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Nitrogen and harvest date affect developmental morphology and biomass yield of warm-season grasses

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Abstract

Information on the growth and development of warm-season grasses in response to management is required to use them successfully as a biomass crop. Our objectives were to determine optimum harvest periods and effect of N fertilization rates on the biomass production of four warm-season grasses, and to investigate if traits of canopy structure can explain observed yields with varying harvest dates and N rates. A field study was conducted at Sorenson Research Farm near Ames, IA, during 2006 and 2007. The experimental design was split-split plot arranged in a randomized complete block with four replications. Big bluestem (*Andropogon gerardii* Vitman), eastern gamagrass (*Tripsacum dactyloides* L.), indiangrass (*Sorghastrum nutrans* L. Nash), and switchgrass (*Panicum virgatum* L.) were main plots. Three N application rates (0, 65, and 140 kg ha^{-1}) were subplots, and 10 harvest dates were sub-sub plots. Biomass of warm-season grasses increased with advanced maturity, but differently among species. The maximum yield of eastern gamagrass occurred at the highest MSC (1.6 and 2.2) when the largest seed ripening tillers were present. Big bluestem, switchgrass, and indiangrass obtained the maximum yields at MSC 3.5, 3.9, and 2.9, respectively when the largest reproductive tillers were present. In terms of a biomass supply strategy, eastern gamagrass may be used during early summer, while big bluestem and switchgrass may be best used between mid- and late- summer, and indiangrass in early fall. Nitrogen fertilization increased yield by increasing tiller development. Optimum biomass yields were obtained later in the season when they were fertilized with 140 kg ha^{-1}.

Keywords: bioenergy, biomass feedstock, big bluestem (*Andropogon gerardii Vitman*), eastern gamagrass (*Tripsacum dactyloides L.)* indiangrass (*Sorghastrum nutrans L. Nash*), switchgrass (*Panicum virgatum L.*), eastern gamagrass (*Tripsacum dactyloides L.*)

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Introduction

Across the Midwest and Great Plains of the USA, native prairie grasses including switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman.), indiangrass (*Sorghastrum nutrans* Nash.), and eastern gamagrass [*Trisacum dactyloides* (L.) L] have been used to supplement the uneven distribution of forage production throughout the grazing season when cool-season grasses are relatively unproductive during the hot summer months (Burns & Bagley, 1996; Massengale, 2000). In many parts of the world, C_4 grasses have recently attracted considerable interest as a source plant biomass to produce energy (Lewandowski *et al.*, 2003). Excellent yield potential and efficient use of resources, especially nitrogen, are important traits which make these grasses desirable for biomass energy production (McKendry, 2002; Heaton *et al.*, 2004).

High concentration of lignocellulosic materials (cellulose, hemicelluloses, and lignin) in biomass is desirable for conversion into different energy or a chemical end product by either biochemical or thermochemical processes (McLaughlin *et al.*, 2002). Lignocelluloses are the main components making up plant cell walls and forming the structural materials of biomass including leaves, stems, and stalks. Therefore, the quantity of biomass produced by warm-season grasses per unit area of production determines the potential energy production capacity of the available land area. Therefore, the more biomass yield produced, the more energy yield is generated.

To maximize dry matter yield with desirable biofuel quality, N fertilization and harvest management are important considerations that can reduce the major costs of producing biomass (Keeney & DeLuca, 1992; Vogel *et al.*, 2002; Lemus *et al.*, 2008), and improve biomass quality by minimizing the concentration of minerals (McKendry, 2002). In Midwest states and the

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Abbreviations: MSC, mean stage count; DM, dry matter; HHV, high heating value.

Central Great Plains of the USA, most studies have reported that significant fertilizer N inputs are required to optimize biomass production by warm-season grasses when managed as forage crops (reviewed by Brejda, 2000). Of the few studies that have assessed the effect of N fertilization on yield of a perennial, warm-season grass managed for bioenergy, most were conducted on switchgrass (Ma *et al*., 2001; Muir *et al*., 2001; Thomason *et al*., 2004; Lemus *et al*., 2008), a model crop for bioenergy feedstock production (Sanderson *et al*., 1996). Alternative species could reduce risks from relying on only single species and extend the range and profitability of biofuel production system. To maximize their yield for biomass at a regional level, different species and management practices have to be determined. According to a review by Parrish & Fike (2005), there is still no clear consensus on best management of N fertility in switchgrass. For example, the optimum biomass yield was achieved in Iowa and Nebraska when switchgrass was fertilized with 120 kg ha^{-1} (Vogel *et al*., 2002), but 168 kg ha^{-1} was required in Texas (Muir *et al*., 2001). In addition, information on the optimum N fertilization rate for other warm-season grasses managed for biomass production is limited or non-existent.

Similar to N fertility, many studies on harvest management of warm-season grasses have been conducted for forage production (Sanderson, 2000), but limited research information is available on harvest schedules for biomass production. For best compromise between forage yield, quality, and plant persistence for hay production, native warm-season grasses should be harvested when grasses are at least 45–60 cm tall and before the boot (Moser & Vogel, 1995). Forage quality of native warm-season grasses is enhanced by cutting more frequently (Brejda *et al*., 1996; Sanderson, 2000). However, harvest management of native warm-season grasses for biomass production may be different from forage production because the objectives of the producer are different. In biomass production, the objective is to obtain high lignocellulose yield with a low mineral concentration. In contrast to forage production, a single late-season harvest may work best for biomass production (Sanderson, 2000). In the Midwest, Vogel *et al*. (2002) demonstrated that maximum first-cut yields were obtained at the 3.3 (R3) to 3.5 (R5) stage of maturity (panicles fully emerged to postanthesis), and depending on the year, sufficient regrowth may be obtained for a second harvest after a killing frost. They suggested that whether or not a second harvest is made will depend on biomass yield and price and cost of harvesting.

Changes in plant morphology occurring during growth can be important determinants of potential productivity and quality in perennial forage grasses (Frank *et al*., 1993; Redfearn *et al*., 1997). Thus, changes in the developmental morphology of grasses will influence management practices such as timing of initial harvest and fertilizer application (Moore *et al*., 1991; Frank *et al*., 1993).

Canopy architecture is important for describing many grass canopy processes influenced by the interaction between plants and the environment (Welles & Norman, 1991). Tiller density is an important trait of canopy architecture, related to relative grass productivity and quality. Redfearn *et al.* (1997) reported that reduced yields of switchgrass were expected to occur as a result of low plant densities. Accumulation of a large number of reproductive tillers provided greater yield for forage species compared with forage species with less reproductive tillers.

Quantification of the developmental morphology of tiller populations indicates the architectural changes in the grass sward. The quantifying system for morphological development of grasses developed by Moore *et al.* (1991) is applicable to most annual and perennial grass swards, and is easily applied in the field.

Little is known about developmental morphology for native warm-season swards in relation to tiller and canopy architecture as affected by the interaction of harvest date and N fertilization. Elementary information on the growth and development of the canopy structure response to management is required to use native warm-season grasses successfully as a biomass crop. Therefore, the objectives of this research were: (i) to determine optimum harvest periods and the effect of N fertilization rates on the production of warm-season grasses as a biomass crop; and (ii) to investigate if traits of canopy architecture can explain observed yields of warm-season grasses with varied harvest dates and N fertilization rates.

Material and methods

Plant establishment

Field experiments were conducted during 2006 and 2007 at the Iowa State University Sorenson Farm, near Ames, IA (42°0′40″ N, 93°44′46″W) on Canisteo silty clay loam (fine-loamy, mixed, superactive, calcareous, mesic, Typic Endoaquoll), Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaquoll), and Clarion loam (fine-loamy, mixed, superactive, mesic, Typic Hapludoll). Weather data were compiled from the Iowa Environmental Mesonet and were collected from a weather station located approximately 4.8 km from the research site (Waramit *et al*., 2010). The experiment was laid out as a split-split plot design with hierarchal classification in the subplots in a completely randomized block with four replications. Four warm-season grass species, big bluestem (‘Roundtree’), indiangrass (‘Rumsey’), eastern gamagrass (‘Pete’), and switchgrass (‘Cave-In-Rock’) were main plots measuring

3×42.8 m. Three N application rates (0, 65, and 140 kg N ha^{-1}) were subplots (3×10.7 m) and the 10 harvest dates were sub-sub plots, all randomly assigned.

Grass plots were established in a fallow managed in a corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] rotation. Based on the size, weight of individual seeds of grass species and soil conditions, proper seeding rates for successful stand establishment were different among grass species (Mitchell & Britton, 2000). Big bluestem, indiangrass, switchgrass, and eastern gamagrass were seeded at 3.6, 3.6, 2.3, and 4.5 kg pure live seed (PLS) ha^{-1} (or at rate 127, 139, 197, and 8 PLS m^{-2}, approximately), respectively, in the fall of 2003. Based on plant size and growth habit of grass species (Barnhart, 1994; Masters *et al*., 2004), the three former species were seeded in 20-cm rows using a 10-row small grain drill (Tye model 2007, AGCO Co., Lockney, TX) while eastern gamagrass was seeded in 76-cm rows using a 2-row corn planter (John Deere model 71 Flexi Planter, John Deere Co., Moline, IL, USA). Uniform plant density was maintained before the experiment was started in spring 2006. Big bluestem and indiangrass plots were overseeded to increase the plant density in the spring of 2005.

Before initiation of spring growth each year, imazapic [(RS)-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2 yl)-5-methylnicotinic acid] was applied at a rate of 140 g a.i. ha^{-1} to indiangrass and atrazine [6-chloro-N-ethyl-N′-(1-methylethyl)-1,3,5,-triazine-2,4-diamine] was applied at a rate of 2.24 kg of a.i. ha^{-1} to all other species for weed control. In early May of 2004 and 2005, nitrogen fertilizer was applied at a rate of 85 kg N ha^{-1}. Eastern gamagrass plots received a single inter-row cultivation in June 2004 and 2005. Standing dead material was annually mowed to a 5 cm stubble height, and the residue was removed from plots using a self-propelled forage harvester (John Deere model 5480, John Deere Co., Moline, IL, USA). In April 2006, prescribed fires were applied in all grass plots in order to decrease weed density and to remove the majority of accumulated above-ground material before the initiation of N treatments, (Moser & Vogel, 1995; Mitchell & Britton, 2000).

Nitrogen treatments

Ammonium nitrate (NH_4NO_3: 0, 65, and 140 kg N ha^{-1}) was preweighed and applied with 1.5 m wide drop spreader (Model 6500, Gandy Co., Owatonna, MN, USA) on each N treatment subplot on 8 May 2006 and 12 May 2007. Based on soil test results in a companion research project conducted by Heggenstaller *et al.* (2009) and the characteristics of native warm-season grasses which have wide range of adaptation and ability to be productive with low soil fertility, no other fertilizer was needed.

Biomass harvest

Biomass samples were harvested on 10 dates between 16 May and 3 October in 2006, and 22 May and 8 October in 2007. The first harvest occurred at an early vegetative growth stage and depended on spring growth in each year. For the first seven harvest dates (at day 136, 151, 164, 178, 192, 206, 220 for 2006; day 142, 155, 169, 183, 197, 211, and 225 for 2007), the samples were collected at approximately 2-week intervals and at approximately 3-week intervals for last three harvest dates (day 234, 255, and 276 for 2006; day 239, 260, and 281 for 2007). At each harvest, the developmental stage of the live grass stands were visually scored using the system of Moore *et al.* (1991).

At each sampling date, the tillers used for morphological classification were hand-clipped at ground level from two quadrats of 0.38-m^2 that were randomly located within each subplot. After recording fresh weights, the samples were dried at 60 °C in a forced-air oven for approximately 72 h and reweighed to determine dry matter yield.

Quantifying developmental morphology

Before oven-drying, samples at each harvest date were examined to determine the morphological development of the tiller populations using the mnemonic scale developed by Moore *et al.* (1991). The life cycle of individual grass tillers was divided into four primary growth stages including: (i) vegetative; (ii) elongation; (iii) reproductive; and (iv) seed ripening. Substages within each primary growth stage describe specific morphological events occurring in most grasses. To quantify the developmental morphology of a population of tillers, the mean stage by count (MSC) was calculated using the following equation:

$$\text{Mean Stage by Count (MSC)} = \sum_{i=0}^{4.9} \frac{S_i N_j}{C}$$

where Si = growth stage, i = 0 to maximum growth stage (4.9), Ni = number of tillers in stage Si, C = total number of tillers. Quantifying morphological development of the tiller population was reported as a decimal value of a primary growth stage. A more morphologically advanced tiller population was indicated by a higher MSC value.

Statistical analysis

Data were analyzed with the SAS MIXED procedure (Littell *et al*., 1996) with grass species and nitrogen application rates considered as fixed effects, and block and interactions with blocks considered as random effects. Least squares means for species, nitrogen application rates, harvest dates, and interactions were separated by the SAS PDIFF option. All differences were considered significant at the 0.05 probability level.

Results

Mean stage count

For MSC of all four warm-season grasses, significant effects were detected for the interaction of the grass species × N rate, the species × harvest date, in both years, and the species × N rate × harvest dated in 2007 (Table 1). Consequently, the effects of harvest date and N rate on MSC were evaluated and reported separately

Table 1 Mean stage counts (MSC) and dry matter yield *F*-values and significances in response to four species, three nitrogen rates and ten harvest times during the growing season of 2006 and 2007 at Ames, IA

Effect	2006	2007
MSC		
Species (S)	231.70**	403.26**
N rate (N)	22.60**	41.06**
S × N	8.62**	4.78**
Harvest (H)	546.32**	814.31**
S × H	83.26**	119.51**
N × H	2.86**	3.16**
S × N × H	1.02 ns	1.76**
Dry matter		
S	16.87**	11.32**
N	9.17**	18.77**
S × N	1.22 ns	1.71 ns
H	107.17**	87.70**
S × H	6.97**	4.28**
N × H	1.92*	1.63 ns
S × N × H	0.81 ns	0.90 ns

ns, non-significant.
*significant at the 0.05 probability level.
**significant at the 0.01 probability level.

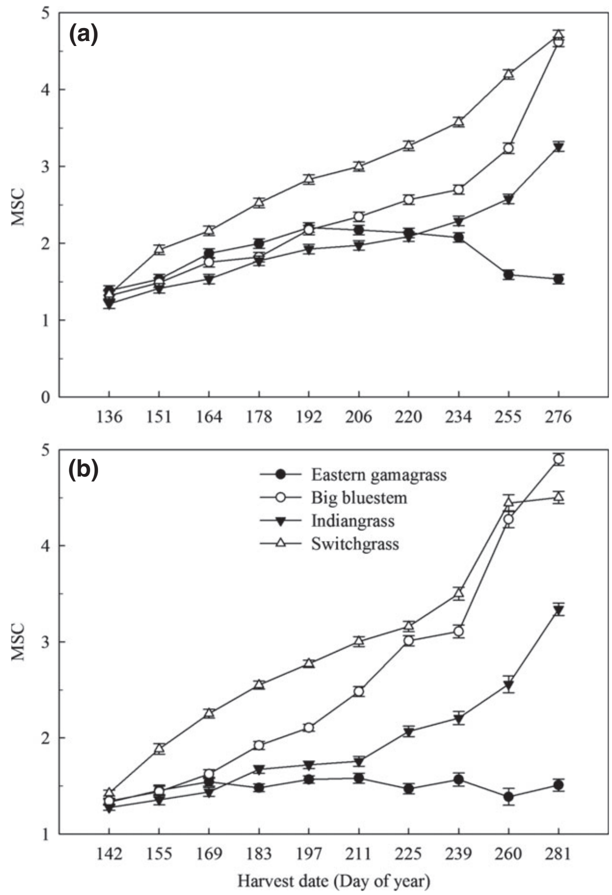

Fig. 1 Mean stage count as influenced by eastern gamagrass (EG), big bluestem (BB), indiangrass (ID), and switchgrass (SW) and ten harvest dates. Data are averaged over four replications and three nitrogen rates, in (a) 2006; and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

by species. The species × harvest date interaction suggested that the MSC for big bluestem, indiangrass, and switchgrass increased until the final harvest in 2006 and 2007 (Fig. 1). The MSC for switchgrass was always greater than that for other species on common harvest dates, illustrating that switchgrass matured more rapidly than other species. Big bluestem and switchgrass had a larger proportion of tillers developing to the seed production stage late in the season than did other two species (Fig. 2 and 3), resulting in the greater MSC for big bluestem and switchgrass in both years (Fig. 1). Few indiangrass tillers reached reproductive maturity before the completion of harvest causing a lower MSC, as indicated by tiller demographics (Fig. 2 and 3). In contrast, little change in the MSC of eastern gamagrass with harvest date was observed in either year. The MSC for eastern gamagrass increased gradually from the first harvest to the fifth harvest (from day 136 to 192 for 2006; from day 142 to 197 for 2007) and decreased until the final harvest (Fig. 1). The species × N rate interaction suggested that the MSC of big bluestem, indiangrass, and switchgrass slightly increased with N fertilization while N fertilization had no effect on the morphological development of eastern gamagrass (Fig. 4). The N rate × harvest date interaction indicated that the increase in the mean maturity of grasses receiving N fertilization advanced MSC late in the season of 2006, and between mid and late in the season of 2007 (Fig. 5). Conversely, the MSC for eastern gamagrass decreased when N was applied at 140 g ha^{-1} in 2006 (Fig. 4).

Dry matter yield

For dry matter yield of the warm-season grasses, the interaction effects of species x harvest date in both years, and N rate × harvest date in 2006 were detected (Table 1). As a result, the effect of harvest date on dry matter yield was evaluated separately by grass species and N rate. The species × harvest date interaction indicated that dry matter yield of all species increased to their maximum as the growing season progressed (Fig. 6). After the maximum-yield harvest periods, biomass yields were reduced. But yield reduction was not significant for switchgrass in 2007 and indiangrass in both years.

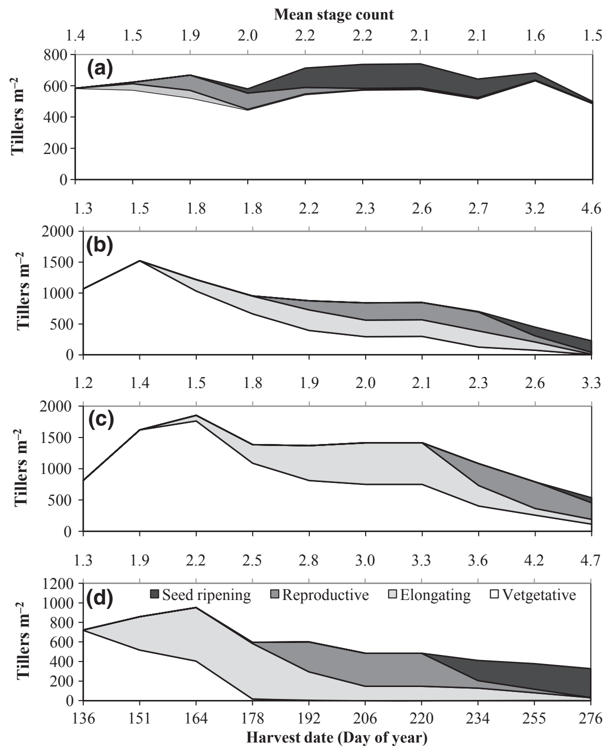

Fig. 2 Tiller demographics and mean stage counts for eastern gamagrass (a), big bluestem (b), indiangrass (c); and switchgrass (d) grown near Ames, IA, in 2006.

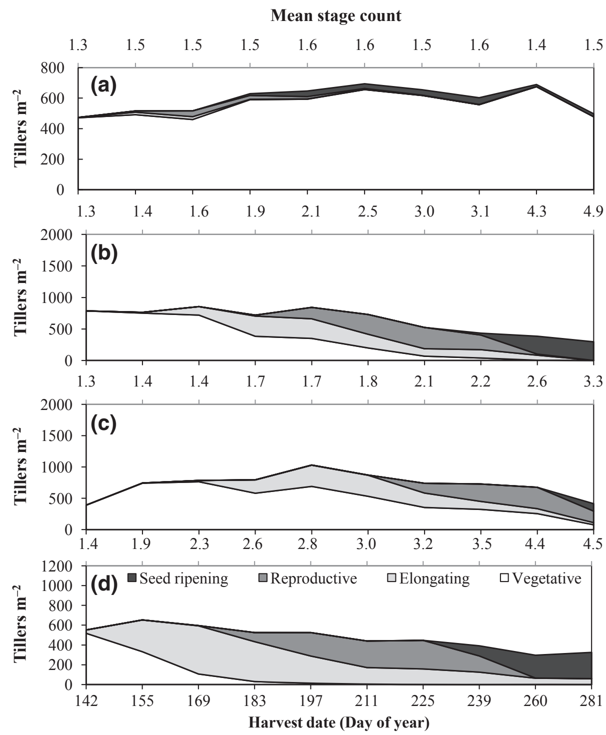

Fig. 3 Tiller demographics and mean stage counts for eastern gamagrass (a), big bluestem (b), indiangrass (c); and switchgrass (d) grown near Ames, IA, in 2007.

Dry matter accumulation of eastern gamagrass was the greatest ranging from 24.4 to 27.8 Mg ha^{-1} at between the fifth harvest (day 192; MSC = 2.2) and eighth harvest (day 234; MSC = 2.1) in 2006 (Fig. 6). For 2007, maximum yield ranged from 17.1 to 20.9 Mg ha^{-1}, occurring between the fifth (day 197; MSC = 1.6) and ninth harvest (day 260; MSC = 1.4) (Fig. 6). For big bluestem, maximum yield (21.9 Mg ha^{-1}) occurred at the eighth harvest (day 234) when swards were at late elongation stage (MSC = 2.7) in 2006. Similar to eastern gamagrass for 2007, maximum yield of big bluestem ranged from 18.0 to 20.5 Mg ha^{-1} occurring between the sixth (day 211) and ninth harvest (day 260) when swards were between mid-elongation (MSC = 2.5) and soft dough stages (MSC = 4.3). Maximum yields of switchgrass occurred between the sixth (day 206) and ninth harvest (day 255) in 2006 when swards were between boot stage (MSC = 3.0) and milk/soft dough stage (MSC = 4.2). They ranged from 15.7 to 19.0 Mg ha^{-1}. In 2007, maximum yields of switchgrass occurred between the fifth (day 211) and final harvest (day 281) when swards were between boot stage (MSC = 3.0) and hard dough stage (MSC = 4.5). Maximum yields obtained at these harvest dates ranged from 11.7 to 15.0 Mg ha^{-1}. Dry matter yield for indiangrass peaked later in the season than did other species in both years. Maximum dry matter accumulation of indiangrass ranged from 15.6 to 19.6 Mg ha^{-1} in 2006 and from 12.3 to 14.7 Mg ha^{-1} in 2007, between the eighth (in September) and final harvest (in October) when the swards were between early elongation (MSC = 2.2–2.3) and early reproductive stage (MSC = 3.3). After peaking, yields decreased up to 19% for switchgrass, 38% for big bluestem, and 61% for eastern gamagrass, at the final harvest in 2006. For the final harvest in 2007, big bluestem and eastern gamagrass yields were reduced by 30% and 54% of the maximum yield, respectively (Fig. 6).

There was no species × nitrogen rate interaction for biomass dry matter yield. Total biomass yields of all four species increased as increased application rate of N in both years. Nitrogen fertilization at 65 and 140 kg ha^{-1} increased total biomass across four species and ten harvest dates by 6.5% and 24%, respectively in 2006, and by 26% and 49%, respectively in 2007 (Fig. 7). In both study years, the N rate × harvest date suggested that the increase in biomass yields with N fertilization occurred between mid-growing season and later season (Fig. 7), when grass swards had a great proportion of elongated tillers (Fig. 2 and 3).

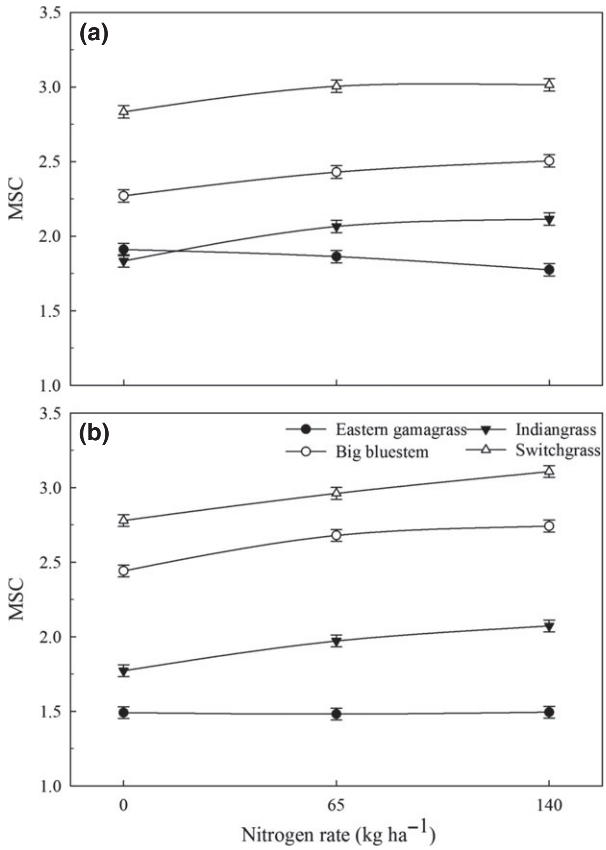

Fig. 4 Mean stage count of eastern gamagrass (EG), big bluestem (BB), indiangrass (ID), and switchgrass (SW) as influenced by nitrogen application rate. Data are averaged over four replications and harvest dates, in (a) 2006; and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

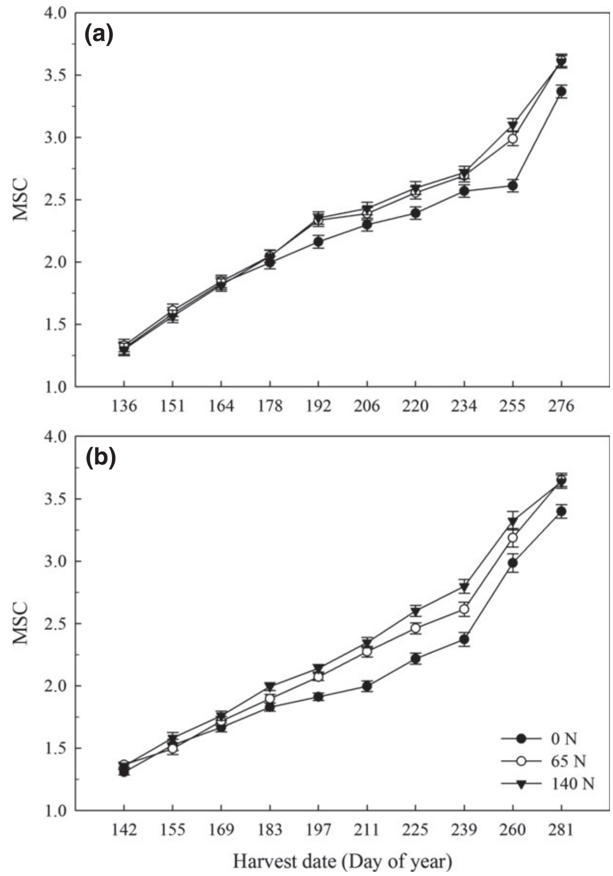

Fig. 5 Mean stage count as influenced by three nitrogen rates at 0 (0°N), 65 (65°N), and 140 (140°N) kg N ha^{-1} and ten harvest dates. Data are averaged over four replications and four species in (a) 2006 and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

Discussion

Morphological development of warm-season grasses varied with harvest date, with potentially important implications for crop management. Improper timing of warm-season grass cutting results in low quality and low yield, and may be detrimental to stand persistence (Moore & Moser, 1995; Mitchell *et al.*, 2001). Waller *et al.* (1985) suggested that if grazing was delayed until day 183 of year or until at least 90% of elongating tillers had appeared, then regrowth would be limited following defoliation. Our study shows that change in the MSC of warm-season grasses with harvest date was different among grass species. Notably, the MSC for eastern gamagrass increased gradually during the first five harvests and decreased until the final harvest which was different from other species (Fig. 1). This is likely due to the appearance of new tillers in grass sward across the season (Fig. 2 and 3). Dewald & Louthan (1979) and Jackson & Dewald (1994) reported that eastern gamagrass is indeterminate with respect to reproductive growth. Appearance of new vegetative tillers and spikes on the same plant occurs over a considerable time period in the season. This makes each plant a multiaged population of vegetative and reproductive tillers. Lemke *et al.* (2003) observed visually during the course of their study that only about 10% of the tillers on a grass advance to reproductive growth, whereas the rest remain vegetative. Lemus *et al.* (2002) suggested that a lower leaf to stem ratio may improve biomass quality because stems have a higher fiber content. Thus, the proportion of stem tissue could be one of the determinants of the biofuel quality of grasses. In this study, the large proportion of elongating, reproductive, and seed ripening tillers after the fourth harvest could reinforce the importance of harvesting warm-season grasses for biomass in early summer. But harvesting at a later stage increases lignocellulose and decreases minerals in biomass (Jung & Vogel, 1992; Madakadze *et al.*, 1999; Mitchell *et al.*, 2001; Adler *et al.*, 2006; Mulkey *et al.*, 2006; Waramit *et al.*, 2010). Thus, a single

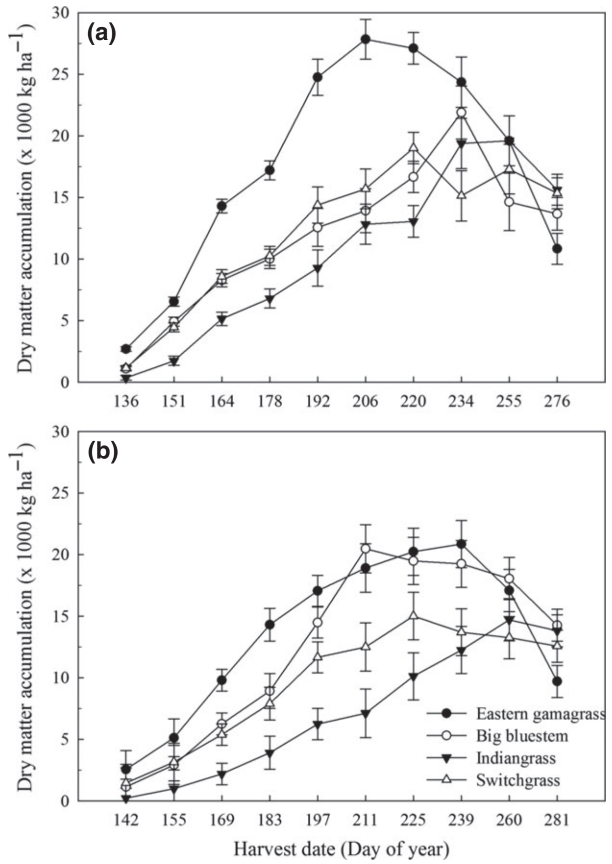

Fig. 6 Mean dry matter accumulation as influenced by eastern gamagrass (EG), big bluestem (BB), indiangrass (ID), and switchgrass (SW) and ten harvest dates. Data are averaged over four replications and three nitrogen rates, in (a) 2006; and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

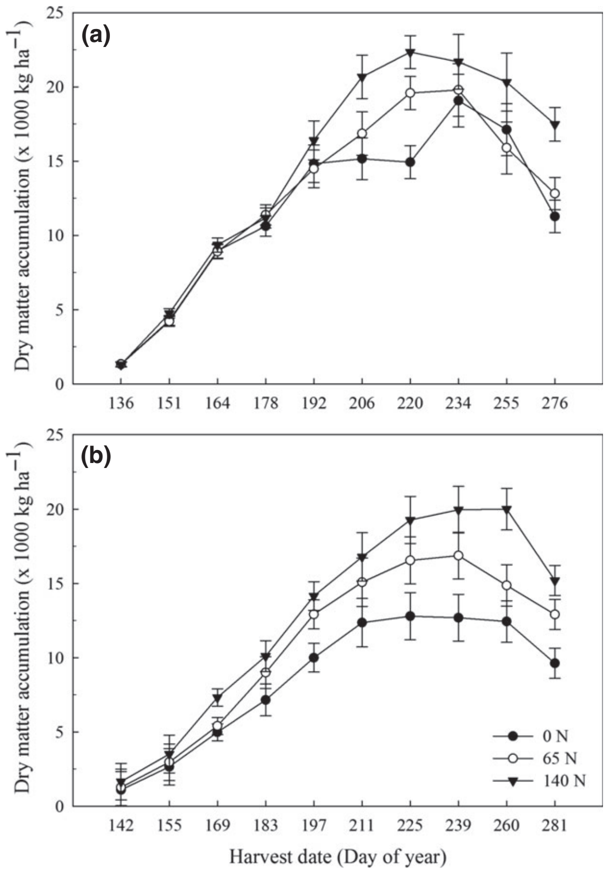

Fig. 7 Mean dry matter accumulation as influenced by three nitrogen rates at 0 (0°N), 65 (65°N), and 140 (140°N) kg N ha^{-1} and ten harvest dates. Data are averaged over four replications and four species in (a) 2006; and (b) 2007, at Ames, IA. Standard error bars are partially covered by graph symbols.

late-season harvest may be more beneficial for bioenergy production. Our data also indicate that the application of N fertilization increased the mean maturity of grasses and advanced MSC (Fig. 5). These results are similar to a study of Hill & Loch (1993) suggested that application of N can increase inflorescence density per unit area. Additionally, Harlan & Kneebone (1953), George & Reigh (1987), and Masters *et al.* (1993) demonstrated that N application significantly increased the density of reproductive tillers and seed produced for big bluestem and switchgrass. This is different from the trend found in this study for eastern gamagrass, in which MSC decreased when N was applied at 140 g ha^{-1} in 2006 (Fig. 4). This is likely because the high rate of N application in eastern gamagrass increased vegetative growth while it suppressed reproductive growth, leading to diluted MSC. Light penetration into the crown area of the plants can be decreased by excessive vegetative growth from N application, resulting in decreased inflorescence formation. The reproductive stem density in grasses was increased as light penetration into the canopy increased (Lemke *et al*., 2003). The increase in reproductive stem density as light penetration into the canopy is also found in other warm-season grasses. Knapp (1984) reported that reproductive stem density for big bluestem was increased when plant debris substantially removed, leading to improving the light environment of emerging shoots. A three-way interaction of species × N rate × harvest date for MSC occurring in 2007 (Table 1) is likely attributed to a small change in the MSC of eastern gamagrass with advanced maturity between mid and final harvests, while for other species MSC significantly increased. However, this three-way interaction and other two-way interactions of species × N rate, N rate × harvest date contributed little variability when compared with the main effects in this study. This assumes that grass species and harvest dates appear to be more important to the MSC index of a biomass crop than the N rate.

Biomass of warm-season grasses increased to their maximum as the growing season progressed. These results are similar to a previous study by Vogel *et al.* (2002) reporting that maximum yields of switchgrass grown in the Midwestern USA occurred in mid-August at full panicle emergence to postanthesis. However, harvesting switchgrass during mid-August may decrease the long-term stand densities as observed in the north-central USA by Casler & Boe (2003) and this is reflected in lower yields. Noticeably, yields for big bluestem, indiangrass, and switchgrass peaked when swards had the largest proportion of reproductive tillers before the onset of seed development (Fig. 2, 3, and 6). These grasses are determinate in growth habit. With inflorescence development, most vegetative growth of them terminates. Generally, when the tillers advance to the seed ripening stages, growth stops, and tiller senescence occurs (Dahl & Hyder, 1977). Therefore, maximum biomass yield occurred when grasses had the lower proportion of vegetative tillers, and the larger proportion of reproductive and seed ripening tillers (Fig. 2 and 3). In contrast, eastern gamagrass is indeterminate in growth habit with the earlier appearance of new reproductive tillers between mid- and late- growing season. This study shows that maximum dry matter yield of eastern gamagrass, therefore, occurred at harvest periods with the highest MSC index. In these periods, the largest proportion of seed tillers was present within swards. Interestingly, delaying harvest after late maximum-yielding periods gave the decreased yield (Fig. 6), but this could provide improved biomass quality (Waramit *et al.*, 2010). Vogel *et al.* (2002) suggested that significant amounts of N are remobilized from the aboveground biomass to underground organs of switchgrass when harvesting was delayed until a killing frost. The nitrogen fertilization requirement for the next season would be reduced with this harvest scheme. They also suggested that the economic value of the yield loss with delayed harvest would be compensated for by the value of decreased fertilizer and application cost. Additionally, the concentration of N and other minerals of warm-season grasses that negatively affect conversion and combustion systems decrease as they mature during the growing season, making them more desirable for biofuel quality (Sanderson & Wolf, 1995; Vogel *et al.*, 2002; Adler *et al.*, 2006). The decreases in biomass yield from maximum-yield harvests to final harvest occurred as a result of senescence. During the senescence periods, some tillers lodged and fell to the ground caused the loss of leaves and stems, and seed shattered. Switchgrass yields in the Midwestern USA decreased 10–20% with harvests after a killing frost in October (Vogel *et al.*, 2002). Frank *et al.* (2004) reported overall, stems contributed 56–60% for total switchgrass above-ground biomass on the peak-yielding harvest, but stems accounted for 42–48% of total biomass at the final harvest. Leaf biomass decreased 4–11% for switchgrass at the final harvest (day 255 of year). Senesced biomass increased from 14–19% for switchgrass on the peak-yielding harvest date to 37–49% at the final harvest. In this study, the large proportion of senesced biomass and litter were left in the field as residue, and not picked up during sampling. Decreased yields for warm-season grasses were consistent with lower tiller density except in eastern gamagrass which senescenced late in the season (Fig. 2 and 3). Adler *et al.* (2006) demonstrated that more than twice as much residue was not picked up by the baler when harvest was delayed from fall to spring. They were left in field, either because it was not cut due to lodging or it was cut but not picked up. In addition, the decrease in biomass yield occurred as a result of a lower standing tiller weight due to loss of leaves and panicles. In terms of a biomass supply strategy, eastern gamagrass could be used as a feedstock in early summer, while big bluestem and switchgrass could be used between mid-and late-summer and indiangrass in the early fall. However, this approach must be balanced with the biomass quality required and costs of production (Nelson *et al.*, 1994; Vogel *et al.*, 2002; Tiffany *et al.*, 2006). In this study, the lack of a species x nitrogen rate interaction for biomass dry matter yield indicates the response of each species to N fertilization is approximately the same (Table 1). The increase in biomass yield with N fertilization found when grass swards were at elongation stage (Fig. 7), is likely because N application in warm-season grasses increases stem development (Brejda *et al.*, 1994). N fertilization effect is attributed to an increase in the proportion of elongating, reproductive, and seed tillers for big bluestem, indiangrass, and switchgrass and an increase in total number of tillers for eastern gamagrass, resulting in raised yields for these grasses. Higher grass yields are associated with the accumulation of a large number of reproductive tillers. The nodes and internodes contribute most dry matter while leaf blades contribute the smallest proportion to dry matter production (Kalmbacher, 1983). But the larger number of vegetative tillers in eastern gamagrass receiving high N fertilization rates may contain high concentrations of N, thus decreasing biomass quality (Waramit *et al.*, 2010). These results are consistent with earlier studies that found that warm-season grasses produce typically higher yields with N application rates ranging from 50 to 120 kg N ha^{-1} in the Central Plains and Midwest states (Balasko & Smith, 1971; George *et al.*, 1995; Brejda, 2000; Vogel *et al.*, 2002). George *et al.* (1990) reported that yield of switchgrass supplied with 90 kg N ha^{-1} increased 61% from May to June. However, this study has shown that as yield of grasses had not reached a plateau at the highest nitrogen application rate (Fig. 4). Therefore, it is possible that higher rates could have led to further increases in biomass yields and further changes in MSC. Additionally, warm-season grasses grown at locations where soil organic carbon concentration is very high, do not respond to N due to N released from mineralization of soil organic carbon (Mulkey *et al.*, 2006). Therefore, the results in this study might not be applicable to the area particularly where different soil properties and climatic conditions prevail. A further study may need to be conducted at other sites with different conditions.

Lastly, this study increases our understanding of how different harvest dates and N fertilization rates affect morphological development and biomass yield of four warm-season grasses. Therefore, we can conclude that there is a difference in optimal time to harvest for biomass yields among the warm-season grasses studied. For the best compromise between biomass yield and quality, the optimal times to harvest big bluestem, switchgrass, and indiangrass with determinate growth habit are late peak-yielding harvest dates occurring at 2.7 in 2006 (E5; seventh node palpable) to 4.3 in 2007 (S2; soft dough), 4.2 in 2006 (S1-2; milk-soft dough) to 4.5 in 2007 (S3; hard dough), and 3.3 (R2; spikelet fully emerged) stage of maturity in both years, respectively. At these morphological stages, a large proportion of reproductive or seed ripening tillers are present. For eastern gamagrass, maximum biomass yield with an indeterminate growth habit is obtained at harvest dates with highest MSC index (2.1–2.2; first to second node palpable in 2006 and 1.4–1.6; mid-vegetative stage in 2007) when the largest proportions of reproductive tillers within sward are present. Biomass yield and mean stage count index for big bluestem, switchgrass, and indiangrass increase with higher rates of N fertilization. N fertilization at 65 and 140 kg ha^{-1} increased total above-ground biomass for four grasses across ten harvest dates by 6.5–26% and 24–49%, respectively. Thus, optimum biomass yields with desirable quality for conversion systems might obtain later in the season other than at early maximum-yielding harvest dates when they received 140 kg N ha^{-1}. The associated morphological modifications occurring in tillers within swards influence on yield. The MSC could be identified as consistent indicators of biomass traits to conversion systems. For biomass traits used as bioenergy crop, canopies should contain tillers with higher stem proportion. In contrast to forage crops, leaf yield would play more important key role than total yield with low quality of stem.

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