to separate the hemicellulose and lignin from the cellulose, break up the crystalline structure of the cellulose fiber, and increase the porosity of the biomass feedstock (Sun and Cheng, 2002). Dilute acid hydrolysis is commonly used as a pre-treatment for biomass in many lignocellulose conversion systems. Dilute sulfuric acid is introduced to the ground lignocellulosic feedstock at a temperature of 100 to 200° C (Kootstra et al., 2009).

After pre-treatment, cellulase enzymes are introduced to disassemble the cellulose polymers. Cellulases come from several bacteria and fungi species, the most extensively researched of which is the fungi genus *Trichoderma* (Sun and Cheng, 2002). A mixture of different types of cellulase enzymes is often utilized. Endoglucanase breaks the cellulose polymer into smaller sections. Exoglucanase then separates the polymer into two-glucose units, called cellobiose, from the ends of the cellulose chain. β-glucosidase then breaks down the cellobiose into glucose (Keshwani and Cheng, 2009). This step of the process usually is carried out at 45-50° C (Sun and Cheng, 2002) for at least 36 h (Aden et al., 2002). After this point, the process is much the same as conventional

ethanol production from corn. Yeast is used to ferment the sugar to produce ethanol and distillation separates the ethanol from the feedstock slurry (Demirbas, 2007).

Rotational Stocking

Because cellulosic ethanol technology has not yet been perfected and few industrial scale plants are currently producing ethanol from cellulose, there is little market for biomass crops at this time (Schubert, 2006). Currently, switchgrass stands are often used as summer grazing in rotational stocking operations (Moore et al., 2004). Therefore, it is likely that these producers will be among the first to take advantage of the biomass market, if such a market develops.

Livestock producers have traditionally used continuous grazing practices in their operations. In continuous grazing, animals are allowed to graze a large pasture and remain in that pasture for a long period of time (Kothman, 2009). Animals randomly graze certain areas of the pasture, while other areas become mature. The large area allows animals to be highly selective in what plants they graze, and since they prefer more digestible vegetative plants over mature plants, they often continue to graze in the same areas. The repeated defoliation of those plants weakens their root structure, reducing stand persistence. Rotational stocking attempts to remedy many of the disadvantages of continuous grazing (Heady, 1961).

Rotational stocking is a livestock management system in which the grazing unit is subdivided into paddocks that are stocked in succession. This stocking method allows most of the paddocks to rest and regrow while only one is being grazed (Kothman, 2009). This rotation and regrowth period allows for more uniform and efficient grazing that

encourages growth of the forage plant both above ground and below ground. This, in turn, improves soil quality, limits weed growth, and enhances forage quality and quantity. A well-managed rotationally stocked pasture can greatly reduce supplemental feed costs and lessen the need for fertilizer (Heady 1961).

Harvesting Switchgrass

Switchgrass for forage must be cut or grazed before becoming overly mature. When used for biomass production, switchgrass usually is harvested at a more mature stage for maximum yield (Sanderson et al., 1999). Many studies have attempted to ascertain the ideal stage at which to harvest switchgrass for biomass.

A method to accurately describe the growth stage of warm season grasses was developed by Moore, et al. (1991). This system divides the plant's life span into five primary growth stages: germination (G), vegetative growth (V), elongation (E), reproduction (R), and seed development (S). These primary stages are then divided into substages marked by specific events occurring in the plants' life and signified by a number that follows the letter of the primary growth stage (such as R1 for inflorescence emergence during the reproductive stage). This method is commonly used to communicate specific maturity stages of switchgrass.

A review by Parrish and Fike (2005) stated that to obtain the greatest possible yields, switchgrass should be harvested once per year, or possibly twice if timed correctly. Three or more harvests annually reduced long term yields.

Vogel et al. (1998) reported that the greatest biomass yields could be realized by harvesting between the R3 (panicle emerged) and R5 (post-anthesis) stages of maturity,

which are usually reached during the first three weeks of August. In favorable years, there may be enough regrowth to allow a post-frost harvest.

Casler and Boe (2003) reported that biomass yield of switchgrass is unstable and difficult to predict, but proposed late summer or early autumn as an optimal harvest date. They also acknowledged that a delayed harvest will reduce plant mortality and improve longevity of switchgrass stands.

Research by Sanderson et al. (1999) looked at the utilization of switchgrass as forage and biomass by cutting once in May, twice in May and June, and thrice in May, June, and July, all followed by a harvest in the fall. They concluded that the "limited regrowth" could be grazed but had low crude protein and high neutral detergent fiber. However, this project did not include a biomass harvest followed by grazing in late summer when switchgrass would be most needed due to inactivity of cool-season forages. Similar to other studies, they found that more harvests led to decreased total growth. They also stated that the greatest amount of biomass resulted from a single harvest in mid-September compared to October or November harvests.

Multiple studies (Sanderson and Wolf, 1995; Burns et al., 1997) have observed that switchgrass nutritive value decreases rapidly through the stem elongation stage and into the reproductive stage. Switchgrass utilized as forage should be grazed or harvested before quality decreases.

Unfortunately, switchgrass reaches the ideal stage for grazing relatively early in the season when cool-season grasses are still producing sufficient yield (George and Oberman, 1989). Research by George and Oberman (1989) indicates that early defoliation of switchgrass may be beneficial by providing regrowth in a better quality

vegetative stage for grazing in late summer. In their study, switchgrass stands were clipped early in the season to simulate grazing, but at that time of year, cool season grasses will still be available for grazing (George and Oberman 1989) and will likely be of higher quality than the switchgrass (Moore et al., 2004). Perhaps early harvest such as this would be better utilized as biomass.

Moore et al. (2004) reported that, in one year of their study, switchgrass was hayed early in the season to delay maturity, which improved livestock gains when the switchgrass was grazed in the summer. The authors did, however, question the sustainability of this management if practiced for several years.

Parrish and Fike (2005) reported that earlier harvests may lead to reduced yields in following seasons, because switchgrass translocates nitrogen and nonstructural carbohydrates from the shoots to the underground portions of the plant during senescence and are thus available to the plant for spring growth the following season. Delaying harvest until later in the fall allows nutrients to be fully transferred and aids in stand persistence. They also stated that shattering and lodging during the winter have little effect on yields if harvest is delayed until early spring.

A study completed by Adler et al. (2006) compared yields between harvesting in autumn and delaying harvest until the following spring. Switchgrass yields were reduced when the harvest was delayed until the following spring. However, most of the lost yield was attributed to biomass that was not picked up by the baler during the spring harvest. Yield loss was greater in years with more snowfall, due to increased lodging. They also reported that greater moisture levels in fall harvested switchgrass may cause more spoilage during storage and raise the cost of transport due to the increased weight. When

looking at biofuel quality, Adler et al. (2006) found that the spring harvested switchgrass contained a larger concentration of both cell wall glucose and lignin. Estimated ethanol production, based on carbohydrate levels, was greater for spring harvested feedstock, but this estimation did not take into account the adverse effects that lignin may have on conversion. Gas production from *in vitro* incubation with mixed ruminal microorganisms, which is an indicator of ethanol production in a simultaneous saccharification and fermentation system, showed that the spring harvested feedstock yielded 25% less than fall harvested.

Studies concur that switchgrass intended for energy production through direct combustion or pyrolysis should be harvested after senescence when nitrogen, potassium and ash levels are least (Sanderson and Wolf, 1995; Heaton et al., 2004). Large levels of inorganic compounds reduce hydrocarbon yield (Sanderson and Wolf, 1995) and increase pollution in thermochemical conversion processes (Heaton et al., 2004). However, the greater lignin content during this stage may reduce efficiency of biochemical conversion by preventing the enzymes' access to the cellulose (Sanderson and Wolf, 1995).

Dien et al. (2006) carried out a hydrolysis efficiency experiment by incubating switchgrass, reed canarygrass, and alfalfa of varying maturities in a cellulase enzyme preparation after dilute acid pretreatment. Switchgrass samples were taken at pre-boot, anthesis, and post-frost stages. Fermentable sugars produced were then measured to determine efficiency of conversion in an enzymatic saccharification system. Increasing Klason lignin levels were reported to reduce conversion efficiency much like increasing lignin reduces digestibility in forages (Moore and Jung, 2001). Glucose conversion efficiency decreased as maturity increased, but the greater amount of carbohydrates

available from high-yielding, though mature biomass, yielded more total glucose. The author went on to stress that these findings were "preliminary and that definitive conclusions on these topics will require analysis of larger sample sets."

Previous studies have thoroughly examined harvest timings of switchgrass for biofuel feedstock (Parrish and Fike, 2005, Vogel et al., 1998, Adler et al., 2006), and some researchers have examined the possibility of using switchgrass for both forage and biomass in a single season (Sanderson et al., 1999), but the utilization of switchgrass as a late summer forage following a harvest for biomass has not been tested. Comparison of conversion efficiency at varying maturities has been studied only over a small set of maturity stages (Dien et al., 2006). A more detailed schedule of saccharification conversion efficiencies across switchgrass growth stages will be beneficial in helping producers and the biofuel industry decide when and how to best harvest biomass.

DUAL USE SWITCHGRASS: MANAGING SWITCHGRASS FOR BIOMASS PRODUCTION AND SUMMER GRAZING

ABSTRACT: A study was conducted during 2010 and 2011 on established switchgrass (*Panicum virgatum L.*) plots to determine the feasibility of harvesting switchgrass for biomass and forage in a single season. Plots were located at Columbia, on Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs) and Mount Vernon, on Viraton silt loam (fine-loamy, siliceous, active, mesic Oxyaquic Fragiudalfs), both in Missouri, USA. Forage/biomass yields and nutritive value of samples intended for forage use, were compared between four harvest management treatments: a single post-frost harvest for biomass, a forage harvest at boot stage followed by a post-frost biomass harvest, biomass harvest at post-anthesis with summer regrowth harvested as forage and biomass harvest at pre-anthesis with summer regrowth harvested as forage. A complementary study was conducted to determine the effects of switchgrass maturity stage on efficiency of conversion to glucose through enzymatic hydrolysis. These maturity stages included boot stage, pre-anthesis, post-anthesis, full seed, and post-frost. Switchgrass regrowth is not a reliable source of summer forage at Mt. Vernon, an effect likely related to shallow rooting depth. The regrowth was also more lignified than initial growth. In this study, hemicellulose had a greater effect on glucose conversion than lignin.

INTRODUCTION

Switchgrass is a perennial, warm-season grass native to North America. It is used for wildlife habitat, erosion control, or forage production (Sanderson and Wolf, 1995). Lately, switchgrass has gained recognition as a feedstock for fuel ethanol because it is a native plant that efficiently uses water and nutrients and is adapted to many soil and climatic conditions (McLaughlin and Kszos, 2005).

Despite its recent popularity as biofuel feedstock, switchgrass traditionally has been used for forage (Keshwani and Cheng, 2009). Producers in the transition zone often utilize warm-season grasses, like switchgrass, to provide forage for livestock during the summer when cool-season grasses are dormant (Moore et al., 2004). Switchgrass forage quality declines rapidly as the plant matures, and should be hayed or grazed before becoming too mature (Burns et al., 1997). However, switchgrass tends to reach ideal maturity for grazing in early summer when cool-season grasses are still producing sufficient forage. This early maturation makes switchgrass undesirable for many producers who have more need of forage later in the season when growth of cool-season grasses declines (Hudson, et al., 2010). Research by George and Oberman (1989) indicate that early defoliation of switchgrass may provide regrowth in a vegetative state which could be used as a high quality forage in late summer. It may be possible to utilize an early harvest of switchgrass for biomass and graze the regrowth in late summer.

Previous studies show that multiple cuttings of a switchgrass stand within a season reduces total yield (Sanderson, et al., 1999) and three or more cuttings reduces stand longevity (Parrish and Fike, 2005). For maximum biomass yield, switchgrass should be harvested once per year (Sanderson et al., 1999). Delaying cutting until after

frost allows the plant to translocate nutrients from senescing stems and leaves to the underground portions of the plant. The nutrients are stored for the next season and enhance stand longevity (Parrish and Fike, 2005). While a three-cutting system is detrimental to switchgrass longevity (Parrish and Fike, 2005), a two-harvest system may be worthwhile to producers if there is no significant reduction of yield, if it provides forage of suitable quality for the intended use, and if the producer gains another way to profitably utilize the crop. This could allow producers to integrate biomass production into a grazing system and take advantage of the biomass market, when and if such a market develops.

After harvest, the biomass must be converted to a usable product. One of the common methods used to convert lignocellulose to fuel is enzymatic hydrolysis, a process in which cellulase enzymes are used to break down cellulose into glucose. The glucose can then be fermented to produce ethanol. Hydrolysis is often preceded by a pretreatment with dilute acid to make the lignocellulose structure more accessible to cellulase enzymes (Sun and Cheng, 2002).

As plants mature and lignin content increases (Jung and Allen, 1995), they become more difficult to digest by ruminant livestock (Jung, 1989). A similar effect may be present in enzymatic hydrolysis of switchgrass. Previous research indicates that lignin inhibits the hydrolysis of lignocellulose by cellulase enzymes (Chang and Holtzapple, 2000), and that less mature plant samples have a greater efficiency of conversion to glucose through enzymatic hydrolysis (Dien et al., 2006).

The first objective of this study was to identify a harvesting scheme in which switchgrass could be used for biomass and grazing within a single season. To this end,

we examined some two-harvest systems that utilized switchgrass for forage and for biomass: spring forage followed by a biomass harvest after frost, a biomass harvest at post-anthesis followed by summer forage, and a biomass harvest at pre-anthesis followed by summer forage. These harvesting systems were compared to a single-cut biomass harvest after frost.

The second objective of this study was to determine if maturity of switchgrass has an effect on its efficiency of conversion to glucose through enzymatic hydrolysis This complementary study examined glucose yields after switchgrass was subjected to dilute-acid pretreatment and enzymatic hydrolysis and if maturity of the switchgrass altered glucose yield.

MATERIALS AND METHODS

Harvest Management Study

A switchgrass harvest study was conducted over two years during the growing seasons of 2010 and 2011. Data were collected from established switchgrass plots at two sites: The University of Missouri Bradford Research Center near Columbia, Missouri, and the University of Missouri Southwest Research Center near Mount Vernon, Missouri. The plots at the Columbia location were mapped as Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualfs) and the Mount Vernon location was Viraton silt loam (Fine-loamy, siliceous, active, mesic Oxyaquic Fragiudalfs). Cultivars present at each location were Cave-in-Rock, WS98-SB, Sunburst, and Blackwell at the Columbia site and Alamo, Kanlow, Cave-in-Rock, and Pathfinder at the Mount Vernon site.

Four harvest management treatments were used in the study (Fig. 1). Treatment I was harvested in autumn after a killing frost, representing a typical management scheme intended only for biomass. Treatment II was harvested at the boot stage to represent grazing, with the regrowth harvested as biomass after frost. Treatment III was harvested as biomass at the post-anthesis stage, and the regrowth was harvested as forage in August. Treatment IV was harvested for biomass at the pre-anthesis stage, and also harvested as forage in August. The two summer regrowth treatments (III and IV) were harvested on the same day in late August. Treatment III regrowth was cut approximately four weeks after the first harvest. Treatment IV regrowth was cut approximately eight weeks after the first harvest. Each treatment had four reps blocked by one of the four

cultivars used at the site. Switchgrass maturity stages were determined as described by Moore, et al. (1991).

The plots were burned in late March at both locations. Two weeks after burning, plots were sprayed with picloram + 2, 4-D (Grazon P+D, Dow AgroSciences, Indianapolis, IN) and pendimethalin (Prowl, BASF Corp., Research Triangle Park, NC) at 2.3 L ha^{-1}. Ammonium nitrate (NH_4NO_3) fertilizer was applied in late March at 67 kg N ha^{-1}.

Switchgrass was harvested using a tractor-mounted, flail-type harvester at the Columbia site and a walk-behind, flail- type plot harvester at the Mount Vernon site. All plots were cut to a 15-cm stubble height. Wet mass was determined in the field and a 400- to 600-g subsample was collected for calculation of moisture content and analysis. Switchgrass sub-samples were dried in a forced air oven at 45° C for at least 48 h and dry matter calculated. The samples were then ground using a cyclone mill (UDY Corp., Ft. Collins, CO) to pass a 1-mm screen. After grinding, samples were analyzed for forage quality.

Nitrogen content was measured by a LECO FP-428 nitrogen analyzer (LECO Corp., St. Joseph, MI) and multiplied by 6.25 to calculate crude protein. The detergent fiber method was used to provide values for NDF, ADF, and ADL following the procedures provided by Ankom Technology (Fairport, NY) as described by Mertens (2002). The method described by Robinson (1999) was used to determine *in vitro* true digestibility. Samples were subjected to *in-vitro* digestion with rumen fluid collected from a cannulated cow and incubated in an Ankom Daisy^{II} for 48 h (Ankom Technology,

Fairport, NY) followed by washing in neutral detergent fiber solution in an Ankom 200 Fiber Analyzer.

Enzymatic Hydrolysis Study

A complementary study was conducted to determine efficiency of conversion of lignocellulose to glucose through enzymatic hydrolysis at various stages of switchgrass maturity. The glucose conversion study utilized samples across four varieties (Cave-in-Rock, WS98-SB, Sunburst, and Blackwell) at Bradford Research Center near Columbia, Missouri. The enzymatic hydrolysis study utilized different plots than the harvest management study to allow analysis of a greater range of maturity stages. The plots were adjacent to and subjected to the same management (weed control, fertility) as the plots used for the harvest study previously described. Samples were collected at boot stage, pre-anthesis, post-anthesis, full seed, and post-frost stages during the years of 2010 and 2011. Switchgrass samples were cut by hand at a height of 15 cm and dried in a forced air oven at 44° C for at least 48 h before calculation of dry matter. Samples were then coarsely ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass a 5-mm screen. These samples were not ground as finely as is common in a laboratory setting. Rather, they were ground coarsely as to be representative of feedstock in an industrial setting, such as an ethanol plant.

Samples were subjected to acid pretreatment by placing 2 g of ground material into a 125 ml Erlenmeyer flask, adding 18 mL of dilute sulfuric acid (2.5% wt/vol), and heating in an oven at 105° C for 30 min. After removal from the oven, the mixture was allowed to cool to room temperature for 2 h. The pretreated biomass was then diluted

with 20 ml H_2O and neutralized with 2.3 ml of 4M KOH. The solution was buffered with 2.5 ml of 1M citric acid, and 40 μL of 50 g L^{-1} thymol in a solution of 70% vol/vol ethanol was added. A mixture of two cellulase enzymes was used for hydrolysis, Novozyme 188 and Celluclast 1.5L (Novozyme, Denmark). Equal volumes of the two enzyme solutions were mixed and 1 mL of the mixture was introduced to the biomass samples. The contents of the flasks were then incubated for 72 h in a water bath shaker (New Brunswick Scientific C76 Water Bath Shaker, Edison, NJ) at 125 rpm and set to a temperature of 45° C. These samples were analyzed in duplicate. Glucose yield was determined using a Sucrose/Fructose/D-Glucose assay kit (Megazyme International, Ireland) following the procedure described by Megazyme International.

In addition to the enzymatic hydrolysis analysis, a second sub-sample from each maturity class was ground in a cyclone mill (UDY Corp., Ft. Collins, CO) to pass a 1-mm screen and analyzed for NDF, ADF, and ADL using the methods described previously.

Statistical Analysis

At each location and year, plots for the harvest management study were arranged in a randomized complete block design with four treatments and four blocks. Data were analyzed using the JMP software (SAS Institute, Cary, North Carolina) in a mixed model with year, location, and block as random effects. Year was considered as a repeated measure since the same plots were used at each location each year. Fisher's protected LSD was used for means separation. P values less than 0.05 were used to define significant differences.

Plots for the maturity and enzymatic hydrolysis study were arranged in a randomized complete block with five treatments (maturity classes) and four blocks. Year was considered a repeated measure. Analysis of these data followed the same methods as those described above.

RESULTS

Harvest Management Study

Yield. Significant location by year interactions were observed; as a result the data were analyzed by year and location. This study differentiates between two types of yield: switchgrass harvested for biomass (Treatment I, second cutting of Treatment II, and first cutting of Treatments III and IV) referred to as "biomass yield", and switchgrass harvested for forage (first cutting of Treatment II, and second cutting of Treatments III and IV) referred to as "forage yield". The total yield for the season (being the sum of the biomass yield and the forage yield) is referred to as "annual yield".

Biomass yields in 2010 ranged from 3090 to 6937 kg ha^{-1} at the Columbia location and from 4700 to 6203 kg ha^{-1} at Mt. Vernon. Biomass yields at Mt. Vernon were not significantly different between treatments, but at Columbia, biomass harvested at the pre-anthesis stage (Treatment IV) yielded significantly less than that harvested at the post-anthesis (Treatment III) or post-frost stage (Treatment I) (Table 1).

In 2011 at Columbia, the single-cut Treatment I had the greatest biomass yield at 9727 kg ha^{-1}. At Mount Vernon in the same year, Treatment I was not statistically different than the early biomass harvests, Treatments III and IV. Treatment II, (regrowth following a forage harvest at boot stage), had the least biomass yield at 2504 kg ha^{-1} (Table 1).

Forage yields were compared between the initial growth cut at boot stage (Treatment II) and the summer regrowth (Treatment III, regrowth after post-anthesis harvest, and Treatment IV, regrowth after a pre-anthesis harvest). At Columbia,

switchgrass regrowth after a biomass harvest provided forage yields equal to or greater than that of the initial growth harvested at boot stage for both 2010 and 2011 (Table 1). At Mt. Vernon, summer regrowth after a biomass harvest was only 8 to 40% of that of initial growth (Table 1).

Annual yields also showed mixed results. In Columbia, there was no significant difference between the single-harvest and the two-harvest treatments during the 2010 season. However, during 2011, the single harvest at 9727 kg ha^{-1} yielded significantly more biomass than any of the two-cut systems (Table 1). Switchgrass at the Mt. Vernon location also showed no difference between the single-harvest yields and the two-harvest yields in 2010. Annual yields at Mt. Vernon during 2011 were greater for the two-harvest treatments than for the single-harvest treatment.

Forage Nutritive Value. Crude protein levels varied by year and location, though in two site-years (Mt. Vernon in 2010 and Columbia in 2011) there were no differences between the treatments (Table 2). For the other site-year combinations, the effects of treatments were inconsistent. For instance, at the Columbia site in 2010, forage from Treatment IV had 20 to 30 g kg^{-1} less crude protein than either Treatment II or III ($P < 0.01$), while nearly the opposite effect was true for Mount Vernon in 2011.

Neutral detergent fiber (NDF) was not greatly influenced by harvest treatment, ranging only from 630 to 690 g kg^{-1} (Table 2). In Columbia, the NDF of forage from Treatment II was less than Treatment III (regrowth after a post-anthesis harvest) but not significantly different than the forage from Treatment IV (regrowth after a pre-anthesis harvest) in 2010. During the 2011 season in Columbia, the opposite trend occurred, as the NDF from forage from Treatment II was greater than that for Treatment IV but was

not significantly different than Treatment III. At Mt. Vernon in 2010, Treatment IV had less NDF than the other two treatments, and there were no treatment effects in 2011 (Table 2).

The ADF of switchgrass intended for forage use do not indicate any clear trend between the initial growth and the summer regrowth (Table 2). Values for ADF were not significantly different between treatments in 2011 at Mt. Vernon and were within a 50 g kg^{-1} range for the other site/year combinations.

The initial growth (Treatment II) tended to have a lower ADL value than at least one of the summer regrowth treatments in each year and location, except that differences were not significant in 2011 at the Columbia site.

In 2010, *in vitro* true digestibility of forage from Treatment II was always equal to or greater than the late summer forage in Treatments III and IV (Table 2).

Enzymatic Hydrolysis Study

In the complementary study, switchgrass at five different maturity stages was subjected to enzymatic hydrolysis and the resulting glucose yield quantified to determine conversion efficiency. Glucose yields were similar across maturity stages in the 2010 samples, except for biomass harvested at full seed, which was significantly greater than the other maturity groups (Table 3). Correspondingly, the full seed sample contained the smallest hemicellulose concentration. In 2011, boot and pre-anthesis stages yielded the least glucose while pre-anthesis and full seed were greatest. Again, the maturity stages with the greatest glucose yield also have the smallest hemicellulose concentration.

The post frost samples contained the most cellulose, NDF, ADF, and ADL and also the least cell solubles. The range in the values of several of the parameters was fairly narrow. Cellulose ranged from 320 to 380 g kg^{-1}, hemicellulose from 290 to 340 g kg^{-1}, NDF from 690 to 770 g kg^{-1}, and ADL from 40 to 70 g kg^{-1} (Table 3).

Additionally, the glucose yields were relatively low in comparison to the glucose levels published by Dien et al. (2006). The 2006 study by Dien et al. reported 233 to 312 g kg^{-1} of glucose released from switchgrass after acid pretreatment and enzymatic hydrolysis. In the present study, glucose yields ranged from 140 to 200 g kg^{-1}.

DISCUSSION

Harvest Management Study

Yield. Later harvest dates tended to yield more biomass because plants harvested later in the growing season simply had more time to accumulate dry matter. This result is not surprising and is consistent with research conducted in Iowa and Nebraska by Vogel et al. (2002), in Iowa by Moore et al. (2004), and in Michigan by Hudson et al. (2010), all of which reported increasing biomass throughout the early part of the growing season.

Sanderson et al. (1999) published results from a study in Texas stating that a four-cut system yielded approximately 50% less annual yield than a one-cut system. While we only tested two-cut systems, multiple harvests reduced total yield in only one location and year (Columbia in 2011). Both Sanderson et al. (1999) and the present study cut switchgrass to a 15-cm stubble height. Sanderson et al. (1999) also commented that switchgrass harvested frequently yielded less the following year. Other studies (Casler and Boe, 2003; Parrish and Fike, 2005) remark that frequent and early harvests reduce switchgrass yields in following years. Multiple harvests and early harvests of switchgrass interfere with translocation of nutrients from the stems and leaves to the roots at the end of the season. Interference with this process does not allow the plant to store energy for the following year, which apparently reduces long-term yields (Sanderson et al., 1999).

The fact that the single-harvest system at Mt. Vernon did not yield more than the two-harvest treatments and the lack of summer regrowth implies that switchgrass growth at that location took place primarily early in the season. Precipitation was less than

normal at Mt. Vernon during both summers (Fig. 2). However, at Columbia, the wetter 2010 season did not yield appreciably more than the drier 2011 season. Soil characteristics at the two sites provide a more plausible explanation. The plots at Mt. Vernon were located on Viraton silt loam (fine-loamy, siliceous, active, mesic Oxyaquic Fragiudalfs). This series is reported to have a fragipan at a depth of 38 to 84 cm (Soil Survey Staff, 2006). The fragipan layer limits the effective rooting depth in this soil, which in turn, limits available water, especially during summer.

At Columbia, the plots were located in Mexico silt loam. This series typically has a rooting depth of at least 200 cm, and thus, greater water holding capacity (Soil Survey Staff, 2006) than the soil at the Mt. Vernon site. The Columbia site, with its presumably deeper rooting depth and greater water holding capacity, supported switchgrass growth late in the summer and thus, greater yields in the summer and autumn. Casler et al. (2007) reported that biomass yield is often a function of plant height, and plants that reach the reproductive stage later in the season have more time in which to increase biomass. If early genetic maturation limits vegetative growth and biomass production, then lack of moisture will similarly limit growth and reduce biomass yields.

Both locations show that the rate of biomass accumulation is greater early in the season than late in the season. This is in agreement with research conducted by Sanderson et al. (1999) where 60 to 80% of the annual yield came from the first two harvests in three-cut and four-cut treatments. Additionally, Vogel et al. (2002) reported that first-cut yields had a greater contribution to total seasonal yield than second-cut yields.

Results in the present study indicate that switchgrass yield increases rapidly early in the season and biomass accumulation slows later in the season. Utilizing switchgrass regrowth as forage after an early season biomass harvest is likely to only be worthwhile on sites with deep rooting depth and/or during years with above-average rainfall in late summer. Sites with a shallower rooting depth will not provide enough regrowth for significant summer grazing in most years.

Forage Nutritive Value. Crude protein data in the present study do not indicate that initial growth or regrowth consistently have greater crude protein levels. Griffin and Jung (1983), Burns et al. (1997) and Mitchell et al. (2001) all observed declining crude protein as maturity of switchgrass increased. Sanderson and Wolf (1995) reported similar findings with crude protein decreasing rapidly early in the growing season followed by a leveling-off later in the season. They noted differences between their crude protein levels and those in previous studies and suggested soil fertility and nitrogen application rates as probable explanations. The present study does not show that an early harvest will maintain greater crude protein levels in the regrowth, even though the stand is kept in a vegetative state.

Several studies (Griffin and Jung, 1983; Sanderson and Wolf, 1995; Burns et al., 1997; Mitchell et al., 2001) have established that NDF increases as switchgrass matures. Moore et al. (2004) in Iowa reported improved livestock gains in summer when animals grazed the regrowth from switchgrass after it had been harvested for hay early in the season. However, in the present study, even though switchgrass was kept in a vegetative state by an early biomass harvest, NDF values in the regrowth were not consistently less than in the initial growth.

Even though the regrowth was less mature than the initial growth cut at boot stage, the regrowth was more lignified. Research published in 2011 by Burns presented similar results in which switchgrass regrowth had more lignin than initial growth. This could be due to increased temperatures later in the summer. Enzymes that synthesize lignin are more active as temperature increases (Moore and Jung, 2001) so plants grown in greater temperature tend to be more lignified (Van Soest et al., 1978).

In vitro true digestibility results reveal that the initial growth is more digestible than the regrowth. These digestibility results match those for lignin content. Lignin in the cell wall inhibits digestion of the forage in the rumen (Jung and Allen, 2005). Burns (2011) also reported reduced IVTD and greater lignin concentrations in switchgrass regrowth when compared to initial growth.

Burns et al. (1997) working in North Carolina, noted a rapid decline in the nutritive value of switchgrass early in the season. The earliest harvest of switchgrass (June 9, vegetative) in their study provided forage that would support 0.9 kg d^{-1} of weight gain on a 272 kg steer. Switchgrass harvested 14 d later (June 24, vegetative) supported daily gains of only 0.2 kg. After this initial period of rapid decline, nutritive value of switchgrass decreased at a slower rate (Burns et al., 1997). Sanderson and Wolf also observed this phenomenon in a 1995 study. They noted a phase of rapid increase in NDF, ADF, and ADL followed by a phase in which the fiber components increase at a much slower rate or remain fairly constant. Furthermore, they were able to link the shift between the phases to the stem elongation stage at both locations in their study.

In the present study, biomass harvests early in the season were used to keep switchgrass in a vegetative state in an attempt to maintain greater forage nutritive value.

This however, did not work. Despite being less mature, switchgrass regrowth does not consistently have less NDF or more crude protein concentration than initial growth switchgrass at boot stage. In fact, the regrowth tends to be more lignified and less digestible. These results suggest that switchgrass is best utilized as a forage early in the season, and regrowth is not a high-quality source of summer forage.

Enzymatic Hydrolysis Study

The small range of cellulose, hemicellulose, and ADL implies that there is little change in cell wall component concentrations of switchgrass after the boot stage. As previously mentioned, Sanderson and Wolf (1995) and Burns et al. (1997) described a rapid increase in NDF, ADF, and ADL and a decrease in digestibility early in the growing season of switchgrass. After an initial period of increasing fiber and decreasing digestibility, the rate of increase of NDF, ADF, and ADL slowed, and more mature switchgrass plants showed less change. Sanderson and Wolf (1995) stated that the phase of rapid fiber increase ended during the stem elongation stage. The stem elongation stage occurs before the boot stage (Moore et al, 1991). The present study analyzed maturity stages beginning with boot stage and proceeding through senescence. If rapid increase in fiber occurred during stem elongation as indicated by Sanderson and Wolf (1995), then the later maturity stages tested in the present study would show less change in levels of cell wall components. This would explain the apparent lack of variation in the cell wall components observed in the present research.

Glucose yields after dilute-acid pretreatment and enzymatic hydrolysis in the present study disagree with the findings of Dien et al. (2006), in which less mature plants

had greater efficiency of glucose conversion. The effect of lignin inhibiting enzymatic digestion (Chang and Holtzapple, 2000) was not shown in this study. In fact, the larger ADL values tend to correspond to the larger glucose yields. Another noticeable trend in these data is that maturity stages with the largest glucose yield have the smallest hemicellulose concentrations, and the maturity stages with the lesser glucose yields tend to correspond to the greater hemicellulose concentrations.

These results were somewhat unexpected. Previous studies (Chang and Holtzapple, 2000; Dien et al., 2006) show clearly that lignin does inhibit enzymatic cellulose conversion. Furthermore, Dien et al. (2006) noted that ADL was not a good estimator of lignin or glucose yield. They suggested Klason lignin as a better measure although ADL and Klason lignin are positively correlated (Moore and Jung, 2001).

The glucose values reported in the present study are less than the glucose values published by Dien et al. (2006). This is possibly due to the cooler temperature during acid pretreatment leading to less complete cell wall breakdown and less efficient enzymatic conversion. Generally, warmer temperatures improve efficiency of acid pretreatment (Kootstra et al., 2009). Previous research shows that a 150° C acid pretreatment allows more complete glucose conversion than biomass pretreated at 120° C (Dien et al., 2006). The present study used a temperature of 105° C during pretreatment. This is at the lower end of the 100 to 200° C range of temperatures used in acid pretreatment (Kootstra et al., 2009), though more likely to be practical in an industrial setting. Less heat during pretreatment could provide a possible explanation for the effects of hemicellulose on glucose yield in the present study. Hemicellulose, as well as lignin, surrounds the cellulose in the cell wall and reduces access to the cellulose (Sun

and Cheng, 2002). Hemicellulose is also the primary component removed during pretreatment (Kootstra et al., 2009). A cooler temperature during pretreatment may not thoroughly remove all the hemicellulose, leaving some to inhibit cellulose conversion, thereby "masking" the effects of lignin. The samples with more hemicellulose at the beginning of the hydrolysis process would likely have more hemicellulose after hydrolysis, explaining the negative relation between hemicellulose and glucose yield.

The results of the present study, contrary to Dien et al. (2006), indicate that hemicellulose concentration has a greater effect of inhibiting cellulose breakdown through enzymatic hydrolysis than does lignin. Cellulose, hemicellulose, and lignin levels in switchgrass did not change much after boot stage, and according to previous research (Sanderson and Wolf, 1995; Burns et al., 1997), the period of greatest change in switchgrass cell wall components occurs before boot stage. Perhaps greater variation of cell wall components would be observed if earlier maturity stages had been included in the study; however, prior to boot stage, switchgrass has likely not produced enough yield to justify harvesting for biomass purposes.

LITERATURE CITED

Burns, J. C., K. R. Pond, D. S. Fisher, and J. M. Luginbuhl. 1997. Changes in forage quality, ingestive mastication, and digesta kinetics resulting from switchgrass maturity. J. Anim. Sci. 75:1368-1379.

Burns, J. C. 2011. Intake and digestibility among caucasian bluestem, big bluestem, and switchgrass compared with bermudagrass. Crop Sci. 51:2262-2275.

Chang, V. S. and M. T. Holtzapple. 2000. Fundamental factors affecting biomass enzymatic reactivity. Appl. Biochem. and Biotechnol. 84-86:5-37.

Dien, B. S., H. G. Jung, K. P. Vogel, M. D. Casler, J. F. S. Lamb, L. Iten, R. B. Mitchell, and G. Sarath. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass Bioener. 30:880-891.

Griffin, J. L., and G. A. Jung. 1983. Leaf and stem forage quality of big bluestem and switchgrass. Agron. J. 75:723-726.

Hudson, D. J., R. H. Leep, T. S. Dietz, A. Ragavendran, and A. Kravchenko. 2010. Integrated warm-and cool-season grass and legume pastures: I. Seasonal forage dynamics. Agron. J. 102:303-309.

Jung, H. G.1989. Forage lignins and their effects on fiber digestibility. Agron. J. 81:33-38.

Jung, H. G. and M. S. Allen. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73:2774-2790.

Keswani, D. R. and J. J. Cheng. 2009. Switchgrass for bioethanol and other value-added applications: a review. Bioresource Tech. 100:1515-1523.

Kothman, M. 2009. Grazing methods: a viewpoint. Rangelands. 31:5-10.

McLaughlin, S. B. and L. A. Kszos. 2005. Development of switchgrass (Panicum virgatum) as a bioenergy feedstock in the United States. Biomass Bioener. 28:515-535

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. of AOAC International 85:1217-1240.

Mitchell, R., J. Fritz, K. Moore, L. Moser, K. Vogel, D. Redfearn, and D. Wester. 2001. Predicting forage quality in switchgrass and big bluestem. Agron. J. 93:118-124.

Moore, K. J., L. E. Moser, K. P. Vogel, S. S. Waller, B.E. Johnson, and J. F. Pederson. 1991. Describing and quantifying growth stages of perennial forage grasses. Agron, J. 83:1073-1077.

Moore, K. J., T. A. White, R. L. Hintz, P. K. Patrick, E. C. Brummer. 2004. Sequential grazing of cool- and warm-season pastures. Agron. J. 96:1103-1111.

Moore, K. J. and H. G. Jung. 2001. Lignin and fiber digestion. J. Range Manage. 54:420-430.

Parrish, D. J. and J. H. Fike. 2005. The biology and agronomy of switchgrass for biofuels. Crit. Rev. Plant Sci. 24:423-459.

Robinson, P. H., M. C. Mathews, and J. G. Fadel. 1999. Influence of storage time and temperature on in vitro digestion of neutral detergent fibre at 48h, and comparison to 48h in sacco neutral detergent fibre digestion. Animal Feed Sci. Tech. 80:257-266.

Sanderson, M. A. and D. D. Wolf. 1995. Switchgrass biomass composition during morphological development in diverse environments. Crop Sci. 35:1432-1438.

Sanderson, M. A., J. C. Read, and R. L. Reed. 1999. Harvest management of switchgrass for biomass feedstock and forage production. Agron. J. 91:5-10.

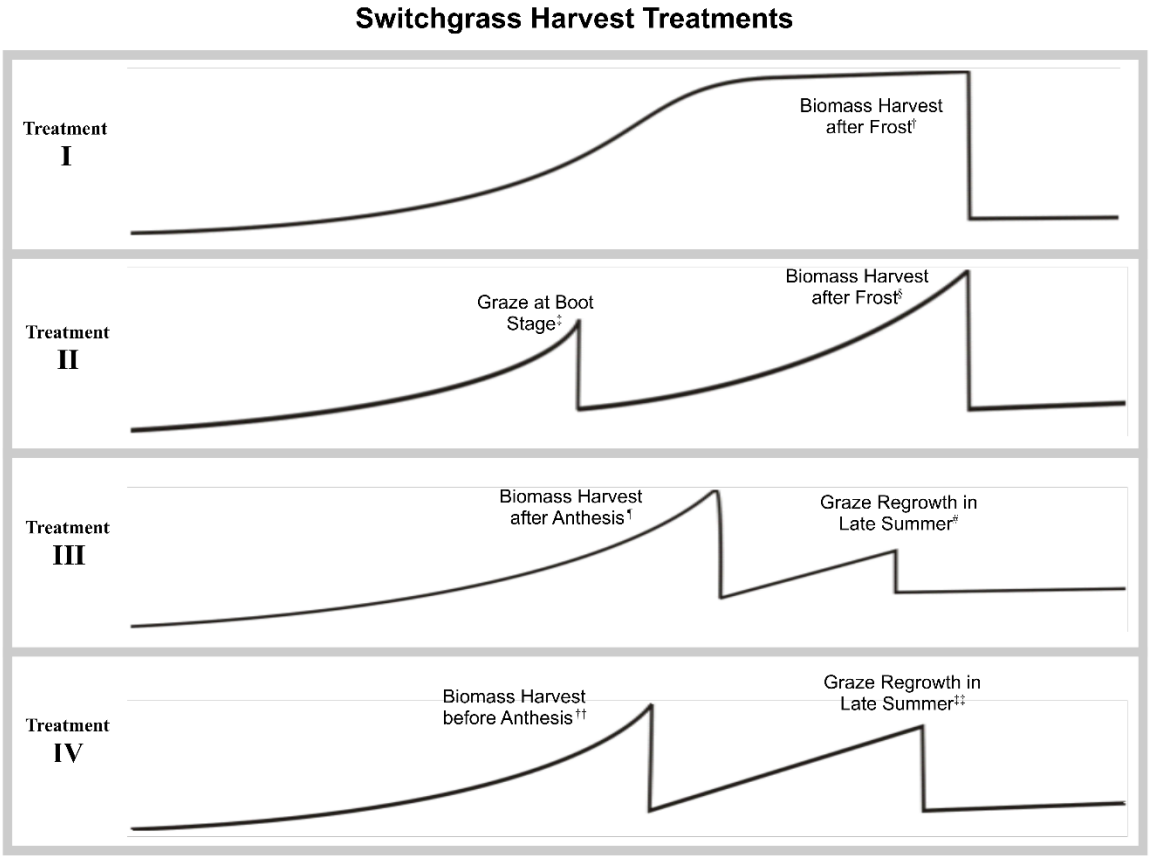
Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Mexico Series. 2006.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Viraton series. 2006.

Sun, Y. and J. Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology 83:1-11.

Van Soest, P. J., D. R. Mertens, and B. Deinum. 1978. Preharvest factors influencing quality of conserved forage. J. Anim. Sci. 47:712-720.

Figure 1. Schedule for treatments used in the harvest management study.



† 18 November 2010 and 29 November 2011 at Columbia, 22 November 2010 and 1 December 2011 at Mt. Vernon
‡4 June 2010 and 9 June 2011 at Columbia, 2 June 2010 and 3 June 2011 at Mt. Vernon
§ 29 November 2011 at Columbia, 1 December 2011 at Mt. Vernon
¶ 22 July 2010 and 1 August 2011 at Columbia, 27 July 2010 and 28 July 2011 at Mt. Vernon
# 19 August 2010 and 29 August 2011 at Columbia, 20 August 2010 and 26 August 2011 at Mt. Vernon
†† 23 June 2010 and 7 July 2011 at Columbia, 28 June 2010 and 8 July 2011 at Mt. Vernon
‡‡ 19 August 2010 and 29 August 2011 at Columbia, 20 August 2010 and 26 August 2011 at Mt. Vernon

Figure 2. Air temperatures and precipitation for 2010, 2011, and the 30-year average at Columbia and Mt. Vernon, Missouri, USA. Air temperatures (lines) represent bi-monthly averages. Precipitation (bars) are averaged by month.

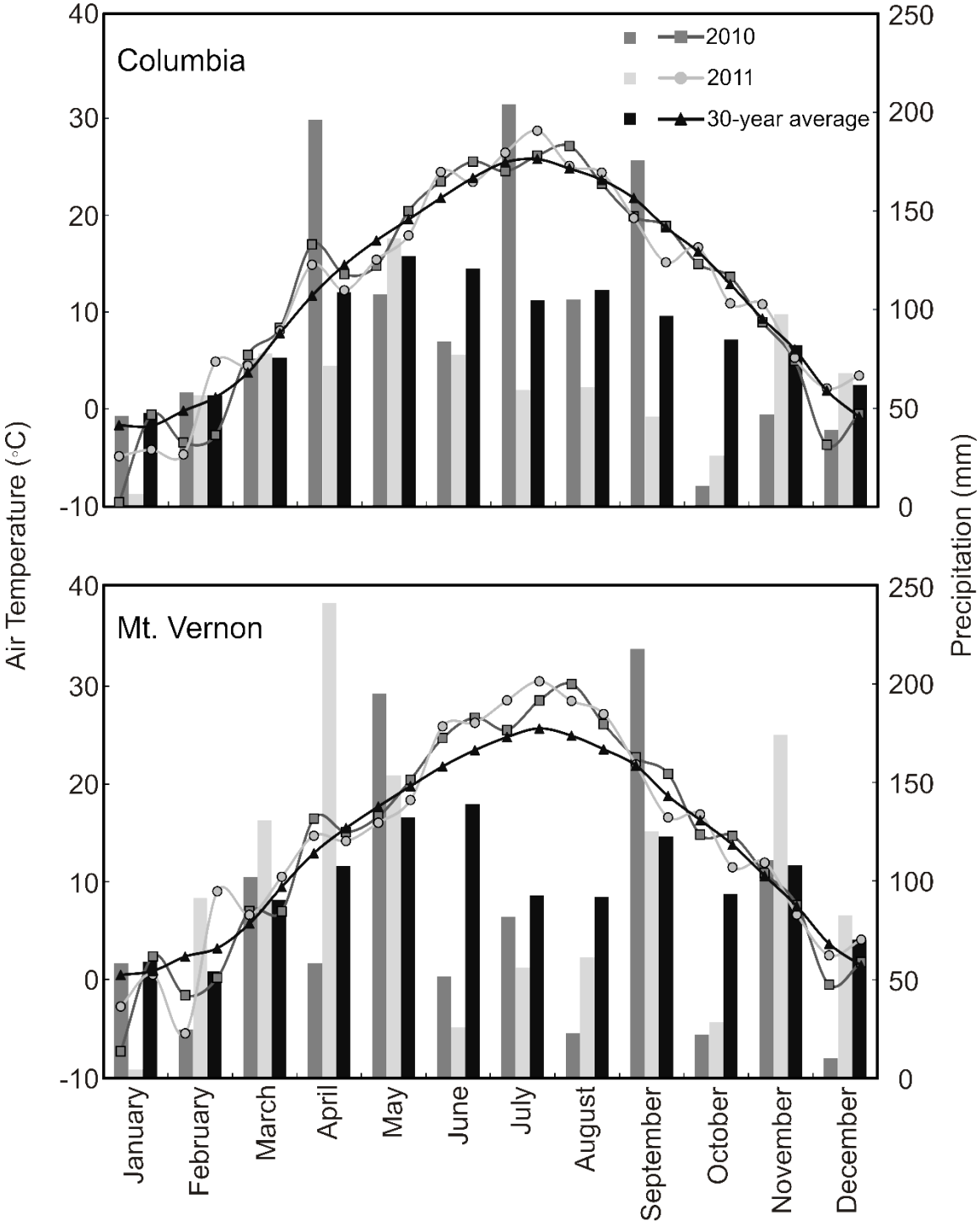


Table 1. Forage, biomass, and total yield of switchgrass harvested under one of four different management treatments. Treatment I was harvested once for biomass in autumn, Treatment II was harvested for forage at boot stage and regrowth was harvested for biomass in autumn, Treatment III harvested for biomass at post-anthesis and regrowth harvested for forage in late summer, Treatment IV harvested for biomass at pre-anthesis and regrowth harvested for forage in late summer. Values within a column within a grouping with different superscripts are significantly different.

	Forage Yield	Biomass Yield	Total Yield
	----------kg ha^{-1}----------		
2010			
Columbia			
Treatment I	0	6937^{a}	6937^{ab}
Treatment II	1875^{b}	-†	-†
Treatment III	1399^{b}	5864^{a}	7263^{a}
Treatment IV	2720^{a}	3090^{b}	5810^{b}
Mt. Vernon			
Treatment I	0	4700	4700
Treatment II	2465^{a}	-†	-†
Treatment III	185^{b}	6203	6388
Treatment IV	994^{b}	5268	6261
2011			
Columbia			
Treatment I	0	9727^{a}	9727^{a}
Treatment II	2169	2784^{c}	4952^{b}
Treatment III	1626	4870^{b}	6497^{b}
Treatment IV	2865	2457^{c}	5323^{b}
Mt. Vernon			
Treatment I	0	5208^{a}	5208^{b}
Treatment II	4197^{a}	2504^{b}	6701^{a}
Treatment III	477^{b}	5794^{a}	6270^{a}
Treatment IV	822^{b}	5249^{a}	6071^{ab}

† Data not available

Table 2. Nutritive values of switchgrass intended for forage use. Forage from treatment II was the initial growth harvested at boot stage, while for treatments III and IV the forage was the regrowth harvested in August after a biomass harvest earlier in the growing season. Treatment I is not included as it was harvested only for biomass after frost and not harvested as forage. Values with different superscripts within a column and within a year x location combination are significantly different.

	Crude Protein	NDF[†]	ADF[‡]	ADL[§]	IVTD[¶]
	----------g kg^{-1}----------				
2010					
Columbia					
Treatment II	100^{a}	670^{b}	320^{b}	30^{b}	750^{a}
Treatment III	110^{a}	690^{a}	330^{b}	60^{a}	680^{b}
Treatment IV	80^{b}	680^{ab}	360^{a}	60^{a}	650^{b}
Mt. Vernon					
Treatment II	90	680^{a}	330^{b}	30^{b}	770^{a}
Treatment III	90	690^{a}	370^{a}	50^{a}	640^{c}
Treatment IV	90	640^{b}	320^{b}	40^{b}	700^{b}
2011					
Columbia					
Treatment II	80	680^{a}	360^{ab}	60	700^{a}
Treatment III	70	670^{ab}	380^{a}	80	580^{b}
Treatment IV	80	660^{b}	360^{b}	100	620^{b}
Mt. Vernon					
Treatment II	70^{c}	630	320	60^{b}	730^{a}
Treatment III	110^{a}	650	350	60^{ab}	730^{a}
Treatment IV	90^{b}	650	340	90^{a}	690^{b}

[†]NDF=Neutral Detergent Fiber
[‡]ADF=Acid Detergent Fiber
[§]ADL=Acid Detergent Lignin
[¶] IVTD=In Vitro True Digestibility

Table 3. Glucose yield after dilute acid pretreatment and enzymatic hydrolysis and plant cell components of switchgrass at various maturity stages as determined by detergent fiber analyses. Switchgrass was harvested near Columbia, Missouri. Values within a column within a grouping with different superscripts are significantly different.

	Glucose Yield	Cell Solubles[†]	NDF[‡]	Hemi-cellulose[§]	ADF[¶]	Cellulose[#]	ADL[††]
	----------g kg^{-1}----------						
2010							
Boot	150^{b}	310^{a}	690^{d}	340^{a}	350^{c}	320^{c}	40^{d}
Pre-Anthesis	140^{b}	280^{bc}	720^{bc}	320^{b}	400^{b}	350^{b}	50^{c}
Post-Anthesis	140^{b}	270^{c}	730^{b}	320^{b}	410^{b}	350^{b}	60^{b}
Full Seed	230^{a}	290^{ab}	700^{cd}	290^{c}	410^{b}	350^{b}	70^{b}
Post-Frost	150^{b}	240^{d}	760^{a}	310^{b}	450^{a}	380^{a}	70^{a}
2011							
Boot	150^{b}	270^{a}	730^{b}	340^{a}	390^{c}	340^{b}	40^{d}
Pre-Anthesis	140^{b}	260^{a}	740^{b}	320^{b}	420^{b}	360^{ab}	60^{c}
Post-Anthesis	200^{a}	280^{a}	720^{b}	300^{c}	410^{b}	350^{b}	60^{b}
Full Seed	190^{a}	280^{a}	720^{b}	300^{c}	410^{b}	350^{b}	60^{ab}
Post-Frost	170^{ab}	230^{b}	770^{a}	330^{ab}	440^{a}	370^{a}	70^{a}

[†] Cell Solubles = portion of forage sample removed by neutral detergent wash
[‡] NDF = Neutral Detergent Fiber
[§] Hemicellulose = portion of forage sample removed by acid detergent wash
[¶] ADF = Acid Detergent Fiber
[#] Cellulose = portion of forage sample removed by ADL procedure
[††] ADL = Acid Detergent Lignin

GENERAL CONCLUSIONS

The premise of this research was to utilize an early-season biomass harvest to keep the switchgrass stand in a vegetative state so that it could be used as forage during late summer. However, regrowth of switchgrass does not necessarily have less fiber than initial growth cut at boot stage, and tends to be more lignified and less digestible. Results also indicate that, due to less available water, switchgrass regrowth is not a reliable source of summer forage on sites with shallow rooting depth. Switchgrass for forage should be harvested prior to boot stage to obtain the best quality.

In this study, efficiency of conversion of lignocellulose to glucose through enzymatic hydrolysis was not decreased by lignin content as was expected based on previous research. Rather, hemicellulose was seen to have a greater effect of inhibiting enzymatic breakdown of cellulose. This is possibly due to the cooler temperature during acid pretreatment allowing some hemicellulose to remain beyond the pretreatment stage and inhibit enzyme activity during the enzymatic hydrolysis stage.

Previous research states that cellulose and lignin rapidly increase during stem elongation and level off after this initial rapid increase. Cellulose, hemicellulose, and lignin levels in this study did not change much after boot stage as the major changes in cell wall composition had apparently already taken place during stem elongation. Prior to boot stage, switchgrass will not have produced enough yield to justify harvesting for biomass even if the plant material at that stage can be more efficiently converted to glucose. Therefore, the primary considerations in deciding when to harvest switchgrass for biomass should be yield and stand persistence.

REFERENCES

Aden, A., M. Ruth, K. Ibsen, J. Jechura, K. Neeves, J. Sheehan, B. Wallace, L. Montague, A. Slayton, and J. Lukas. 2002. Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. NREL/TP-510-32438 (DE-AC36-99-GO10337) US DOE, Golden, CO.

Adler, P. R., M. A. Sanderson, A. A. Boateng, P. J. Weimer, and H. G. Jung. 2006. Biomass yield and biofuel quality of switchgrass harvested in fall or spring. Agron. J. 98:1518-15-25.

Burns, J. C., K. R. Pond, D. S. Fisher, and J. M. Luginbuhl. 1997. Changes in forage quality, ingestive mastication, and digesta kinetics resulting from switchgrass maturity. J. Anim. Sci. 75:1368-1379.

Burns, J. C. 2011. Intake and digestibility among caucasian bluestem, big bluestem, and switchgrass compared with bermudagrass. Crop Sci. 51:2262-2275.

Casler, M. D. and A. R. Boe. 2003. Cultivar X environment interactions in switchgrass. Crop Sci. 43:2226-2233.

Casler, M. D., K. P. Vogel, C. M. Taliaferro, and R. L. Wynia. 2004. Latitudinal adaptation of switchgrass populations. Crop Sci. 44:293-303.

Casler, M. D., K. P. Vogel, C. M. Taliaferro, N. J. Ehlke, J. D. Berdahl, E. C. Brummer, R. L. Kallenbach, C. P. West, and R. B. Mitchell. 2007. Latitudinal and longitudinal adaptation of switchgrass populations. Crop Sci. 47:2249-2260.

Chang, V. S. and M. T. Holtzapple. 2000. Fundamental factors affecting biomass enzymatic reactivity. Appl. Biochem. Biotechnol. 84-86:5-37.

Demirbas, A. 2007.Producing and using bioethanol as an automotive fuel. Energy Sources 2:391-401.

Dien, B. S., H. G. Jung, K. P. Vogel, M. D. Casler, J. F. S. Lamb, L. Iten, R. B. Mitchell, and G. Sarath. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass Bioener. 30:880-891.

Griffin, J. L., and G. A. Jung. 1983. Leaf and stem forage quality of big bluestem and switchgrass. Agron. J. 75:723-726.

Heady, H. F. 1961. Continuous vs. specialized grazing systems: a review and application to the California annual type. J. Range Manage. 14:182-193.

Hopkins, A. A. and C. M Taliaferro. 1997. Genetic variation within switchgrass populations for acid soil tolerance. Crop Sci. 37:1719-1722.

Hsu, F. H., C. J. Nelson, and A. G. Matches. 1985. Temperature effects on seedling development of perennial warm-season forage grasses. Crop Sci. 25:249-255.

Hudson, D. J., R. H. Leep, T. S. Dietz, A. Ragavendran, and A. Kravchenko. 2010. Integrated warm-and cool-season grass and legume pastures: I. Seasonal forage dynamics. Agron. J. 102:303-309.

Jeoh, T. 1998. Steam explosion pretreatment of cotton gin waste for fuel ethanol production. M.S. thesis. Virginia Polytechnic Inst. and State Univ. Blacksburg.

Jung, H. G. 1989. Forage lignins and their effects on fiber digestibility. Agron. J. 81:33-38.

Jung, H. G. and M. S. Allen. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73:2774-2790.

Keswani, D. R. and J. J. Cheng. 2009. Switchgrass for bioethanol and other value-added applications: a review. Bioresource Tech. 100:1515-1523.

Kootstra, A. M. J., H. H. Beeftink, E. L. Scott, J. P. M. Sanders. 2009. Comparison of dilute mineral and organic acid pretreatment for enzymatic hydrolysis of wheat straw. Biochem. Eng. J. 46:126-131.

Kothman, M. 2009. Grazing methods: a viewpoint. Rangelands. 31:5-10.

Martinex-Reyna, J. M. and K. P. Vogel. 2002. Incompatibility systems in switchgrass. Crop Sci. 42:1800-1805.

McLaughlin, S. B. and M. E. Walsh. 1998. Evaluating environmental consequences of producing herbaceous crops for bioenergy. Biomass Bioener. 14:317-324.

McLaughlin, S. B. and L. A. Kszos. 2005. Development of switchgrass (Panicum virgatum) as a bioenergy feedstock in the United States. Biomass Bioener. 28:515-535

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. J. of AOAC International 85:1217-1240.

Mitchell, R., J. Fritz, K. Moore, L. Moser, K. Vogel, D. Redfearn, and D. Wester. 2001. Predicting forage quality in switchgrass and big bluestem. Agron. J. 93:118-124.

Moore, K. J., L. E. Moser, K. P. Vogel, S. S. Waller, B.E. Johnson, and J. F. Pederson. 1991. Describing and quantifying growth stages of perennial forage grasses. Agron, J. 83:1073-1077.

Moore, K. J. and H. G. Jung. 2001. Lignin and fiber digestion. J. Range Manage. 54:420-430.

Moore, K. J., T. A. White, R. L. Hintz, P. K. Patrick, E. C. Brummer. 2004. Sequential grazing of cool- and warm-season pastures. Agron. J. 96:1103-1111.

Parrish, D. J. and J. H. Fike. 2005. The biology and agronomy of switchgrass for biofuels. Crit. Rev. Plant Sci. 24:423-459.

Porter, C. L., Jr. 1966. An analysis of variation between upland and lowland switchgrass *Panicum virgatum* L. in central Oklahoma. *Ecology* 47: 980–992.

Robinson, P. H., M. C. Mathews, and J. G. Fadel. 1999. Influence of storage time and temperature on in vitro digestion of neutral detergent fibre at 48h, and comparison to 48h in sacco neutral detergent fibre digestion. Animal Feed Sci. Tech. 80:257-266.

Sanderson, M. A. and D. D. Wolf. 1995. Switchgrass biomass composition during morphological development in diverse environments. Crop Sci. 35:1432-1438.

Sanderson, M. A., J. C. Read, and R. L. Reed. 1999. Harvest management of switchgrass for biomass feedstock and forage production. Agron. J. 91:5-10.

Sarath, G., L. M. Baird, K. P. Vogel, R. B. Mitchell. 2007. Internode structure and cell wall composition in maturing tillers of switchgrass. Bioresource Tech. 98:2985-2992.

Schmer, M. R., K. P. Vogel, R. B. Mitchell, and R. K. Perrin. 2008. Net energy of cellulosic ethanol from switchgrass. PNAS 105:464-469.

Schubert, C. 2006. Can biofuels finally take center stage. Nature Biotech. 24:777-784.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Mexico Series. 2006.

Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Official Soil Series Descriptions. Viraton series. 2006.

Somerville, C., H. Youngs, C. Taylor, S. C. Davis, and S. P. Long. 2010. Feedstocks for cellulosic biofuels. Science (Washington DC) 329:790-792.

Sun, Y. and J. Cheng. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Tech. 83:1-11.

Van Soest, P. J., D. R. Mertens, and B. Deinum. 1978. Preharvest factors influencing quality of conserved forage. J. Anim. Sci. 47:712-720.

Vogel, K. P., J. J. Brejda, D. T. Walters, and D. R. Buxton. 1998. Switchgrass biomass production in the Midwest USA: harvest and nitrogen management. Agron. J. 94:413-420.