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Warm-Season Grass Monocultures and Mixtures for Sustainable Bioenergy Feedstock Production in the Midwest, USA

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Abstract

Biomass yield and adaptability to a broad range of environments are important characteristics of dedicated energy crops for sustainable bioenergy feedstock production. In addition to yield potential, the role of species diversity on ecosystem services is also growing in importance as we seek to develop sustainable feedstock production systems. The objective of this study was to compare the biomass yield potential of the commercially available germplasm of native warm-season grasses in monocultures and in blends (mixture of different cultivars of the same species) or mixtures of different species across an environmental gradient (temperature and precipitation) in the Midwest, USA. Warm-season grasses including switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans*[L.] Nash), sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.) and *Miscanthus* × *giganteus* (Greef and Deu.) were planted in 2009. Biomass was annually harvested from 2010 through 2015 for Urbana, IL and Mead, NE but only in 2010 and 2011 for Ames, IA. The effect of species in monocultures and mixtures (or blends) on biomass yields was significant for all locations. In monocultures, the annual biomass yields averaged over a 6-year period were 11.12 Mg ha−1 and 10.98 Mg ha−1 at Urbana and Mead, respectively, while the annual biomass yield averaged over a 2-year period was 7.99 Mg ha−1 at Ames, IA. Also, the annual biomass yields averaged across the different mixtures and blends at each location were 10.25 Mg ha−1, 9.88 Mg ha−1, and 7.64 Mg ha−1 at Urbana, Mead, and Ames, respectively. At all locations, *M.* × *giganteus* and 'Kanlow N1' produced the highest biomass yield in monocultures while mixtures containing switchgrass and big bluestem had the greatest mixture yield. The results from this multi-environment study suggest mixtures of different species provided no yield advantage over monocultures for bioenergy feedstocks in Illinois and Nebraska and both systems consistently produced biomass as long as April–July precipitation was near or above the average precipitation (300 mm) of the regions.

Keywords

Bioenergy crop, Warm-season grasses, Monoculture, Mixture, Precipitation

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ORIGINAL RESEARCH

Warm-Season Grass Monocultures and Mixtures for Sustainable Bioenergy Feedstock Production in the Midwest, USA

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Abstract

Biomass yield and adaptability to a broad range of environments are important characteristics of dedicated energy crops for sustainable bioenergy feedstock production. In addition to yield potential, the role of species diversity on ecosystem services is also growing in importance as we seek to develop sustainable feedstock production systems. The objective of this study was to compare the biomass yield potential of the commercially available germplasm of native warm-season grasses in monocultures and in blends (mixture of different cultivars of the same species) or mixtures of different species across an environmental gradient (temperature and precipitation) in the Midwest, USA. Warm-season grasses including switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman), indiangrass (*Sorghastrum nutans* [L.] Nash), sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.) and *Miscanthus* × *giganteus* (Greef and Deu.) were planted in 2009. Biomass was annually harvested from 2010 through 2015 for Urbana, IL and Mead, NE but only in 2010 and 2011 for Ames, IA. The effect of species in monocultures and mixtures (or blends) on biomass yields was significant for all locations. In monocultures, the annual biomass yields averaged over a 6-year period were 11.12 Mg ha^{-1} and 10.98 Mg ha^{-1} at Urbana and Mead, respectively, while the annual biomass yield averaged over a 2-year period was 7.99 Mg ha^{-1} at Ames, IA. Also, the annual biomass yields averaged across the different mixtures and blends at each location were 10.25 Mg ha^{-1}, 9.88 Mg ha^{-1}, and 7.64 Mg ha^{-1} at Urbana, Mead, and Ames, respectively. At all locations, *M.* × *giganteus* and 'Kanlow N1' produced the highest biomass yield in monocultures while mixtures containing switchgrass and big bluestem had the greatest mixture yield. The results from this multi-environment study suggest mixtures of different species provided no yield advantage over monocultures for bioenergy feedstocks in Illinois and Nebraska and both systems consistently produced biomass as long as April–July precipitation was near or above the average precipitation (300 mm) of the regions.

Keywords Bioenergy crop · Warm-season grasses · Monoculture · Mixture · Precipitation

Introduction

As the need for alternative, renewable energy sources increases, lignocellulosic feedstocks for second-generation bioenergy have gained attention [13, 24, 36]. Warm-season perennial grasses have been identified as potential bioenergy feedstocks due to high biomass yields, low inputs, and greater ecosystem services compared to annual crops and cool season perennial grasses [22, 24, 25]. Additionally, perennial energy feedstocks grown on marginally productive croplands could minimize competition with food production, maximize producer resources through renovation of unproductive lands, and have significant environmental benefits [6, 10, 33].

Switchgrass (*Panicum virgatum* L.) and *Miscanthus* × *giganteus* (Greef and Deu.: denoted as M. × giganteus for the remainder of the paper) have received considerable interest

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Moon-Sub Lee and Rob Mitchell contributed equally to this work.

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as bioenergy feedstocks due to their high yield potential. Numerous studies have reported on the productivity of switchgrass and *M.* × *giganteus*, with advancements made in genetics and agronomic practices, cultivar development, and best management practices [24, 46]. However, switchgrass and *M.* × *giganteus* may not perform well across a broad environmental gradient and over multiple years [1, 7, 34]. In contrast, relatively little research has been conducted on big bluestem (*Andropogon gerardii* Vitman) and indiangrass (*Sorghastrum nutans* [L.] Nash), although they have comparable biomass yield potentials to switchgrass [1, 23, 34]. The majority of information on yield potentials resulted from short-term studies and is limited to certain perennial warm-season grasses. Also, there has been relatively little research on management practices for sustainable bioenergy feedstock productions. Therefore, it is necessary to evaluate and enhance many species and cultivars for specific environments within different agro-ecoregions (Gonzalez- Hernandez et al., 2009; [1, 2]).

Environmental fluctuations such as variable temperature and precipitation patterns can have significant impacts on agricultural production [16, 34]. The use of polycultures or multi-species swards may aid in stabilizing production across years and soil conditions [5, 16, 54]. Species diversity can enhance resilience in agricultural systems and may provide advantages for disease resistance [19, 55], increased production [30, 43], climate change resilience [42], and expanded ecosystem services [22].

Biomass production is the primary factor influencing selection of monocultures or mixtures for bioenergy feedstock production []. However, there are still uncertainties regarding the relationship between biomass production and species diversity. As mentioned above, Tilman et al. [43] observed that the highly diverse prairie grass mixtures produced more biomass than monocultures. Russelle et al. [35] contradicted some of the observations presented by Tilman et al. [43], specifically that difficulties in establishment and maintenance of native prairie grasses grown in high-diversity mixtures were disregarded. Schmer et al. [37] reported that switchgrass monocultures managed for high yield showed 93% higher biomass production and equivalent net energy yield when compared to low-input high-diversity mixtures of native grassland perennials. These discrepancies may be because both studies were deficient in multi-location comparative evaluations against other high yielding perennial monocultures [21]. Therefore, the objective of this study was to compare the biomass production potential of commercially available germplasms and experimental lines of native warm-season grasses in monocultures and in blends or mixtures at three locations in the Midwest, USA, where a large portion of warm-season grasses for bioenergy feedstocks are likely to be grown. Specifically, we addressed the questions: (1) Which grass species and cultivars produce the most biomass across the sites? (2) Do monocultures produce more biomass than mixtures at each site?

Materials and Methods

The study was conducted from 2009 to 2015 in Urbana, Illinois at the University of Illinois research farm (40° 04′ 04.0″ N, 88° 11′ 43.7″ W) on Flanagan silt loam soil (Fine, smectitic, mesic Aquic Argiudolls) and in Mead, Nebraska at the University of Nebraska research farm (40° 10′ 3.08″ N, 96° 25′14.05″ W) on Tomek silt loam soil (Fine, smectitic, mesic Pachic Argiudolls). The study was also conducted at a site in Ames, Iowa at the Iowa State University research farm (42° 00′ 43.9″ N, 93° 44′ 33.7″ W), on Clarion loam soil (Fine-loamy, mixed, superactive, mesic Typic Hapludolls) but data was only collected from 2009 to 2011. All soils at each location were considered as moderate to well drained soils with a slope of less than 4% and major crops in all locations were corn and soybean until 2008. Warm-season grasses including big bluestem (‘Bonanza’ and ‘Goldmine’), indiangrass (‘Scout’, ‘Warrior’, and ‘Chief’), switchgrass (‘Shawnee’, ‘NE2K’, ‘Kanlow N1’, and ‘Cave-in-rock’), and sideoats grama (*Bouteloua curtipendula* [Michx.] Torr. ‘Butte’) were planted as monocultures and as blends or mixtures (Table 4). *Miscanthus* × *giganteus* Greef et Deu ex. Hodkinson et Renvoize was only used as a monoculture. The mixtures were developed based on US Dept. of Agriculture Plant Hardiness Zone (PHZ) adaptations (http://planthardiness.ars.usda.gov) and on the top yielding mixtures from previous research [44, 47–49]. ‘Bonanza’ big bluestem, ‘Scout’ indiangrass, and ‘Shawnee’ switchgrass were used in mixtures for PHZ 4 and 5, while ‘Goldmine’ big bluestem, ‘Warrior’ indiangrass, and ‘Shawnee’ switchgrass were used in mixtures for PHZ 5 and 6. ‘Butte’ sideoats grama was used for both PHZs. We also included a two-cultivar blend for big bluestem and indiangrass and although these two blends are technically monocultures (as they were mixtures of the same species) for the purpose of this paper, blends were analyzed as mixtures because they have wider genetic diversity than a monoculture of a single cultivar. ‘Bonanza’ and ‘Goldmine’ were used in a big bluestem blend and ‘Warrior’ and ‘Scout’ were used in an indiangrass blend. An experimental lowland switchgrass, ‘NE2K’, a precursor to ‘Liberty’ [51], was used at all sites. A total of 28 monocultures and mixtures were included in each location. Of the 18 mixtures (including the two blends) evaluated in the study, 15 of those mixtures included big bluestem and henceforth those mixtures will be denoted as the big bluestem-based mixtures.

The experimental design was a randomized complete block design with four replications at each location, and blocks were separated by alleys (1.5 m). The entire plot area was tilled and packed to develop a firm seedbed prior to planting. The individual plots (1.5 m × 4.5 m) were seeded at a rate of 325 pure live seeds (PLS) m^2 at a depth of 1.5 cm by a plot drill in Urbana, IL (Great Plain Plot planter, Salina, KS, USA), Mead, NE (Hege Inc., Waldenburg, Germany), and Ames, IA (Cole Planet JR, Cole Planter Co., GA, USA) in the spring of 2009. Also, *M.* × *giganteus* rhizomes were transplanted with 0.6 m row spacing and a 0.9 m gap between plants, at the recommended depth of 10 to 20 cm [31]. Quinclorac (Paramount®, (3,7-dichloro-8-quinolinecarboxylic acid)) was applied at a rate of 560 g ha^{-1} immediately after planting for pre-emergent weed control. Plots were fertilized with 45 kg P ha^{-1} prior to planting and with 112 kg N ha^{-1} annually beginning in the second year. In the spring of 2010, stand frequency was measured to determine grass establishment success by following the method of Vogel and Masters [45], while species composition was measured to estimate the change of species composition in monocultures and mixtures at the IL and NE sites in autumn of 2016.

From 2010 to 2015, biomass was harvested in a 1.4 m wide × 4.0 m long area once annually after a killing frost. A biomass plot harvester (Cibus S, Wintersteiger, Salt Lake City, UT) was used in Urbana, IL during early December and a self-propelled forage flail chopper (Carter Manufacturing, Brookston, IN) was employed in Mead, NE during early November at a cutting height of 10 cm. Biomass was cut by hand in a harvest area (3.72 m^2) per plot in Ames, IA during early December of 2010 and 2011. Fresh plot weight was measured with a combine in Urbana, IL and Mead, NE or by hand in Ames, IA where a subsample (approximately 0.5 kg) was directly collected from the combine or from hand harvesting for moisture content calculation. Subsamples were dried in a forced-air oven at 55 °C for 5 days to determine dry matter.

Weather data from stations near Urbana, IL; Mead, NE; and Ames, IA were obtained from the Illinois State Water Survey, High Plains Regional Climate Data Center, and the Iowa Environmental Mesonet, respectively. Precipitation and temperature records are shown in Tables 1 and 2 for each location for the duration of the study.

Biomass yield data were analyzed in a mixed model analysis of variance using PROC MIXED and GLIMMIX procedures in SAS (SAS institute, Cary, NC). Monoculture and mixture (including blends) treatments and year were considered as fixed effects, while block was considered to be random. Locations were analyzed separately due to interactions between location and treatments ($P < 0.001$) such as weather characteristics at each site. Contrast statements were used to compare mean biomass yields between a species and its two- and three-way mixtures using the PROC MIXED procedure in SAS. The coefficient of variance of biomass yield was calculated as the standard deviation (σ) divided by the mean (μ) × 100. To find the critical biomass production time period relative to precipitation availability for each monoculture and mixture, a correlation coefficient was calculated across all months throughout the growing season (April to September) for each location using the CORR procedure in SAS. The monthly period with the highest correlation coefficient was deemed as the most critical time period for biomass production. All statistical significances were determined at $\alpha = 0.05$.

Results

Monthly precipitation and temperature during the experimental period included a record drought in 2012 at Urbana and Mead (Tables 1 and 2). Precipitation in June and July of 2012 at Urbana was 57.9 and 15.5 mm, respectively, which was 47% and 87% below the 30-year average. Precipitation in July and August of 2012 at Mead was 8.4 mm and 7.6 mm, which was 90% less than the 30-year average. At Ames, IA, the growing season precipitation during 2009 through 2011 did not deviate markedly from the 30-year average, but there was approximately 5 days of flooding in 2010 at the experimental area.

The monoculture and mixtures (denoted henceforth as such) included in the study and the percentages of each species within the mixtures are outlined in Table 3. The effect of species monoculture and mixture on biomass yield was significant in Urbana and Mead through the 6-year time period (Table 4). During the 2-year time period in Ames, the significant effect of species monoculture and mixture was also observed. The interaction between treatment × harvest year significantly affected biomass yield at Urbana and Mead, but not at Ames, while biomass yield was significantly influenced by harvest year at all locations (Table 4). However, the ratio of species composition in the mixtures did not have a significant impact on biomass yield (Table 5). The annual biomass yields in monoculture were 11.12 Mg ha^{-1}, 10.89 Mg ha^{-1}, and 7.99 Mg ha^{-1}, at Urbana, Mead, and Ames, respectively, while 10.25 Mg ha^{-1}, 9.88 Mg ha^{-1}, and 7.64 Mg ha^{-1} were harvested in mixture at Urbana, Mead, and Ames, respectively (Table 5). *M.* × *giganteus* was the highest yielding grass grown in monoculture in all three locations across all years with an average annual yield of 17.12 Mg ha^{-1}, 17.89 Mg ha^{-1}, and 13.45 Mg ha^{-1} at Urbana, Mead, and Ames, respectively. 'Kanlow N1', an experimental selection from 'Kanlow' switchgrass for improved winter survival at Mead, was the second highest yielding grass grown in monoculture with mean biomass yields of 13.86 Mg ha^{-1} at Urbana, 12.56 Mg ha^{-1} at Mead, and 9.13 Mg ha^{-1} at Ames. Relative yields of the other treatments varied across years and locations (Table 5).

The coefficient of variation among monocultures and mixtures was different depending on location (Suppl. Table 1). Averaged across harvest years, the coefficient of variation in

Table 1 Precipitation conditions from 2009 to 2015 with the 30-year average for Urbana, IL; Mead, NE; and Ames, IA

Month	2009	2010	2011	2012	2013	2014	2015	30-year average
(A) Urbana, IL								
Jan.	17.3	31.5	16.8	80.5	65.3	40.6	36.3	52.1
Feb.	42.7	40.9	95.8	28.7	81.5	76.7	31.2	54.1
March	66.5	73.9	34.5	41.4	33.8	35.3	43.2	72.6
April	176.3	52.8	188.5	67.5	179.1	100.1	91.9	93.5
May	145.0	86.6	125.2	79.0	95.0	111.3	154.2	124.2
June	112.3	211.6	106.2	57.9	159.3	208.5	232.8	110.2
July	159.8	95.3	40.1	15.5	89.7	221.0	107.2	119.4
Aug.	142.7	41.6	44.7	141.2	9.1	38.6	80.3	99.8
Sept.	20.3	81.3	69.3	145.0	9.7	87.4	163.6	79.5
Oct.	223.3	27.9	62.5	138.7	91.2	126.0	31.5	82.8
Nov.	99.6	98.0	119.9	27.2	39.1	61.5	112.0	93.5
Dec.	95.8	64.8	69.6	52.6	56.6	46.0	189.7	69.3
(B) Mead, NE								
Jan.	9.7	20.8	27.2	4.1	18.5	6.1	23.1	16.3
Feb.	16.3	25.1	20.1	53.3	13.7	15.7	23.6	19.6
March	4.6	45.0	16.8	22.6	53.8	3.3	19.6	49.0
April	38.6	64.3	83.1	88.6	102.1	88.9	50.5	68.8
May	29.7	94.0	152.4	76.2	214.4	133.6	276.9	109.0
June	157.0	251.5	87.4	90.7	63.2	149.9	194.6	110.2
July	46.7	148.1	39.4	8.4	25.4	13.0	60.7	86.4
Aug.	81.3	71.4	175.0	7.6	28.2	191.5	96.0	88.6
Sept.	31.8	94.7	33.8	43.9	50.5	175.0	125.2	76.7
Oct.	107.7	3.3	23.6	48.8	66.8	62.5	12.7	50.0
Nov.	2.5	50.0	42.2	3.81	31.0	11.9	50.3	36.3
Dec.	61.5	6.1	40.1	38.1	5.6	31.0	112.3	24.1
(C) Ames, IA								
Jan.	24.1	29.7	17.8					24.6
Feb.	6.4	19.1	26.9					25.9
March	103.4	52.6	20.1					50.3
April	115.8	93.0	112.3					74.9
May	96.0	92.5	117.3					104.6
June	104.4	283.5	128.3					119.6
July	69.9	171.2	99.1					98.8
Aug.	122.9	284.7	91.2					96.5
Sept.	24.4	166.9	51.3					94.0
Oct.	186.2	9.7	21.8					59.2
Nov.	35.1	56.6	69.1					45.0
Dec.	50.0	20.3	56.6					27.4

monoculture was 26% and 24% at Urbana and Mead, while the coefficient of variance in mixture was 28% and 20% at Urbana and Mead. There were no relationships between the coefficient of variance and species richness at any of the locations (Suppl. Fig. 2). For big bluestem-based mixtures, the two- and three-way mixture (or blends) had higher yield than the 'Bonanza' big bluestem monoculture at Urbana, while there was no yield difference between a 'Bonanza' monoculture and a mixture at Mead (Table 4). At both Urbana and Mead, the 'Goldmine' monoculture yielded the same as the mixtures (Table 4). In addition, regardless of the big bluestem and indiangrass cultivar, mixtures of big bluestem and indiangrass with 'Shawnee' switchgrass were not different from the 'Shawnee' monoculture at Urbana and Mead (Table 4). At Ames, the big bluestem two-way and three-way mixtures, similar to Urbana, had higher yields than

Table 2 Average minimum and maximum air temperature at each location from 2009 to 2015 with the 30-year average

	Average maximum (°C)								Average minimum (°C)							
Month	2009	2010	2011	2012	2013	2014	2015	30-year average	2009	2010	2011	2012	2013	2014	2015	30-year average
	Urbana, IL															
January	−4.1	−5.1	−3.7	3.3	1.3	−3.6	−1.6	−1.2	−13.9	−11.7	−11.7	−7.2	−8.8	−14.2	−10.3	−10.2
February	3.1	−2.1	0.8	4.6	1.3	−4.2	−3.9	1.5	−7.3	−9.7	−7.3	−5.1	−7.3	−13.8	−14.4	−8.2
March	10.9	10.3	9.0	17.8	3.8	5.8	6.9	8.3	−1.6	−0.6	−1.7	4.2	−3.0	−6.2	−4.2	−2.8
April	14.4	19.7	16.2	17.2	14.3	16.1	16.5	15.4	3.4	5.9	4.3	4.1	2.6	3.6	4.4	3.4
May	21.6	22.1	20.6	25.4	22.3	22.2	22.9	21.3	9.8	10.8	9.3	11.7	10.2	9.9	11.0	9.2
June	27.5	27.3	26.8	27.8	26.0	26.4	25.7	26.4	16.4	16.9	15.5	13.6	14.3	15.8	15.4	14.9
July	24.6	28.8	31.4	33.5	26.1	24.8	26.6	27.8	14.3	17.8	19.4	18.9	15.6	13.8	16.1	16.6
August	25.3	29.7	29.4	29.0	27.3	26.4	26.4	27.1	14.1	17.1	15.9	14.4	15.0	16.3	14.5	15.6
September	23.0	24.7	22.2	22.7	26.4	22.7	25.9	24.0	12.2	11.3	10.2	10.4	12.1	10.1	12.8	10.7
October	12.8	19.2	18.4	14.6	17.2	15.7	18.0	16.8	3.6	4.6	4.2	3.6	4.7	5.0	5.5	4.2
November	11.4	10.4	10.8	8.9	7.2	4.9	11.7	8.7	1.0	−2.4	0.8	−2.3	−3.6	−5.6	0.2	−1.7
December	0.3	−3.1	4.4	4.9	−0.1	1.8	6.6	0.9	−7.7	−10.5	−3.8	−3.2	−9.4	−4.4	−0.9	−7.7
	Mead, NE															
January	0.9	−5.6	−3.7	4.7	0.5	0.4	3.3	0.2	−13.3	−13.4	−14.7	−10.2	−11.4	−14.6	−11.0	−11.8
February	5.2	−2.6	2.0	3.5	3.1	−0.7	−0.8	2.8	−9.5	−11.2	−11.1	−8.3	−8.8	−13.2	−14.1	−9.4
March	10.2	9.2	8.6	19.2	6.3	9.5	14.0	9.6	−4.6	−2.6	−3.7	3.0	−6.4	−7.1	−4.8	−3.9
April	15.6	18.8	16.2	19.3	13.2	17.3	17.2	16.3	1.2	4.1	2.3	4.1	−0.9	2.4	3.9	2.1
May	23.4	20.2	21.4	25.3	20.9	24.0	20.1	21.8	8.7	8.1	8.3	10.2	8.4	9.6	9.3	8.6
June	26.5	27.8	27.2	29.4	26.7	27.9	27.7	27.3	14.9	15.7	14.9	15.2	13.9	15.3	15.1	14.4
July	27.2	29.0	31.6	34.4	29.3	29.3	29.7	30.0	14.7	18.6	20.2	19.0	16.1	15.2	17.1	17.3
August	27.5	30.9	28.4	30.2	28.8	28.7	28.0	28.8	14.0	16.9	16.6	13.4	16.8	16.9	15.1	16.0
September	23.2	24.4	22.3	26.2	27.0	23.4	26.4	24.3	9.4	10.1	7.2	6.7	12.6	10.5	14.1	10.2
October	11.5	20.3	19.6	15.5	16.6	18.7	19.4	17.1	0.9	2.3	3.2	0.7	2.0	3.7	5.2	3.1
November	12.6	9.0	9.8	11.8	8.3	6.4	11.7	8.5	−2.6	−4.4	−4.3	−4.7	−6.4	−6.9	−0.7	−4.1
December	−3.6	0.3	3.1	2.5	−0.2	2.3	4.3	1.2	−13.5	−11.4	−9.5	−10.4	−14.0	−5.4	−5.8	−10.3
	Ames, IA															
January	−6.0	−8.2	−6.1					−0.6	−16.8	−15.3	−15.7					−10.0
February	1.7	−5.7	−0.9					2.2	−9.4	−14.9	−10.7					−7.2
March	8.0	6.6	6.1					9.4	−3.7	−2.9	−4.2					−1.1
April	13.9	18.7	14.0					16.7	0.8	5.3	1.7					5.0
May	20.3	20.7	20.3					22.2	8.5	8.7	7.8					11.1
June	24.5	26.1	25.3					27.8	13.8	15.1	14.8					16.7
July	24.4	27.4	29.3					30.0	13.4	17.3	18.8					19.4
August	24.6	28.1	26.5					28.9	13.8	17.0	15.4					18.3
September	22.6	23.2	21.0					24.4	10.2	10.4	8.8					12.8
October	10.7	19.6	18.5					17.2	1.7	3.6	3.8					6.1
November	10.6	7.7	9.1					8.9	0.1	−3.1	−2.1					−0.6
December	−3.7	−3.7	2.4					1.1	−12.9	−12.6	−6.7					−7.8

IA, Iowa; IL, Illinois; NE, Nebraska

a ‘Bonanza’ monoculture. In addition, biomass yield of the big bluestem three-way mixtures were not different from the ‘Shawnee’ monoculture at Ames.

Biomass moisture content for the monocultures and mixtures at each location are shown in Table 6. The monocultures ranged from 17% to 32% at Urbana, whereas at Mead moisture content varied between 24 and 43%. At both Urbana and Mead, *M.* × *giganteus* had the highest moisture content and ‘Chief’ indiangrass had the lowest compared to the other grasses across the years. At Ames,

Table 3 The warm-season grass species and cultivars evaluated in the field experiment during 2009–2015 at Urbana, IL; Mead, NE; and Ames, IA

Cultivar		Abbreviation	Species				
Monoculture	*Miscanthus* × *giganteus*[‡]	MG	Miscanthus				
	Cave-in-Rock	CIR	Switchgrass				
	Kanlow N1	KA	Switchgrass				
	NE2K	NE2K	Switchgrass				
	Shawnee	SH	Switchgrass				
	Bonanza	BO	Big bluestem				
	Goldmine	GO	Big bluestem				
	Chief	CH	Indiangrass				
	Scout	SC	Indiangrass				
	Warrior	WA	Indiangrass				
			% in mixture[§]				
Blend			BB[†]	IN	SW	SO	MG
	Bonanza/Goldmine	BO + GO	100	0	0	0	0
	Warrior/Scout	WA + SC	0	100	0	0	0
Mixture	Bonanza/Scout	BO + SC-1	40	60	0	0	0
	Bonanza/Scout	BO + SC-2	50	50	0	0	0
	Bonanza/Scout	BO + SC-3	60	40	0	0	0
	Bonanza/Scout/Shawnee	BO + SC + SH-1	20	60	20	0	0
	Bonanza/Scout/Shawnee	BO + SC + SH-2	40	40	20	0	0
	Bonanza/Scout/Shawnee	BO + SC + SH-3	60	20	20	0	0
	Bonanza/Scout/Butte	BO + SC + BU	40	20	0	20	0
	Goldmine/Warrior	GO + WA-1	40	60	0	0	0
	Goldmine/Warrior	GO + WA-2	50	50	0	0	0
	Goldmine/Warrior	GO + WA-3	60	40	0	0	0
	Goldmine/Warrior/Shawnee	GO + WA + SH1	20	60	20	0	0
	Goldmine/Warrior/Shawnee	GO + WA + SH2	40	40	20	0	0
	Goldmine/Warrior/Shawnee	GO + WA + SH3	60	20	20	0	0
	Goldmine/Warrior/Butte	GO + WA + BU	40	20	0	20	0
	Scout/Shawnee/Butte	SC + SH + BU	0	40	20	20	0
	Warrior/Shawnee/Butte	WA + SH + BU	0	40	20	20	0

[†] BB, big bluestem; IN, indiangrass; SW, switchgrass; SO, sideoats grama

[‡] Miscanthus × giganteus clone obtained from the Chicago Botanic Garden (Glencoe, IL, USA)

[§] The number followed by abbreviation indicates different ratio of each species in the seeding mixture

grasses in monoculture had moisture levels ranging from 12 to 22% in 2010 and 2011.

Biomass had no consistent response to precipitation variation across the research period or across the locations. One exception was in 2012, where across many of the monocultures and mixtures, low biomass production was associated with low precipitation. Precipitation from April to July in 2012 was 50% lower than the 30-year average in Urbana and 70% lower in Mead (Fig. 1). However, at the monoculture or mixtures level, there were some feedstocks that did have significant responses to change in precipitation. At Urbana, the biomass yield for big bluestem was positively correlated with April–July precipitation (*p* values for 'Bonanza' 0.076 and 'Goldmine' 0.005), whereas no relationship was observed at Mead during the entire growing season. *M.* × *giganteus* biomass yield was positively correlated with the April–July precipitation at Mead (*p* value 0.014) though not significantly correlated at Urbana (*p* value 0.179). Switchgrass and indiangrass biomass yield were not correlated with growing season precipitation at either location.

Discussion

Biomass Yield in Monocultures and Mixtures

In previous experiments, warm-season perennial grasses have required two or more years to achieve full yield potential [17,

Table 4 Summary of ANOVA for biomass yields of warm-season grasses and mixtures, with significant effects indicated by *P* values, at Urbana, IL; Mead, NE; and Ames, IA. Species grown in monocultures and mixtures were considered treatments

Source of variation	Urbana, IL	Mead, NE	Ames, IW[‡]
	-----*P* > *F*-----		
Treatment[†]	< .0001	< .0001	< .0001
Harvest year	< .0001	0.0011	0.0003
Treatment × harvest year	< .0001	0.0004	0.1874
Contrast			
BO vs. BO + SC[§]	0.0180	0.2883	0.0147
BO vs. BO + SC + SH	< .0001	0.2472	0.0004
SH vs. BO + SC + SH	0.6191	0.8007	0.8593
BO + SC vs. BO+SC + SH	0.0095	0.8901	0.0958
BO + SC vs. BO + SC + BU	0.0006	0.3217	0.1682
GO vs. GO + WA	0.0790	0.3770	0.0020
GO vs. GO + WA + SH	0.1085	0.6661	0.0021
SH vs. GO + WA + SH	0.6382	0.8049	0.0849
GO + WA vs. GO + WA + SH	0.8260	0.5234	0.9813
GO + WA vs. GO + WA + BU	0.0036	0.8197	0.0225

[†] Biomass yield was averaged across species composition rates due to no differences among rates within each mixture, and analysis was tested at $\alpha = 0.05$

[‡] IA biomass yield data was collected in 2010 and 2011

[§] BO, 'Bonanza' big bluestem; BU, 'Butte' sideoats grama; GO, 'Goldmine' big bluestem; SC, 'Scout' indiangrass; SH, 'Shawnee' switchgrass; WA, 'Warrior' indiangrass

27, 29]. To maximize profits, it is important to shorten the establishment phase to achieve full yield potential as quickly as possible. In the current experiment, grass monocultures and mixtures were successfully established and reached their full production the year after planting (Suppl. Table 1). Our results indicated that warm-season grasses can be fully established in the planting year and full production could be achieved from the second year with good establishment practices including seed quality, seed bed preparation, and weed control along with favorable soil moisture conditions. In monocultures, *M.* × *giganteus* (17 Mg ha^{-1}) and 'Kanlow N1' (13 Mg ha^{-1}) had greater yield than other species and cultivars at Urbana, Mead, and Ames. These results agree with previous research [8, 15]. On the other hand, Goldmine + Warrior + Shawnee (20/60/20) had greater biomass yields when compared to other mixtures at Urbana and Mead and yielded more than 10 Mg ha^{-1}.

Biomass Moisture Content

Biomass moisture content is critical to effective storage and transport of herbaceous feedstocks, because higher moisture content causes higher storage losses and transportation costs [39]. In our study, moisture levels were not dramatically different between monocultures and mixtures; it is noteworthy that relatively high moisture contents might result from weather conditions prior to harvesting. Recommended moisture content for storage is less than 20% [3, 50], and if moisture content of biomass is above 30%, harvested biomass should be windrowed to promote drying and ensure optimum moisture content for storage [50]. Danalatos et al. [4] observed an approximately 15% decrease in moisture content resulting from a two-week delayed harvest timing of *M.* × *giganteus*. Similarly, at Urbana in this study, *M.* × *giganteus* and switchgrass moisture content decreased when 2013 harvest and 2015 harvest biomass were compared, while big bluestem moisture content increased during the same experimental period. The reason for these observations could be that the 2013 biomass was harvested in November of 2013, while the 2015 biomass was collected in January of 2016. Therefore, the delayed harvest timing could lower the moisture content of *M.* × *giganteus* and switchgrass. However, in the case of big bluestem, lodging was caused by rain and winter snow cover before the 2015 harvest, which could have affected the moisture content in the 2015 biomass. Tahir et al. [41] also reported that early spring harvest management might be vulnerable to snow cover, lodging, and unfavorable soil conditions and therefore is a factor to take into consideration.

Relationship between Biomass Yield and Seasonal Precipitation

Previous studies have found that grassland biomass production responds positively to mean annual precipitation [28] as precipitation is one of the most important factors impacting aboveground biomass production in terrestrial ecosystems

Table 5 Least squares means of biomass dry matter yields of warm-season grass cultivars grown in monoculture and mixtures from 2010 to 2015 at Urbana, IL and Mead, NE and from 2010 to 2011 at Ames, IA

Treatment	% in mixture[§]	Biomass yield (Mg ha^{-1})			
		Urbana, IL	Mead, NE	Ames, IA	Mean
Miscanthus × *giganteus*	100	$17.12^{a\dagger}$	17.89^{a}	13.45^{a}	16.15
Cave-In-Rock	100	10.33^{cde}	9.60^{b}	7.14^{bcd}	9.02
Kanlow N1	100	13.86^{b}	12.56^{b}	9.13^{b}	11.85
NE2K	100	11.52^{bc}	9.94^{b}	6.99^{bcd}	9.48
Shawnee	100	11.15^{cd}	10.49^{b}	7.43^{bcd}	9.69
Bonanza	100	8.56^{de}	8.87^{b}	4.98^{cd}	7.54
Goldmine	100	10.01^{cde}	9.83^{b}	6.43^{bcd}	8.76
Chief	100	8.50^{de}	9.14^{b}	7.16^{bcd}	8.27
Scout	100	9.75^{cde}	10.89^{b}	8.48^{b}	9.71
Warrior	100	10.43^{cde}	9.76^{b}	8.71^{b}	9.63
Bonanza/Goldmine	50/50	8.96^{cde}	9.02^{b}	4.60^{bcd}	7.53
Bonanza/Scout	40/60	9.99^{cde}	9.47^{b}	6.97^{bcd}	8.81
	50/50	9.56^{cde}	9.62^{b}	6.52^{bcd}	9.21
	60/40	10.33^{cde}	9.43^{b}	6.67^{bcd}	8.71
Bonanza/Scout/Shawnee	20/60/20	11.10^{cd}	9.95^{b}	7.64^{bcd}	9.56
	40/40/20	10.69^{cd}	10.54^{b}	8.14^{bc}	9.79
	60/20/20	10.85^{cd}	10.24^{b}	6.87^{bcd}	9.32
Bonanza/Scout/Butte	40/20/40	7.93^{e}	9.18^{b}	5.75^{bcd}	7.62
Goldmine/Warrior	40/60	10.73^{cd}	10.21^{b}	9.21^{b}	10.77
	50/50	11.53^{bc}	9.83^{b}	8.75^{b}	10.04
	60/40	10.67^{cd}	9.88^{b}	8.00^{bcd}	9.52
Goldmine/Warrior/Shawnee	20/60/20	11.28^{bc}	10.88^{b}	9.20^{b}	10.45
	40/40/20	10.90^{cd}	9.98^{b}	7.75^{bcd}	9.54
	60/20/20	10.50^{cde}	9.70^{b}	8.98^{b}	9.79
Goldmine/Warrior/Butte	40/20/40	9.35^{cde}	10.47^{b}	7.03^{bcd}	8.95
Warrior/Scout	50/50	10.57^{cde}	9.76^{b}	9.39^{b}	9.87
Scout/Shawnee/Butte	40/20/40	8.50^{de}	10.27^{b}	7.27^{bcd}	8.68
Warrior/Shawnee/Butte	40/20/40	11.00^{cd}	9.53^{b}	8.70^{b}	9.74

[†] Different letters represent significant differences in Bonferroni test, $\alpha = 0.05\%$

[§] The number followed by abbreviation indicates different ratio of each species in the seeding mixture

[12]. In this present study, each warm-season grass responded differently to precipitation early in the growing season (cumulative precipitation from April–July) at Urbana and Mead (Fig. 1). The biomass productivity of *M.* × *giganteus* at Mead, NE had a high positive correlation with April and July precipitation. At Urbana, *M.* × *giganteus's* biomass production was still positively correlated, although not significantly. This is in agreement with Heaton et al. [8] and Richter et al. [32] in showing that growing season (April–September) precipitation a critical factor for perennial grasses to produce their full biomass yield potential. Shiflet and Dietz [38] also indicated that May–July precipitation can be used as an indicator to estimate big bluestem production and Hong et al. [9] found high correlation between 'Bison' big bluestem biomass yield and April–September precipitation in the northern Great Plains. In this current study, biomass yield of big bluestem was positively correlated with growing season precipitation at Urbana. However, both cultivars produced consistent yield at Urbana and Mead as long as precipitation from April–July was above 250 mm. For switchgrass and indiangrass, however, biomass yield was not correlated with precipitation during the growing season at either location. Results indicated that switchgrass and indiangrass biomass yields did not significantly respond to seasonal precipitation as long as April–July precipitation was more than 200 mm and 300 mm, respectively, for each species. Previous work by Lee and Boe [14], however reported the importance of April–May precipitation for switchgrass biomass yield when the precipitation was below the 30-year average (125 mm) in central South Dakota. In the current study, 2012 was the only year

Table 6 Biomass moisture content of warm-season grass cultivars grown in monoculture from 2010 to 2015 at Urbana, IL and Mead, NE and from 2010 to 2011 at Ames, IA. Moisture content was measured at harvest after a killing frost each year

	Moisture content (g kg^{-1})						
Species	2010	2011	2012	2013	2014	2015	Mean
(a) Urbana, IL							
M. × *giganteus*	29.4	39.5	33.0	33.6	34.1	21.9	31.9
Cave-in-Rock	28.8	31.0	22.7	26.7	18.0	17.9	24.2
Kanlow N1	26.0	28.2	23.2	23.7	17.8	16.5	22.6
NE2K	22.5	28.6	18.9	22.4	18.3	16.9	21.3
Shawnee	27.4	32.2	22.7	26.3	19.0	19.0	24.4
Bonanza	11.8	40.2	15.4	29.8	17.7	35.6	25.1
Goldmine	13.4	35.2	14.4	30.4	19.9	38.2	25.3
Chief	12.5	24.3	15.0	16.1	16.6	17.8	17.1
Scout	13.0	23.8	16.2	17.2	18.0	21.2	18.2
Warrior	16.4	25.5	16.7	20.5	18.1	16.9	19.0
Mean	20.4	30.8	19.8	24.7	19.9	22.2	
LSD ($\alpha = 0.05$)	2.04	5.59	2.28	6.17	3.22	4.47	
(b) Mead, NE							
M. × *giganteus*	53.0	42.9	31.9	36.9	37.5	55.4	42.9
Cave-in-Rock	47.0	39.1	25.8	34.1	38.9	49.6	39.1
Kanlow N1	49.4	4.06	24.3	33.6	36.6	59.2	34.5
NE2K	35.6	31.2	24.8	24.8	30.7	40.2	31.2
Shawnee	45.8	36.1	22.7	31.6	34.6	45.8	36.1
Bonanza	40.9	24.6	17.4	18.5	23.9	33.4	26.5
Goldmine	45.1	31.5	20.3	23.1	29.6	39.2	31.5
Chief	27.9	24.1	23.6	18.2	24.0	26.8	24.1
Scout	33.4	26.6	22.6	16.6	26.3	34.1	26.6
Warrior	36.4	28.3	23.4	23.4	25.0	33.4	28.3
Mean	41.4	32.5	23.7	26.1	30.7	41.7	
LSD ($\alpha = 0.05$)	6.46	3.48	4.80	6.24	5.85	12.0	
(c) Ames, IA							
M. × *giganteus*	17.0	11.9					14.5
Cave-in-Rock	25.2	19.5					22.4
Kanlow N1	11.8	12.7					12.3
NE2K	15.1	11.6					13.4
Shawnee	11.8	11.8					11.8
Bonanza	16.1	21.1					18.6
Goldmine	23.4	16.2					19.8
Chief	22.5	21.5					22.0
Scout	11.0	12.7					11.9
Warrior	12.0	11.9					12.0
Mean	16.6	15.1					
LSD ($\alpha = 0.05$)	6.65	5.99					

in which Urbana and Mead had a severe drought that resulted in a significant reduction in biomass yield across feedstocks, with the exception of switchgrass (Suppl. Table 1). Biomass yields of indiangrass and big bluestem in Urbana and Mead were 35–47% and 11–39% below average in 2012 when April–July precipitation was 50% and 70% below the 30-year average, respectively. In the two- and three-way mixtures of indiangrass, big bluestem, and switchgrass, no clear response to precipitation was observed. This may be because individual species within a mixture may respond differentially to water received during different times throughout the growing season. As a result, there was no relationship between total biomass yield and precipitation in mixed species stands as was also seen in a study by Wang et al. [52].

Biomass Production and Species Diversity

According to Picasso et al. [30], the relationship between biomass production and species diversity might be affected by the presence or absence of "driver" species, which are defined as certain species from which most of the biomass yield is achieved, either in monocultures or in mixtures. Hong et al. [9] reported that switchgrass yielded more than indiangrass and big bluestem in the northern Great Plains, and the biomass yield of switchgrass in monoculture and the two-way or three-way mixture of indiangrass and big bluestem with switchgrass were higher than the mixture that did not include switchgrass. This was also found in the current study. 'Bonanza' big bluestem mixtures containing 'Shawnee' switchgrass resulted in yields that were closer to the 'Shawnee' monoculture rather than either 'Bonanza' or 'Scout' indiangrass monocultures at Urbana, while a binary mixture of 'Bonanza' and 'Scout' produced a yield that was not significantly different from 'Scout' indiangrass (Table 4). However, there was no consistent difference in biomass yields between big bluestem monocultures and two- or three-way mixtures at Urbana and Mead. Although a three-way mixture containing side oats grama produced significantly lower biomass than other mixtures at Urbana (Table 4).

Compatibility in two- and three-way mixtures, which indicates the ability of different species to survive and yield when planted together, was different depending on grass species (Suppl. Table 2). Although indiangrass is one of the dominant warm-season perennial grasses in the tallgrass prairie [18, 53], studies found the ratio of indiangrass declined in mixtures containing big bluestem or switchgrass [9, 40]. In eastern Nebraska tallgrass prairies, big bluestem was found to comprise 42–62% of the total herbaceous standing crop, while indiangrass comprised only 14–16% of the total herbaceous standing crop [20]. Mulkey et al. [26] reported that switchgrass was compatible with big bluestem in South Dakota and big bluestem was likely to be more competitive than switchgrass, while switchgrass out-performed big bluestem in a big bluestem-switchgrass mixture in Oklahoma [40]. In addition, three-way mixtures, which were composed of big bluestem, indiangrass, and sideoats grama, produced lower biomass yields than other mixtures at Urbana, while big bluestem,

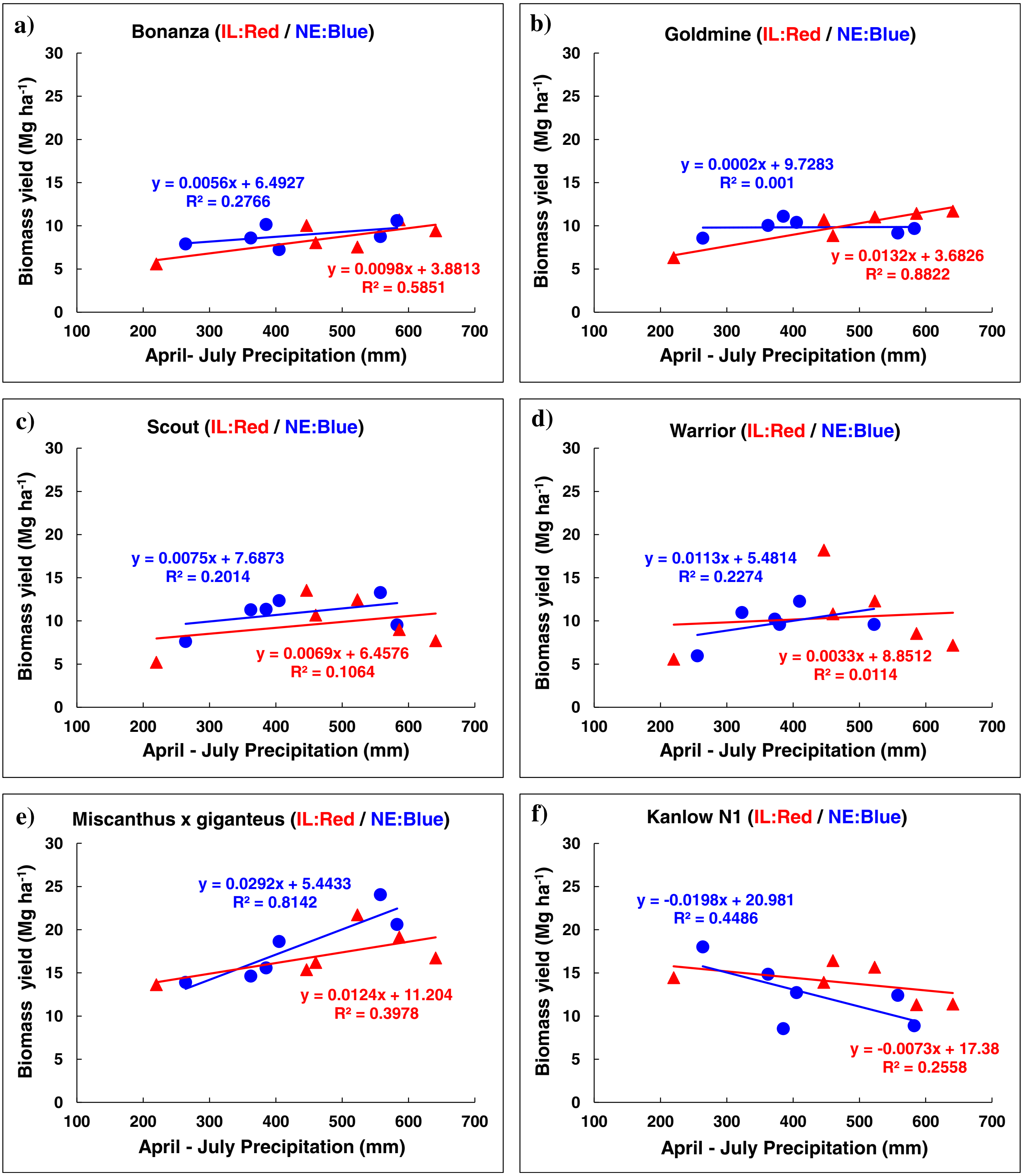

Fig. 1 Relationships between annual biomass dry matter yield and April and July precipitation from 2010 through 2015 at Urbana, IL and Mead, NE. (**A**) Big bluestem: (a) Bonanza and (b) Goldmine. (**B**) Indiangrass: (a) Scout and (b) Warrior. (**C**) Miscanthus × giganteus. (**D**) Switchgrass: Kanlow N1

indiangrass, and sideoats grama produced yields comparable to other mixtures at Mead. This is due to the species composition change which was observed in the three-way mixture at Urbana and Mead. As species compositions shifted after establishment with harvest management, big bluestem was a dominant species in two-way mixture plots and big bluestem and switchgrass were dominant species in three-way mixture plots.

Stand age and environmental conditions influenced the pattern of biomass production change of individual species over the 6-year study (Suppl. Table 1 and Suppl. Fig. 1). The biomass yields of *M.* × *giganteus* and big bluestem did not decrease at Urbana or Mead, while switchgrass and indiangrass yields tended to decrease as the stands aged (Suppl. Fig. 1). A yield decline in switchgrass was observed in 2013 and 2014 at Mead. The reason may have been due to the combination of a water deficit (due to drought conditions) and a late frost, which may have caused damage to Kanlow N1 switchgrass. July through August of 2012 was the second driest year recorded during a period from 1887 to 2014 in Mead, NE. A lack of precipitation resulted in lower soil moisture and subsequent soil moisture depletion in the root zone, which impacted the following year. Moreover, March of 2014 at Mead was the fifth driest month during 1887–2014 and frost damage occurred on May 15 and 17 in 2014.

Conclusion

The 6-year field experiment improves our understanding of the biomass production potential of native warm-season grasses in monocultures and mixtures in the Midwest. At all locations, *M.* × *giganteus* and 'Kanlow N1' produced the highest biomass yields in monocultures, and mixtures containing switchgrass and big bluestem had the highest mixture yields. All warm-season species tested in either monoculture or mixture conditions in this study consistently produced biomass as long as April–July precipitations were near or above the average precipitation (300 mm) of the regions. The 6-year field study results suggest mixtures provided no yield advantage over monocultures for bioenergy feedstocks in Illinois and Nebraska. However, compatibility and seasonal precipitation should be considered when developing warm-season grass mixtures to produce consistent biomass yields, confer resilience to environmental fluctuation, and improve biodiversity.

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