Published September 4, 2015

The Effects of Hybrid Relative Maturity on Corn Stover for Ethanol Production and Biomass Composition

Steve Wilkens, Paul J. Weimer, and Joseph G. Lauer*

ABSTRACT

Full-season corn (*Zea mays* L.) hybrids take advantage of more of the growing season than shorter-season hybrids often leading to greater grain and biomass yield. Many agronomic experiments aimed at corn stover production have been performed at forage harvest rather than later when stover is normally harvested for biofuel measurements. The objective of this research was to evaluate the influence of hybrid relative maturity (days RM) on stover ethanol production, ruminant digestibility, and biomass composition. Hybrids selected were high-yielding commercial grain hybrids grown throughout Wisconsin and ranged from 85 to 115 d RM in 10 d RM increments during 2009, and in 5 d RM increments during 2010. Hybrids were harvested at physiological maturity or after a killing frost. Overall, stover and theoretical ethanol yields increased as RM increased at a linear rate of 0.211 Mg ha^{-1} RM^{-1} and 67.1 L ha^{-1} RM^{-1}. Stover nutritional and biomass composition improved as RM increased, but yield variability was greater than nutritional and biomass compositional variability. Increasing ethanol yields will likely occur by increasing stover yields rather than by altering stover composition. Therefore, until price premiums for stover composition are made available to farmers for ethanol production, the adoption of full-season or longer maturing hybrids should be implemented for increased stover and ethanol yields.

Published in Agron. J. 107:2303–2311 (2015)
doi:10.2134/agronj15.0123
Received 12 Mar. 2015
Accepted 12 July 2015

Large increases in cellulosic biofeedstocks are required for the United States to displace a significant fraction of its petroleum consumption with biofuels under the current agriculture framework (Lorenz et al., 2009; Perlack et al., 2005). Corn stover, which is the aboveground non-grain portion of the plant, is the largest agricultural source of collectable biomass and is predicted to make up a quarter of the 1.24 billion Mg of biomass needed to accomplish the alternative fuels mandate (Perlack et al., 2005). To achieve the output set forth it is estimated that stover production will need to increase by a minimum of 50% (USDOE, 2005).

Over time increases in stover yield have been achieved through the adoption of full-season hybrids and breeding for stronger, denser stalks and delayed senescence, allowing less remobilization of dry matter to the ear during grain fill (Lorenz et al., 2010b). On average, later maturing plants have delayed senescence, or better "stay green" (Duvick, 2005) meaning plants retain their leaves, stay healthier, and are better able to withstand growing season environmental stresses (Tollenaar et al., 1994). Plants with longer photosynthetic durations have the potential to capture more light for photosynthesis and produce more biomass (Richards, 2000). Sarlangue et al. (2007) noted that shorter-season hybrids intercept less radiation and produce less biomass than full-season hybrids. Zeng et al. (2012) reported that stay green is of keen interest when considering corn stover as a source of cellulosic feedstock.

Maturity is defined as that point of time at the end of the grain-filling period when maximum kernel weight has occurred and black layer has developed near the tip of the kernel. Hybrid maturity is determined using four different systems: (i) growing degree days (Wang, 1960); (ii) crop heat units (Brown and Bootsma, 1993); (iii) Minnesota RM rating (Peterson and Hicks, 1973), and (iv) general thermal index (Dwyer et al., 1999a). Only the RM system is based on grain moisture content of hybrids at harvest relative to previously ranked hybrids. The other systems are based on a quantitative response of corn development rate to temperature. All systems can be used as a general guideline for determining corn maturity differences (Dwyer et al., 1999b).

S. Wilkens and J.G. Lauer, Dep. of Agronomy, Univ. of Wisconsin-Madison, Madison, WI 53706; and P.J. Weimer, USDA-ARS, U.S. Dairy Forage Research Center, Madison, WI 53706. *Corresponding author (jglauer@wisc.edu).

Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; NDF, neutral detergent fiber; NDFD, neutral detergent fiber digestibility; NIRS, near infrared reflectance spectroscopy; RM, relative maturity; SSF, simultaneous saccharication and fermentation; TEP, theoretical ethanol potential; TEY, theoretical ethanol yield.

Hybrid RM has been shown to positively influence both grain and silage yields. In a study examining forage yield from hybrids bred in different eras, Lauer et al. (2001) showed that hybrids ranging from 100 to 115 d RM yielded 3.61 Mg ha^{-1} more stover than 80 to 95 d RM hybrids. Capristo et al. (2007) tested 11 hybrids ranging from 1221 to 1656 growing degree days (°C d) from emergence to physiological maturity at three locations in Argentina and observed a curvilinear relationship in stover yield with increasing hybrid relative maturity at one location, but a linear increase of grain yield with hybrid relative maturity in the two remaining locations. They concluded that full-season hybrids generally produced greater grain yields than earlier maturing hybrids.

Moderate to strong associations between stover yield and maturity-related traits are common in the literature, which is expected because plants with longer duration of photosynthesis have the potential to produce more biomass (Hay and Porter, 2006; Richards, 2000). Research has shown that hybrid RM can influence stover production, biomass, and nutritional composition at harvest for ensiling, but no known research has been found examining the effects of RM on these measurements for biomass at physiological maturity. Stover harvested for biofuel production is harvested several weeks after harvest for silage, during which time the plant is in a period of grain fill and subjected to many complex source-sink relationships influenced by grain that affects stover composition (Bertoia et al., 2006; Coors et al., 1997; Lorenz et al., 2010b). Lauer et al. (2001) evaluated hybrid RM on stover yields at forage harvest and found mean stover yields of the full-season RM were 9.06 Mg ha^{-1} while yields of the shorter-season RM trial were 7.18 Mg ha^{-1}. Fairey (1980) concluded that as maturities increased there was an overall trend to higher yields from 6.45 to 7.62 Mg ha^{-1}. Darby and Lauer (2002) reported no significant increases in stover yield between short- and full-season hybrids at silage harvest but noted that in general, full-season hybrids were higher yielding with yields ranging from 7.30 to 10.4 Mg ha^{-1}. As maturity increases, stover ruminant digestibility and the hemicellulose to cellulose ratio decreased (Coors et al., 1997).

No known studies have been conducted to specifically evaluate the effects of hybrid RM on biofuel production potential. The objective of this study was to characterize how hybrid RM affects mature stover yield, composition, and ethanol production. Our hypothesis is that, like grain and silage yield, increasing hybrid RM will increase stover ethanol yield.

MATERIALS AND METHODS

Field Evaluation

Experiments were conducted during the 2009 and 2010 growing season at the University of Wisconsin Research Stations at Arlington, Lancaster, and Marshfield, and at a cooperator field located at Seymour, WI. The experimental design at all locations was a randomized complete block design with three replications. Treatments were hybrids differing in RM (Table 1). All plots were managed using practices similar to those used by producers for commercial fields in the surrounding area of the location. Plots were planted with an Almaco precision planter (Almaco, Nevada, IA). Plot size was 7.62 by 3.10 m with four rows per plot. Plots were seeded at 79,200 plants ha^{-1} and stand counts were taken at the V4 growth stage (Ritchie et al., 2000). Plots were not thinned because observed stand counts were within 5% of each other.

Killing frost and harvest dates are found in Table 1. The target harvest time was after physiological maturity or after a killing frost occurred. During the cool growing season of 2009 and the extreme hybrid relative maturity of 115 d RM for northern Wisconsin, not all plots reached physiological maturity. Therefore, plots were not harvested until after the first killing frost (≤–2.2°C). Above average temperatures during 2010 allowed all plots to reach physiological maturity and were harvested before a killing frost, except for corn grown at Marshfield.

Before harvest, final plant populations were taken on each plot. Five representative plants from the middle two rows of each plot were harvested by cutting the plant 15.0 cm above ground level and removing the whole plant from the field (Darby and Lauer, 2002; Daynard and Hunter, 1975; Weaver et al., 1978). Ears were removed and discarded, with the ear shank and husks remaining on the plant for analysis. Each treatment was weighed for a total stover weight (minus ear) and chopped with a Troy-Built Tomahawk Pro-chipper (Troy, NY). Chopped stover was mixed and a subsample (~500 g) of stover was collected for moisture determination (weighing fresh, drying at 60°C for 7 d, and reweighing), dry matter yield, and subsequent compositional analysis. Since plots were hand harvested by selecting five representative plants, yields were expressed in g $plant^{-1}$ and then extrapolated to Mg ha^{-1} by multiplying the average weight of the plants by the measured population.

Composition Analysis

Stover samples were not pretreated to maximize potential differences in conversion potential among the hybrids, and allow the in vitro digestible dry matter data to reflect digestibility of the stovers to ruminants, which is an alternative use for the stover. All stover samples were ground with a 20.3 cm hammer mill (Christy Hunt Corp, Ipswich, Suffolk, England) through a 1.00-mm screen. Ground samples were scanned on a NIRSystems 6500 near-infrared reflectance spectrophotometer (Silver Spring, MD) to predict crude protein (CP), acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent fiber digestibility (NDFD), cellulose and hemicellulose concentrations. Separate calibration sets were derived from the 2009 and 2010 data. The NIRS calibration was based on analysis of representative stover samples.

Samples for wet chemistry were selected using the SELECT (Shenk and Westerhaus, 1994) procedure with a standardized H value of 1.0. Wet chemistry analysis on each calibration set were analyzed for CP, ADL, ADF, NDF, NDFD, cellulose, and hemicellulose at the University of Wisconsin Soil and Forage Analysis Lab located in Marshfield, WI. Analysis was performed in duplicates. Sources documenting the procedures used by the lab for each analysis were: CP (AOAC Official Methods of Analysis [984.13] [AOAC,1990b]; AOAC Official Methods of Analysis [988.05] [AOAC, 1990c], ADF and ADL (AOAC Official Methods of Analysis [973.18] [AOAC,1990a]), NDF (Goering and Van Soest, 1971; Mertens, 1992; Van Soest et al., 1991), and NDFD (Goering and Van Soest, 1970; Mertens, 1992). Estimated cellulose concentrations were determined by subtracting ADL from ADF,

Table 1. General plot management characteristics and descriptors of four Wisconsin locations for experiments conducted during the 2009–2010 growing seasons.

Descriptor	Arlington	Lancaster	Marshfield	Seymour
Latitude	43°17' 49.9" N	42° 50' 6" N	44° 45' 34" N	44° 49' 36" N
Soil Series	Plano silt loam	Rozetta silt loam Fayette silt loam	Withee silt loam	Onaway silt loam
Previous crop†				
2009	Alfalfa	Soybean	Soybean	Soybean
2010	Alfalfa	Soybean	Corn	Soybean
Soil fertility				
pH	6.2	6.9–7.3	6.3–6.7	7.5
P g kg^{-1}	39–48	21–31	29–78	22–41
K g kg^{-1}	110–110	72–78	66–210	110–120
Fertilizer applied				
N kg ha^{-1}	0–26	74	90–100	58–85
P kg ha^{-1}	4.1	4.1	4.1	4.1
K kg ha^{-1}	–	–	–	51
Temperature‡				
2009	–2.1	–1.8	–0.44	–0.71
2010	+0.28	+1.95	+1.54	+1.50
Precipitation§				
2009	-0.6	+8.1	+1.7	-0.4
2010	+27	+32	+32	+30
Planting date				
2009	2 May	4 May	7 May	11 May
2010	30 April	29 April	10 April	3 May
Fall frost date				
2009	1 October	10 October	10 October	10 October
2010	4 October	29 October	3 October	22 October
Harvest date				
2009	19 October	21 October	25 October	26 October
2010	1 October	3 October	7 October	5 October
Hybrids (RM¶)				
2009	Dahlman D4356VT3 (85), Renk RK570VT3 (95), Pioneer 35F40 (105), Nu-Tech 3T-514VT3 (115)			
2010	Dahlman D4356 (85), Great Lakes 4041 (90), Renk RK570VT3 (95), Pioneer 37Y14 (100), Pioneer 35F40 (105), Dairyland Seed ST9009 (110), Dekalb DKC65-44VT3 (115)			

† Corn, *Zea mays* L.; alfalfa, *Medicago sativa* L.; soybean, *Glycine max* (L.) Merr.

‡ Temperature departure deviation from 30 yr mean from April through October, °C.

§ Precipitation departure deviation from 30 yr mean from April through October, cm.

¶ Denotes hybrid relative maturity (days RM).

and estimated hemicellulose concentrations were determined by subtracting ADF from NDF. In all tests in-house standards were run as a measure of quality control.

From the data obtained in the laboratory, prediction equations were developed that related NIR wavelengths to each of the quality responses following the guidelines of Shenk and Westerhaus (1994). Modified partial least squares calibrations were developed with NIRS software (Infrasoft International v. 3.11, 1995) and the mathematical treatment used was 1-4 4-1. The NIR equations used to predict constituent concentrations of all samples were based on high R^2 and low standard error of cross-validation (SECV) values (Lorenz, 2008). With the exception of NDFD, NIRS equations with R^2 values >0.70 and SECV values being <3.50% of constituent means were chosen for calibration (criteria chosen based on personal communication with Ed Wolfrum, Kevin Silveria, and Aaron Lorenz, 2011; Table 2).

The National Renewable Energy Laboratory (NREL, Golden, CO) developed a broad-based calibration for predicting constituents of corn stover, including various polysaccharides (glucan, xylan, arabinan, mannan, and galactan), total lignins (sum of acid-soluble and acid-insoluble lignin), structural inorganics (mainly silica), protein, acetyl, and uronic acids (Hames et al., 2003). Predictions of constituent values were performed using the PLS1 Thermo Antaris autosampler (Markes International Inc, Wilmington, DE). Values were acceptable for the larger constituents of glucan, xylan, and lignin, but were too large for the other minor constituents (Hames et al., 2003). Predictions with a deviation greater than two times the mean standard error for each constituent were deemed unacceptable (Edward Wolfrum and Amie Sluiter, personal communication). Structural carbohydrate concentrations were used in NREL's theoretical ethanol yield calculator to calculate theoretical ethanol potential (TEP, L Mg^{-1}) for

Table 2. Statistics relating to stover near infrared reflectance spectroscopy (NIRS) composition predictions derived by using partial least squares.†

Measurement‡	No.	Mean	SEC	R^2	SEV(C)
			2009		
CP, g kg^{-1}	29	56.7	0.23	0.94	0.43
ADF, g kg^{-1}	30	484	1.04	0.77	1.44
NDF, g kg^{-1}	30	784	0.84	0.93	1.32
NDFD, g kg^{-1}	12	563	2.57	0.81	1.72
Lignin, g kg^{-1}	12	34.7	0.42	0.84	0.47
Cellulose, g kg^{-1}	30	421	0.92	0.78	1.38
Hemicellulose, g kg^{-1}	28	287	0.89	0.86	1.06
			2010		
CP, g kg^{-1}	51	51.7	0.49	0.80	0.77
ADF, g kg^{-1}	51	491	1.98	0.80	2.20
NDF, g kg^{-1}	49	774	0.64	0.98	1.04
NDFD, g kg^{-1}	50	419	2.09	0.80	3.48
Lignin, g kg^{-1}	51	50.0	1.07	0.80	1.48
Cellulose, g kg^{-1}	52	436	1.92	0.72	2.25
Hemicellulose, g kg^{-1}	49	283	1.81	0.76	2.53

† No., corresponds to the final number of data points used to develop NIRS calibration; SEC, standard error of calibration; R^2, coefficient of determination; SEV(C), standard error of cross validation.

‡ CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; NDFD, neutral detergent fiber digestibility.

each sample (see http://www.nrel.gov/biomass/analytical_procedures.html#).

Theoretical ethanol potential assumes that 100% of structural carbohydrates are converted to ethanol. Actual ethanol yields were generated through a modified simultaneous saccharication and fermentation procedure. A loopful of a single colony of *Saccharomyces cerevesiae* $D_5\alpha$ was inoculated into a 500 mL Erlenmeyer flask that contained 250 mL of PYEG medium (per liter, 20.0 g peptone, 10.0 g yeast extract, 50.0 g D-glucose). After 16.0 h incubation (35.0°C, 150 rev min^{-1}) cultures were centrifuged at 10,000 × *g* for 20.0 min, and resuspended in 30.0 mL of sterile peptone solution (10.0 g L^{-1}) to obtain an OD_{525} of ~90. Medium for simultaneous saccharication and fermentation (SSF) procedure was prepared by aseptic combination of 50.0 mL of 1.00 M citric acid/Na citrate buffer (pH 4.80); 15.0 g of Bacto-Peptone and 20.0 g of yeast extract in 920 mL of deionized water; 4.00 mL tetracycline solution (10.0 mg mL^{-1}); 9.00 mL of Celluclast (50.0 FPU mL^{-1}) and 2.21 mL of b-glucosidase (Novozyme 188, 403 units mL^{-1}), and 30.0 mL of the above resuspended yeast culture. Ten milliliters of the resulting diluted enzyme/culture suspension was added aseptically to previously autoclaved, aluminum foil-covered 60.0 mL serum vials (Wheaton Scientific) that contained 1.00 g of feedstock. Immediately after inoculation, each vial was aseptically sealed with a sterile silicone rubber septum. Vials were vented with a sterile 26-gauge, 1.3 cm hypodermic needle covered with foil and then were incubated in a platform shaking incubator (150 rev min^{-1}, 32.0°C). After 72 h incubation, the liquid phase of each vial was sampled and prepared for high performance liquid chromatography (HPLC) analysis (Weimer et al., 1991). Experiments were performed in duplicate. Actual ethanol yield from each sample was expressed as the amount of glucan converted into ethanol as a percent of maximum theroretical ethanol (Isci et al., 2008).

Statistical Analysis

Analysis of variance was performed using Proc Mixed (SAS Institute, Cary, NC) to analyze treatment differences. Mean separation used Fisher's Protected LSD at $P \leq 0.05$. Data were first analyzed by year. To derive the relationship between hybrid relative maturity and stover production, ethanol yield and stover composition measurements replicate data were used in a covariate analysis. Data were pooled into northern and southern production zones and then pooled overall. The northern zone included data from the Marshfield and Seymour locations while the southern zone included data from the Arlington and Lancaster locations. Hybrid relative maturity was considered a continuous covariate variable and data were analyzed using Proc Mixed covariate analysis procedures of SAS (Littell et al., 1996). Linear and quadratic coefficients were analyzed. Random effects for the combined analyses were environment and replication within environment. The final model was determined using backward stepwise selection. This procedure starts with the full model and sequentially removes nonsignificant factors and their interactions. The highest order nonsignificant *F* value is deleted at each stage and the model is complete when the coefficients remaining in the model produce a value of $P \leq 0.05$. The goal of this procedure was to include in the regression equation only those terms which contribute significantly to the variation in the dependent variable (Gomez and Gomez, 1984). The coefficient of determination (R^2) was derived using the predicted values calculated by PROC MIXED ($R^2 = 1 - [(y_{ij} - y_{(\text{Pred})})^2/(y_{ij} - y_{(\text{grand mean})}{}^2]$).

RESULTS AND DISCUSSION

Growing season temperature and precipitation deviations for the 2009 and 2010 growing season are found in Table 1. Conditions during 2009 were cool and wet the entire growing season. Temperatures from April to October were 3°C below the 30-yr normal, with July being one of the coldest on record at 5°C below normal. September provided above average heat, but October was wet and cool and many plants struggled to reach physiological maturity before the first killing frost. Precipitation for the year was 3 cm below the 30-yr normal. Weather in 2010 had above normal heat and precipitation.

Corn harvested in 2009 was less mature. Whole plant stover moistures in 2009 ranged from 500 to 700 g kg^{-1}, while in 2010 moisture ranged from 260 to 700 g kg^{-1}. In August of 2009 the Lancaster location experienced adverse weather and extreme hail defoliated and injured many plants. As the season progressed, injured plants became infected with Goss' bacterial wilt (*Clavibacter michiganensis* subsp. *nebraskensis*) and increased amounts of stalk lodging were experienced. Due to wet conditions in 2010, plots at the Marshfield location experienced high amounts of root lodging in mid- to late July. In both cases, plots were harvested and data were included in the analysis.

Year Effects

In both years stover yield increased with longer season RM (Table 3). Longer season hybrid RM decreased TEP, however, the magnitude of the decrease was not as great as the effect of RM on yield. Thus TEY, increased dramatically in both years primarily due to greater yields of longer season hybrids.

Table 3. Corn stover production and ethanol yield of hybrids differing for relative maturity in Wisconsin during 2009 and 2010. Values are averaged across four locations each year.

Relative maturity	Moisture	Stover yield		Theoretical ethanol potential	Theoretical ethanol yield	Actual ethanol yield
d	g kg^{-1}	g $plant^{-1}$	Mg ha^{-1}	L Mg^{-1}	L ha^{-1}	%
				2009		
85	546	75	6.5	362	2370	20.1
95	590	100	8.9	364	3220	20.2
105	614	110	9.6	365	3490	20.8
115	607	179	15.7	357	5440	20.1
LSD (0.05)	50	23	2.1	5	810	ns†
				2010		
85	488	75	6.2	365	2230	21.4
90	519	88	7.5	369	2880	20.9
95	538	92	8.1	358	2900	21.0
99	594	82	6.5	368	2490	20.4
105	635	119	9.6	354	3390	22.1
111	569	130	10.5	355	3880	21.9
115	623	131	10.9	353	4410	22.4
LSD (0.05)	26	13	1.1	8	540	0.4

† ns, not significant.

Stover nutritional composition and biomass composition improved as RM increased (Table 4). Crude protein tended to have a quadratic response with a maximum value at about 105 d RM. The nutritional composition measurements of ADF, NDF, and ADL decreased as RM increased, while NDFD increased as RM increased. Although statistical differences were observed for most biomass composition measurements, the changes were generally small.

Production Zone Effects

The relationship between stover moisture and dry matter yield (g $plant^{-1}$ and Mg ha^{-1}) was linear and positive in both production zones Table 5. Observed dry matter yields were similar in both zones (data not shown). In the northern zone, yields increased 93%, from 73.2 to 141 g $plant^{-1}$ at a rate of 2.21 g $plant^{-1}$ RM^{-1}. On an area basis, yield increased from 6.41 to 12.4 Mg ha^{-1} at a rate of 0.201 Mg ha^{-1} RM^{-1}. Likewise, in the southern zone, yields increased 118%, from 66.0 to 144 g $plant^{-1}$ at a rate of 2.59 g $plant^{-1}$ RM^{-1}. On an area basis, yield increased from 5.50 to 12.1 Mg ha^{-1} at a rate of 0.221 Mg ha^{-1} RM^{-1}

Ethanol yield is best described as a positive relationship with RM in both zones. In the northern zone, TEY increased by 71%, from 2490 to 4270 L ha^{-1} at a rate of 59.3 L ha^{-1} RM^{-1} (Table 5). In the southern zone, TEY increased 113% from 1980 to 4210 L ha^{-1} at a rate of increase of 74.5 L ha^{-1} RM^{-1}. The difference in the rate of increase RM^{-1} for TEY between the two zones might be explained by the rate of dry matter increase, as it was higher in the southern zone than the northern zone. The relationship between TEP and RM is best described by a negative linear response in each zone.

Table 4. Corn stover nutritional composition and biomass composition of hybrids differing for relative maturity in Wisconsin during 2009 and 2010. Values are averaged across four locations each year.

Relative maturity	CP†	ADF	NDF	NDFD	ADL	Lignin	Glucan	Xylan	Cell	Hem
d	g kg^{-1}									
					2009					
85	53.5	492	800	556	35.7	121	345	204	425	286
95	56.7	489	801	559	35.5	114	345	207	425	288
105	60.6	476	776	573	33.7	112	350	205	414	287
115	56.8	479	772	567	34.4	111	336	203	418	281
LSD (0.05)	6.5	11	19	9	1.2	9	7	3	8	4
					2010					
85	50.0	497	802	459	49.3	153	354	198	434	293
90	53.2	509	797	487	43.3	151	357	201	440	292
95	55.8	499	789	461	50.9	149	347	195	430	284
99	53.7	520	805	486	48.5	151	361	202	455	273
105	57.1	477	767	496	39.5	142	345	191	420	280
111	55.4	492	775	517	37.3	147	350	197	432	300
115	54.6	472	761	494	43.7	144	336	192	420	278
LSD (0.05)	4.9	14	15	17	5.5	6	9	4	11	11

† CP, crude protein; ADF, acid detergent fiber; NDF; neutral detergent fiber; NDFD, neutral detergent fiber digestibility; ADL, acid detergent lignin; Cell, cellulose; Hem, hemicellulose.

Crude protein was nonresponsive in the southern zone and exhibited a quadratic response in the northern zone where concentrations increased through 109 d RM, and then decreased (Table 5). The ADF and NDF concentrations were at the lowest concentrations at the highest RM tested, and each demonstrated a negative linear response in both zones. The NDFD demonstrated a similar positive response and increased at a rate of 1.31 g kg^{-1} in the northern zone and 1.66 g kg^{-1} RM^{-1} in the southern zone as maturity increased. Glucan and ADL decreased with increasing RM in each zone. Lignin and xylan were unresponsive in the southern zone and cellulose and hemicellulose failed to show a response in either zone.

The northern production zone had greater variability for most measurements, which might be attributed to less mature plants as moistures for the northern zone ranged from 565 to 697 g kg^{-1} and the southern zone ranged from 401 to 613 g kg^{-1}. Crude protein, lignin, and xylan were nonresponsive in the southern zone, and cellulose and hemicellulose were nonresponsive in each zone. The target harvest moisture for corn silage is typically 60 to 70% whole plant moisture (Wiersma et al., 1993). Stover biomass composition is influenced by grain fill (Bertoia et al., 2006; Coors et al., 1997). Results from Darby and Lauer (2002) indicated that, when stover was harvested at immature stages (high silage moisture), recorded measurements were more responsive and declined with increased plant maturity. Similarly, Wiersma et al. (1993) and Cummins (1970) both reported that after 40% dry matter accumulation, digestibility plateaus. Coincidently, these results also suggest that as plants mature the ability to manage for biomass compositional measurements decreases.

Table 5. The relationship between corn stover yield, nutritional composition, biomass composition, and hybrid relative maturity (days RM). Data are pooled by zone (2009–2010).

Measurement†		Regression equation‡	R^2
		Northern zone§	
Yield	Moisture, g kg^{-1}	241 + 3.81RM	0.77
	DM yield, g $plant^{-1}$	–115 + 2.21RM	0.85
	DM yield, Mg ha^{-1}	–10.6 + 0.201RM	0.86
	TEY, L ha^{-1}	–2550 + 59.3RM	0.81
	Ethanol yield, %	18.2 + 0.1176RM	0.84
Nutritional composition	CP, g kg^{-1}	$-227 + 5.44RM - 0.0263RM^2$	0.72
	ADF, g kg^{-1}	578 – 0.911RM	0.70
	NDF, g kg^{-1}	952– 1.65RM	0.51
	NDFD, g kg^{-1}	379 + 1.31RM	0.91
	ADL, g kg^{-1}	72.4 – 0.351RM	0.76
	Lignin, g kg^{-1}	183 – 0.432RM	0.94
Biomass composition	TEP, L Mg^{-1}	414 – 0.553RM	0.62
	Glucan, g kg^{-1}	409 – 0.602RM	0.71
	Xylan, g kg^{-1}	221 – 0.231RM	0.76
	Cellulose, g kg^{-1}	no significant coefficients	–
	Hemicellulose, g kg^{-1}	no significant coefficients	–
		Southern zone¶	
Yield	Moisture, g kg^{-1}	–210 + 7.16RM	0.86
	DM yield, g $plant^{-1}$	–154 + 2.59RM	0.72
	DM yield, Mg ha^{-1}	–13.2 +0.222RM	0.65
	TEY, L ha^{-1}	–4350 + 74.5RM	0.65
	Ethanol yield, %	19.9 + 0.079RM	0.72
Nutritional composition	CP, g kg^{-1}	no significant coefficients	–
	ADF, g kg^{-1}	593 – 0.961RM	0.58
	NDF, g kg^{-1}	886 – 1.09RM	0.68
	NDFD, g kg^{-1}	288 + 1.66RM	0.98
	ADL, g kg^{-1}	109 – 0.532RM	0.92
	Lignin, g kg^{-1}	no significant coefficients	–
Biomass composition	TEP, L Mg^{-1}	398 – 0.438RM	0.78
	Glucan, g kg^{-1}	391 – 0.432RM	0.66
	Xylan, g kg^{-1}	no significant coefficients	–
	Cellulose, g kg^{-1}	no significant coefficients	–
	Hemicellulose, g kg^{-1}	no significant coefficients	–

† DM, dry matter; TEP, theoretical ethanol potential; TEY, theoretical ethanol yield; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; NDFD, neutral detergent fiber digestibility; ADL, acid detergent lignin

‡ RM = Hybrid relative maturity (days).

§ Marshfield and Seymour.

¶ Arlington and Lancaster.

Overall Results

In the overall pool, significant relationships between all stover measurements and RM were found, except for hemicellulose (Table 6). As hybrid RM increased stover and ethanol yields increased. With the exception of CP and NDFD, fiber and biomass compositional measurements decreased. Stover yield increased linearly by 2.41 g plant^{-1} RM^{-1} increase. Based on the regression equation, this corresponds to a yield range prediction of 71.5 to 143 g plant^{-1} or 6.13 to 12.4 Mg ha^{-1} with an increase of 0.211 Mg ha^{-1} RM^{-1} increase. This observed yield increase is in agreement with several researchers who have found that later maturity hybrids produce larger amounts of dry matter yields (Darby and Lauer, 2002; Hay and Porter, 2006; Lauer et al., 2001; Richards, 2000; Sarlangue et al., 2007; Wolf et al., 1993). Research has shown that later maturity hybrids tend to possess additional leaves plant^{-1}, which can help explain the increase in stover yields (Fairey, 1980).

Ethanol yield increased linearly at a rate of 0.098% RM^{-1} increase (Table 6). Ethanol yields were low but the SSF processes used to make ethanol did not include a pretreatment due to cost, time, and lab constraints. Weimer et al. (2005) noted that experimental evaluation of cellulosic materials to ethanol by SSF is not amenable to rapid processing of large numbers of samples, due to the requirements of setup and operation under aseptic conditions and of sample processing for ethanol analysis via gas chromatograph or high performance liquid chromatography methods.

Theoretical ethanol yield also demonstrated a positive linear response where yields increased at the rate of 67.1 L ha^{-1} RM^{-1}, from 2220 to 4230 L ha^{-1} (Table 6). Theoretical ethanol potential displayed a negative linear response and declined at a rate of 0.491 L Mg^{-1} as RM increased. A 4.5% decrease in TEP, from 365 to 349 L Mg^{-1} was observed as maturity increased to 115 d RM. Overall, the range of TEP was small while differences in TEY were substantial and were primarily due to variability in stover yield rather than TEP. This increased variability indicates that the shortest avenue to increasing ethanol yields will be through increasing stover yields and not TEP. Another important observation was the much stronger relationship between stover yield and TEY compared to stover yield and TEP. Since TEY is a function of TEP and stover yield, this result reflects the larger amount of variation of stover yield over stover composition.

The ADF and NDF concentrations decreased linearly as RM increased (Table 6). The ADF concentrations decreased by 6% and NDF concentrations decreased by 6.5% as RM increased. These findings are similar to those found by others (Darby and Lauer, 2002; Wiersma et al., 1993) who noted that as the corn progressed toward physiological maturity, stover fiber concentrations increased. The NDFD demonstrated a positive response and increased 8.75% as maturity increased. Concentrations ranged from 459 to 503 g kg^{-1} and increased linearly at 1.48 g kg^{-1} RM^{-1}. Only CP exhibited a quadratic relationship where concentrations increased to 96 RM and then decreased at increasing RM.

Lignin concentrations decreased 8.1% from 149 to 137 g kg^{-1} as maturity increased (Table 5). The ADL concentrations decreased 25% from 53.8 to 40.6 at a rate of 0.442 g kg^{-1} RM^{-1}. The ability to manage lignin concentrations is imperative for the cellulosic industry as strong negative correlations have been observed between lignin concentration and stover hydrolysis and digestibility (Dien et al., 2006; Isci et al., 2008). By adopting full-season and later maturing hybrids, not only can dry matter and ethanol yields be increased, lignin concentrations can be decreased. However, glucan, xylan, and cellulose concentrations each decreased at a rate of 0.521, 0.242, and 0.511 g kg^{-1} RM^{-1}. Hemicellulose, was unresponsive to RM.

Table 6. The relationship between corn stover yield, nutritional composition, biomass composition, and hybrid relative maturity (days RM). Data are pooled over all locations (2009–2010).

Measurement†		Regression equation‡	R^2
		All locations	
Yield	Moisture, g kg^{-1}	–15.2 + 5.51RM	0.88
	DM yield, g plant^{-1}	–134 + 2.41RM	0.78
	DM yield, Mg ha^{-1}	–11.8 + 0.211RM	0.76
	TEY, L ha^{-1}	–3490 + 67.1RM	0.74
	Ethanol yield, %	19.1 + 0.098RM	0.74
Nutritional composition	CP, g kg^{-1}	–118 + 3.34RM – 0.016RM^2	0.57
	ADF, g kg^{-1}	586 – 0.912RM	0.65
	NDF, g kg^{-1}	919 – 1.65RM	0.64
	NDFD, g kg^{-1}	333 + 1.48RM	0.96
	ADL, g kg^{-1}	91.2 – 0.442RM	0.92
	Lignin, g kg^{-1}	180 – 0.371RM	0.95
Biomass composition	TEP, L Mg^{-1}	406 – 0.491RM	0.73
	Glucan, g kg^{-1}	400 – 0.521RM	0.67
	Xylan, g kg^{-1}	217 – 0.242RM	0.84
	Cellulose, g kg^{-1}	482 – 0.511RM	0.65
	Hemicellulose, g kg^{-1}	no significant coefficients	–

† DM, dry matter; TEP, theoretical ethanol potential; TEY, theoretical ethanol yield; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; NDFD, neutral detergent fiber digestibility; ADL, acid detergent lignin.

‡ RM = Hybrid relative maturity (days).

CONCLUSION

Collectively, these results suggest that stover and ethanol yields and stover nutritional composition can be improved by adopting longer, full-season hybrids provided that those hybrids reach physiological maturity. Stover dry matter and TEY were responsive to hybrid relative maturity and differences in these two measurements were substantial when compared to other measurements. Dry matter increased linearly at a rate of 0.211 Mg ha^{-1} RM^{-1} as hybrid RM increased from 85 to 115 d RM. Likewise stover nutritional composition improved with longer season hybrids. The findings are in agreement with several researchers who have reported improved stover nutritional composition for longer season hybrids at corn silage harvest (Coors et al., 1997; Darby and Lauer, 2002; Fairey, 1980; Wiersma et al., 1993). Ethanol yield and TEY increased linearly by 0.098% RM^{-1} and 67.1 L ha^{-1} RM^{-1} while the effects of RM on most biomass composition were best described by negative linear responses. The measurements of cellulose, glucan, xylan, and TEP declined linearly as RM increased, but the range in data was small indicating that the shortest avenue to increasing ethanol yields will be through increasing stover yields and not through improving stover biomass quality. More importantly, lignin and ADL concentrations decreased by 8.1 and 25%, respectively, as maturity increased. The ability to manage these concentrations is imperative for the cellulosic industry as lignin greatly reduces the hydrolysis and digestibility of stover (Dien et al., 2006; Isci et al., 2008). Lorenz et al. (2010a) noted a strong negative correlation between lignin concentrations and ethanol yield. Therefore, until biomass composition becomes more important for ethanol production than do yield or nutritional composition, producers should consider adopting fuller season hybrids to maximize production.

ACKNOWLEDGMENTS

The authors thank Ed Wolfrum and Amie Sluiter of the National Renewable Energy Laboratory, Kevin Silveria (University of Wisconsin), and Aaron Lorenz (University of Nebraska) for advice and assistance with this project. Mention of trade names or commercial products in this article is soley for the purpose of providing specific information and does not imply recommendation of endorsement.

REFERENCES

AOAC. 1990a. AOAC Official Methods of Analysis [973.18]. Fiber (acid detergent) and lignin in animal feed. 15th ed. Assoc. of Official Analytical Chemists, Rockville, MD.

AOAC. 1990b. AOAC Official Methods of Analysis [984.13]. Protein (crude) determination in animal feed: Copper catalyst Kjeldahl method. 15th ed. Assoc. of Official Analytical Chemists, Rockville, MD.

AOAC. 1990c. Official Methods of Analysis [988.05]. Protein (crude) determination in animal feed: CuSO4/TiO2 mixed catalyst Kjeldahl method. 15th ed. Assoc. of Official Analytical Chemists, Rockville, MD.

Bertoia, L., C. Lopez, and R. Burak. 2006. Biplot analysis of forage combining ability in maize landraces. Crop Sci. 46:1346–1353. doi:10.2135/cropsci2005.09-0336

Brown, D.M., and A. Bootsma. 1993. Crop heat units for corn and other warm-season crops in Ontario. OMAF Factsheet Agdex 111/31. Ontario Ministry Agric. Food, Toronto.

Capristo, P.R., R.H. Rizzalli, and F.H. Andrade. 2007. Ecophysiological yield components of maize hybrids with contrasting maturity. Agron. J. 99:1111–1118. doi:10.2134/agronj2006.0360

Coors, J.G., K.A. Albrecht, and E.J. Bures. 1997. Ear-fill effects on yield and quality of silage corn. Crop Sci. 37:243–247. doi:10.2135/cropsci1997.0011183X003700010043x

Cummins, D.G. 1970. Quality and yield of corn plants and component parts when harvested for silage at different maturity stages. Agron. J. 62:781–784. doi:10.2134/agronj1970.00021962006200060030x

Darby, H.M., and J.G. Lauer. 2002. Harvest date and hybrid influence on corn forage yield, quality, and preservation. Agron. J. 94:559–566. doi:10.2134/agronj2002.5590

Daynard, T.B., and R.B. Hunter. 1975. Relationships among whole-plant moisture, grain moisture, dry matter yield, and quality of whole-plant corn silage. Can. J. Plant Sci. 55:77–84. doi:10.4141/cjps75-010

Dien, B.S., H.-J.G. Jung, K.P. Vogel, M.D. Casler, J.F.S. Lamb, L. Iten et al. 2006. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass Bioenergy 30:880–891. doi:10.1016/j.biombioe.2006.02.004

Duvick, D.N. 2005. The contribution of breeding to yield advances in maize (*Zea mays* L.). In: L.S. Donald, editor, Advances in agronomy. Vol. 86. Academic Press, San Diego, CA. p. 83–145.

Dwyer, L.M., D.W. Stewart, L. Carrigan, B.L. Ma, P. Neave, and D. Balchin. 1999a. A general thermal index for maize. Agron. J. 91:940–946. doi:10.2134/agronj1999.916940x

Dwyer, L.M., D.W. Stewart, L. Carrigan, B.L. Ma, P. Neave, and D. Balchin. 1999b. Guidelines for comparisons among different maize maturity rating systems. Agron. J. 91:946–949. doi:10.2134/agronj1999.916946x

Fairey, N.A. 1980. The effects of hybrid maturity, date of planting, and date of harvesting on growth and development of forage maize. Can. J. Plant Sci. 60:1367–1375. doi:10.4141/cjps80-191

Goering, H.K., and P.J. Van Soest. 1971. Forage fiber analysis (Apparatus, reagents, procedures, and some applications). Agric. Handb. 379 ed. U.S. Gov. Print Office, Washington, DC.

Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analyses. Agric. Handb. 379. Agric. Res. Serv., USDA, Washington, DC.

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. John Wiley & Sons, New York.

Hames, B.R., S.R. Thomas, A.D. Sluiter, C.J. Roth, and D.W. Templeton. 2003. Rapid biomass analysis-New tools for compositional analysis of corn stover feedstocks and process intermediates from ethanol production. Appl. Biochem. Biotechnol. 105:5–16. doi:10.1385/ABAB:105:1-3:5

Hay, R.K.M., and J.R. Porter. 2006. The physiology of crop yield. Wiley-Blackwell, Oxford, UK.

Isci, A., P.T. Murphy, R.P. Anex, and K.J. Moore. 2008. A rapid simultaneous saccharification and fermentation (SSF) technique to determine ethanol yields. Bioenergy Res. 1:163–169. doi:10.1007/s12155-008-9015-9

Lauer, J.G., J.G. Coors, and P.J. Flannery. 2001. Forage yield and quality of corn cultivars developed in different eras. Crop Sci. 41:1449–1455. doi:10.2135/cropsci2001.4151449x

Littell, R.C., G.A. Milliken, W.W. Stroup, and W.W. Wolfing. 1996. SAS System for mixed models. SAS Inst., Cary NC.

Lorenz, A.J. 2008. Characterization, inheritance, and covariation of maize (*Zea mays* L.) traits relevant to cellulosic biofuels production. Ph.D. thesis.Univ. of Wisconsin, Madison.

Lorenz, A.J., R.P. Anex, A. Isci, J.G. Coors, N.d. Leon, and P.J. Weimer. 2009. Forage quality and composition measurements as predictors of ethanol yield from maize (*Zea mays* L.) stover. Biotechnol. Biofuels 2:5–13. doi:10.1186/1754-6834-2-5

Lorenz, A.J., J.G. Coors, C.N. Hansey, S.M. Kaeppler, and N. de Leon. 2010a. Genetic Analysis of Cell Wall Traits Relevant to Cellulosic Ethanol Production in Maize (Zea mays L.). Crop Sci. 50:842–852. doi:10.2135/cropsci2009.04.0168

Lorenz, A.J., T.J. Gustafson, J.G. Coors, and N.d. Leon. 2010b. Breeding maize for a bioeconomy: A literature survey examining harvest index and stover yield and their relationship to grain yield. Crop Sci. 50:1–12. doi:10.2135/cropsci2009.02.0086

Mertens, D.R. 1992. Critical conditions in determining detergent fiber. In: Proceedings of NFTA Forage Analysis Workshop, Denver, CO.

Perlack, R.D., L.L. Wright, A.F. Turhollow, R.I. Graham, B.J. Stokes, and D.C. Erbach. 2005. Biomass as a feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion ton annual supply. DOE/GO 102005 2135 and ORNL/TM 2005/66. NTIS, Springfield, VA. http://feedstockreview.ornl.gov/pdf/billion_ton_vision.pdf (accessed 15 July 2009).

Peterson, R.H., and D.R. Hicks. 1973. Minnesota relative maturity rating of corn hybrids. Agron. no. 27. Univ. of Minnesota Agric. Ext. Serv., St. Paul.

Richards, R.A. 2000. Selectable traits to increase crop photosynthesis and yield of grain crops. J. Exp. Bot. 51:447–458. doi:10.1093/jexbot/51.suppl_1.447

Ritchie, N.J., M.E. Schutter, R.P. Dick, and D.D. Myrold. 2000. Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. Applied Environ. Microbiol. 66:1668–1675.

Sarlangue, T., F.H. Andrade, P.A. Calvino, and L.C. Purcell. 2007. Why do maize hybrids respond differently to variations in plant density? Agron. J. 99:984–991. doi:10.2134/agronj2006.0205

Shenk, J.S., and M.O. Westerhaus. 1994. The application of near infrared reflectance spectroscopy (NIRS) to forage analysis. In: G.C. Fahey, editor, Forage quality, evaluation, and utilization. ASA, CSSA, and SSSA, Madison, WI. p. 406–449.

Tollenaar, M., D.E. McCullough, and L.M. Dwyer. 1994. Physiological basis of the genetic improvement of corn. In: G.A. Slafer, editor, Genetic improvement of field crops. Marcel Dekker, Inc., New York. p. 183–236.

USDOE. 2005. Breaking the biological barriers to cellulosic ethanol: A joint research agenda. Workshop Proceedings. U.S. Dep. of Energy, Office of Sci. http://www.genomicscience.energy.gov/biofuels/b2bworkshop.shtml (accessed 9 Feb. 2011) Rockville, MD.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2

Wang, J.Y. 1960. A critique of the heat unit approach to plant response studies. Ecology 41:785–789. doi:10.2307/1931815

Weaver, D.E., C.E. Coppock, G.B. Lake, and R.W. Everett. 1978. Effect of maturation on composition and in vitro dry matter digestibility of corn plant parts. J. Dairy Sci. 61:1782–1788. doi:10.3168/jds.S0022-0302(78)83803-X

Weimer, P.J., B.S. Dien, T.L. Springer, and K.P. Vogel. 2005. In vitro gas production as a surrogate measure of the fermentability of cellulosic biomass to ethanol. Appl. Microbiol. Biotechnol. 67:52–58. doi:10.1007/s00253-004-1844-7

Weimer, P.J., Y. Shi, and C.L. Odt. 1991. A segmented gas/liquid delivery system for continuous culture of microorganisms on solid substrates, and its use for growth of *Ruminococcus flavefaciens* on cellulose. Appl. Microbiol. Biotechnol. 36:178–183. doi:10.1007/BF00164416

Wiersma, D.W., P.R. Carter, K.A. Albrecht, and J.G. Coors. 1993. Kernel milkline stage and corn forage yield, quality, and dry matter content. J. Prod. Agric. 6:94–99. doi:10.2134/jpa1993.0094

Wolf, D.P., J.G. Coors, K.A. Albrecht, D.J. Undersander, and P.R. Carter. 1993. Agronomic evaluations of maize genotypes selected for extreme fiber concentrations. Crop Sci. 33:1359–1365. doi:10.2135/cropsci1993.0011183X003300060047x

Zeng, M., E. Ximenes, M.R. Ladisch, N.S. Mosier, W. Vermerris, C.-P. Huang, and D.M. Sherman. 2012. Tissue-specific biomass recalcitrance in corn stover pretreated with liquid hot-water: Enzymatic hydrolysis (part 1). Biotechnol. Bioeng. 109:390–397. doi:10.1002/bit.23337