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Harvest date effects on biomass quality and ethanol yield of new energycane (*Saccharum* hyb.) genotypes in the Southeast USA

Joseph E. Knoll [a,*], *William F. Anderson* [a], *Edward P. Richard Jr.* [b], *Joy Doran-Peterson* [c], *Brian Baldwin* [d], *Anna L. Hale* [b], *Ryan P. Viator* [b]

[a] USDA-ARS, Crop Genetics and Breeding Research Unit, P.O. Box 748, Tifton, GA 31793, USA
[b] USDA-ARS, Sugarcane Research Unit, 5883 USDA Road, Houma, LA 70360, USA
[c] University of Georgia, Dept. of Microbiology, 527 Biological Sciences Bldg., 1000 Cedar St., Athens, GA 30602, USA
[d] Mississippi State University, Dept. of Plant and Soil Sciences, P.O. Box 9555, Mississippi State, MS 39762, USA

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ABSTRACT

Energycane (*Saccharum* hyb.) is a perennial bioenergy crop derived from sugarcane, but with higher fiber, greater biomass yields, and better cold tolerance than typical sugarcane. Two commercial sugarcanes, two high-sugar (Type I) energycanes, and five high-fiber (Type II) energycanes were planted at Tifton, GA, USA in a randomized complete block design with four replications. Beginning in October, 2008 (plant-cane crop year) five monthly samples were taken to assess the effects of delaying harvest on biomass composition and quality for ethanol production. The monthly harvests were repeated in the winter of 2010–2011 (second-ratoon crop year). Delaying harvest into the winter months resulted in minimal reductions in biomass moisture and N mass fractions, while K mass fraction decreased significantly. Free sugar mass fraction also decreased, thus causing the biomass to have an apparent increase in relative mass fractions of ash and neutral detergent fiber (NDF). The reduction in free sugars was more pronounced in the colder harvest season (2010–2011). The composition of biomass fiber (cellulose, hemicellulose, and lignin) was generally stable over time. A bench-top partial saccharification and co-fermentation (PSCF) protocol employing xylose-fermenting *Escherichia coli* was used to assess ethanol yields from the sequentially harvested biomass. Ethanol yield from sugarcanes and Type I energycanes was more variable over time, due to degradation of free sugars. Thus, early harvest is recommended to avoid loss of fermentable sugars. Type II energycanes can be harvested later during the winter months with little change in conversion properties.

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Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; DM, dry matter; DNS, 3,5 dinitrosalicylic acid; NDF, neutral detergent fiber; PSCF, partial saccharification and co-fermentation.

* *Corresponding author*. Tel.: +1 229 386 3189; fax: +1 229 391 3701.
E-mail addresses: Joe.Knoll@ars.usda.gov, joe_knoll@yahoo.com (J.E. Knoll), bill.anderson@ars.usda.gov (W.F. Anderson), edward.richard@ars.usda.gov (E.P. Richard), joydoranpeterson@gmail.com (J. Doran-Peterson), bbaldwin@pss.msstate.edu (B. Baldwin), Anna.Hale@ars.usda.gov (A.L. Hale), Ryan.Viator@ars.usda.gov (R.P. Viator).

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1. Introduction

Modern sugarcane cultivars have been developed by crossing cultivated sugarcane (*Saccharum officinarum* and *Saccharum* hybrids) with their wild relatives to include other *Saccharum* species such as *Saccharum spontaneum* and *Saccharum robustum*, as well as species in the genera *Miscanthus* and *Erianthus*. These wild relatives generally do not accumulate high sugar levels in the stem like cultivated sugarcane, but may harbor useful traits such as disease resistance or cold tolerance and must be crossed back to cultivated sugarcane several times before a commercial sugarcane cultivar can be developed. Compared to sugarcane cultivars, F_1 and BC_1 generation hybrids are more vigorous, have higher fiber content, and have improved cold tolerance. In addition they tend to have better ability to ratoon, or regrow after multiple harvests [1]. Such early-generation sugarcane hybrids have been selected from the USDA sugarcane breeding program at Houma, LA as today's modern energycanes, specifically for the bioenergy industry. Exceptional yields of biomass have been reported for energycanes in Florida [2,3], Louisiana [4], and Georgia [5].

Tew and Cobill [6] classify energycanes into two types depending on their sugar and fiber (cell wall) components and intended uses. Type I energycanes have sugar mass fraction similar to sugarcanes grown for commercial sugar production, around 130 g kg^{-1} fresh mass, but they also have higher fiber mass fraction (approx. 170 g kg^{-1} fresh mass) than sugarcane. Thus, on a dry-mass basis the sugar mass fraction is around 520 g kg^{-1} for sugarcanes, while Type I energycanes have around 430 g kg^{-1} of sugars and 470 g kg^{-1} of fiber. Type I energycanes are intended to produce lignocellulosic biomass in addition to sugar for ethanol production. Type II energycanes have relatively low sugar mass fraction, and are intended to be grown primarily for lignocellulosic biomass. On a fresh-mass basis, free sugar is only around 50 g kg^{-1}, while fiber averages 300 g kg^{-1}. Type II energycanes also have lower water mass fraction (approx. 650 g kg^{-1}) than sugarcane or Type I energycanes. Thus, on a dry mass basis Type II energycanes average 860 g kg^{-1} of fiber.

Many new energycane selections have been developed, and need to be evaluated in various environments for biomass yield and quality. In addition, the optimal time to harvest energycane biomass has not been determined. In several other perennial grass species grown for bioenergy, it has been shown that delaying harvest into the winter months, or even into the following spring, can have beneficial effects on improving biomass quality by decreasing moisture and nutrient mass fractions in the biomass [7–10]. However, previous studies were conducted in colder climates using grasses that are physiologically very different from energycanes. Thus, it is likely that harvest timing will have different effects on biomass quality in energycane than in other grasses. Its close relative, sugarcane, is generally harvested before senescence, so there has been little research on the changes in sugar and fiber composition of sugarcanes over the winter on which to draw comparisons. The purpose of this study is to evaluate and compare the yield and biomass quality of several promising new energycane genotypes, and to determine the effects of delayed harvest on nutrient mass fractions, biomass quality, and cellulosic ethanol yield of energycane.

2. Materials and methods

2.1. Plant material and study site

Nine energycane entries were planted from cane cuttings on 27 September 2007 in a randomized complete block design with four replications. The study site was located near Tifton, GA, USA, on a Clarendon loamy sand (fine-loamy siliceous semiactive thermic Plinthaquic Paleudult). Two entries (Ho 95-988 and L 99-233) have been released as commercial sugarcane cultivars [11,12]. One entry (Ho 00-961) is considered a Type I energycane [13]. Entry Ho 01-07 was developed as a Type II energycane, but at this location it displays more Type I characteristics, and will be considered Type I in this study. All other entries (Ho 06-9001, Ho 06-9002, Ho 02-144, Ho 02-147, and Ho 72-114) are Type II energycanes. Plots were single rows 6 m long and 1.8 m apart. A border row of sugarcane (Ho 95-988) was planted on both ends of each block, and the blocks were separated by 2 m alleys. Irrigation was applied only after planting to establish the stand. On 23 April 2008 and 12 May 2010 the plots were fertilized with 90, 22, and 45 kg ha^{-1} of N, P, and K, respectively.

2.2. Biomass yield, moisture mass fraction, and sequential harvests

At the end of the 2008 (plant-cane) and 2010 (second-ratoon) growing seasons, biomass was sampled at monthly intervals, beginning the first week of October, and continuing to February (plant-cane 2008–2009: 1 Oct, 3 Nov, 4 Dec, 5 Jan, and 10 Feb; second-ratoon 2010–2011: 4 Oct, 1 Nov, 3 Dec, 4 Jan, and 1 Feb; Fig. 1). Each sample, consisting of three representative stalks with leaves and tops attached, was chopped, weighed fresh, dried to completion in an oven at 65 °C, and was then weighed again to determine mass fractions of moisture (w_{H_2O}) and dry matter (w_{DM}). The dried biomass samples were then ground in a Wiley mill to pass a 2 mm screen prior to storage and further analyses. Total aboveground biomass was mechanically harvested and weighed to determine plot yields after completion of the sequential harvests in February (plant-cane and second-ratoon crops). For purposes of calculating yield on a per-hectare basis, the plot area was adjusted to 7 m × 1.8 m (12.6 m^2). Although sequential harvests were not performed on the first-ratoon crop, the total biomass yield was harvested and measured in February 2010. At harvest, a sample of biomass from each plot was taken for determination of moisture and dry matter (DM) mass fractions as described above.

2.3. Evaluation of biomass composition

Nitrogen mass fraction (w_N) in the biomass samples was determined by dry combustion in a Vario EL-III Universal CHNOS Elemental Analyzer (Elementar Analysensysteme, Hanau, Germany). Mass fraction of ash (w_{ash}) was determined by combustion in a Muffle furnace at 450 °C for 6 h. Potassium

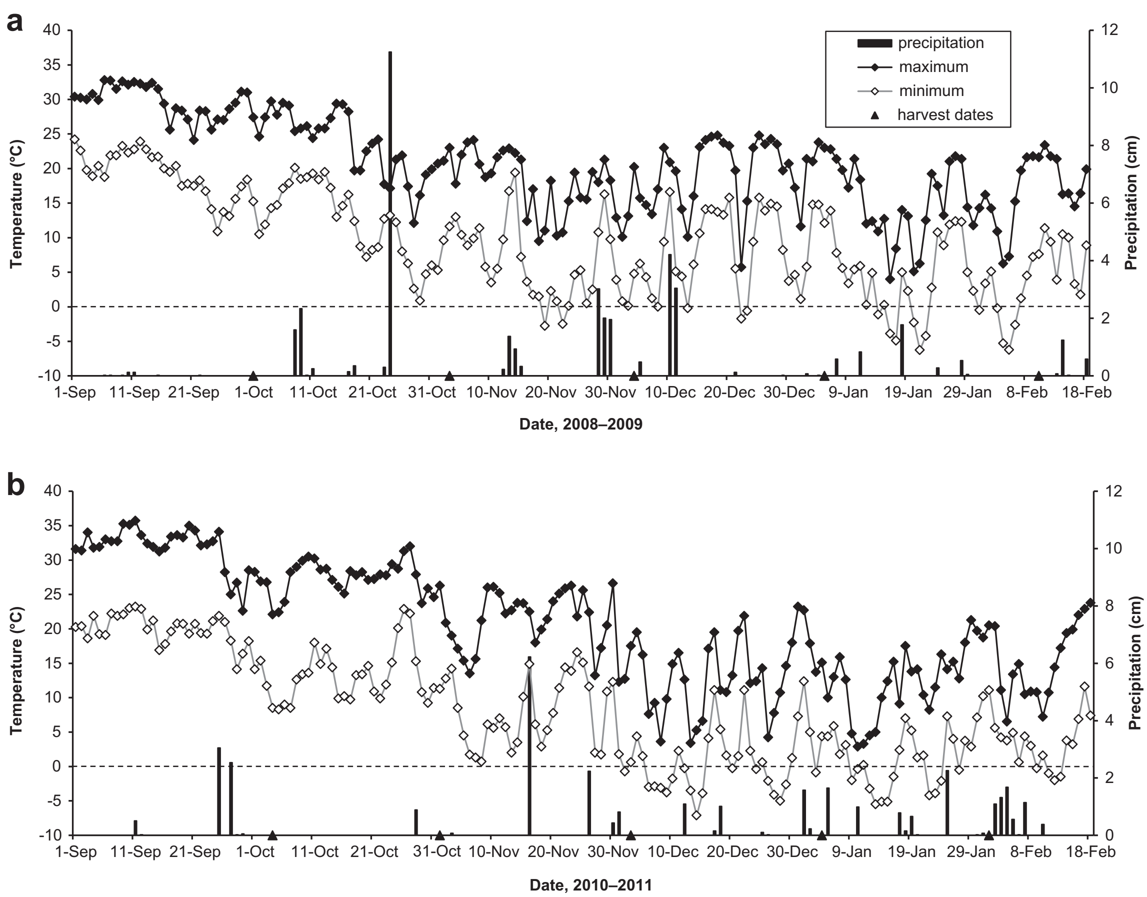

Fig. 1 – Daily maximum and minimum air temperatures and precipitation recorded at Tifton, GA [26] during (a) the plant-cane harvest season in 2008–2009 and (b) the second-ratoon harvest season in 2010–2011. Harvest dates are indicated by the black triangles. The dotted horizontal line denotes 0 °C.

mass fraction (w_K) was determined by inductively-coupled plasma (ICP) spectrometry at the University of Georgia Agricultural and Environmental Services Laboratories, on samples from October, December, and February only. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) mass fractions were determined using F57 Fiber Filter Bags (ANKOM Technology, Macedon, NY). NDF and ADF were determined using either an A200 or A2000 Fiber Analyzer (ANKOM Technology), while ADL determination was carried out in a DaisyII incubator (ANKOM Technology), all according to the manufacturer's protocols. Hemicellulose mass fraction was approximated by subtracting ADF from NDF, and cellulose mass fraction was estimated by subtracting ADL from ADF [14]. Mass fraction of free sugars (w_{sugar}) in the biomass was determined by placing 500 mg ground DM in 5 mL deionized water, mixing with a vortex mixer, and then incubating for 1 h at 50 °C. One-milliliter samples were then centrifuged at 16,000 × g for 10 min. In PCR plates, 100 μL supernatant was incubated with 10 μL of 2 mol L^{-1}HCl at 95 °C for 5 min in a PTC-100 thermocycler (MJ Research, Waltham, MA) to hydrolyze the non-reducing sugar sucrose into reducing sugars [15]. Total reducing sugars were then quantified using the dinitrosalicylic acid (DNS) method [16], modified for PCR plates as described by King et al. [17]. Twenty microliters of each reaction was diluted into 160 μL deionized water in clear-bottom microplates, and absorbance at 540 nm was measured in a SpectraFluor Plus (Tecan Group, Ltd., Männedorf, Switzerland) fitted with a 540 nm bandpass filter (Omega Optical, Inc., Brattleboro, VT). A standard curve was constructed using known concentrations of sucrose.

2.4. *Partial saccharification and co-fermentation (PSCF)*

Biomass from harvests in October, December, and February (plant-cane and second-ratoon) was tested for conversion to ethanol. Ethanol yield from the biomass was determined using a bench-scale PSCF scheme, similar to that described by Doran-Peterson et al. [18] with minor modifications. A negative control containing only deionized water as the substrate was included for comparison in each run; all PSCF experiments were run in triplicate. Two grams DM energycane biomass was placed in Pyrex culture tubes with 11.3 mL deionized water, fitted loosely with a screw cap, and then autoclaved for 1 h at 110 °C. After cooling to room temperature, the pH was adjusted to 4.5 and enzymes were added: 5 FPU g^{-1} Novo 13 cellulase (Novozymes, Franklinton, NC) and 60 CBU g^{-1} Novo 188 cellobiase (Novozymes). A 1 mL sample was immediately taken, centrifuged to remove insoluble material, filtered through a CoStar Spin-X centrifuge tube 0.22 μm nylon filter (Corning, Inc., Corning, NY), and then frozen (−20 °C) until analysis. The culture tubes were then placed in a shaking water bath at 45 °C for 24 h. The pH was then raised to 5.5 with KOH, and xylose-fermenting *Escherichia coli* strain LY40A in LB broth was added to an OD_{550} of 1.0, and then another 1 mL sample was taken. The final fermentation volume was 20 mL and the total solids concentration was 100 g L^{-1}. The tubes were then placed on a shaking incubator at 37 °C for 120 h. Every 24 h another sample was taken. Reducing sugars in the samples were quantified by the DNS method [16]. Ethanol was quantified by gas chromatography as described by Doran-Peterson et al. [18] using isopropanol as an internal standard.

2.5. *Statistical procedures*

All data were analyzed using the GLIMMIX procedure in SAS v. 9.2 (SAS Institute, Cary, NC). Biomass yield data showed overdispersion and were thus log-transformed prior to analysis using the DIST = LOGNORMAL option in the MODEL statement to obtain a better fitting model. For the combined analyses across years, year was considered as a fixed effect because of the perennial nature and aging of the crop stand. The year by entry interaction was included in the model. Because of the different responses observed across sequential harvest dates in the two winters, the plant-cane harvest and the second-ratoon harvest were analyzed separately when assessing the harvest date responses. In some analyses all nine genotypes were compared, while in others, they were grouped according to type (sugarcane and Type I versus Type II) for comparison. Interactions between entry or type with harvest date were included in all models. Simple effect comparisons within interactions were conducted using the SLICE option in the LSMEANS statement. In all analyses, the plots were identified as subjects within the RANDOM statement, thus these were analyzed as repeated measures designs. All tests were conducted at a significance level of $\alpha = 0.05$ with Tukey's adjustment for multiple comparisons.

3. Results

3.1. *Biomass yield*

Averaged over the February harvests of the plant-cane, first-ratoon, and second-ratoon crops of the test, entries Ho 06-9002 and Ho 06-9001 both yielded over 34 Mg ha^{-1} y^{-1} (Fig. 2). High DM yields were also observed for Ho 02-147, Ho 72-114, and Ho 02-144. These are all Type II energycanes. The lowest yielding entries were the sugarcanes L 99-233 and Ho 95-988, both averaging less than 15 Mg ha^{-1} y^{-1}. Intermediate yields were observed for the Type I energycanes Ho 00-961 (20.1 Mg ha^{-1} y^{-1}) and Ho 01-07 (22.6 Mg ha^{-1} y^{-1}). Overall, yields were lowest in the second-ratoon year, but all Type II energycanes still produced over 20 Mg ha^{-1} in the second-ratoon. Of the type II energycanes only Ho 02-144 showed a significant yield decline in the second-ratoon. Yields of both sugarcanes and Ho 01-07 (Type I) also declined after the plant-cane crop year (Fig. 2).

3.2. *Biomass moisture*

In both the plant-cane (2008–2009) and second-ratoon (2010–2011) harvest seasons, biomass moisture mass fraction (w_{H_2O}) of the various entries was affected by 'Type' of energycane. The sugarcanes and Type I energycanes had the highest w_{H_2O} (>700 g kg^{-1}), followed by Ho 72-114, Ho 02-147, and Ho 02-144. The highest-yielding entries, Ho 06-9001 and Ho 06-9002, tended to have the lowest w_{H_2O} (Table 1). In the plant-cane harvest season biomass w_{H_2O} decreased significantly between October and February for most entries, except the two sugarcane entries Ho 95-988 and L 99-233, which averaged 733 g kg^{-1} and 720 g kg^{-1}, respectively. The w_{H_2O} of the Type I energycanes decreased only slightly (Fig. 3a). Also in the plant-cane harvest season the Type II entries showed greater decrease in biomass w_{H_2O}, with the greatest decrease between January and February (Fig. 3a). In the second-ratoon harvest season the average biomass w_{H_2O} decreased slightly between October and November, but did not decrease

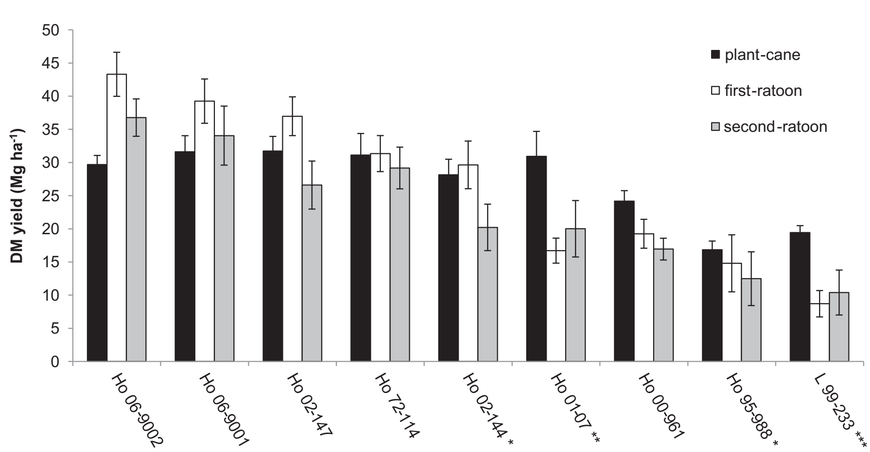

Fig. 2 – Mean annual dry matter (DM) yields from plant-cane through second-ratoon years for nine energycane entries grown at Tifton, GA. Error bars represent one standard error of the mean. Statistical comparisons were performed on log-transformed data; *, **, and * indicate significant year by entry interaction at $\alpha = 0.05$, 0.01, and 0.001, respectively.**

Table 1 – Mean biomass moisture mass fraction, fiber compositions, and estimated ethanol yields for nine energycane entries of three different types [6] grown at Tifton, GA, USA.

Type	Entry	w_{H_2O} [a]		Fiber components: Hemicellulose [a]		Cellulose [a]		Lignin [a]		Estimated ethanol yield [b]
		g kg^{-1}		g kg^{-1} NDF						L ha^{-1}
II	Ho 06-9002	599	c	388	d	547	a	64.3	ab	2800
II	Ho 06-9001	600	c	389	d	545	ab	66.4	a	2500
II	Ho 02-147	659	b	415	bc	520	c	65.4	a	3200
II	Ho 72-114	661	b	399	cd	533	abc	68.4	a	2400
II	Ho 02-144	628	bc	441	a	496	d	64.2	ab	2000
I	Ho 01-07	711	a	398	cd	540	ab	62.5	ab	2700
I	Ho 00-961	714	a	403	cd	535	abc	62.0	ab	2000
Sugarcane	Ho 95-988	706	a	429	ab	521	c	50.4	b	2000
Sugarcane	L 99-233	705	a	418	bc	527	bc	54.9	ab	1500

Within columns, means with the same letter are not significantly different.
a Mean of five monthly harvests (Oct.–Feb) in both the plant-cane and second-ratoon crops.
b Ethanol yield is based on three-year mean DM yields and mean ethanol production from partial saccharification and co-fermentation experiments in this study.

significantly over the following months. A slight increase in w_{H_2O} between January and February (Fig. 3b) could be attributed to the biomass absorbing some moisture from recent rainfall. On the two days preceding this harvest, 0.3 mm and 0.8 mm rain was recorded.

3.3. Biomass composition

All mass fractions for biomass composition other than moisture were calculated on a dry mass basis. Biomass N

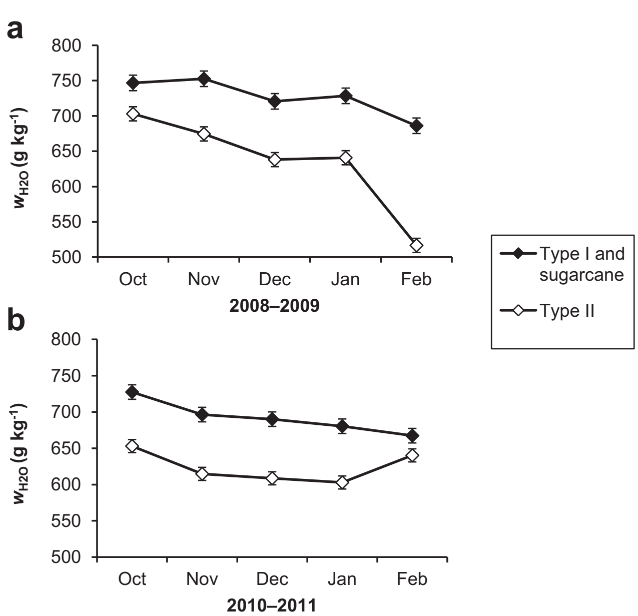

Fig. 3 – Mean monthly biomass moisture mass fraction (w_{H_2O}) for three classes of energycane (sugarcane, Type I, and Type II) measured over (a) plant-cane (2008–2009) and (b) second-ratoon (2010–2011) harvest seasons at Tifton, GA. Because sugarcanes and Type I energycanes performed similarly, they are presented together. Error bars represent one standard error of the mean.

mass fraction (w_N) decreased from 4.78 g kg^{-1} in October to 3.33 g kg^{-1} in November in 2008, after which it did not significantly change. In contrast, during the second-ratoon harvest season biomass w_N increased significantly from 2.86 g kg^{-1} in November to 4.06 g kg^{-1} in January (Fig. 4a). Only minor differences in biomass w_N were measured between entries and types. In both the plant-cane and second-ratoon harvests, biomass K mass fraction (w_K) decreased significantly between October and December, and then did not significantly change (Fig. 4b). There were only minor differences between entries. Biomass ash mass fraction (w_{ash}) showed a more complex pattern of change over time. Generally, w_{ash} in Type II energycanes was greater than in Type I energycanes and sugarcanes (Fig. 5a,b). For Type I and sugarcanes in both harvest years w_{ash} decreased to its lowest point in December, and then increased. The same pattern was observed for Type II energycanes in the plant-cane harvest (Fig. 5a). Ash mass fraction tended to increase after November in 2010 for the Type II energycanes (Fig. 5b).

Biomass free sugar mass fraction (w_{sugar}) was highest among the sugarcanes and Type I energycanes, as expected. Sugar mass fraction was affected by harvest time, with the greater effect in the second-ratoon harvest. In the plant-cane harvest season, w_{sugar} remained relatively stable until February, when there was a significant decrease in all types of energycane (Fig. 6a). In the second-ratoon season free sugars showed a much greater decline between December and January, particularly among the sugarcanes and Type I energycanes (−263 g kg^{-1}; Fig. 6b). Neutral detergent fiber mass fraction (w_{NDF}) tended to increase slightly over time, but the increase was usually not statistically significant (Fig. 6c,d). However, among Type I and sugarcanes a significant increase in w_{NDF} occurred in the second-ratoon harvest season between December 2010 and January 2011 (Fig. 6d), corresponding to the decrease in w_{sugar} (Fig. 6b).

The proportion of hemicellulose, cellulose, and lignin in the total fiber (NDF) did not change over the course of the plant-cane harvest season for all entries. However, in the

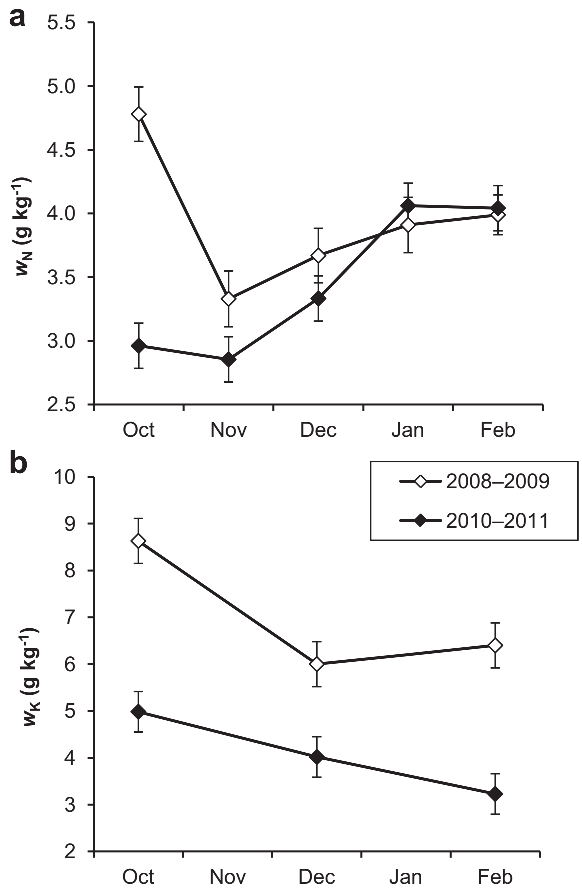

Fig. 4 – Mean monthly mass fractions of (a) N and (b) K in harvested energycane biomass, measured over plant-cane (2008–2009) and second-ratoon (2010–2011) harvest seasons at Tifton, GA. Error bars represent one standard error of the mean.

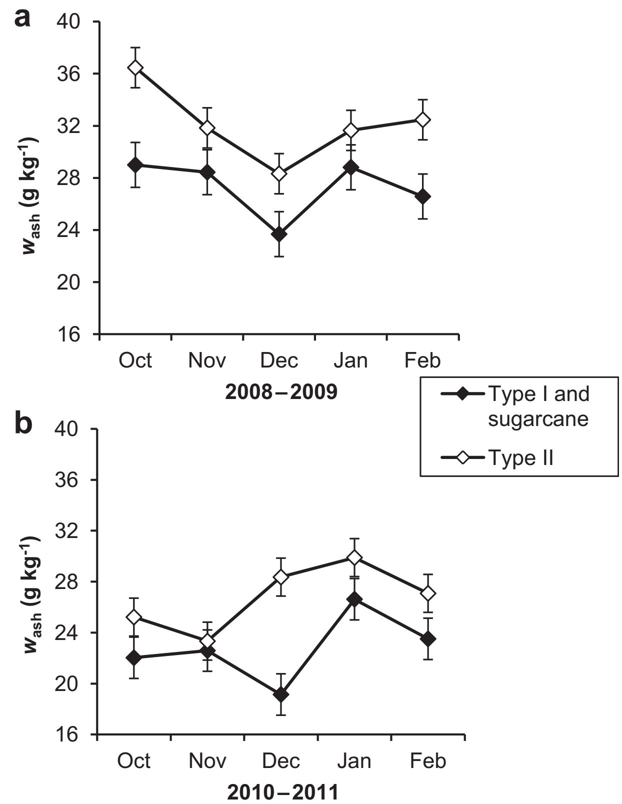

Fig. 5 – Mean monthly ash mass fraction (w_{ash}) in harvested biomass for three classes of energycane (sugarcane, Type I, and Type II) measured over (a) plant-cane (2008–2009) and (b) second-ratoon (2010–2011) harvest seasons at Tifton, GA. Because sugarcanes and Type I energycanes performed similarly, they are presented together. Error bars represent one standard error of the mean.

second-ratoon harvest season, a small but significant decrease in the average proportion of cellulose (−20 g kg^{-1} NDF) did occur between October and February. This was accompanied by an increase in the proportion of hemicellulose (+24 g kg^{-1} NDF). As a proportion of total fiber, lignin peaked slightly in November (average 73.2 g kg^{-1} NDF; data not shown). There were some differences between entries in fiber composition as well. Ho 02-144 fiber had the highest proportion of hemicellulose (441 g kg^{-1} NDF), and consequently the lowest proportion of cellulose (496 g kg^{-1} NDF). The lowest proportion of hemicellulose, and highest of cellulose was measured in Ho 06-9001 and Ho 06-9002. Overall, the proportion of lignin in total fiber was lower in sugarcanes and Type I energycanes than in Type II energycanes (Table 1).

3.4. PSCF

A preliminary test of the PSCF with dilute sulfuric acid pretreatment did not yield sufficient quantities of ethanol, suggesting that the free sugars in the biomass may have contributed to excess formation of inhibitory compounds [19,20]. Thus, a gentler hot water pretreatment was used. Following pre-treatment the enzymatic saccharification was allowed to proceed for 24 h, after which the *E. coli* was added. Reducing sugar concentration began to decline after 24 h of fermentation, and usually reached its minimum level by 48 h of fermentation (Fig. 7a). Ethanol concentration usually reached near maximum after 48 h of fermentation by *E. coli* (Fig. 7b). A typical PSCF profile is shown in Fig. 7a,b. Reducing sugar concentration usually fell to below 5 g L^{-1} upon completion of fermentation, with a few notable exceptions. In October 2008, for example, the sugars from Ho 95-988 did not completely ferment; the reducing sugar concentration for this entry remained around 20 g L^{-1} even after 120 h of fermentation (Fig. 7a). From this same cultivar in February 2011 some residual sugars (5.08 g L^{-1}) remained after 120 h. In December 2010 some unfermented sugars remained from L 99-233 and Ho 01-07 (8.14 and 10.38 g L^{-1}, respectively) after 120 h of fermentation. It is not clear why the incomplete fermentations occurred at different times in the harvest sequence, for different entries. However, these entries are all

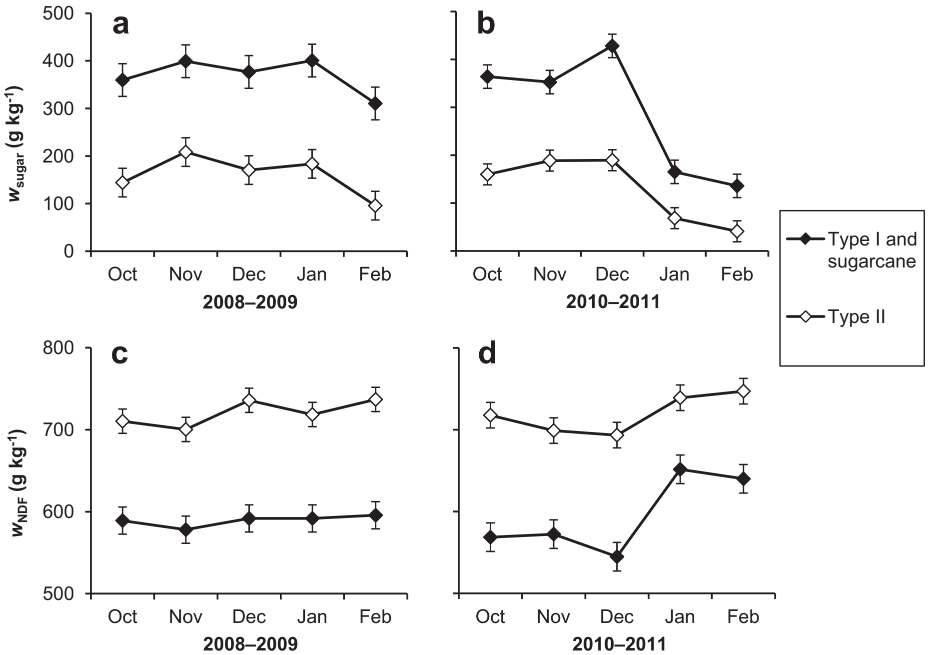

Fig. 6 – Mean monthly mass fractions of (a,b) free sugars and (c,d) neutral detergent fiber (NDF) in harvested biomass for three classes of energycane (sugarcane, Type I, and Type II) measured over (a,c) plant-cane (2008–2009) and (b,d) second-ratoon (2010–2011) harvest seasons at Tifton, GA. Because sugarcanes and Type I energycanes performed similarly, they are presented together. Error bars represent one standard error of the mean.

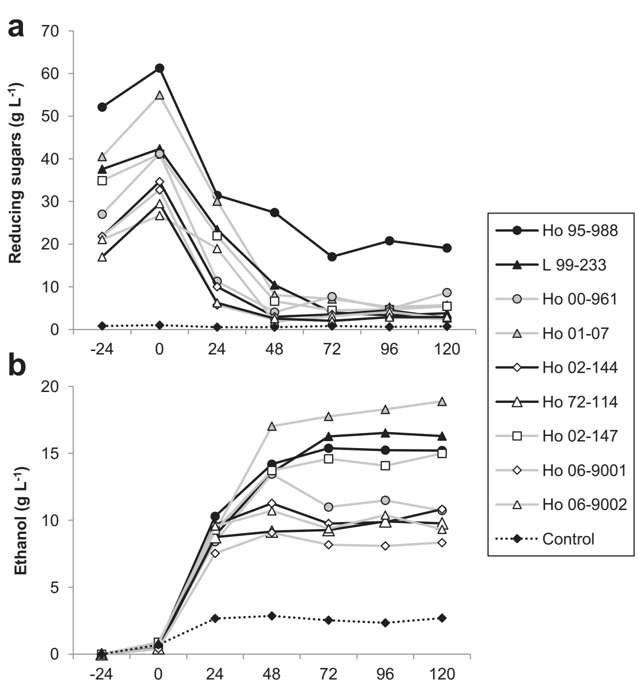

Fig. 7 – Partial saccharification and co-fermentation (PSCF) profile for nine energycane entries, harvested in October 2008 at Tifton, GA, and a negative control (no biomass). Partial saccharification began at −24 h, and fermentation began at 0 h. (a) Reducing sugar concentration (b) ethanol concentration.

high-sugar types, suggesting that even the gentler hot water pre-treatment is likely to produce fermentation-inhibiting compounds when a high concentration of free sugars is present.

Harvest timing did affect the fermentability of energycane biomass, but the pattern differed between the two crop years. In the plant-cane harvest season, peak reducing sugar concentration (0 h) was not affected by harvest timing for the sugarcanes L 99-233 and Ho 95-988, or the Type I energycane Ho 01-07 (Fig. 8a). Reducing sugars released from type II energycanes Ho 06-9002 and Ho 72-114 were also not affected by harvest timing. Overall, the lowest reducing sugar concentrations were measured in the December harvest. Despite the differences in reducing sugar concentrations, the concentration of ethanol was not affected by harvest date in the plant-cane season for any entries (Fig. 8a). A much clearer trend was observed in the second-ratoon season in 2010–2011. Reducing sugars were virtually unchanged between October and December and then dropped considerably in February for all lines tested. The ethanol yield also dropped significantly from December to February, but only for the Type I energycanes and sugarcanes (Fig. 8b). The sugarcanes and Type I energycanes tended to have the greatest ethanol yield per unit of biomass. Among the Type II energycanes, Ho 02-147 had the greatest ethanol yield per unit of biomass (Fig. 8a,b).

4. Discussion

Several studies have reported yield reductions associated with late harvesting of *Miscanthus* [7,21], reed canarygrass

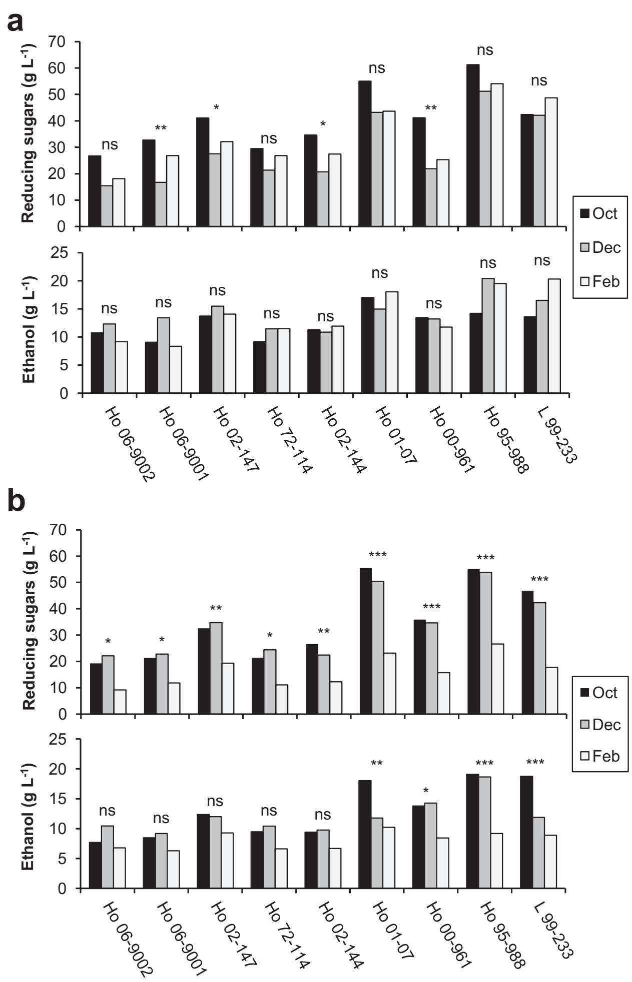

Fig. 8 – Peak reducing sugar concentrations at 0 h and ethanol concentrations at 48 h from partial saccharification and co-fermentation (PSCF) of biomass from nine energycane entries harvested in October, December, or February over (a) plant-cane (2008–2009) and (b) second-ratoon (2010–2011) harvest seasons at Tifton, GA. Significance of harvest time effect on individual entries is denoted by * (0.05), ** (0.01), and * (0.001), while ns denotes no significant effect of harvest time.**

(*Phalaris arundinacea* L.) [9,10], and switchgrass (*Panicum virgatum* L.) [8], mostly attributed to leaf drop and breakage of brittle stem tops. The small plot size in this study prevented evaluation of yield losses over the winter, so it is not yet known to what extent this occurs in late-harvested energycane. Lewandowski et al. [7] reported that late winter or early spring harvest of various species and hybrids of *Miscanthus* resulted in reduced moisture, ash, and K concentrations in harvested biomass compared to autumn harvest. Similar observations were reported for spring-harvested switchgrass [8] and reed canarygrass [9,10]. However, most of these studies were conducted in colder climates, with temperate grasses that are physiologically very different from energycane. Temperate grasses are able to flower and then begin a natural process of senescence in which N is actively translocated from the shoots to the roots after flowering, whereas most energycanes do not flower, and senescence is abrupt, usually the result of freezing temperatures, and the N appears to be trapped in the shoots. This has been observed in late-flowering types of *Miscanthus* compared to early-flowering types [7]. Although w_{ash} tended to increase after December, the mass fraction of K, a major component of ash, still showed a consistent decrease. It has been observed that K is readily leached from wheat and barley straw by rain [22], and it appears to also leach from senesced energycane biomass. This study demonstrated that the stalks of energycane, which are much thicker than those of other grasses, dry very little over the course of the winter. Even as late as February in 2009, the lowest w_{H_2O} was 425 g kg^{-1} (Ho 06-9002). Mean temperatures were much lower in the 2010–2011 winter season than in the 2008–2009 season (Fig. 1), which may have contributed to the lack of observed drying in the second-ratoon year. In the 2010–2011 harvest season, the lowest w_{H_2O} was only 556 g kg^{-1} (Jan 2011, Ho 06-9002). Clearly, delayed harvest does little to reduce moisture in energycane biomass.

The drop in w_{sugar} was most prominent among Type I energycanes and sugarcanes in the 2010–2011 (second-ratoon) harvest season, when temperatures were lower and there were more freeze events recorded (Fig. 1). Freezing temperatures can cause cracks and other damage to sugarcane tissue, allowing invasion by sugar-degrading microbes, particularly lactic-acid bacteria and yeasts [23]. Nights with freezing temperatures, but with days above freezing, would have facilitated the degradation of free sugars in the second-ratoon harvest season, leading to loss of some dry matter in the form of CO_2 and ethanol. This appears to be the reason for the apparent increases in NDF and ash mass fractions that were observed. During ensiling of sugarcane Alli et al. [24] reported that the proportion of ADF increased by 121–132 g kg^{-1} DM due to the fermentation of sugars to ethanol.

Although biomass yields were measured on relatively small plots in this study, the yields are similar to those reported by Knoll et al. [5] for napiergrass [*Cenchrus purpureus* (Schumach.) Morrone, formerly *Pennisetum purpureum* (Schumach.)] and L 79-1002 energycane in a similar environment on larger plots. Bischoff et al. [25] reported that plot size had relatively little effect on yield estimates of combine-harvested sugarcane, though standard errors were reduced by using larger plots. Nonetheless, it is clear that the Type II energycanes produced more biomass than typical sugarcanes, with Type I energycanes producing intermediate yields. Under the conditions in these experiments using minimal pretreatment of whole biomass, Ho 02-147 could produce about 3200 L ha^{-1} y^{-1} ethanol, the highest estimated in this test. If more of the sugars could be released through pretreatment, the ethanol yield would be much greater. Ethanol yield from this entry was not affected by harvest dates. The lowest ethanol yield would be expected from L 99-233, at 1500 L ha^{-1} y^{-1}. Although this entry produced more ethanol per unit of biomass due to high w_{sugar}, its overall DM yield was very low. Also, as a sugarcane it was more greatly affected by harvest timing, particularly in the second-ratoon harvest season. High w_{sugar} also appears to interfere with the pretreatment, probably by forming inhibitory compounds [20]. For high-sugar types, it would be preferable to harvest early to avoid loss of fermentable sugars. It could also be beneficial to separate out the free sugars prior to pretreatment and fermentation of the lignocellulose fraction. In contrast, low-sugar Type II energycanes can be harvested late in the season and processed as whole biomass.

4.1. *Conclusions*

Delayed harvest of energycane biomass into the winter months resulted in only minimal reduction in w_{H_2O}. Delaying harvest did not decrease dry biomass w_N, except from October to November in the plant-cane crop year. Dry biomass w_K showed a consistent decrease with delayed harvest, while w_{ash} tended to decrease into mid-winter, and then increased. The reduction in w_{sugar} over the winter caused an apparent increase in mass fractions of ash and total fiber in the harvested biomass. The reduction in free sugars was more prominent in the colder of the two winter harvest seasons in this test. Because they contain less free sugar, ethanol yield from Type II energycanes was less affected by harvest time than that of sugarcanes and Type I energycanes. Biomass fiber composition was found to be quite stable over winter harvest dates.

Disclaimer

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