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2012

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Schmer, Marty R.; Vogel, Kenneth P.; Mitchell, R. B.; Dien, B. S.; Jung, H. G.; and Casler, M. D., "Temporal and Spatial Variation in Switchgrass Biomass Composition and Theoretical Ethanol Yield" (2012).

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Temporal and Spatial Variation in Switchgrass Biomass Composition and Theoretical Ethanol Yield

M. R. Schmer,* K. P. Vogel, R. B. Mitchell, B. S. Dien, H. G. Jung, and M. D. Casler

ABSTRACT

Information on temporal and spatial variation in switchgrass (*Panicum virgatum* L.) biomass composition as it affects ethanol yield (L Mg^{-1}) at a biorefinery and ethanol production (L ha^{-1}) at the field-scale has previously not been available. Switchgrass biomass samples were collected from a regional, on-farm trial and biomass composition was determined using newly developed near-infrared reflectance spectroscopy (NIRS) prediction equations and theoretical ethanol yield (100% conversion efficiency) was calculated. Total hexose (cell wall polysaccharides and soluble sugars) concentration ranged from 342 to 398 g kg^{-1} while pentose (arabinose and xylose) concentration ranged from 216 to 245 g kg^{-1} across fields. Theoretical ethanol yield varied significantly by year and field, with 5 yr means ranging from 381 to 430 L Mg^{-1} . Total theoretical ethanol production ranged from 1749 to 3691 L ha^{-1} across fields. Variability (coefficient of variation) within established switchgrass fields ranged from 1 to 4% for theoretical ethanol yield (L Mg^{-1}) and 14 to 38% for theoretical ethanol production (L ha^{-1}). Most fields showed a lack of spatial consistency across harvest years for theoretical ethanol yield or total theoretical ethanol production. Switchgrass biomass composition from farmer fields can be expected to have significant annual and field-to-field variation in a production region, and this variation will significantly affect ethanol or other liquid fuel yields per ton or hectare. Cellulosic biorefineries will need to consider this potential variation in biofuel yields when developing their business plans.

CELLULOSIC REFINERIES WILL require substantial amounts of biomass on a year-round basis and are expected to have higher capital costs than similar sized grain ethanol plants based on first-generation biomass refining technology (Wright and Brown, 2007). A reliable feedstock supply will be essential in maintaining stable operational costs. Further, cellulosic refineries will be required to convert biomass with potentially greater feedstock quality variability than existing corn (*Zea mays* L.) grain ethanol plants. Switchgrass is being developed as a biomass energy crop for the temperate regions of North America (Vogel and Mitchell, 2008). Temporal and spatial variation information across production years and fields for biomass yield and quality will be needed for establishing reliable feedstock supply areas for a cellulosic biorefinery. Information on field-scale spatial and temporal variation for biomass yield of switchgrass is becoming available (Schmer et al., 2010). Switchgrass biomass composition and theoretical ethanol production at the field-scale have thus far not been reported.

Biomass conversion to transportation fuels by biochemical methods will be dependent on efficient cellulose and hemicellulose polymer hydrolysis to simple sugars and then conversion to oxygenated hydrocarbons (Himmel et al., 2007). First generation cellulosic biorefineries, using biochemical methods, will produce primarily ethanol by converting cellulose, hemicellulose, and noncell wall carbohydrates into simple sugars which are then fermented to ethanol by genetically engineered organisms (Lynd et al., 1991; Perlack et al., 2005). Lignin, an abundant phenolic polymer in cell walls, can be used for combined heat and power generation (Demirbas, 2001; Lynd and Wang, 2003; Sheehan et al., 2003). Biochemical methods involve a pretreatment to reduce cell wall recalcitrance and increase cell wall porosity, a saccharification process to hydrolyze complex polysaccharides to monosaccharides, and a fermentation process to convert monosaccharides to a biofuel (Stephanopoulos, 2007). Near-term commercialized efforts to convert lignocellulosic feedstocks to biofuels through biochemical methods will likely involve simultaneous saccharification and fermentation (SSF). Alternative conversion systems such as consolidated bioprocessing which combines the enzymatic production, hydrolysis, and fermentation process into one reactor, thus reducing capital costs and increasing biorefinery efficiency, are expected to be commercially available as well (Lynd et al., 2005).

Cellulosic biomass conversion to biofuels via biochemical or thermochemical methods require more complex and

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Published in *Agron. J.* 104:54–64 (2012)

Posted online 23 Nov 2011

doi:10.2134/agronj2011.0195

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Abbreviations: ARA, arabinose; CRP, Conservation Reserve Program; ETOHTLT, total theoretical ethanol yield from all biomass sugars; FRU, fructose; GAL, galactose; GLC, cell wall glucose; GLCS, soluble glucose; HEX, total hexose; HEXEL, theoretical ethanol yield from all biomass hexoses; MAN, mannose; NIRS, near-infrared reflectance spectroscopy; NSC, nonstructural carbohydrates; PENTETL, theoretical ethanol yield from pentose sugars; PDSI, Palmer drought severity index; SSF, simultaneous saccharification and fermentation; STA, starch; SUC, sucrose; XYL, cell wall xylose.

comprehensive analyses of feedstock composition determinations than typical forage quality analyses (Dien, 2010; Dien et al., 2006; Dowe and McMillan, 2001). Estimated costs of feedstock composition analyses using wet laboratory methods are approximately \$300 per sample without including equipment or laboratory overhead costs (Vogel et al., 2011). Near-infrared reflectance spectroscopy is a nondestructive technology that can be used to obtain rapid, low-cost, high-throughput and accurate estimates of agricultural product composition if suitable prediction equations are available. The NIRS technology has been widely used in the food and pharmaceutical sector including recent advances to accurately predict corn grain quality and total fermentable carbohydrate content at dry-grind ethanol plants (Bothast and Schlicher, 2005). Advances in NIRS technology have made it feasible and analytically acceptable to determine if predicted spectral profile samples are within the spectral profile of the calibration set without continual wet laboratory verification (Murray and Cowe, 2004; Shenk and Westerhaus, 1991; Westerhaus et al., 2004). This study was made feasible by USDA-ARS development of NIRS calibrations for switchgrass biomass composition including cell wall and soluble sugars which enables hundreds of biomass samples to be economically evaluated for biomass composition (Vogel et al., 2011). These NIRS calibrations are being made available for use by other laboratories, both public and private, through the NIRS Consortium (<http://nirsconsortium.org/default.aspx>; verified 18 Nov. 2011).

Cell wall and soluble carbohydrate biomass composition estimates at the field-scale for multiple years across a diverse geographical region have not been previously reported for switchgrass. The effect of variation in biomass composition on potential ethanol yield via SSF ($L\ mg^{-1}$) and ethanol production per unit area ($L\ ha^{-1}$) over years and fields in a large potential production region have likewise not been previously reported. Primary objectives of this study were to quantify temporal and spatial variation in biomass composition that can occur in switchgrass biomass over years and fields in a production region and to determine the effects of this variation on theoretical ethanol yield ($L\ Mg^{-1}$) and production ($L\ ha^{-1}$).

MATERIALS AND METHODS

The study was conducted on farm fields in North Dakota (two fields), South Dakota (four fields), and Nebraska (four fields) as a component of a large-scale, multipurpose experiment which included studies on switchgrass establishment, economics, yield modeling, net energy calculations, soil carbon sequestration, and spatial and temporal biomass yield variation (Kiniry et al., 2008; Liebig et al., 2008; Perrin et al., 2008; Schmer et al., 2006, 2008, 2010). Detailed information on switchgrass biomass production for each farm can be found in the previous reports cited above. Farms are identified by the nearest town (Fig. 1). The 10 fields were located in a major agroecoregion where previous economic model analyses indicated switchgrass grown as a biomass energy crop would be economically feasible (Walsh, 1998). Fields were chosen based on characteristics of the region and qualifications in the Conservation Reserve Program (CRP). Nebraska fields were planted in 2000. The Atkinson, NE, field was replanted in 2001 because of stand failure caused by drought. The South Dakota and North

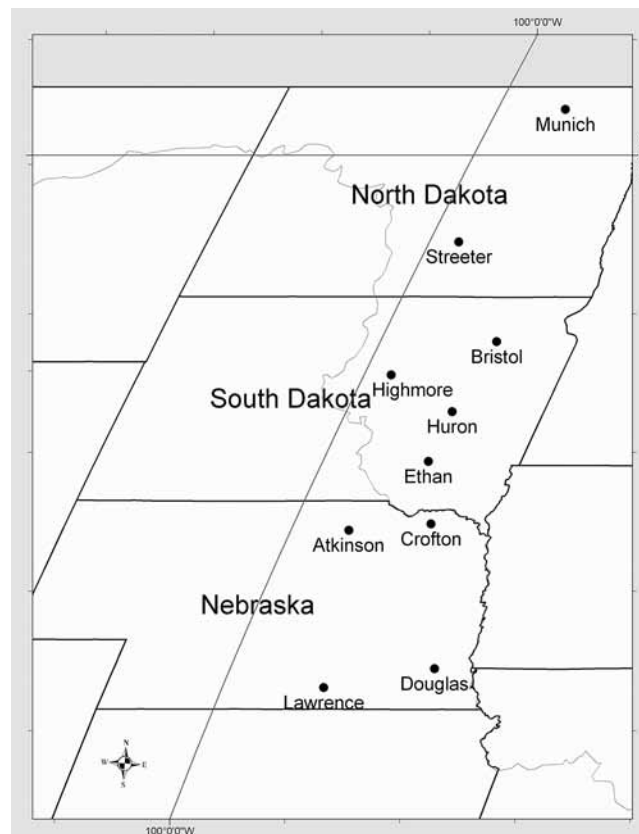


Fig. 1. Location of switchgrass fields managed for bioenergy in the Great Plains Region.

Dakota fields were planted in 2001. Field size ranged from 3 to 9.5 ha with an average of 6.7 ha. Farm cooperators managed all aspects of switchgrass production and harvest using a set of recommended management practices based on previous small plot research which included fertilization and harvesting procedures (Vogel, 2004).

Cultivars selected for each field were based on prior research within respective geographical regions. Switchgrass cultivars used in the study were 'Cave-in-Rock', 'Trailblazer', 'Shawnee', and 'Sunburst'. The selected cultivars were primarily developed for use in livestock pastures. Soil descriptions, previous cropping history and field size by location have been described previously (Liebig et al., 2008; Schmer et al., 2006). Nitrogen fertilization rates were based on previous research in the Central Plains which showed that at current switchgrass yield levels, approximately $10\ to\ 12\ kg\ N\ ha^{-1}$ is required for each $Mg\ ha^{-1}$ of expected biomass yield (Vogel et al., 2002). Nitrogen application varied across sites based on biomass yield expectations. Over the 5 yr period of the switchgrass stands, site averages of applied N ranged from $31\ to\ 104\ kg\ N\ ha^{-1}\ yr^{-1}$ (mean = $74\ kg\ N\ ha^{-1}$) (Schmer et al., 2010).

Fields were mechanically harvested and baled by cooperators. Based on previous research, optimal harvest times are when switchgrass is at anthesis which occurs in early to mid-August in the Great Plains or after a killing frost (Parrish and Fike, 2005; Vogel et al., 2002). Most cooperators chose to harvest at emerged inflorescence to postanthesis (early to mid-August) in postestablishment years, except for the Bristol and Munich fields, which were harvested after a killing frost. Six fields were mechanically harvested in the establishment year while

the Lawrence, Crofton, and Douglas fields were burned the following spring. The establishment year biomass at Ethan was neither removed nor burned the following spring but rather left standing due to lodging and subsequent switchgrass early spring growth in 2002.

Before mechanical harvest, fields were sampled each year at multiple quadrat sites to determine within-field variation for biomass yield and composition. Quadrat sample sites were randomly chosen by stratification based on cultivar and/or topography within each field (Schmer et al., 2010). A 12-channel global position system receiver (Lowrance Globalmap 1001; Catoosa, OK¹) was used to georeference each quadrat site within each switchgrass field. Biomass yields were estimated at 16 quadrat sites within a field using a 1-by 1-m quadrat in 2000 and a 0.3-by 3.66-m frame (1.1 m²) in 2001 through 2005 at the plant maturity stage of R1 to R5 (panicle fully emerged from boot to postanthesis) except for the establishment year which were sampled after a killing frost (Moore et al., 1991). Total plant biomass within the frame was clipped to a 10-cm stubble height and weighed with a portable electronic scale (Intercomp CS750, Minneapolis, MN). A subsample was dried at 55°C until a constant weight was reached to determine dry matter yield.

Switchgrass biomass subsamples from each sample site were ground through a 2-mm screen in a Wiley mill and then reground in a cyclone-type mill (Udy Corp., Ft. Collins CO) to pass a 1-mm screen. Ground samples were scanned using a Model 6500 near-infrared spectrometer (NIRSystems, Silver Springs, MD; now FOSS NIRSystems, Inc., Laurel, MD). A comprehensive set of switchgrass NIRS prediction equations were used to predict the composition of the harvested biomass samples (Vogel et al., 2011). The calibration equations were based on a set of switchgrass samples selected from several thousand switchgrass samples in a two-tiered process which represented a wide range of plant maturities, cultivars, ecotypes, fertility rates, and environments (Vogel et al., 2011). The NIRS calibration set of samples ($n = 112$) was analyzed for chemical composition, ethanol and pentose sugar yields following pretreatment, and SSF using commercial cellulases and *Saccharomyces cerevisiae*, and forage quality traits using wet laboratory methods (Dien et al., 2006; Vogel et al., 2011). The results of the wet laboratory analyses were then used to develop the NIRS prediction equations with specific calibration statistics reported between the wet laboratory value and NIRS predicted value (Vogel et al., 2011). No samples from this study were used in the development of the NIRS calibrations. Sample fitness from this study was determined using the Global H statistic (Murray and Cowe, 2004). The Global H statistic (Mahalanobis distance) is used to compare spectral profile of calibration samples and the samples to be analyzed. Samples with Global H values greater than three were excluded from the analysis (<4% of total samples). The NIRS calibrations are considered valid in estimating composition when Global H statistic values from analyzed samples are three or less (Murray and Cowe, 2004; Shenk and Westerhaus, 1991).

¹ Trade and company names or commercial products mentioned is solely for the purpose of providing specific information and does not imply recommendation of endorsement by the U.S. Department of Agriculture.

Although the available NIRS prediction equations can estimate 20 compositional components and 13 complex feedstock traits (Vogel et al., 2011), the focus in this report will be on the major biomass sugars that can be converted into ethanol and Klason lignin concentration. These carbohydrate traits include cell wall glucose (GLC), cell wall xylose (XYL), total hexose (HEX) which includes cell wall hexoses (GLC, mannose [MAN], galactose [GAL]) and nonstructural carbohydrates (NSC) of the biomass. The NSC consists of the soluble carbohydrates (glucose [GLCS], sucrose [SUC], and fructose [FRU]) and starch (STA) present in switchgrass biomass.

Total hexoses were calculated as per Vogel et al. (2011) as follows:

1. $HEX (g\ kg^{-1}) = [(MAN + GAL + GLC)(180/162)] + NSC$, where NSC is:
2. $NSC (g\ kg^{-1}) = GLCS + FRU + SUC + STA$.

Total pentose includes XYL and arabinose (ARA).

Theoretical ethanol yield and ethanol production was calculated using the following equations (Vogel et al., 2011):

3. Theoretical ethanol yield from all biomass hexoses:
 $HEXEL (L\ Mg^{-1}) = \{[(MAN + GAL + GLC + STA) \times 0.57] + [(GLCS + FRU) \times 0.51] + (SUC \times 0.537)\} \times 1.267$; assuming 100% conversion.
4. Theoretical ethanol yield from pentose sugars:
 $PENTETL (L\ Mg^{-1}) = (ARA + XYL) \times 0.579 \times 1.267$.
5. Total Theoretical ethanol yield from all biomass sugars:
 $ETOHTLT (L\ Mg^{-1}) = HEXEL + PENTETL$.
6. Total theoretical ethanol production ($L\ ha^{-1}$) =
 $ETOHTLT \times \text{biomass production yield } (Mg\ ha^{-1})$.

Theoretical ethanol production values ($L\ ha^{-1}$) for each sample site by field, harvest, and year were calculated based on quadrat yields and the composition of the biomass sample from each quadrat using the procedures described above.

Statistical Analysis

The study was analyzed as a repeated-measure experiment, by field, with stand age (establishment year through Year 5) as a fixed effect while calendar year (2000 through 2005) was treated as a random effect to differentiate between the normal stand maturation trend over time and random weather effects (Loughin, 2006). Data were analyzed by cultivar and harvest year for Crofton, Douglas, and Lawrence where different cultivars were planted in the same field. Data were analyzed using the mixed procedure in SAS (Littel et al., 1996). Main effects and any interactions were considered significant when $P \leq 0.05$. Mean separations was performed using Fisher's LSD.

Pearson correlations were calculated among the cell wall composition traits. Variability in theoretical ethanol yield and theoretical ethanol production were determined using coefficient of variation by field and established harvest years (harvest years three through five). Spearman rank correlations were used to quantify year-to-year spatial consistency for theoretical ethanol yield ($L\ Mg^{-1}$) and theoretical ethanol production ($L\ ha^{-1}$) within a field. Rank correlations were based on the ranking of the sample sites within a field for all harvest years. Spearman rank

correlations as suggested by Florin et al. (2009) above $r = 0.5$ would indicate that spatial patterns exist for sample sites within a field over harvest years while correlations below $r = 0.5$ would indicate a lack of spatial pattern consistency over harvest years.

RESULTS

Mean annual precipitation for the fields sites during the 5 yr of study ranged from 430 mm in the west to 779 mm in the east, while mean annual temperature ranges from 2.7°C in the north to 12.8°C in the south (Schmer et al., 2010). Drought conditions were prevalent at most fields for the study duration as indicated by the Palmer drought severity index (PDSI) (Fig. 2). Negative PDSI values indicate degree of drought severity. Switchgrass fields in North Dakota and South Dakota tended to have above average precipitation conditions at establishment phase and at the end of the study (Fig. 2). Switchgrass fields in

Nebraska were under moderate drought conditions during the establishment phase with above average precipitation in 2001 (Fig. 2). Drought conditions were more prevalent at the southern and western fields (Fig. 1 and 2).

Total hexose components differed by harvest year for all fields (Table 1). Hexose sugars ranged from 342 g kg⁻¹ at Munich to 398 g kg⁻¹ at Lawrence across fields (Tables 2 and 3). Hexose concentrations differed by cultivar at Douglas and Lawrence (Table 1) with Cave-in-Rock and Shawnee having higher hexose concentrations than Trailblazer (Table 3). Pentose concentrations differed across harvest years at all fields with average concentrations ranging from 216 g kg⁻¹ at Munich to 245 g kg⁻¹ at Crofton. Cell wall glucose, found primarily in cellulose, comprised 66 to 82% of the total switchgrass hexose concentration while XYL was the most abundant pentose carbohydrate, found in hemicellulose, comprising 77

Table 1. Analysis of variance *F* values and statistical significance for estimated switchgrass biofuel quality parameters from fields managed for bioenergy.

Interaction	Field locations									
	Munich	Streeter	Bristol	Highmore	Huron	Ethan	Atkinson	Crofton	Douglas	Lawrence
	<u>Total hexose, g kg⁻¹</u>									
Harvest year (HY)	81.6***	28.8***	38.4***	102.9***	119.0***	4.63***	112.2***	34.9***	9.6***	34.8***
Cultivar (C)								0.75	6.8*	11.9***
HY × C								0.26	1.6	1.7
	<u>Glucose, g kg⁻¹</u>									
HY	147.7***	34.4***	89.5***	112.7***	86.7***	1.65	36.7***	25.4***	8.61***	102.6***
C								7.10***	23.3***	26.6***
HY × C								0.9	0.6	3.4*
	<u>Pentose, g kg⁻¹</u>									
HY	83.6***	48.1***	88.5***	51.0***	95.2***	3.23***	39.4***	45.9***	13.8***	4.7**
C								14.7***	34.7***	18.7***
HY × C								3.4*	5.41***	2.3*
	<u>Xylose, g kg⁻¹</u>									
HY	110.8***	32.5***	38.9***	36.9***	68.6***	3.71**	22.2***	45.4***	6.8***	24.9***
C								11.3***	24.0***	23.8***
HY × C								5.31***	2.64*	1.5
	<u>Nonstructural carbohydrates, g kg⁻¹</u>									
HY	27.4***	31.3***	105.8***	36.4***	18.0***	53.1***	51.1***	54.7***	15.3***	51.4***
C								25.8***	30.5***	25.8***
HY × C								2.29	0.97	0.87
	<u>Klason lignin, g kg⁻¹</u>									
HY	0.61	6.32***	14.9***	30.3***	13.1***	20.4***	2.28	1.63	13.7***	93.5***
C								0.06	0.07	10.1***
HY × C								0.30	2.41	0.57
	<u>Theoretical ethanol yield, L Mg⁻¹</u>									
HY	120.5***	17.8***	28.8***	99.2***	72.0***	3.49**	14.31***	6.5***	20.4***	14.6***
C								5.4*	2.2	0.83
HY × C								2.65*	0.5	0.2
	<u>Theoretical ethanol production, L ha⁻¹</u>									
HY	37.9***	81.2***	13.2***	52.6***	55.1***	23.1***	43.9***	18.0***	7.4***	16.0***
C								10.7**	4.6*	0.3
HY × C								1.0	5.0**	0.8

*Significance at the 0.05 *P* level.

** Significance at the 0.01 *P* level.

*** Significance at the 0.001 *P* level.

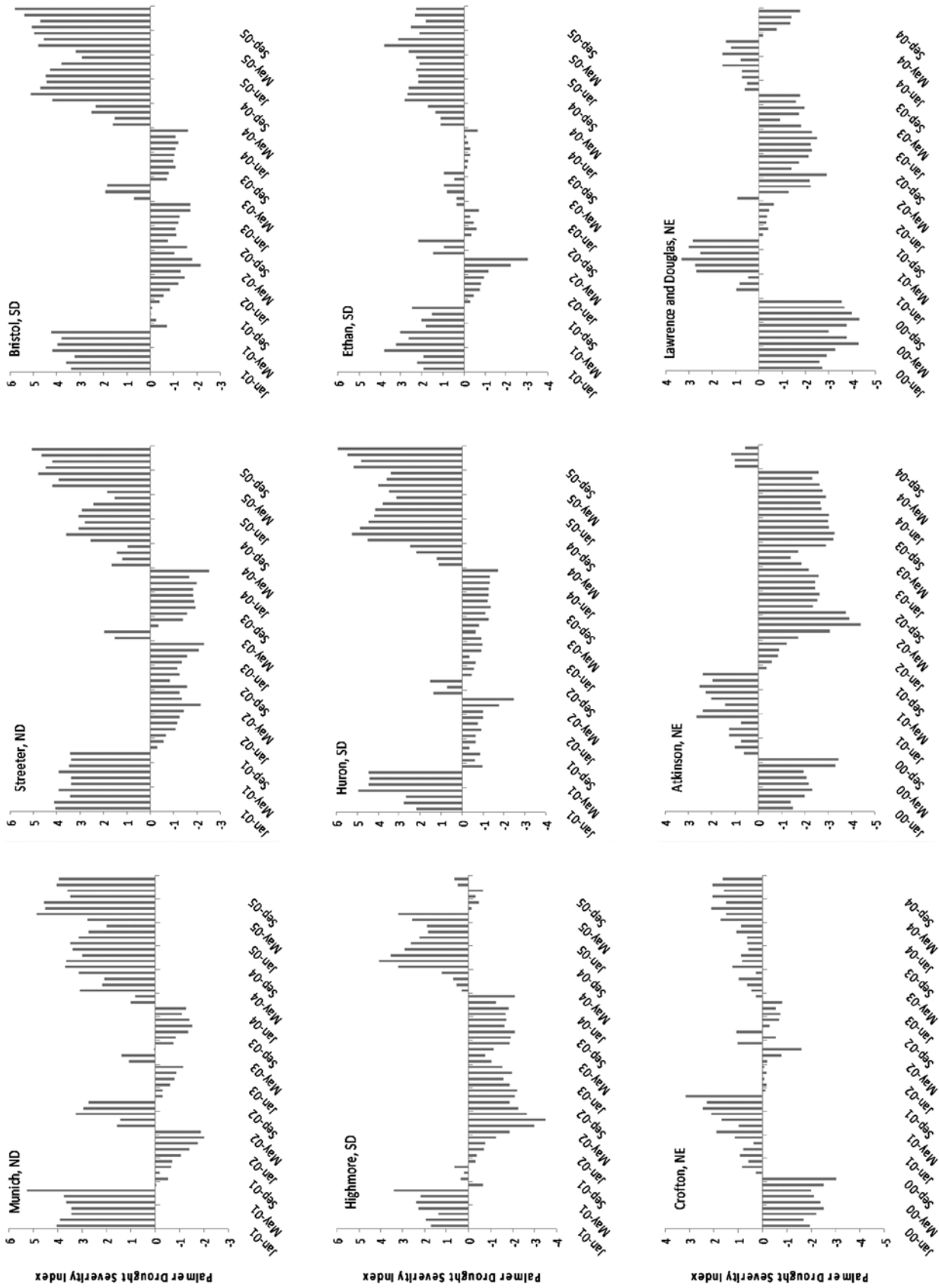


Fig. 2. Palmer drought severity index for regions in the Great Plains where switchgrass fields were managed for bioenergy (2000–2005). Negative values indicate drought conditions.

Table 2. Composition of switchgrass from a regional on-farm bioenergy trial in the Great Plains. Field samples (n = 16) per location were taken at panicle emergence to postanthesis.

Year	Munich	Streeter	Bristol	Highmore	Huron	Ethan	Atkinson
<u>Total hexose, g kg⁻¹</u>							
2001	283	–†	362	284	355	411	397
2002	337	341	351	312	333	363	323
2003	361	358	374	369	–‡	375	276
2004	357	380	381	373	385	372	391
2005	374	373	388	381	381	402	–§
Mean	342	363	371	344	364	385	347
LSD 0.05	9	6	5	8	4	18	6
<u>Glucose, g kg⁻¹</u>							
2001	200	–	253	192	267	279	263
2002	266	270	281	235	253	254	243
2003	295	271	295	279	–	281	275
2004	281	290	308	291	305	264	282
2005	297	274	306	277	287	277	–
Mean	268	276	289	255	278	271	266
LSD 0.05	8	7	5	8	5	–	6
<u>Pentose, g kg⁻¹</u>							
2001	189	–	211	197	222	217	224
2002	215	220	246	235	243	240	254
2003	230	228	242	235	–	239	246
2004	214	235	243	237	253	213	239
2005	230	226	245	234	240	230	–
Mean	216	227	237	228	240	228	241
LSD 0.05	4	5	3	5	3	14	4
<u>Xylose, g kg⁻¹</u>							
2001	146	–	190	163	192	189	196
2002	185	188	216	205	213	205	219
2003	200	202	211	203	–	201	210
2004	183	199	209	202	216	175	202
2005	199	197	208	199	208	190	–
Mean	183	197	207	194	207	192	207
LSD 0.05	4	5	3	6	3	12	4
<u>Non-structural carbohydrates, g kg⁻¹</u>							
2001	49	–	68	52	37	84	88
2002	27	26	28	33	35	67	38
2003	19	46	28	40	–	40	42
2004	20	37	19	27	27	21	60
2005	25	50	29	59	46	75	–
Mean	28	40	34	42	36	57	57
LSD 0.05	7	10	5	6	5	10	9
<u>Klason lignin, g kg⁻¹</u>							
2001	116	–	110	123	111	100	102
2002	116	109	111	108	103	94	102
2003	114	102	103	101	–	106	101
2004	118	112	112	114	111	118	107
2005	117	111	102	102	104	107	–
Mean	116	109	108	106	108	105	103
LSD 0.05	ns¶	6	3	5	4	7	ns

† Mechanical hay harvest was done mid-summer before quadrat sampling to remove volunteer oats.

‡ Mechanical hay harvest was done before quadrat sampling.

§ Study completed spring of 2005.

¶ ns, not significant.

Table 3. Switchgrass cell wall composition from Nebraska locations planted with cultivars Cave-in-Rock (CIR), Shawnee (SH), and Trailblazer (TB). Field samples ($n = 16$) per location were taken at panicle emergence to postanthesis.

Location	Year							Cultivar			
	2000	2001	2002	2003	2004	Mean	LSD	CIR	SH	TB	LSD
	<u>Total hexose, g kg⁻¹</u>										
Crofton	405	376	368	371	389	382	7	–	383	381	ns†
Douglas	370	357	384	382	397	378	14	382	–	374	6
Lawrence	416	374	393	413	394	398	8	399	405	390	7
	<u>Glucose, g kg⁻¹</u>										
Crofton	325	276	268	282	296	289	6	–	285	294	4
Douglas	280	285	268	280	296	282	10	277	–	287	4
Lawrence	308	266	262	267	291	279	8	275	276	286	7
	<u>Pentose, g kg⁻¹</u>										
Crofton	247	229	260	246	245	245	5	–	243	248	3
Douglas	221	239	246	235	239	236	7	231	–	241	3
Lawrence	243	238	245	237	245	242	5	238	238	248	4
	<u>Xylose, g kg⁻¹</u>										
Crofton	218	193	222	207	206	209	5	–	207	212	3
Douglas	190	210	211	197	198	201	10	196	–	207	4
Lawrence	222	203	211	199	206	208	7	205	204	216	4
	<u>Nonstructural carbohydrates, g kg⁻¹</u>										
Crofton	8	46	49	36	35	35	6	–	40	30	4
Douglas	26	25	70	49	43	43	13	51	–	34	6
Lawrence	31	62	86	88	51	64	9	68	73	49	8
	<u>Klason lignin, g kg⁻¹</u>										
Lawrence	121	112	87	87	109	103	5	105	99	106	4
Douglas	117	118	98	106	118	111	7	111	–	112	ns
Crofton	109	107	106	104	108	107	ns	–	107	107	ns

† ns, not significant.

to 90% of the total pentose concentration (Tables 2 and 3). Total hexose and GLC concentrations were positively correlated ($r = 0.69$, $P < 0.01$) as were XYL and total pentose concentration ($r = 0.94$, $P < 0.01$). Cell wall glucose concentration was 38% higher than XYL concentration averaged across fields and harvest years. Concentrations of GLC and XYL were positively correlated ($r = 0.59$; $P < 0.001$). Cell wall glucose concentrations differed by harvest year for all fields except for Ethan (Table 1). Differences in 5-yr-mean GLC concentrations by fields ranged from 255 g kg⁻¹ at Highmore to 289 g kg⁻¹ at Bristol (Table 2 and 3). Cultivars differed for GLC concentration at Crofton, Douglas, and Lawrence with a harvest year \times cultivar interaction at Lawrence (Table 1). Trailblazer had higher 5-yr-mean GLC concentrations than either Cave-in-Rock or Shawnee at Crofton, Douglas, and Lawrence, respectively (Table 3). Xylose concentrations differed by harvest year at all fields with 5-yr-mean concentrations ranging from 183 g kg⁻¹ at Munich to 209 g kg⁻¹ at Crofton (Tables 2 and 3). Annual biomass yields and annual GLC concentrations were positively correlated ($r = 0.54$, $P < 0.01$) across all fields. Correlations between total hexose, total pentose, XYL, NSC, and Klason lignin concentrations were not correlated ($P > 0.05$) with annual biomass yields across all fields (data not shown).

Nonstructural carbohydrate concentrations differed by harvest year at all locations (Table 1). Field sites that were established in 2001 showed above average NSC concentrations during the establishment year while fields established in 2000 tended to have higher NSC concentration in 2002 (Tables 2 and 3). Trailblazer had lower NSC concentrations averaged

across harvest years than either Cave-in-Rock or Shawnee (Table 3). Nonstructural carbohydrates accounted for 12% of the total hexose concentration when averaged across fields and harvest years with a range from 2 to 22% by harvest year and field. Cell wall glucose was negatively correlated ($r = -0.38$; $P < 0.01$) with NSC concentration. Klason lignin concentrations differed by harvest year for 7 out of 10 locations (Table 1). Cultivar differences were found at Lawrence with Shawnee having lower 5-yr-mean values than Cave-in-Rock or Trailblazer (Table 3). Klason lignin 5-yr-mean values ranged from 103 g kg⁻¹ at Atkinson and Lawrence to 116 g kg⁻¹ at Munich. Klason lignin was negatively correlated with XYL and NSC ($r = -0.45$ and -0.44 , respectively; $P < 0.01$), but no significant correlations were found between Klason lignin and the other cell wall polysaccharide component sugars (data not shown).

Theoretical ethanol yield (L Mg⁻¹) differed by harvest years for all fields (Table 1). Ethanol yield 5-yr-means across fields ranged from 381 L Mg⁻¹ at Munich to 430 L Mg⁻¹ at Lawrence (Tables 4 and 5). Theoretical ethanol yields differed between fields by as much as 122 L Mg⁻¹ for individual harvest years. Ethanol yield differences between Trailblazer and Shawnee were present at Crofton (Table 5) but cultivars were similar at Douglas and Lawrence. Theoretical ethanol production (L ha⁻¹) values differed by harvest years for all fields (Table 1) with cultivar differences present at Crofton and Douglas (Table 5). Theoretical ethanol production 5-yr-mean values ranged from 1749 at Highmore to 3691 L ha⁻¹ at Bristol (Table 4). In 2002, drought conditions (Fig. 2) resulted in low switchgrass mean biomass yields (Schmer et al., 2010), with lower than average GLC levels for all fields

Table 4. Switchgrass theoretical ethanol yield (100% conversion) and ethanol production means from a regional on-farm bioenergy trial in the Great Plains. Field samples (n = 16) per location were taken at panicle emergence to postanthesis.

Year	Munich	Streeter	Bristol	Highmore	Huron	Ethan	Atkinson
<u>Theoretical ethanol yield, L Mg⁻¹</u>							
2001	318	–†	401	333	397	427	425
2002	381	387	419	378	401	417	400
2003	409	411	425	414	–‡	415	416
2004	386	414	423	412	431	395	427
2005	410	408	431	420	426	422	–§
Mean	381	405	420	392	414	415	418
LSD 0.05	8	7	4	8	4	11	7
<u>Theoretical ethanol production, L ha⁻¹</u>							
2001	819	–	2962	737	1739	1898	1570
2002	1795	1790	3039	428	1952	1024	622
2003	4012	2411	5085	2734	–	3267	3037
2004	2152	3069	4064	3502	3724	2343	3212
2005	2646	1891	3307	1178	1930	2050	–
Mean	2311	1887	3691	1749	2336	2116	2194
LSD 0.05	380	230	491	388	251	330	396

† Mechanical hay harvest was done mid-summer before quadrat sampling to remove volunteer oats.

‡ Mechanical hay harvest was done before quadrat sampling.

§ Study completed spring of 2005.

Table 5. Switchgrass theoretical ethanol yield (100% conversion) and ethanol production means from Nebraska locations planted with cultivars Cave-in-Rock (CIR), Shawnee (SH), and Trailblazer (TB). Quadrant samples (n = 16) per field were taken at panicle emergence to postanthesis.

Location	2000	2001	2002	2003	2004	Mean	LSD	CIR	SH	TB	LSD
<u>Theoretical ethanol yield, L Mg⁻¹</u>											
Crofton	436	406	426	416	426	422	7	–	420	425	4
Douglas	394	410	429	413	420	413	14	411	–	415	ns†
Lawrence	440	414	436	433	428	430	8	428	431	432	ns
<u>Theoretical ethanol production, L ha⁻¹</u>											
Crofton	1315	1854	2231	2851	3184	2287	505	–	2032	2542	312
Douglas	1943	2878	2984	3746	3902	3091	814	3286	–	2895	365
Lawrence	1259	1918	2329	3485	2620	2322	591	2310	2247	2409	ns

† ns, not significant.

Table 6. Coefficient of variation for theoretical ethanol yield and theoretical ethanol production from established switchgrass fields in North Dakota, South Dakota, and one field in Nebraska.

Year	Munich	Streeter	Bristol	Highmore	Huron	Ethan	Atkinson
<u>Theoretical ethanol yield</u>							
2003	2	3	2	2	–	2	2
2004	3	2	2	3	1	3	3
2005	3	4	2	3	2	3	–
<u>Theoretical ethanol production</u>							
2003	23	20	14	29	–	19	31
2004	17	19	21	38	17	34	27
2005	35	28	19	23	14	29	–

and above average XYL concentrations for 9 out of the 10 fields (Tables 2 and 3). Ethanol production values in 2002 were lower than the 5-yr-average for 9 out of the 10 fields (Tables 4 and 5).

Coefficients of variation, which were used as indicators of production stability, were smaller for theoretical ethanol yield than theoretical ethanol production (Tables 6 and 7). Coefficient of variation for established switchgrass fields ranged from 1 to 4% for theoretical ethanol yield (Tables 6 and 7).

Table 7. Coefficient of variation for theoretical ethanol yield and theoretical ethanol production for years and cultivars in established switchgrass fields in Nebraska.

Location	2002	2003	2004	CIR	SH	TB
<u>Theoretical ethanol yield</u>						
Crofton	3	3	2	–	4	3
Douglas	3	3	2	5	–	4
Lawrence	3	2	2	3	3	3
<u>Theoretical ethanol production</u>						
Crofton	26	38	18	–	39	42
Douglas	37	16	22	35	–	38
Lawrence	28	24	24	42	48	45

Theoretical ethanol production had CV values ranging from 14 to 38% across fields and harvest years (Tables 6 and 7). Although theoretical ethanol yield varied by year for each field, within field theoretical ethanol yields showed minimal variation.

Rank correlations for theoretical ethanol yields were generally low ($r < 0.5$) for most fields (Table 8). Low correlation coefficients that are either positive or negative indicates theoretical ethanol yield were not predictable by harvest years. A similar result was found for theoretical ethanol production (Table

Table 8. Spearman rank correlations for theoretical ethanol yield (L Mg⁻¹) within fields over harvest years. Ranked correlations above $r = |0.5|$ indicate that sample sites within a field had some form of spatial pattern persistence while correlations below $r = |0.5|$ indicates a lack of spatial patterns over harvest years.

Location	Harvest years	Harvest years				
		H1	H2	H3	H4	H5
Munich	H1	-	0.11	0.13	-0.15	0.17
	H2		-	-0.25	-0.37	-0.25
	H3			-	0.05	0.37
	H4				-	-0.10
Streeter	H1	-	-	-	-	-
	H2		-	0.18	0.60	0.19
	H3			-	0.51	0.14
	H4				-	0.29
Bristol	H1	-	0.05	0.21	-0.17	0.12
	H2		-	0.19	-0.14	-0.32
	H3			-	0.33	0.36
	H4				-	0.21
Highmore	H1	-	0.11	0.30	-0.15	0.12
	H2		-	0.17	0.60	-0.27
	H3			-	-0.17	0.12
	H4				-	-0.21
Huron	H1	-	0.12	-	0.17	-0.08
	H2		-	-	0.79	0.09
	H3			-	-	-
	H4				-	0.28
Ethan	H1	-	0.17	0.50	-0.36	0.02
	H2		-	-0.31	-0.40	0.52
	H3			-	0.26	-0.16
	H4				-	-0.33
Atkinson	H1	-	-0.18	0.03	0.16	-
	H2		-	0.29	0.47	-
	H3			-	0.28	-
	H4				-	-
Crofton	H1	-	0.11	-0.21	0.10	0.19
	H2			0.18	0.16	0.70
	H3			-	0.31	0.10
	H4				-	0.37
Douglas	H1	-	0.17	0.15	0.45	-0.44
	H2		-	0.14	0.16	-0.07
	H3			-	0.34	-0.18
	H4				-	-0.13
Lawrence	H1	-	-0.23	0.19	0.17	0.20
	H2		-	0.33	0.20	0.18
	H3			-	0.10	-0.11
	H4				-	0.23

Table 9. Spearman rank correlations for theoretical total ethanol production (L ha⁻¹) within fields over harvest years. Ranked correlations above $r = |0.5|$ indicate that sample sites within a field had some form of spatial pattern persistence while correlations below $r = |0.5|$ indicates a lack of spatial patterns over harvest years.

Location	Harvest years	Harvest years				
		H1	H2	H3	H4	H5
Munich	H1	-	0.28	0.35	-0.03	-0.03
	H2		-	0.53	-0.17	0.16
	H3			-	0.15	0.49
	H4				-	0.02
Streeter	H1	-	-	-	-	-
	H2		-	0.34	-0.19	0.17
	H3			-	0.39	0.53
	H4				-	0.52
Bristol	H1		0.68	0.69	0.16	0.43
	H2			0.47	0.33	0.52
	H3				0.26	0.39
	H4					0.49
Highmore	H1	-	0.20	0.23	0.02	-0.50
	H2		-	0.57	0.40	-0.11
	H3			-	0.39	-0.09
	H4				-	0.50
Huron	H1	-	0.34	-	0.08	-0.09
	H2		-	-	-0.23	-0.03
	H3			-	-	-
	H4				-	0.02
Ethan	H1	-	0.52	0.02	0.00	-0.29
	H2		-	0.65	-0.08	0.40
	H3			-	0.16	0.55
	H4				-	-0.07
Atkinson	H1	-	-0.17	-0.17	0.21	-
	H2		-	0.59	0.09	-
	H3			-	0.14	-
	H4				-	-
Crofton	H1	-	0.56	0.63	0.59	0.52
	H2		-	0.27	0.54	0.56
	H3			-	0.57	0.39
	H4				-	0.44
Douglas	H1	-	0.04	-0.62	-0.15	0.18
	H2		-	0.19	0.09	-0.01
	H3			-	0.33	-0.05
	H4				-	-0.21
Lawrence	H1	-	0.41	0.06	0.09	0.14
	H2		-	0.13	0.08	0.11
	H3			-	0.54	0.34
	H4				-	0.43

9). Crofton showed a high positive correlation for theoretical ethanol production between harvest Year 1 and harvest Year 2 through 5 (Table 9). Theoretical ethanol yields were not highly correlated at Crofton for most years (Table 8), indicating ethanol production rates were the probable result of correlated biomass yields. High positive correlations indicate that spatial patterns exist within fields for ethanol production and these values are predictable between harvest years. Negative rank correlations ($r > 0.5$) indicate that spatial patterns for ethanol

yield and ethanol production are inverted by growing season as a result of weather conditions.

DISCUSSION

The results of this study demonstrate that biorefineries can expect significant annual variation in biomass composition as it affects potential ethanol yield (L Mg⁻¹) and for ethanol production (L ha⁻¹) from individual fields within a production region. This variation was not unexpected because year-to-year climatic variation due to variation in solar radiation,

temperature, and precipitation can affect plant growth and development of perennial grasses and thereby affect biomass yield and quality. Growth temperatures above the optimum growth range for perennial plants has been shown to accelerate plant maturation, lower the leaf/stem ratio, and promote lignification (Buxton and Casler, 1993). Rainfall limitations and variable growing season temperatures in the study region (Fig. 2) likely influenced biofuel quality traits evaluated in this study. Drought conditions increased cell wall hemicellulose concentration in switchgrass (Table 2 and 3) similar to previous findings in tobacco (*Nicotiana tabacum* L.) cell cultures and white spruce [*Picea glauca* (Moench) Voss] needles (Iraki et al., 1989; Zwiazek, 1991). Management practices such as harvest dates and methods fertilization rates, and cultivars have resulted in biomass yield and forage quality variation (Adler et al., 2006; Buxton and Casler, 1993; Dien et al., 2006; Mulkey et al., 2006) which contributed to variation in this study. Improved and more consistent management practices in a production region could reduce management sources of variation in biomass conversion quality. Climatic variation is not controllable by management but it will be possible to model the effects of climatic variation during a growing season on biomass quality so that optimal harvest dates could be predicted based on desired quality traits. Previous research has demonstrated that switchgrass forage quality parameters can be predicted using growing degree day units or morphological development (Mitchell et al., 2001).

Theoretical ethanol yields obtained in this study assume 100% conversion of selected cell wall carbohydrates and storage polysaccharides similar to conversion formulas developed by the Department of Energy (USDOE, 2010). Actual ethanol yields obtained by a biorefinery will be less but at the present time are difficult to predict because biochemical conversion efficiency will be dependent on the pretreatment process, enzyme loading requirements, conversion rate, and ability to reduce inhibitors at all conversion stages (Lau and Dale, 2009). Cellulose polymers tend to be more recalcitrant than hemicellulose and overall conversion efficiency is sensitive to pretreatment method. Nonstructural carbohydrates were a significant portion of HEX concentrations but NSC recovery will be dependent on the pretreatment method used (Dien et al., 2006). Regardless of the pretreatment method and biochemical conversion technology, conversion efficiency will be dependent on the composition of the biomass being processed. Further, compositional knowledge of incoming switchgrass might allow for process conditions to be adjusted for increased conversion efficiency. The positive correlation we observed between GLC and XYL concentrations may require higher enzyme-loading requirements to increase conversion rates for switchgrass lots with these characteristics. Increased pretreatment severity may be required for switchgrass lots with high Klason lignin concentrations but may reduce XYL conversion because of the positive correlation we found between Klason lignin and XYL. The low negative correlation between GLC and NSC would suggest that ethanol yield from hexoses fractions would not vary greatly among switchgrass lots.

The within-field variation for switchgrass biomass quality is important because it is an indicator of the amount of bale-to-bale variation in feedstock composition that can be expected

from a single field. Variability in ethanol yield was relatively consistent with CV values ranging from 1 to 4% indicating minimal spatial variation within fields for potential conversion quality (Tables 6 and 7). This indicates that bales from a single harvest year from a field should be consistent in conversion quality and this consistent quality can be maintained using recommended storage practices. However, this limited variation in conversion quality could be compromised if bales become degraded because of variable on-farm storage effects before processing (Digman et al., 2010; Sanderson et al., 1997).

Switchgrass ethanol production is influenced by both biomass yields and biomass quality. Our results indicate that there is significantly more spatial and temporal variation for ethanol production ($L\ ha^{-1}$) than theoretical ethanol yield ($L\ Mg^{-1}$). Weather-related stresses on annual crops have caused overall yield variability (CV) within fields to vary by year (Kravchenko et al., 2005) which is similar to switchgrass theoretical ethanol production values presented here. In addition, most fields showed a lack of spatial pattern consistency over harvest years for ethanol yield or production (Tables 8 and 9). Improvements in ethanol yield ($L\ Mg^{-1}$) and ethanol production ($L\ ha^{-1}$) and stability should be feasible via additional breeding and management research (Digman et al., 2010; Mitchell et al., 2008; Vogel and Jung, 2001; Vogel et al., 2011).

In summary, switchgrass biomass composition from farmer fields can be expected to have significant annual and field-to-field variation in a production region and this variation will significantly affect ethanol or other liquid fuel yields per ton or hectare. Because of large differences in potential liquid fuel yields, it will be advisable for cellulosic biorefineries to test switchgrass for biomass quality before conversion. Rapid and economical testing of switchgrass for biomass composition is feasible using NIRS technology. Cellulosic biorefineries will need to consider this potential variation in biofuel yields when implementing their biochemical conversion technology.

ACKNOWLEDGMENTS

USDA is an equal opportunity provider and employer. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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