BIOMASS YIELD AND COMPOSITION OF POTENTIAL BIOENERGY FEEDSTOCKS

By

JEFFREY ROBERT FEDENKO

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2011

To my parents, for their continuing support in my quest to be a perpetual student

ACKNOWLEDGMENTS

I wish to thank Dr. John Erickson, Chairman of the Supervisory Committee, for his guidance, support and assistance throughout my graduate program, and for his willingness to allow me to continue as a PhD student with him.

I would also like to thank Dr. Lynn Sollenberger, Dr. Zane Helsel and Dr. Gary Peter for their assistance throughout the research and in answering my questions, and for serving on my Supervisory Committee.

Also, I would like to thank Richard Fethiere and Robert Querns for their assistance with nutrient and fiber analyses, as well as the HPLC analyses, and Jonathan Holland and Andrew Schreffler for their assistance in prepping and running samples.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	Acid detergent fiber; equivalent to cellulose and lignin
ADL	Acid detergent lignin
Extractives	All non-structural components of the plant removed by autoclaving at 121°C and 15 psi for 60 minutes in water in a sealed pressure tube.
NDF	Neutral detergent fiber, the structural material of a plant composed primarily of cellulose, hemicellulose and lignin
TEP	Theoretical Ethanol Potential, expressed as either L Mg^{-1} of dry biomass or L ha^{-1} as specified

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

BIOMASS YIELD AND COMPOSITION OF POTENTIAL BIOENERGY FEEDSTOCKS

By

Jeffrey Robert Fedenko

August 2011

Chair: John E. Erickson
Major: Agronomy

The identification of sources of alternative energy to reduce dependence on fossil fuels has become increasingly important due to shrinking supplies and rising prices. Perennial grasses including sugarcane (*Saccharum* spp.), energycane (*Saccharum* spp.), sweetcane (*Saccharum arundinaceum* Retz. IK76-110), elephantgrass (*Pennisetum purpureum* Schum.), giant reed (*Arundo donax* L.*)* and giant miscanthus (*Miscanthus x giganteus* Greef and Deuter ex Hodkinson and Renvoize), as well as the annual crop sweet sorghum [*Sorghum bicolor* (L.) Moench], are all potential candidates for bioenergy production. The objectives of this study were to evaluate biomass yield and tissue composition by identifying structural sugars, extractives, ash and lignin for the plant and first ratoon crops of the perennials; by identifying extractives, cellulose, hemicellulose and lignin for sweet sorghum; and calculating theoretical ethanol yields for each of these species when grown at three sites across Florida. Maximum theoretical ethanol yields for the perennial plant crop averaged 16 000 L ha^{-1} for sugarcane, energycane, sweetcane and elephantgrass, as well as giant reed at one site. In the ratoon crop maximum yields were approximately 20 900 L ha^{-1} for sugarcane across sites. Differences in tissue composition were relatively minor, with

lignin on a structural basis varying by only three percentage units between species. Nonstructural sugars as a component of extractives varied significantly, and were highest in sugarcane and lowest for giant miscanthus. Whole plant ash was lowest at Ona for the plant crop, and was typically lower in sugarcane than other species due to high levels of extractives. Maximum theoretical ethanol yields from a single crop of sorghum were 12 000 L ha^{-1}, of which 71% was from structural biomass. Maximum ethanol yields from sorghum juice were 3400 L ha^{-1}, and M-81E was a better suited cultivar for ethanol conversion than Dale due to higher biomass production and theoretical ethanol yields. When plant crop perennial grasses were compared with a single crop of sorghum, the highest yielding perennials, such as energycane, had significantly higher total theoretical ethanol potentials than sorghum. Compared to the perennial grass ratoon crop, a single crop of sorghum only produced 57% as much ethanol as the highest yielding perennial grasses, although the sorghum growth period was about half as much as well. Future research on harvest management, post harvest storage, nutrient requirements, water use, invasion potential and industrial conversion processes will further help to determine which species are most suited for lignocellulosic ethanol production in the southeastern USA.

CHAPTER 1
LITERATURE REVIEW OF SORGHUM AND PERENNIAL GRASSES

Background

Increasing concern over rising fossil fuel prices, dwindling reserves and instability in major oil producing regions has led to an increased interest in the use of biomass for energy. Currently, biomass provides approximately 13% of global energy, mainly as a fuel source in rural areas (Sims et al., 2006). Commercial production and use of biomass for energy is generally separated into biofuels, or liquid fuels for transport, and biopower, using crops to generate heat or power (Karp and Shield, 2008). Biofuels represent a significantly smaller but increasing fraction of biomass-derived energy, but at present rely primarily on starch or simple sugars produced by plants for fermentation to ethanol.

In Brazil, sugarcane has been used for ethanol production for decades, and supplies 40 to 50% of the country's transportation fuel (Somerville, 2010; Pohit et al., 2011). In the USA, corn (*Zea mays* L.) has recently gained widespread use as a feedstock for first-generation starch ethanol production via grain fermentation. At present, Brazil produces about 23 billion liters of ethanol from sugarcane and the US produces about 45 billion liters from corn grain (RFA, 2011). To produce 45 billion liters of ethanol requires about ~12 million hectares or approximately 1/3 of annual U.S. corn production. Therefore, the use of corn and to lesser extent sugarcane, as fuels has raised several issues, including concern over the use of food as a fuel, and in the case of corn, the relatively small amount of petroleum that can be displaced with corn-based ethanol (Byrt et al., 2011). These concerns have led to capping U.S. corn ethanol production at 57 billion liters of ethanol (EISA, 2007; Fletcher et al., 2011). However,

plans have been developed to supply as much as 30% of the US demand (in 2004) for transportation fuels with biofuels by 2030, or an increase to 227 billion liters of biofuels such as ethanol (Himmel et al., 2007).

Current ethanol production systems based on simple sugars and starch are first-generation systems that rely on established processes but make limited use of a plant for fuel, and are inadequate for largely displacing fossil fuel consumption (Karp and Shield, 2008). Somerville et al. (2010) lists average corn grain ethanol yields of 2900 L ha^{-1}, with estimated sugarcane ethanol yields of 6900 L ha^{-1} from sugar, 3000 L ha^{-1} from bagasse and 9950 L ha^{-1} total (Table 1-1).

Therefore, more advanced second-generation technologies that can produce renewable fuels from lignocellulosic material have received considerable interest and are the focus of research concerning potential feedstocks and their suitability for lignocellulosic conversion. Saha et al. (2003) has reported ethanol yields as high as 388 L Mg^{-1} dry biomass at a lab scale using an alkaline pretreatment with wheat (*Triticum aestivum* L.) straw, although most technologies currently produce significantly less than theoretical yields. As an example, the application of lignocellulosic conversion of giant miscanthus could produce 4600 to 12 400 L ha^{-1} (Table 1-1). The USA, through the Energy Independence and Security Act (EISA. 2007), has mandated the production of 21 billion gallons of cellulosic ethanol in addition to the current 15 billion gallons of corn ethanol by 2022 (Fletcher Jr. et al., 2011).

Tissue Composition

Biomass composition, in addition to yield, determines biofuel yield potential from second- and third-generation conversion technologies. Cellulosic ethanol production makes use of the structural sugar polymers found in the cell wall of plants (Fig. 1-1),

cellulose and hemicellulose, as sugar sources for microbial fermentation. Cellulose is composed solely of linked six-carbon glucose monomers (Fig. 1-2), while hemicellulose (Fig. 1-3) is composed primarily of the five-carbon sugar xylose with a significant percentage of glucose and minor amounts of other five- and six-carbon sugars. The ratios of hemicellulose and cellulose, as well as of the sugar component of hemicellulose, can vary significantly among species, within species depending on maturity, and even among growing sites (Vermerris et al., 2007).

Lignin is also present in lignocellulosic material. Lignin is a large (> 10,000 Da) relatively hydrophobic and aromatic three-dimensional polymer. It is imbedded in the cell wall among the cellulose, hemicellulose and pectin components, and acts as a binding agent through covalent linkages with hemicellulose and crosslinks with other lignin subunits and cell wall components. These bonds strengthen the plant cell wall, provide rigidity and resistance to hydrolysis (Davison et al, 2006; Fu et al., 2011). Lignin is a complex molecule consisting of subunits formed from three different phenyl-propane precursor monomers: *p*-coumaryl, coniferyl and sinapyl alcohols. These monomers are linked via three types of ether bonds, and repeat in a non-uniform fashion and can vary among plants. These subunits are incorporated into lignin in the form p-hydroxyphenyl (H), guaiacyl (G), and syringal (S) phenylpropanoids (Himmel et al., 2007; Fu et al., 2011; Vermerris, 2008) (Fig. 1-4).

Biomass Conversion

The process for converting lignocellulosic biomass to simple sugars and ultimately fuels and bio-based products is more difficult than direct microbial fermentation of the parent material. The biomass must first be pretreated, typically using heat, alkaline materials and/or acid, to convert hemicellulose to monomeric sugars or

oligosaccharides, as well as to partially break down the structure of the biomass. The biomass is then hydrolyzed or enzymatically degraded to release monomeric sugars and free these sugars from the lignin. Hydrolysis/degradation can be accomplished through a variety of methods, including heat, acid and/or enzymes, individually or in conjunction with each other. Fermentation of hydrolyzed biomass to produce ethanol is also more difficult because of the presence of both 5- and 6-carbon sugars. To produce ethanol efficiently both types of sugars must be fermented, but microbes usually preferentially ferment specific types of sugars. Therefore, engineered microbes that can ferment both 5-carbon (xylose) and 6-carbon (glucose) sugars are being developed, as well as the possibility of mixing different microbial species in the same fermentation tanks to simultaneously ferment both types of sugars (Foyle et al., 2007; Himmel et al., 2007; Casler et al., 2009).

After pre-treatment and hydrolysis to release the structural sugars there are still issues associated with microbial conversion, such as: efficient conversion of 5-carbon sugars to ethanol, inhibitory effects of lignin on fermentation, and the ability to withstand treatment conditions (Himmel et al., 2007; Ingram et al., 1999) Glucose is easily fermented by yeast (*Saccharomyces cerevisiae)* and many bacteria (e.g., *Bacillus* spp. and *Escherichia coli*) to ethanol with little or no modification, but efficient xylose fermentation pathways are less common. Fermentation pathways for minor hemicellulose sugars, such as arabinose (a component of arabinoxylans) and mannose (mannans), are even more uncommon and not widely researched when compared with xylose fermentation pathways (Ingram et al., 1999; Casler et al., 2009).

Total lignin concentration and/or the ratio of subunits can affect the release of structural sugars and the efficiency of enzymes and microbes in both hydrolysis and fermentation (Chen and Dix, 2007; Walford et al., 2008; Dien et al., 2009; Studer et al., 2011). Recent research with a variety of species has shown that even minor differences in lignin concentration can have significant effects on conversion efficiency. For instance, a two percentage unit difference in total plant lignin in switchgrass had a significant impact on the efficiency of biomass conversion to ethanol (Fu et al., 2011). In trees of the *Populus* genus, glucose release was correlated with lignin concentration (Studer et al., 2011). According to Davison et al. (2006), a two percentage unit reduction in total lignin from 24.8 to 22.7% increased xylose yield under partial hydrolysis from 40 to 55% of total theoretical yield.

Finally, distillation (isolation of ethanol from water) is necessary to produce fuel grade ethanol, as anhydrous ethanol is necessary for blending with gasoline (Goldemberg, 2007). Consequently, there are more challenges associated with using lignocellulosic material for biofuel production. Structural sugars cannot be directly fermented, are difficult and/or expensive to hydrolyze and ferment, and can vary across cultivars and species (Vermerris, 2008; Walford, 2008).

Any lignocellulosic material, such as sugarcane bagasse, wood chips or crop residues, can be converted to its structural components and the sugars fermented, but sustainable feedstock production is vital for long-term feasibility (Summerville et al., 2010; Karp and Shield, 2008). For this reason, tall grass species, especially perennial warm-season (C4) grasses, are excellent candidates for biomass production at low latitudes in the USA. Warm-season grasses have high dry matter production and

require reduced inputs for equivalent DM production compared to cool-season grasses due to higher nitrogen and water use efficiency, as well as greater overall rates of conversion of solar energy into biomass (Karp and Shield, 2008; Byrt et al., 2011; Fletcher et al., 2011). Gerbens-Leenes et al. (2009) showed that the global average water requirement for grain sorghum used as a biofuel feedstock was 419 m^3 per GJ of ethanol produced, compared with 108 m^3 per GJ of ethanol for sugarcane, a perennial grass. Additionally, perennial grasses can help increase soil quality, sequester carbon in root systems and increase biodiversity (Somerville et al., 2010). The use of native perennial grass systems for biomass production may increase biodiversity relative to row crop or plantation tree production (Fletcher Jr. et al., 2011). Groom et al (2008) compared the water use, fertilizer inputs and greenhouse gas emissions (GHG) of corn, switchgrass and sugarcane (perennial monocultures) and woody species (poplar and willow). Comparatively, corn had the highest water and fertilizer requirements and GHG emissions. Switchgrass had the lowest GHG emissions, with woody species and sugarcane having intermediary levels of emissions. Switchgrass and woody species also had low water and fertilizer requirements, while sugarcane requirements were closer to those of corn.

Potential Tall Grass Bioenergy Crops for the Southeastern USA

Elephantgrass (*Pennisetum purpureum* Schum.)

Elephantgrass is a large tropical perennial C4 bunchgrass native to Africa that was introduced to the U.S. A. in 1913, and can grow to 5.5 m in height during the warm growing season in Florida (Woodard and Sollenberger, 2011). Small seeds of short-lived viability are produced in the fall, but because of genetic variability and limited viability are not used for propagation. Elephantgrass is vegetatively propagated in

summer, or from late October until freezing conditions occur. Additionally, elephantgrass is potentially invasive in South Florida, but has not been classified as such elsewhere (Woodard and Sollenberger, 2011).

The 'Merkeron' cultivar was registered in 1989 (Burton) and had average reported plant crop yields of 32 Mg ha^{-1} yr^{-1}, dropping to 22.3 Mg ha^{-1} for the second ratoon, comparable to other varieties of elephantgrass including PI 300086, N51 and N43 (Woodard and Prine, 1991). Later research by Woodard and Prine (1993) showed significantly higher yields of 46 to 47 Mg ha^{-1} yr^{-1} for PI 300086 and N51 grown in north Florida in 1989 and 1990. Mislevy et al. (1989) showed even higher average yields of 56.5 Mg ha^{-1} yr^{-1} for PI 300086 grown over a 4-yr-period in Central Florida. High biomass yields require significant fertilization with recommended rates of 225 to 340 kg N ha^{-1} yr^{-1} applied in the spring or as split applications (Woodard and Sollenberger, 2011).

Giant Reed (*Arundo donax* L.)

Giant reed is a tall, perennial C3 grass that is native to subtropical environments from the Mediterranean through India, is found throughout much of the world, and was introduced to California in the 1820s. It can grow to over 7 m, and is considered invasive in some states because of its ability to vegetatively propagate via rhizomes and viable nodes of mature cane, as well as its dense and fibrous root system. Giant reed produces very few seeds, which are considered sterile. Propagation is accomplished by planting large rhizomes or stems with two or more nodes in early spring. Fertilization with 60 kg N ha^{-1} per harvest as well as sufficient water is necessary to produce high yields (Gilbert et al., 2008). There are few known diseases or insect pests that affect giant reed, though this may change with widespread cultivation (Gilbert et al., 2008).

Giant reed is regularly cultivated in Europe, both for the production of musical instruments and as a potential bioenergy crop. Angelini et al. (2005) reported dry biomass yields of 22 Mg ha^{-1} for plant crop and 47 Mg ha^{-1} for first ratoon giant reed that was well fertilized, harvested in winter and grown in Central Italy.

Sugarcane (*Saccharum* spp.)

Sugarcane is a widely cultivated tropical perennial C4 crop that is native to Asia and characterized by having very high levels of sugar (primarily sucrose, greater than 20%) in its sap, and can grow to over 5 m in height (Karp and Shield, 2008; Rainbolt and Gilbert, 2008). Sugarcane is used for the commercial production of both sugar and ethanol, and in 2005 was cultivated on 165,000 ha in Florida. Production is concentrated in South Florida in the Everglades Agricultural Area, and CP89-2143, the cultivar used in this research, was the second most widely grown cane at 20% of total acreage (Glaz and Gilbert, 2010). Sugarcane can be grown in most of the southern U.S.A, but is extremely cold sensitive and grows best with temperatures above 21°C. Sugarcane is vegetatively propagated from mature stem cuttings, ideally in the fall, as seeds are extremely small and not typically viable. Fertilization requirements are dependent on production conditions; on the muck soils of southern Florida, minimal fertilization is needed, but otherwise fertilization is similar to other C4 perennial grasses, typically 200 kg N ha^{-1} yr^{-1} (Rice et al., 2010). Sugarcane cultivars are typically resistant to common plant diseases, and insect pests include wireworms, grubs, a stalk borer and aphids (Rainbolt and Gilbert, 2008; Stricker et al., 2009). Somerville et al. (2010) lists average yields of 11 Mg ha^{-1} yr^{-1} of sugar and 10 Mg ha^{-1} yr^{-1} of bagasse.

Energycane (*Saccharum* spp.)

Energycanes are *Saccharum* species that store relatively low levels of sucrose in the stalk, but tend to have greater fiber and biomass production. Energycane is propagated and fertilized the same as sugarcane, but generally has greater cold tolerance. Cultivar L 79-1002 was released in 2007, and is a high-fiber, low sucrose hybrid of *S. spontaneum, S. officinarum, S. barberi Jeswiet* and *S. sinense Roxb.* amend *Jeswiet* (Bischoff et al., 2008). It has an average fiber concentration of 257 g kg^{-1} on a fresh weight basis, more than twice that of sugarcane. Woodard and Prine (1993) showed average yields over 2 yr of 49 Mg ha^{-1} yr^{-1} for L 79-1002 grown in Central Florida compared with average 4-yr yields in Louisiana of 67 Mg ha^{-1} yr^{-1}. L 79-1002 is moderately susceptible to smut and susceptible to ratoon stunting disease, and is resistant to the mosaic virus, brown rust, leaf scald and sugarcane borer (Bischoff et al., 2008)

Giant Miscanthus (*Miscanthus x giganteus* Greef and Deuter ex Hodkinson and Renvoize)

Giant miscanthus is a perennial C4 grass native to Africa and Asia. It can reach heights of 4 m and must be vegetatively propagated because it is a sterile hybrid of *M. sacchariflorus* and *M. sinensis*is. It is photoperiod sensitive but can survive significantly colder weather than most other C4 species, and can be cultivated as far north as the Canadian border (Heaton et al., 2003). It can spread via rhizomes, and will gradually expand to form dense stands with deep roots. At present, giant miscanthus is not considered invasive, but there are some concerns regarding its use since it is not native to the USA and can spread vegetatively via rhizomes. Fertilization is recommended at a rate of 55 to 85 kg N ha^{-1} yr^{-1} after establishment (Erickson et al., 2008).

Propheeter et al. (2010) reported average yields of 3.3 and 12.8 Mg ha^{-1} yr^{-1} for giant miscanthus grown in Kansas in 2007 and 2008. First ratoon yields were significantly greater than plant crop yields and were expected to increase in the following 3 to 4 yr. Somerville et al. (2010) lists average yields from a variety of studies in the range of 15 to 40 Mg ha^{-1} yr^{-1} with estimated ethanol yields of 4600 to 12 400 L ha^{-1}.

Sweetcane (*Saccharum arundinaceum* Retz. IK76-110)

Sweetcane, formerly referred to as *Erianthus arundinaceum*, is a tropical C4 bunchgrass that is tolerant to most pests, and can produce high biomass yields, and was (Mislevy et al., 1997). It has a spreading growth habit and is generally more difficult to establish than elephantgrass or sugarcane. It is vegetatively propagated via stem cuttings, and the best time for planting in the Southeast USA is from early November until a killing frost. It is relatively tolerant to saturated soil conditions, and on phosphatic clays should be fertilized with 180 to 220 kg N ha^{-1} yr^{-1} (Stricker et al., 2009).

Byrt et al. (2011) reported peak dry matter yields of approximately 50 Mg ha^{-1} yr^{-1} for sweetcane, consistent with reported yields by Mislevy et al. (1997). Mislevy reported yields of 51.5 Mg ha^{-1} yr^{-1} over a 4-yr period for well fertilized sweetcane grown in Central Florida and harvested once per year in December.

Sweet Sorghum (*Sorghum bicolor* (L.) Moench)

Sweet sorghum is an annual C4 row crop that can grow to over 5 m in height. It is capable of producing a ratoon crop after harvesting in the same year, provided the growing season is long enough, and can be cultivated throughout the continental USA. It also produces viable seed and is primarily self-pollinating. Sweet sorghum cultivars are characterized by having a high concentration of easily fermented sugars in the juice,

which can be extracted by pressing or squeezing in the same way sugarcane is processed. Sweet sorghum also produces high biomass yields (Broadhead et al., 1981). Planting should occur when soil temperatures are over 18°C using seed in widely spaced rows, and fertilization with 170 kg N ha^{-1} and 55 kg P_2O_5 and K_2O ha^{-1} is typically recommended. Sorghum is susceptible to a variety of disease and insect problems, but it is not a potential invasive species (Vermerris et al., 2008).

Propheeter et al. (2010) reported average yields of 28.2 and 32.6 Mg ha^{-1} yr^{-1} for M-81E grown in Kansas in 2007 and 2008, with estimated ethanol yields of 9660 L ha^{-1} and 10 200 L ha^{-1}, which were greater than corn, forage sorghum or giant miscanthus estimated ethanol yields. Mislevy et al. (1989) showed total average yields of 28.9 Mg ha^{-1} yr^{-1} for M-81E grown over a 2-yr period in central Florida, with an average of 20.4 Mg ha^{-1} yr^{-1} produced on average produced by the plant crop and 8.5 Mg ha^{-1} yr^{-1} by the ratoon crop.

Optimal Fuel Production

Total ethanol yields will be influenced by a variety of factors, including yields and tissue composition. Higher yields increase the amount of biomass available for hydrolysis, but ratios of cellulose, hemicellulose and lignin are also important. Currently, the glucose monomers of cellulose are more easily fermented to ethanol using traditional commercial processes than pentose sugars, and therefore higher cellulose yields relative to hemicellulose may produce more ethanol. However, research is under way to apply xylose-fermenting bacteria and yeast to commercial production systems to increase conversion efficiency of pentose sugars on an industrial scale. Also, some sugars, such as arabinose, are not currently fermented to ethanol in existing systems; thus, higher levels of these sugars reduce the amounts of other

sugars that can be fermented. Lignin concentration is important for conversion as well, both because of direct mass balance as well as other effects of lignin. For direct mass balance, less lignin on a percent basis may equate to a higher percentage of fermentable sugars as either hemicellulose or cellulose. Additionally, lignin ratios and overall lignin levels affect the efficiency of hydrolysis and conversion (Davison et al., 2006). Therefore, optimal fuel production from lignocellulosic biomass is likely to occur with low levels of lignin, high biomass yields and relatively high levels of cellulose and to a lesser extent hemicellulose.

Therefore, the overall objective of this thesis is to characterize biomass yields, composition and theoretical ethanol production of potential tall grass bioenergy crops in Florida. This will be accomplished by quantifying lignin, ash and structural sugar composition of each species, and calculating theoretical ethanol yields based on equations developed in prior research.

Table 1-1. Reported ethanol yields for various crops.

Biofuel crop	Ethanol yield (L ha^{-1})
Corn (grain) †	2900
Sugar cane (juice) †	6900
Sweet sorghum (juice) ‡	3500
Giant miscanthus (theoretical)†	4600-12 400

†Adapted from Somerville et al., 2010

‡Adapted from Brown, L.R., 2006

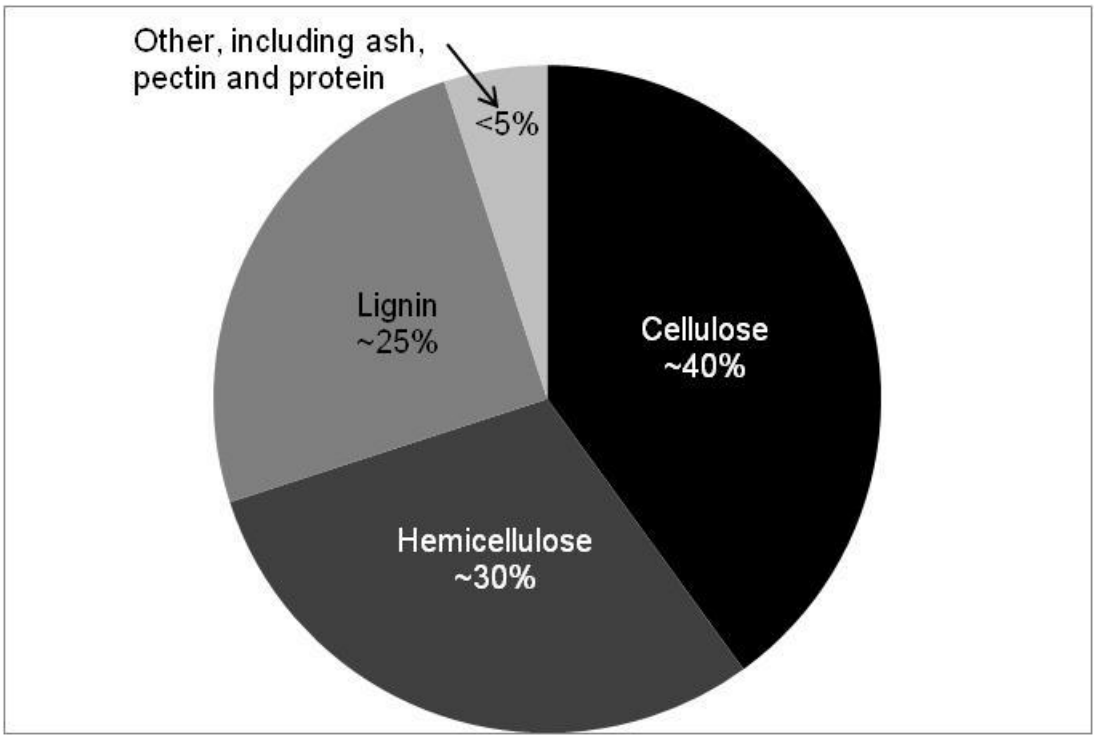

Figure 1-1. Approximate structural tissue composition of grasses (adapted from Saha et al., 2003; and Vermerris et al., 2008).

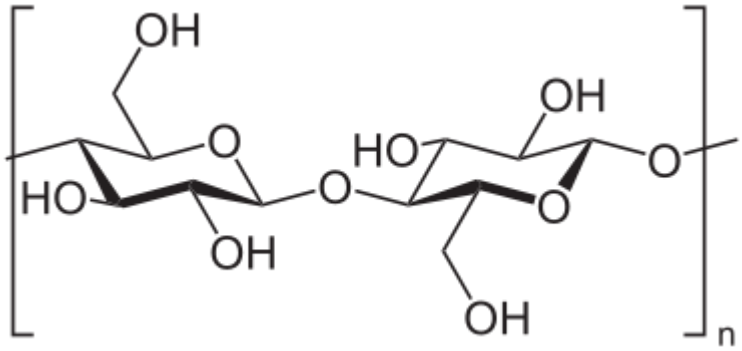

Figure 1-2. Structure of cellulose.

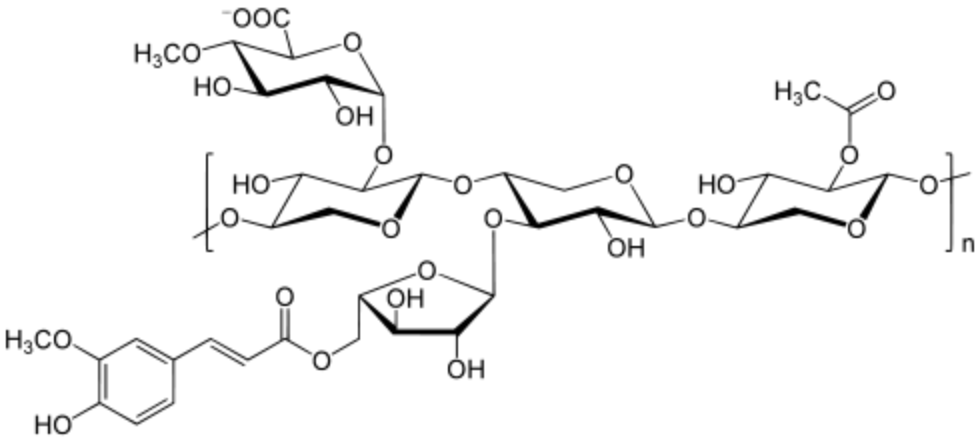

Figure 1-3. Possible structure of xylan, a component of hemicellulose. Xylose backbone with glucuronate (top) and pentose sugar (bottom) side chains and pentose sugar substitution (far right) in the backbone.

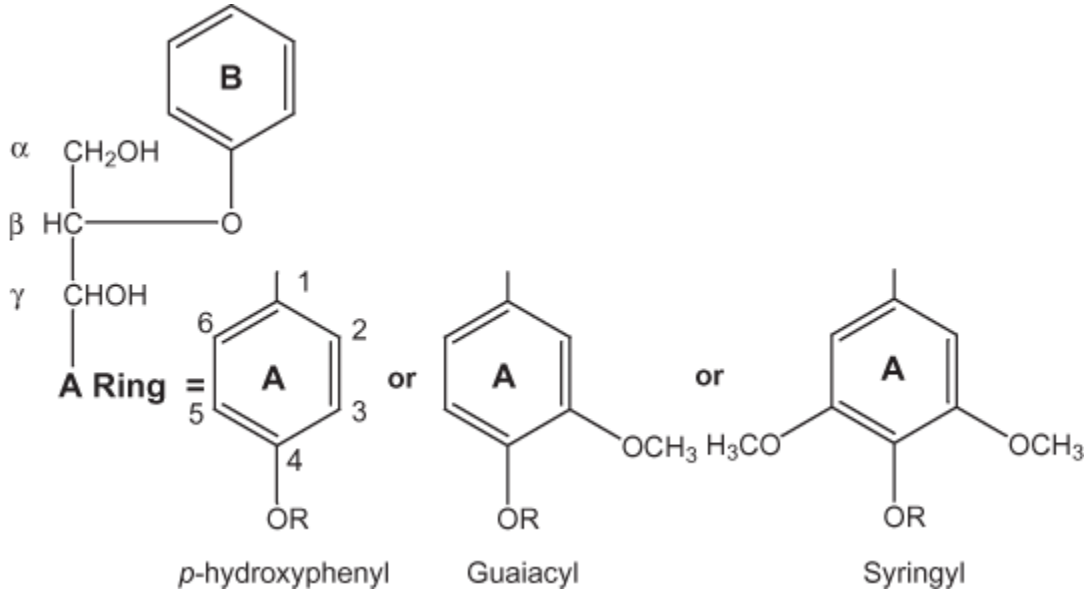

Figure 1-4. Structure of lignin subunits.

CHAPTER 2
TISSUE COMPOSITION OF PERENNIAL GRASSES

Background

Declining reserves of fossil fuels, increasing consumer energy prices and impacts of fossil fuel exploration and combustion on the environment have led to increased demand for renewable, domestic energy resources in the USA. In order to meet this growing pressure, mandates have been set to increase the use of bioenergy crops to produce alternative transportation fuels. Most alternative fuels are supplied by corn ethanol at present, however, production of corn ethanol has been capped at approximately 25% of the estimated 2030 demand for liquid biofuels (EISA, 2007; Fletcher et al., 2011). Additionally, biomass can be used in a variety of other energy production systems, such as co-firing with coal (Sami et al., 2001). Thus, new sources of biomass for energy production are necessary, a demand which may possibly be met in part by perennial grasses.

Perennial grasses are excellent candidates as feedstocks for renewable energy production at low latitudes in the USA for a variety of reasons, including high dry matter yields, lower cost and more efficient use of inputs compared to annual crops (Karp and Shield, 2008; Byrt et al., 2011; Fletcher et al., 2011). Additionally, perennial grasses can provide annual or multiple harvests per year, and for comparable yields may have more favorable tissue compositions for conversion, water use, and fertilizer use when compared with woody biomass (Woodard and Prine, 1991; Groom et al., 2007). Higher lignin concentrations are negatively correlated with sugar release and conversion efficiency (Chen and Dixon, 2007), and perennial grasses typically have lower lignin

concentrations than woody biomass (161 to 192 mg g^{-1} vs. 157 to 279 mg g^{-1}) (Fu et al., 2011; Studer et al., 2011).

Dry matter yields of elephantgrass [*Pennisetum purpureum* (Schum.) cv. 'Merkeron'] and sugarcane (*Saccharum* spp. cv. 'CP89-2143') species have been studied in the southeastern USA. 'Merkeron' elephantgrass had average reported plant crop yields of 32 Mg ha^{-1} yr^{-1}, dropping to 22.3 Mg ha^{-1} for the second ratoon, comparable to other introductions and breeder's lines of elephantgrass including PI 300086, N51 and N43 (Woodard and Prine, 1991). Even higher yields of 46 to 47 Mg ha^{-1} yr^{-1} have been reported for elephantgrass grown in North Florida (Woodard and Prine, 1993). Similar yields have been reported for energycane (*Saccharum* spp. cv. 'L79-1002'). Woodard and Prine (1993) showed average yields over 2 yr of 49 Mg ha^{-1} yr^{-1} for L 79-1002 grown in Central Florida compared with average 4-yr yields in Louisiana of 67 Mg ha^{-1} yr^{-1} (Bischoff et al., 2008). Average sugarcane yields across studies and production systems of 21 Mg ha^{-1} were reported by Somerville et al. (2010), but yields as high as 80 Mg ha^{-1} have been reported with 12 mo of growth by Karp and Shield (2008). Sweetcane [*Saccharum arundinaceum* (Retz.) Jesw.] (formerly *Erianthus arundinaceum* (Retz.) Jesw.) yields of 52 Mg ha^{-1} have been reported by Mislevy et al. (1986). Plant crop yields have ranged from 47 to 65 Mg ha^{-1}, and 29 to 40 Mg ha^{-1} in first ratoon crops (Mislevy et al., 1997).

Giant miscanthus [*Miscanthus x giganteus* (Greef and Deuter ex Hodkinson and Renvoize)] and giant reed [*Arundo donax* (L.)] are two perennial grasses that have received considerable attention for use as a bioenergy feedstock in Europe (Sanderson et al., 2004; Angelini, 2005), but they have received little attention in the USA until

recently (Heaton et al., 2003; Anderson et al., 2008). Giant miscanthus yields reported in Europe have been highly variable, ranging from 0.3 Mg ha^{-1} in England to almost 30 Mg ha^{-1} in Germany, but typically average 9 to 24 Mg ha^{-1} yr^{-1} (Sanderson et al., 2004). Angelini et al. (2005) have reported dry matter yields for giant reed, a C3 grass, of 18 Mg ha^{-1} in Italy for plant crop and as high as 47 Mg ha^{-1} for first ratoon crop.

There is much less is known about biomass composition than biomass yield of candidate energy grasses. Due to mass balance, higher levels of cellulose and hemicellulose per unit of biomass will theoretically provide greater amounts of hexose and pentose sugars for cellulosic conversion after hydrolysis. Lignin levels can significantly impact both total sugars, due to mass balance (more lignin means less structural sugars per unit of biomass), and because higher lignin concentrations can negatively affect conversion efficiency (Davison et al., 2006; Fu et al., 2011). However, lignin levels and structural sugar composition are variable across the life cycle of the plant, are affected by site and within species differences, and are not well known (Vermerris et al., 2007).

Thai Hoa et al. (2008) reported elephantgrass as composed of 18.2% extractives, 43.3% cellulose, 19.5% pentose sugars and 17.3% acid insoluble lignin, all on an oven dry weight basis. Anderson et al. (2008) reported neutral detergent fiber percentages of 69.4% and 74.2% for mature Merkeron elephantgrass leaf and stem tissues respectively. Acid detergent fiber was measured as 36.0% and 48.1%, and acid detergent lignin was 3.04% and 6.95%. Anderson et al. (2008) reported neutral detergent fiber percentages of 65.5 to 67.6% and 71.9 to 75.4% for mature giant reed leaf and stem tissues respectively, which varied with cultivar. Acid detergent fiber was

reported to be 33.7 to 36.7% and 45.9 - 49.9%, and acid detergent lignin was 3.82 to 4.14% and 8.67 to 8.98%.

Energycane is a higher fiber variety of *Saccharum* spp*.* produced by crossing traditional sugarcane with lower soluble sugar yielding related cultivars and wild types. Bischoff et al. (2008) reported plant crop fiber concentrations of 250 mg g^{-1} and total fiber yields of 19.2 Mg ha^{-1}, while ratoon crop fiber concentration was 257 mg g^{-1} and yields were 21.4 Mg ha^{-1}. In comparison, sugarcane fiber concentrations were reported as 141 and 193 mg g^{-1} for plant and ratoon crops respectively, and brix values were 60% higher in sugarcane than energycane. Consequently, the potential for perennial grasses to be used for bioenergy depends not only on biomass yield, but also on biomass composition, including nonstructural carbohydrates and lignin.

Although a number of candidate bioenergy grasses have been evaluated primarily for biomass yield in the southeastern USA, a comparative study of biomass yield and composition including several of the leading candidate species side by side across a relatively wide geographic range is lacking. The objectives of the present study were therefore to characterize biomass yield and composition for plant and first ratoon crops of six perennial grass species across three locations in Florida differing in soil and climate characteristics, and to estimate theoretical ethanol potential (TEP) from structural, non-structural and total biomass.

Materials and Methods

Experimental Site and Design

A replicated field experiment was conducted at three sites in North, Central, and South Florida. The North Florida location was at the UF Plant Science Research and Education Unit (29°24’N 82°10’W) in Citra, Florida. The soil at Citra was a relatively

well-drained Arredondo fine sand (loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults). The Central Florida location was at the UF Range Cattle Research and Education Center (27°23'N 81°55'W) in Ona, Florida. The soil at Ona was a relatively poorly-drained flatwoods soil of the Pomona fine sand series (sandy, siliceous, hyperthermic Ultic Alaquod). The South Florida location was at the UF Everglades Research and Education Center (26°39'N 80°37'W) in Belle Glade. The soil at Belle Glade was an organic Pahokee muck soil (euic, hyperthermic Lithic Haplosaprist).

At each of the sites, six perennial grasses, including elephantgrass, giant reed, sugarcane, energycane, giant miscanthus and sweetcane were established in a randomized complete block design (RCBD) with four replicate plots per species. Plots were established from stem cuttings in November 2008, except for giant miscanthus, which was established from commercial micropropagules (Speedling, Inc., Sun City, FL) in March 2009. At Citra and Ona, plots were six rows each (7 x 6 m) planted on 1-m row centers for all species. Similarly at Belle Glade, 6-row plots were used, but row spacing differed to facilitate harvest with sugarcane equipment. Sugarcane, energycane, sweetcane and elephantgrass were planted on 1.5-m row centers, making plots 7 x 9 m, while giant reed and giant miscanthus were planted on 1-m row centers.

Cultural Practices and Harvest Management

At Citra and Ona all plots were fertilized at a rate of 280 kg N ha^{-1} yr^{-1} with a 16-4-8 blended granular fertilizer that included minor nutrients in split applications of 90 kg N ha^{-1} yr^{-1} in mid-April and 190 kg N ha^{-1} yr^{-1} in June. Limited irrigation was applied to plots during establishment and at sign of visual drought stress (e.g., leaf rolling) via

overhead irrigation at Citra and Ona. Irrigation at Belle Glade was maintained via canal levels. Weeds were removed during establishment mechanically by rotary hoe and subsequently by hand as needed.

Plots were harvested once per year in the fall around late November, prior to anticipated frost. A 4-m section from the middle of one of the two inner rows was cut at a 7.5-cm stubble height using a gasoline powered trimmer (Echo, Inc., Lake Zurich, IL) and harvested by hand. The 4-m section was immediately weighed green in the field to provide estimates of green yield. A 4-stalk whole plant subsample was collected, weighed fresh in the field and then dried at 50°C until a constant dry weight was achieved to determine dry matter concentration and estimate dry biomass yield. Dry matter concentrations can be found in Table A-1. Dried tissue samples were run through a commercial chipper (DEK Chipper Shredder Model CH1; GXI Outdoor Power, Clayton, NC) and then ground with a Thomas-Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 2-mm screen.

Biomass Composition

Dried biomass samples were analyzed for non-structural extractives, structural carbohydrates and lignin using a modified National Renewable Energy Laboratory procedure (Sluiter et al., 2010). Briefly, non-structural extractives were removed from ground plant tissue by autoclaving 0.8 g of dried sample in 100 mL of deionized water in a 100-mL sealed pressure tube (ACE Glass, Inc., Vineland, NJ) at 121°C for 1 hour. Samples were then vacuum filtered through coarse porosity (>25 µm) filter paper of a known weight to capture all structural biomass, but allow non-structural extractives to pass through.

The captured structural biomass was dried at 50°C, weighed, and then a 0.3 g sample was hydrolyzed for lignin and structural carbohydrates (Sluiter et al., 2010). Hydrolysis was conducted as a two-stage process in pressure tubes. First, concentrated sulfuric acid (72%, Fluka Alalytical, Sigma-Aldrich, St. Louis, MO) was used for 60 min in a 30°C water bath. This was then followed by dilution with 84 mL of deionized water to 4% sulfuric acid and autoclaved for one hour in sealed tubes at 121°C. Hydrolyzed samples were vacuum filtered through 25 mL medium porosity porcelain filtering crucibles (Coors #60531, CoorsTek, Golden, CO). Crucibles were then dried at 105°C for a minimum of 12 h. Hydrolysis liquor was pH balanced to between 5 and 6 using analytical grade calcium carbonate, and after precipitation and settling the supernatant had a pH of approximately 7. The supernatant was then diluted 1:100 using deionized water and passed through a 0.22 µm syringe filter (Fisher Scientific, Pittsburgh, PA) for HPLC analysis.

For all hydrolyzed samples, acid soluble lignin determination was done at a wavelength of 240 nm using a UV-Vis Spectrophotometer (StellarNet, Inc., Tampa, FL). Acid soluble lignin was calculated using the NREL equation

$$\% \text{ ASL} = \frac{\text{UVabs} \times \text{Volume}_{filtrate} \times \text{Dilution}}{\varepsilon \times \text{ODW}_{sample}} \times 100$$

where UVabs is the average UV absorbance of 2 measurements at 240 nm, ODW is the oven dry weight of the sample and ε is the absorptivity of the biomass at 240 nm (40 L/g cm). Insoluble lignin was determined gravimetrically as total solids remaining in the crucible after vacuum filtration of hydrolyzed biomass.

Structural sugars were determined by HPLC (Perkin-Elmer Flexar system, Waltham, MA) using a refractive index detector and a Biorad Aminex HPX-87P column

maintained at 65°C. HPLC grade water was used as the mobile phase at a flow-rate of 0.3 mL min^{-1} with a 10 µL injection and 60 minute run time. Perkin-Elmer's Chromera software was used to identify peaks and determine peak area based on standard sugar solutions of 10, 25, 50 and 100 ppm made from high purity sugar standards (Fischer Scientific, Pittsburgh, PA). Linear regressions between peak area standard sugar concentrations were determined and used to calculate unknown sugar concentrations in the biomass samples. Whenever possible multiple points were used to calculate equations, and R-squared values were greater than 0.95 for all equations.

A subsample of the extractives (n=2 per species) was also analyzed via HPLC using the same procedure described above in order to calculate the percentage of extractives that are fermentable sugars for total theoretical ethanol yield calculations.

Ash determination was conducted for all samples by determining absolute dry matter (drying at 105°C for 15 h) and then heating samples to 500°C for a minimum of 4 h, cooling in desiccators to room temperature and re-weighing. Ash determination was conducted on both whole-plant samples and acid insoluble lignin.

Ethanol Calculations

Ethanol yields from both structural biomass and extractives were calculated separately based on modified equations from Goff et al. (2010) and are reproduced below.

$$H = [\%\text{Glucose} + \%\text{Mannose}] \times 172.82$$
$$P = [\%\text{Xylose} + \%\text{Arabinose}] \times 176.87$$
$$\text{Theoretical Ethanol Potential [TEP]} \ (\text{L Mg}^{-1}) = [\text{Hexose} + \text{Pentose}] \times 4.17$$

H and *P* are theoretical ethanol production from the conversion of hexose and pentose sugars. Theoretical ethanol yields were then calculated by multiplying the theoretical

ethanol yields per Mg by tissue yields on a per ha basis. Total theoretical ethanol yields were determined by adding theoretical structural and extractive ethanol yields.

Statistical Analysis

Analyses of variance for all biomass composition and yield, as well as structural and extractive ethanol yield data, were performed for a randomized complete block design using the generalized linear mixed models procedure (PROC GLIMMIX) of SAS (SAS Inc., 1996). Given multiple interactions, data were analyzed separately for plant (2009) and first ratoon (2010) crops, with site and species as fixed effects and block as a random effect. All treatment effects were considered significant at $P < 0.05$, and pairwise comparisons were made using the lsmeans statement with the Tukey method. Main effects for site and species, as well as the interaction, were analyzed for all biomass composition and yield data, as well as for ethanol yield data.

Results

Ethanol Yields

Theoretical ethanol potential per hectare from structural sugars in the plant crop ranged from 4700 to 13 900 L ha^{-1}, and was affected by the site by species interaction (Table 2-1, 2-2). At Citra, structural TEP (L ha^{-1}) was similar among elephantgrass, energycane and sweetcane, all of which were greater than giant reed and giant miscanthus (Table 2-2). At Ona, structural TEP (L ha^{-1}) for energycane and elephantgrass was greater than giant reed and giant miscanthus. Finally, at Belle Glade, structural TEP (L ha^{-1}) was similar among giant reed, elephantgrass, energycane and sweetcane, all of which were greater than giant miscanthus (Table 2-2). TEP from nonstructural sugars was only affected by species (Table 2-1), and ranged from 232 L ha^{-1} for giant miscanthus to 8,500 L ha^{-1} for sugarcane (Table 2-3). Notably, TEP from

nonstructural sugars was greater for energycane compared to elephantgrass, sweetcane and giant reed, which did not differ (Table 2-3). At Citra, total (structural and nonstructural) TEP (L ha^{-1}) was similar among elephantgrass, energycane, sugarcane and sweetcane, all of which were greater than giant reed and giant miscanthus (Table 2-2). At Ona, total TEP (L ha^{-1}) for energycane and elephantgrass were greater than giant reed and giant miscanthus, while at Belle Glade, total TEP (L ha^{-1}) was similar among giant reed, elephantgrass, energycane, sugarcane, and sweetcane, all of which were greater than giant miscanthus (Table 2-2).

Theoretical ethanol potential (TEP) from structural sugars in the ratoon crop ranged across species from 7800 to 16 500 L ha^{-1}, and was not affected by the site by species interaction (Table 2-4, 2-5). Sweetcane produced the greatest average structural TEP across sites, followed by elephantgrass and energycane, then giant reed and sugarcane, with giant miscanthus producing the lowest TEP (Table 2-5). Structural TEP by site was greatest at Belle Glade, lower at Citra and lowest at Ona (18 600 vs 12 800 vs 8400 L ha^{-1}) (Table 2-6). TEP from nonstructural sugars were affected by the site by species interaction (Table 2-4), but followed the same pattern at all sites: TEP was greatest for sugarcane, followed by energycane, with sweetcane, giant reed and elephantgrass producing even lower yields, and giant miscanthus having the lowest ethanol potential from extractable sugars (Table 2-7). Total TEP was not affected by the site by species interaction (Table 2-4). Sugarcane produced the greatest average total TEP across sites, followed by energycane, then sweetcane, elephantgrass and giant reed, with giant miscanthus producing the lowest total TEP (Table 2-5). Total TEP

by site was greatest at Belle Glade, lower at Citra and lowest at Ona (21 800 vs 15 700 vs 10 200 L ha^{-1}) (Table 2-6).

Biomass Yields

Total dry biomass yields for the plant crop were highest in and comparable for sugarcane, energycane, sweetcane and elephantgrass at all sites, and for giant reed at Belle Glade, averaging approx. 33 Mg ha^{-1} (Fig. 2-1). Giant miscanthus produced the lowest biomass yields (9.4 Mg ha^{-1}), which did not differ across sites. Ratoon crop yields were highest for sugarcane (38 Mg ha^{-1} average across sites) and again lowest for giant miscanthus (17 Mg ha^{-1} averaged across sites), and were not affected by the site by species interaction (Fig. 2-2). For the ratoon crop, Belle Glade produced the highest average dry biomass yields across species, followed by Citra, and lowest biomass yields were obtained at Ona (44 vs 32 vs 21 Mg ha^{-1}) (Fig. 2-2).

Plant Crop Tissue Composition

Extractives, which were all non-structural components of plant tissue removed during extraction, on a whole plant basis ranged from 176 to 486 mg g^{-1} for the plant crop and were greatest for sugarcane (474 mg g^{-1}), which did not differ by site (Tables 2-8, 2-9). Sugars as a component of total extractives as determined by HPLC were 769 mg g^{-1} for sugarcane, 453 mg g^{-1} for energycane, 275 mg g^{-1} for giant reed, 267 mg g^{-1} for sweetcane, 248 mg g^{-1} for elephantgrass and 147 mg g^{-1} for giant miscanthus (data not shown). This resulted in greater extractive sugar concentrations for sugarcane, which was greater than energycane, both of which were greater than elephantgrass, sweetcane, and giant reed, all of which were greater than giant miscanthus (Table 2-10). Ash concentration was lowest at Ona and greatest at Belle Glade, but was not affected by species (Table 2-11).

Glucose concentration from structural carbohydrates was not affected by site (Table 2-8), and was comparable among all species except sugarcane, which had lower structural glucose as a fraction of total plant biomass (Table 2-10). Xylose concentrations were not affected by site, species or their interaction (Table 2-8), and averaged 184 mg g^{-1} across all species. Arabinose concentration ranged from 0 to 44 mg g^{-1}, and was greatest in giant miscanthus at Ona. Mannose was not detected in enough samples to be analyzed statistically, but average values for samples in which it was detected are reported in Table 2-9.

Total whole plant lignin as a component of total plant biomass ranged from 117 to 210 mg g^{-1}, and was lowest in sugarcane, which averaged 125 mg g^{-1} across all three sites, but did not differ for other species (Table 2-9). Total lignin on a structural fiber (extractives-free) basis was greater in sweetcane compared to sugarcane and giant miscanthus, and greater in giant reed than giant miscanthus, but was similar among all other species (Table 2-12). Additionally, total lignin on a fiber basis was higher for Belle Glade compared to Ona and Citra, which did not differ (Table 2-13).

Ratoon Crop Tissue Composition

On a whole-plant basis, ratoon crop total extractives and sugars in the extractives were greatest in sugarcane compared to the other species (Tables 2-14, 2-15). Sugarcane extractives and extractives sugars were greater at Citra than Belle Glade. Extractives in giant miscanthus were lower than all other species at Citra and Ona, but at Belle Glade they only differed from sugarcane (Table 2-15). Energycane extractives were greater than elephantgrass and sweetcane at Citra and Ona. Additionally, energycane extractives sugars were greater than all other species except sugarcane. Ash concentrations ranged from 18.2 to 42.7 mg g^{-1} across all treatments (Table 2-15).

At Citra, ash concentration was greater in elephantgrass compared to giant miscanthus and sugarcane, whereas at Ona species did not affect ash concentration, and at Belle Glade, ash concentration was greater in giant reed than sugarcane.

Glucose and xylose concentrations from structural carbohydrates as a component of total plant biomass differed for species across sites (Table 2-14). At Citra, structural glucose concentrations were greater for giant miscanthus compared to giant reed and sugarcane, but at Ona glucose concentrations were greater for giant miscanthus, elephantgrass, and sweetcane than sugarcane (Table 2-15). Xylose concentrations were higher for giant reed compared to all other species at Citra, but no differences were seen in species at Ona. No minor structural sugars (i.e., arabinose, mannose, etc.) were detected by HPLC in the ratoon crop.

A site by species interaction (Table 2-14) affected total lignin concentration as a component of total plant biomass. Concentrations were lowest for sugarcane at all sites (Table 2-15). Overall, whole-plant lignin concentrations ranged from 135 mg g^{-1} in sugarcane at Ona to 227 mg g^{-1} in giant miscanthus at Citra. Total lignin on a structural fiber basis was lower for elephantgrass than sweetcane or giant reed at Citra (Table 2-15). At Ona structural lignin was lower in sugarcane and energycane than giant reed, while at Belle Glade, structural lignin was lower for elephantgrass and giant miscanthus than giant reed.

Discussion

Theoretical Ethanol Yields

This study demonstrated that total TEP of approximately 16 000 L ha^{-1} was attainable in the Southeast USA from sugarcane, energycane, elephantgrass, sweetcane, and giant reed (only at Belle Glade) from a plant crop, and as high as 20

900 L ha^{-1} were possible with the first ratoon of sugarcane. In comparison, average US corn grain yields of about 10 Mg ha^{-1} (Lee and Tollenar, 2007) can produce approximately 4000 to 5000 L ha^{-1}. Switchgrass is estimated to produce 2000 to 4500 L ha^{-1} (Varvel et al., 2008), and poplar and willow (woody species) are estimated to produce 5500 to 9000 L ha^{-1} yr^{-1} (Groom et al., 2008; Schmer et al., 2008). Additionally, the energy conversion efficiencies (based on energy output compared to fossil energy inputs for planting, fertilization, harvesting, transportation, conversion, etc), are significantly higher for the perennial grasses at 8 to 10 for sugarcane juice and 2 to 6 for cellulosic ethanol production versus 1.1 to 1.25 for corn grain (Groom et al., 2008). Given these estimates, several of the perennial grasses investigated in the present study offer great potential as bioenergy crops.

Plant crop TEP yields were driven primarily by total biomass produced, and influenced to a lesser degree by tissue composition. Biomass yields and total theoretical ethanol potential were greatest in sugarcane, energycane, sweetcane and elephantgrass across sites. All of these species had comparable tissue composition as well, except for sugarcane, which had lower whole plant lignin levels and in some cases lower lignin concentrations on a structural basis as well. The lowest yielding specie, giant miscanthus, also produced the lowest total TEP. The TEP of first ratoon crops was also primarily dependent on biomass yield, as the highest and lowest biomass producers also had the highest and lowest total TEP. Higher levels of extractable sugars in some species, notably energycane and sugarcane, helped compensate for lower structural biomass yields, resulting in relatively consistent ethanol potential among species with similar biomass yields. While giant reed biomass yields were competitive

with some of the higher yielding warm-season grasses at Belle Glade, biomass composition was generally less favorable due to higher structural lignin concentrations.

The variability among species in extractable sugars also influenced TEP. In sugarcane, ~80% of extractives (non-structural material) were fermentable sugars, which compensated for extremely low structural TEP per unit of biomass. In contrast, low levels of fermentable sugars in extractives of elephantgrass moderated high biomass and structural TEP. High levels of fermentable sugars in the extractives may be desirable for production systems that first press the biomass to remove extractives, but could potentially reduce the efficiency of cellulosic conversion by producing inhibitors during the pretreatment process (Ingram et al., 1999; Geddes et al., 2011).

Groom et al. (2008) reported average worldwide ethanol yields of 5300 to 6500 L ha^{-1} from sugarcane juice. These numbers are lower than extractive (essentially juice) TEP reported here of 8400 L ha^{-1}. These results are not significantly higher though, and are reasonable given that commercial production does not extract all non-soluble sugars. Sugarcane juice ethanol production is competitive with corn grain ethanol based on these results, and based on the higher energy conversion efficiency of sugarcane juice.

Propheter et al. (2010) reported plant crop TEP for giant miscanthus of 1040 and first ratoon yields of 3960 L ha^{-1}, but they assumed an intermediate conversion efficiency, as opposed to the optimum efficiency assumed here, for lignocellulosic processing.

Biomass Yields

Biomass yield for many of these grasses has been reported previously, but there are no studies where all have been grown together at multiple locations. Total dry

matter yields averaged across sites significantly increased from plant to first ratoon crop for both giant miscanthus and giant reed, which was more than the ~10% increase seen in sugarcane, energycane, sweetcane and elephantgrass. However, most of the yield increase observed from plant to ratoon crop for giant miscanthus is attributable to a significant increase in dry biomass yields at Belle Glade, while Citra only experienced a minor increase in dry biomass. All species were harvested together at each site, but were at different stages of maturity. Giant reed was two to three months post-flowering, while giant miscanthus was three to four months post-flowering. All other species were reaching maturity, which may account for greater biomass yields in sugarcane, sweetcane, energycane and elephantgrass, and lowest yields in giant miscanthus. Additionally, both giant reed and giant miscanthus continued to fill in row centers to a greater degree in the first ratoon compared with the plant crop, whereas the other perennial species established and rapidly filled in row centers during the plant year, which may have accounted for the markedly greater ratoon yield increases seen for giant reed and giant miscanthus. Although sweetcane was among the highest yielding species, it was the most difficult to establish and required some replanting in the plant year.

Propheter et al. (2010) reported plant crop yields of 3.3 and first ratoon yields of 12.8 Mg ha^{-1} for giant miscanthus grown in Kansas, an increase of almost 400%. Nevertheless, overall biomass yields of giant miscanthus were generally less than those seen for elephantgrass, energycane, sugarcane and sweetcane in the present study. Increasing biomass yields from plant to first ratoon crop appear to be primarily responsible for increasing potential ethanol yields of giant miscanthus from plant to first

ratoon. Lower giant miscanthus yields here than those observed in Illinois (Heaton et al., 2003) are likely due to early flowering and maturity in Florida, as giant miscanthus flowering was observed in July at the Citra location (data not shown). Multiple harvests per year have been shown to increase total biomass accumulation of some potential perennial bioenergy crops, and may increase yields for giant miscanthus in the region (Thomason et al., 2004; Sanderson and Adler, 2008). Similarly, yields of giant reed were generally less than elephantgrass, energycane, sugarcane and sweetcane. Giant reed plant crop at Belle Glade was an exception to this, however, as it produced yields there that were comparable to sugarcane, sweetcane, elephantgrass and energycane. Ratoon yields at Belle Glade were higher than at other sites, but not plant crop yields. This was likely due to wider row spacing in energycane, sugarcane, sweetcane and elephantgrass used at Belle Glade resulting in late and/or incomplete canopy closure and lower plant crop yields. Additionally, muck soils and canal irrigation at Belle Glade provided significantly higher nutrient and water availability than the mineral sand soils and management practices used at Citra and Ona, which would also contribute to higher yields at Belle Glade.

Tissue Composition

Walford (2008) reviewed several methods for determining fiber composition of sugarcane, and reported average yields of sugarcane bagasse. Assuming 57% of total dry matter is bagasse, cellulose (therefore glucose) concentration was 148 to 268 mg g^{-1}, hemicellulose concentration (primarily xylose) 108 to 188 mg g^{-1}, lignin concentration of 80 to 131 mg g^{-1}, and ash concentration of 6 to 29 mg g^{-1}. These results are consistent with the results obtained in this research, especially when variability in extractives concentration is taken into account.

Burner et al. (2009) measured the tissue composition of giant miscanthus grown in Arkansas, and they reported cellulose concentrations of 400 to 440 mg g^{-1} and ash concentrations of ~40 mg g^{-1} for stalks. These data are consistent with obtained glucose concentrations of 370 to 525 mg g^{-1} and ash values of 20 to 45 mg g^{-1}.

Anderson et al. (2008) reported extractives of 240 to 330 mg g^{-1} in giant reed, and neutral detergent fiber concentration (roughly equivalent to total glucose) of 370 to 410 mg g^{-1}, which are comparable to results obtained here.

Thai Hoa et al. (2008) reported elephantgrass to be composed of 180 mg g^{-1} extractives, 430 mg g^{-1} glucose, 200 mg g^{-1} xylose and other pentose sugars and 173 mg g^{-1} acid insoluble lignin. Pentose sugar and insoluble lignin concentrations are similar between this work and Thai Hoa, but extractives concentrations here were greater and glucose concentrations ~20% lower. These differences may be due to harvesting at a different growth stage or after senescence by Thai Hoa, or may simply be natural variation the cultivar. In any case, the tissue composition of elephantgrass for cellulosic conversion in their study was significantly more favorable than that exhibited here. Woodard and Prine (1991) reported comparable elephantgrass extractive concentrations of 190 to 210 mg g^{-1}. Anderson et al. (2008) reported extractive concentrations of 260 mg g^{-1}, glucose concentration of 410 mg g^{-1}, and pentose concentration of ~250 mg g^{-1}, which are again similar to results obtained here.

Comparable whole-plant structural xylose concentrations amongst all species but significantly lower structural glucose concentrations for sugarcane for the plant crop indicated that sugarcane had a significantly higher hemicellulose component as a percentage of total structural material. This is important for cellulosic conversion

because xylose is not as easily fermented and can produce significant inhibitory products during pretreatment that can reduce the efficiency of conversion (Ingram et al., 1999; Vermerris et al., 2008). Higher minor hemicellulose sugars in giant miscanthus and elephantgrass at some sites may also slightly reduce efficiency of conversion due to a lack of efficient fermenting microbes (Vermerris et al., 2008).

Total structural lignin only varied by 2.6 percentage units between species in the plant crop, and 3.2 percentage units in the first ratoon crop. These differences are fairly minor, but may have significant impacts on efficiency of conversion as shown in prior research (Fu et al., 2011; Studer et al., 2011). A small difference may be especially important given the similarities in biomass yields and total sugar concentrations amongst sugarcane, energycane, sweetcane and elephantgrass. Lignin in switchgrass was slightly lower than lignin concentrations found in this research (210 vs. 230-260 mg g^{-1} on a structural basis) (Sathitsuksanoh et al., 2011), while NREL determined lignin of woody biomass ranged from 260 to 280 mg g^{-1} on a pulp basis, which is higher than lignin concentrations obtained for these grasses (Iakovlev and van Heiningen, 2011)

Overall, the predominant components of structural plant fiber were glucose, xylose and lignin. Minor sugars that are possible components of hemicellulose, such as arabinose and mannose, were detected in small quantities in the plant crop but not in the ratoon crop. Additionally, mannose was detected in so few samples that the differences could not be analyzed statistically. These results are consistent with prior research, which has shown that the hemicellulose component of grasses is composed primarily of glucurono-arabinoxylans (a xylose backbone with arabinose and glucuronic acid substitutions), with arabinose concentrations declining significantly as the plants

mature (Vermerris et al., 2007; Vermerris, 2008). As all plots were harvested during or after flowering, the low concentrations of arabinose and mannose are consistent with prior research. It should be noted though that not detecting these minor sugars does not indicate that they are not present at some level in the plant, but that they are simply not present at high enough levels to be identified. Additionally, there was no reliable, comparably produced data found for comparison for sweetcane tissue composition. This lack of data, along with relatively little data available for giant reed in the US, emphasizes the importance of the current research.

Higher concentrations of extractive sugars, which are more readily and efficiently fermented, coupled with lower structural lignin levels than the other high yielding species (e.g., sweetcane, energycane and elephantgrass) make sugarcane a strong candidate for ethanol production using current technologies, in areas where the climate is favorable for its production. The fiber (bagasse) also possessed relatively low lignin concentration, favorable for cellulosic conversion to ethanol. However, where low extractives are desirable or where the climate is not favorable for sugarcane, the present study indicated that elephantgrass and energycane were good candidate bioenergy grasses based on biomass yield and composition. Elephantgrass and energycane possessed relatively low lignin levels compared to sweetcane and giant reed and generally greater (giant reed) or similar (sweetcane) biomass yield. If extractive sugars are detrimental or not used, however, then elephantgrass had the more favorable composition. Additionally, difficulties in establishment of sweetcane, as well as lodging, may decrease its usefulness as a bioenergy crop. Although biomass composition of giant miscanthus was favorable for cellulosic conversion to ethanol

(relatively low extractives, low lignin, and high glucose to xylose ratio), the low biomass yield observed for giant miscanthus across all sites did not make it a very suitable candidate bioenergy grass in Florida compared to the other grasses used in the study. While the present study identified strong candidate bioenergy grasses for the Florida based on biomass yield and composition, further research on nutrient requirements, water use, environmental impacts, disease resistance, persistence, post-harvest storage and harvesting technologies, among others, will help to further elucidate sustainable bioenergy crops for the region.

Table 2-1. ANOVA table for plant crop (2009) total ethanol potential (TEP), both per unit of dry biomass and per unit land area basis.

Sources of variation	Structural TEP		Extractive TEP		Total TEP
	L Mg^{-1}	L ha^{-1}	L Mg^{-1}	L ha^{-1}	L ha^{-1}
Site	ns	ns	ns	ns	ns
Species	***	***	***	***	***
Site*Species	*	*	ns	ns	*

ns denotes no significant effects

* Significant effect at $P \leq 0.05$

*** Significant effect at $P \leq 0.001$

Table 2-2. Theoretical ethanol potential (TEP) from structural sugars, both in liters per unit of dry biomass and on a land area basis, and total whole plant theoretical ethanol potential on a land area basis as affected by the site by species interaction for the plant crop (2009) perennial grass biomass. Data are means across four replications (n = 4).

Site	Species	Structural TEP		Total TEP
		L Mg^{-1}	L ha^{-1}	L ha^{-1}
Citra	Giant reed	396bc[†]	5700c-e	6400cd
	Sugarcane	290d	10 100a-d	19 500a
	Sweetcane	439a-c	14 700a	16 100ab
	Energycane	412a-c	15 600a	19 200a
	Elephantgrass	398bc	14 700ab	16 500ab
	Giant Miscanthus	465ab	5100c-e	5300cd
Ona	Giant reed	417a-c	6200c-e	6900b-d
	Sugarcane	303d	10 000a-d	18 300a
	Sweetcane	400a-c	10 700a-d	12 000a-d
	Energycane	393bc	13 100ab	16 200ab
	Elephantgrass	422a-c	13 200ab	14 400ab
	Giant Miscanthus	472a	4400e	4600d
Belle Glade	Giant reed	385c	11 200a-c	12 800a-c
	Sugarcane	282d	8310b-e	16 100ab
	Sweetcane	398bc	14 500ab	16 100ab
	Energycane	410a-c	13 000ab	15 400ab
	Elephantgrass	409a-c	14 000ab	15 400ab
	Giant Miscanthus	386bc	4600de	4900d

[†] Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-3. Main effect of grass species on the theoretical ethanol potential (TEP) of extractive sugars, both in liters per unit of dry biomass and on a land area basis for plant crop (2009) perennial grass biomass. Data are means across three sites and four replications (n = 12).

	Extractive TEP	
	L Mg^{-1}	L ha^{-1}
Giant reed	51.6c†	957cd
Sugarcane	263a	8500a
Sweetcane	46.7c	1400c
Energycane	89.3b	3100b
Elephantgrass	42.6c	1400c
Giant Miscanthus	22.5d	232d

† Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-4. ANOVA table for first ratoon crop (2010) theoretical ethanol potential (TEP), both in liters per unit of dry biomass, and on a land area basis.

Sources of variation	Structural TEP		Extractive TEP		Total TEP
	L Mg^{-1}	L ha^{-1}	L Mg^{-1}	L ha^{-1}	L ha^{-1}
Site	*	***	**	***	***
Species	***	***	***	***	***
Site*Species	**	ns	*	***	ns

* Significant effect at $P \leq 0.05$

** Significant effect at $P \leq 0.01$

*** Significant effect at $P \leq 0.001$

Table 2-5. Main effect of grass species on the theoretical ethanol potential (TEP) of structural and total sugars, both in liters per unit of dry biomass and on a land area basis for the first ratoon crop (2010) perennial grass biomass. Data are means across three sites and four replications (n = 12).

	Structural TEP	Total TEP
	L ha^{-1}	L ha^{-1}
Giant reed	12 600bc†	13 800c
Sugarcane	11 800c	20 900a
Sweetcane	16 500a	17 600a-c
Energycane	15 200abc	18 100ab
Elephantgrass	15 700ab	16 900bc
Giant Miscanthus	8000d	8000d

† Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-6. Main effect of site on the theoretical ethanol potential (TEP) of structural and total sugars, both in liters per unit of dry biomass and on a land area basis for the first ratoon crop (2010) perennial grass biomass. Data are means across six species and four replications (n = 24).

	Structural TEP	Total TEP
	L ha^{-1}	L ha^{-1}
Citra	12 800b[†]	15 700b
Ona	8400c	10 200c
Belle Glade	18 600a	21 800a

[†] Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-7. Theoretical ethanol potential (TEP) from extractive sugars, both in liters per unit of dry biomass and on a land area basis, and from structural sugars in liters per unit of dry biomass, as affected by the site by species interaction for the first ratoon crop (2010). Data are means across four replications (n = 4).

Site	Species	Structural TEP	Extractives TEP	
		L Mg^{-1}	L Mg^{-1}	L ha^{-1}
Citra	Giant reed	423ef[†]	41.8de	1200d-f
	Sugarcane	290i	257a	9900a
	Sweetcane	434c-f	34.5ef	1100d-f
	Energycane	390g	86.9c	3600c
	Elephantgrass	431d-f	36.7ef	1400d-f
	Giant Miscanthus	474ab	11.6f	160f
Ona	Giant reed	423e-g	41.6de	680f
	Sugarcane	314hi	239ab	6000b
	Sweetcane	441b-e	34.8ef	970e-f
	Energycane	407fg	83.7c	2300c-e
	Elephantgrass	439b-e	32.5ef	610f
	Giant Miscanthus	480a	12.1f	100f
Belle Glade	Giant reed	434c-f	36.7ef	1600d-f
	Sugarcane	327h	215b	11 300a
	Sweetcane	458a-d	28.9ef	1500d-f
	Energycane	431d-f	64.5cd	2700cd
	Elephantgrass	451a-e	28.5ef	1400d-f
	Giant Miscanthus	461a-c	15.0f	420f

[†] Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-8. ANOVA table for plant crop (2009) biomass composition.

Sources of variation	Extractives			Structural Sugars				Lignin	
	Total	Sugars	Ash	Glucose	Xylose	Arabinose	Mannose	Whole plant	Structural
Site	ns	ns	*	ns	ns	ns	ns	ns	*
Species	***	***	ns	***	ns	*	ns	***	***
Site*Species	*	ns	ns	ns	ns	**	ns	*	ns

ns denotes no significant effect ($P > 0.05$)

* Significant effect at $P \leq 0.05$

** Significant effect at $P \leq 0.01$

*** Significant effect at $P \leq 0.001$

Table 2-9. Concentrations of extractives, structural arabinose and mannose, and total lignin of whole plant crop (2009) perennial grass biomass as affected by the site x species interaction. Data are means across four replications (n = 4).

Site	Species	Extractives	Structural Sugars		Lignin
		Total	Arabinose	Mannose[†]	Whole plant
		mg g^{-1}			
Citra	Giant reed	268bc[‡]	5.9b	ND[§]	177a
	Sugarcane	486a	7.5b	ND	117d
	Sweetcane	205bc	11.2ab	ND	189a
	Energycane	287bc	7.1b	ND	167a-c
	Elephantgrass	255bc	9.9ab	ND	170a-c
	Giant Miscanthus	182c	8.1b	ND	193a
Ona	Giant reed	231bc	6.6b	2.51	194a
	Sugarcane	454a	3.9b	0.51	128cd
	Sweetcane	262bc	4.8b	ND	188a
	Energycane	290b	2.2b	ND	169a-c
	Elephantgrass	227bc	0.5b	1.8	190a
	Giant Miscanthus	176c	44.2a	5.37	172ab
Belle Glade	Giant reed	282bc	ND	0.56	189a
	Sugarcane	481a	4.1b	0.41	130b-d
	Sweetcane	260bc	ND	ND	210a
	Energycane	244bc	2.3b	ND	195a
	Elephantgrass	231bc	12.4ab	2.41	207a
	Giant Miscanthus	270bc	7.6b	1.95	182a

[†] Mannose not present in enough species for pairwise comparisons
[‡] Means within a column not followed by the same letter are different ($P \leq 0.05$)
[§] ND represents none detected

Table 2-10. Main effect of species on the concentration of extractives and structural glucose and xylose on a whole plant dry matter basis for plant crop (2009) perennial grass biomass. Data are means across three sites and four replications (n = 12).

	Extractives Sugars	Structural Sugars Glucose
	mg g^{-1}	
Giant reed	72c[†]	355a
Sugarcane	365a	235b
Sweetcane	65c	378a
Energycane	124b	366a
Elephantgrass	59c	374a
Giant Miscanthus	31d	369a

[†] Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-11. Main effect of site on the concentration of ash and the structural sugar xylose on a dry matter basis for plant crop (2009) perennial grass biomass. Data are means across six species and four replications (n = 24).

	Ash
	- mg g^{-1} -
Citra	32.9b[†]
Ona	25.2c
Belle Glade	45.9a

[†] Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-12. Main effect of species on the concentration of total lignin on a structural fiber (extractives-free) basis for plant crop (2009) perennial grass biomass. Data are means across three sites and four replications (n = 12).

	Structural Lignin
	----- mg g^{-1} -----
Giant reed	253ab[†]
Sugarcane	237b-c
Sweetcane	258a
Energycane	243a-c
Elephantgrass	247a-c
Giant Miscanthus	232c

[†] Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-13. Main effect of site on the concentration of total lignin on a structural fiber (extractives-free) basis for plant crop (2009) perennial grass biomass. Data are means across six species and four replications (n = 24).

	Structural Lignin
	----- mg g^{-1} -----
Citra	234b[†]
Ona	239b
Belle Glade	262a

[†] Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 2-14. ANOVA table for first ratoon crop (2010) biomass composition.

Sources of	Extractives			Structural Sugars		Lignin	
variation	Total	Sugar	Ash	Glucose	Xylose	Whole plant	Structural
Site	**	**	***	***	***	***	***
Species	***	***	***	***	***	***	***
Site*Species	***	*	***	**	***	***	***

* Significant effect at $P \leq 0.05$

** Significant effect at $P \leq 0.01$

*** Significant effect at $P \leq 0.001$

Table 2-15. Concentrations of extractives, extractive component sugars and ash, structural glucose and xylose, whole plant total lignin and lignin on a structural basis of the first ratoon crop (2010) perennial grass biomass as affected by the site x species interaction. Data are means across four replications (n = 4).

Site	Species	Extractives			Structural Sugars		Lignin	
		Total	Sugars†	Ash‡	Glucose	Xylose	Whole plant	Structural
		---------- mg g^{-1} ----------						
Citra	Giant reed	211c-e§	58.0de	32.6a-e	355cd	227a	207cd	263a-c
	Sugarcane	463a	356a	27.0c-e	278de	122c	137h	256b-e
	Sweetcane	180ef	47.9ef	39.3a-c	497ab	106c	217a-d	265a-c
	Energycane	266c	121c	38.6a-c	459ab	83c	188ef	256b-e
	Elephantgrass	203de	50.3ef	42.7a	-	-	200de	251de
	Giant Miscanthus	109g	16.0f	21.8de	525a	133c	227a	254c-e
Ona	Giant reed	210de	57.7de	28.2b-e	435a-c	148bc	207cd	262a-c
	Sugarcane	430ab	331ab	21.6de	341cd	93c	135h	238f
	Sweetcane	181ef	48.2ef	22.7de	489ab	120c	209a-d	255b-e
	Energycane	256cd	116c	21.9de	416b-c	145c	183f	246ef
	Elephantgrass	182ef	45.1ef	20.9e	472ab	135c	208cd	254c-e
	Giant Miscanthus	114g	16.8f	18.2e	510a	152bc	224ab	253c-e
Belle Glade	Giant reed	183ef	50.5ef	40.5ab	325d	272a	220a-c	270a
	Sugarcane	388b	298b	26.7c-e	233e	216ab	163g	266ab
	Sweetcane	151e-g	40.2ef	37.2a-c	352cd	280a	220a-c	259a-d
	Energycane	198ef	89.5cd	34.8a-d	-	-	208b-d	259a-d
	Elephantgrass	160e-g	39.6ef	37.2a-c	-	-	218a-c	259b-d
	Giant Miscanthus	142fg	20.9f	38.5a-c	-	-	222a-c	259b-d

†Sugars are a component of extractives

‡Ash is a component of extractives

§ Means within a column not followed by the same letter are different ($P \leq 0.05$)

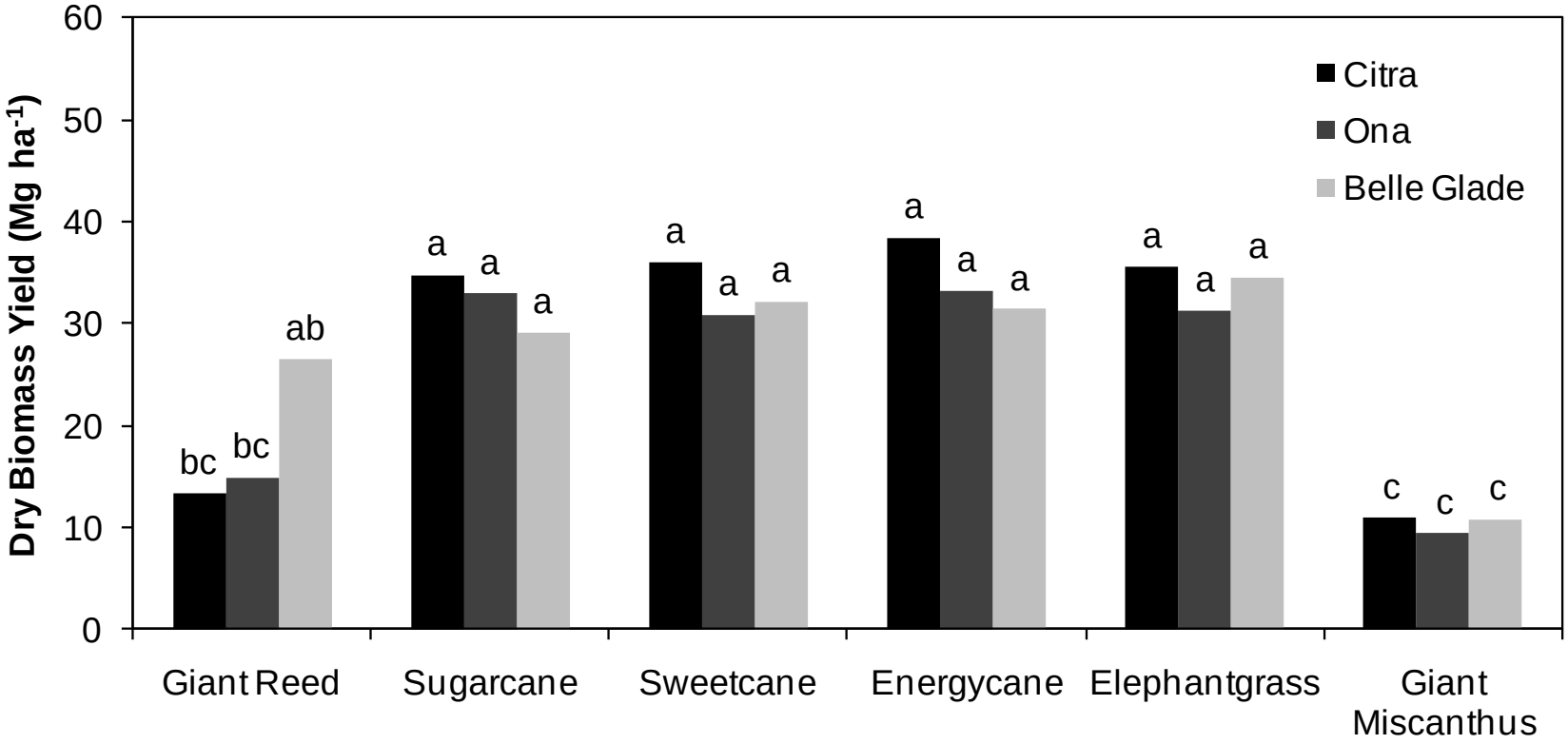

Figure 2-1. Plant crop dry biomass yields by site and species. Bars not accompanied by the same letter are different ($P \leq 0.05$).

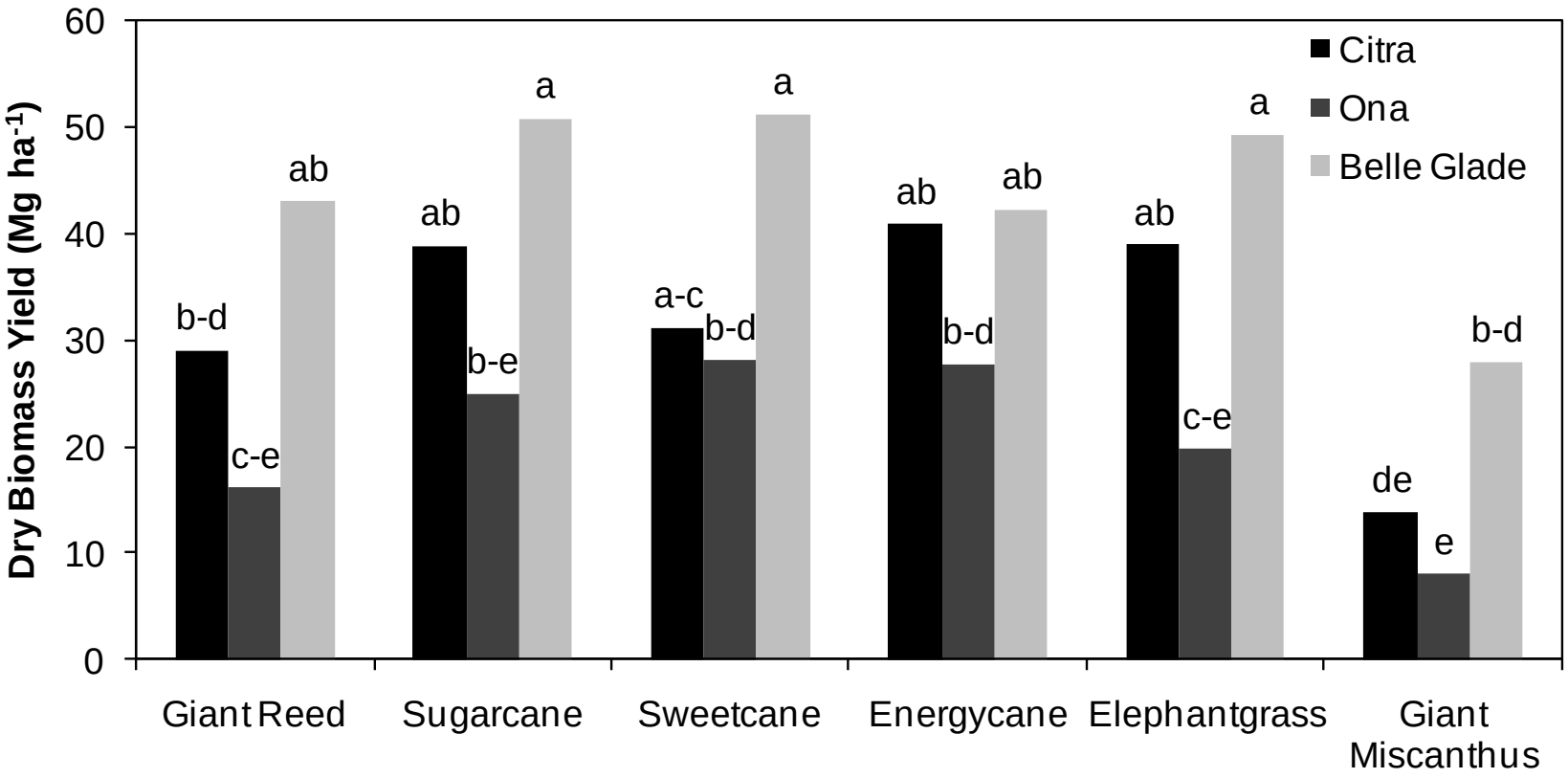

Figure 2-2. First ratoon crop dry biomass yields by site and species. Bars not accompanied by the same letter are different ($P \leq 0.05$).

CHAPTER 3
BIOMASS YIELD, COMPOSITION, AND PARTITIONING OF SWEET SORGHUM GROWN FOR BIOFUEL IN FLORIDA

Background

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a warm-season grass that has received considerable attention as a renewable source of sugar and/or biomass for energy and other bio-based compounds. Easily fermentable sugars accumulate in the stem of the plant and can be removed via milling in the same way that sugarcane is processed. The remaining bagasse can be used either as a biopower source in combustion or as a feedstock for lignocellulosic conversion (Amaducci et al., 2004; Goff et al., 2010). Sorghum is also a relatively short duration annual crop planted from seed that could be double cropped from seed or it has the ability to ratoon, potentially providing two harvests per growing season in regions where the climate is favorable (Ferraris, 1981). Unlike maize (*Zea mays* L.), sweet sorghum is not typically viewed as a food or feed crop in the USA, minimizing concerns over using food as fuel (Amaducci et al., 2004; Vermerris, 2008). Despite the many attractive features of sweet sorghum as a bioenergy crop, there are currently limited data on sweet sorghum biomass yield, composition, and partitioning in the Southeast USA, all of which are important for estimating energy yields from sweet sorghum production.

Biomass yield is an important factor underlying biofuel yield potential from sweet sorghum and is influenced by many factors including climate (Wortmann et al., 2010), disease (Bandyopadhyay et al., 1998), planting date (Broadhead, 1969), fertility (Erickson et al., 2011; Tamang et al., 2011), irrigation (Miller and Ottman, 2010), and cultivar (Zhao et al., 2009; Goff et al., 2010). For example, single-crop dry yields of 12.3 Mg ha^{-1} were reported for ‘M-81E’ sweet sorghum grown in a double cropping system

with winter triticale (x *Triticosecale* Wittmack) in Iowa, while 'Topper 76-6' produced significantly lower yields of approximately 8 Mg ha^{-1} under the same system (Goff et al., 2010). In China, Zhao et al. (2009) reported dry biomass yields of 13.2 to 35.2 Mg ha^{-1} across five sweet sorghum cultivars. Propheter et al. (2010) reported total dry biomass yields of 28.2 to 32.6 Mg ha^{-1} for M-81E grown in Kansas over 2 yr. Mislevy et al. (1989) showed total average dry yields of 28.9 Mg ha^{-1} yr^{-1} for M-81E grown over a 2-yr period in Central Florida, with 20.4 dry Mg ha^{-1} yr^{-1} produced on average by the plant crop and 8.5 dry Mg ha^{-1} yr^{-1} by the ratoon crop.

Although biomass yield is important, the concentration of nonstructural carbohydrates, primarily in the stem, is also important at present, since they are easily converted to biofuels using current technologies (Wu et al., 2009). Readily convertible sugars (sucrose, glucose and fructose) are up to 20% of the juice of sweet sorghum, and can be directly converted to fuels and bio-based products. Thus, several recent studies have looked at potential ethanol yield from sugars in expressed sweet sorghum juice. Miller and Ottman (2010) reported ethanol yields of 2730 L ha^{-1} for M-81E grown in Arizona under varied levels of water inputs, which did not affect their estimates of ethanol yield. Wortman et al. (2010) reported yearly theoretical ethanol yields of 2090 L ha^{-1} from M-81E grown at dryland sites in Nebraska with no fertilizer or irrigation inputs. Under higher input conditions, potential ethanol yields of 3530 to 5410 L ha^{-1} from juice sugars have been reported for M-81E (Zhao et al., 2009).

Sweet sorghum also produces grain, although it generally represents less than 15% of the dry biomass (Zhao et al., 2009). Starch is found as 65 to 80% of the dry weight of the grain, and can be fermented after treatment with amylase to cleave the

starch into monomeric sugars (Clark et al., 2001). However, Zhao only obtained grain starch concentrations of 390 to 480 mg g^{-1} for M-81E harvested at grain maturity, and total grain yields of 2.6 to 3.3 Mg ha^{-1}. Propheter et al. (2010) reported grain yields of 0.7 to 2.1 Mg ha^{-1} for M-81E grown in Kansas over 2 yr. Theoretical ethanol yields from these grain yields ranged from 588 to 682 L ha^{-1} for Zhao (2009), but were not reported separately by Propheter (2010). Murray et al. (2008a) reported starch concentrations of 600 to 680 mg g^{-1} for Rio, a sweet sorghum cultivar similar to M-81E, with grain dry matter yields as high as 2.6 Mg ha^{-1}.

In addition to conversion of nonstructural carbohydrates, conversion of structural sugars (cellulose and hemicellulose) to fuels and bio-based products is expected to become increasingly important as technologies develop and mature (cellulosic references here). Limited data are available on fiber composition and resultant sugars, but recent estimates of total theoretical ethanol potential (TEP) from a single sweet sorghum crop ranged from 4900 to 11 400 L ha^{-1} depending on cultivar and total biomass yield (Zhao et al., 2009). For M-81E, cellulose ranged from 302 to 320 g kg^{-1} of dry biomass, hemicellulose ranged from 237 to 284 g kg^{-1} and lignin ranged from 18 to 24 g kg^{-1}, resulting in TEP yields of 560 to 610 L Mg^{-1} of dry biomass (Goff et al., 2010). Combining ethanol potential from nonstructural and structural carbohydrates resulted in total theoretical ethanol potential for M-81E when double cropped with winter triticale of 9850 L ha^{-1}, which increased to 11 900 L ha^{-1} if M-81E was grown as a single crop per season.

Thus, the overall potential for sweet sorghum to be used for bio-based fuels and other products depends not only on nonstructural carbohydrates in expressed juice and

grain, but also on mass and structural carbohydrates found in leaf, stem, and grain. There are very limited data in Florida regarding the effect of different sweet sorghum cultivars and growth locations on sweet sorghum responses. The objectives of the present study were to characterize i) biomass yield and partitioning (leaf, stem, and grain), ii) biomass composition and iii) potential ethanol yield based on biomass yield and composition for two sweet sorghum cultivars differing in maturity and growing at two locations in Florida during 2 yr.

Materials and Methods

Experimental Site and Design

A replicated field experiment was conducted at two sites in North and Central Florida. The North Florida location was the University of Florida Plant Science Research and Education Unit (29°24'N 82°10'W) in Citra, Florida. The soil at Citra was a relatively well-drained Arredondo fine sand (loamy, siliceous, semiactive, hyperthermic Grossarenic Paleudults). The Central Florida location was the University of Florida Range Cattle Research and Education Center (27°23'N 81°55'W) in Ona, Florida. The soil at Ona was a relatively poorly-drained flatwoods type soil of the Pomona series (sandy, siliceous, hyperthermic Ultic Alaquod).

At each of the sites, two cultivars of sweet sorghum, cultivars 'Dale' and M-81E, were established in a randomized complete block design (RCBD) with four replicate plots per species. Plots were established from seed (source: MAFES Foundation Seed Stocks, MS State Univ., MS) in late March to early April of each year at a rate of 20 to 23 seeds m^{-1} row using a hand seeder (Precision Garden Seeder 1001-B, EarthWay, Bristol, IN). Plots were six rows each (5 x 7 m) planted on 0.76-m row centers for both cultivars.

Cultural Practices and Harvest Management

All plots were fertilized at a rate of 135 kg N ha^{-1} yr^{-1} with a 11-37-0 blended liquid fertilizer in split applications, 19 kg N ha^{-1} at planting and 116 kg N ha^{-1} when plants were 30- to 60-cm tall. Plots were treated with the herbicide Atrazine at a rate of 2.3 L a.i. ha^{-1} at planting. Subsequently, weeds were removed mechanically by rotary hoe or hand. Overhead irrigation of 2 cm was provided at planting, and limited overhead irrigation was applied to plots at sign of visual drought stress (e.g., leaf rolling).

Plots were harvested when approximately half of the grain heads had reached the soft dough stage, which is optimal for sugar recovery (Lingle, 1987; Tarpley et al., 1994). A 4-m section from the middle of one of the two inner rows was harvested by hand at a 7.5-cm stubble height using a gasoline powered trimmer (Echo, Inc., Lake Zurich, IL). The 4-m section was immediately weighed green in the field to provide estimates of green yield, and then a six-stalk subsample was collected and weighed fresh. Each stalk in the subsample was then partitioned into leaf blade (sheath remained with stem), grain head (everything above the flag leaf) and stem. The six-stalk subsamples of leaf, grain head and stem were weighed fresh in the field and then dried at 50°C until a constant dry weight was achieved to determine dry matter concentration and estimate dry biomass yield. Dried tissue samples were run through a commercial chipper-shredder (DEK, MODEL CH1) and then ground with a Thomas-Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 2-mm screen. Samples were then analyzed for composition as described below.

Another subsample of 15 stalks was collected in the field for juice extraction and total soluble solids brix values. Grain heads and leaves were removed and stems were

weighed fresh in the field. Stems were then pressed at 12.4 Mpa of pressure twice in a grooved, two-roller mill powered by a 1.5-HP electric motor, and the expressed juice was then weighed. Extracted juice was then mixed thoroughly and three subsamples were collected for brix (g kg^{-1}) measurements using a portable refractometer after samples had equilibrated to room temperature, 23°C (ATAGO PAL-1, ATAGO USA, Inc., Bellevue, WA).

Biomass Composition

Dried biomass samples were analyzed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) using ANKOM procedures (ANKOM Technology, Macedon, NY). The NDF was analyzed in an ANKOM 200 Fiber Analyzer using the Neutral Detergent Fiber in Feeds Filter Bag Technique. Samples (0.50 ± 0.05 g) were sealed in ANKOM filter bags and digested at 100°C and 15 psi for 75 min in a neutral detergent solution with alpha-amylase and sodium sulfite. Samples were then rinsed three times with 90°C water, the first two times with additional alpha-amylase. The ADF concentration was analyzed in an ANKOM 200 Fiber Analyzer using the Acid Detergent Fiber in Feeds Filter Bag Technique. Samples (0.50 ± 0.05 g) were sealed in ANKOM filter bags and digested at 100°C and 15 psi for 60 min in an acid detergent solution (1.00 *N* H_2SO_4 solution). Samples were then rinsed three times with 90°C water. The ADL was determined using the Method for Determining Acid Detergent Lignin in Beakers (ANKOM Technology, Macedon, NY). Samples that had undergone ADF determination were soaked in 72% by weight H_2SO_4 solution for 3 h with mixing every 30 min, and then rinsed with water until pH was 7. All samples were soaked in acetone for 5 min after each analysis to remove water and then dried at 105°C for 4 h. Samples were then placed in desiccators, allowed to cool to room

temperature and weighed to determine percent NDF, ADF and ADL. All samples were corrected for moisture concentration, and all results are expressed on an absolute dry matter basis.

Ash determination was conducted for all samples by determining absolute dry matter (oven-dried at 105°C for 15 hours) and then samples were heated to 500°C for a minimum of 4 h, cooling in desiccators to room temperature and re-weighing.

Ethanol Yield Calculations

Ethanol yields from structural material were calculated according to the equations of Goff et al. (2010) based on NDF, ADF and ADL values corrected for moisture concentration.

$$H = [\%\text{Cellulose} + (\%\text{Hemicellulose} \times 0.07)] \times 172.82$$
$$P = [\%\text{Hemicellulose} \times 0.93] \times 176.87$$
$$\text{Theoretical Ethanol Potential (L Mg}^{-1}) = [H + P] \times 4.17$$

H and *P* are theoretical ethanol production from the conversion of hexose and pentose sugars, cellulose is ADF minus ADL, and hemicellulose is NDF minus ADF. Theoretical ethanol yields were then calculated by multiplying the theoretical ethanol yields per Mg by tissue yields on a per ha basis.

Ethanol yields for simple sugars in juice were calculated based on equations and conversion values from Wortmann et al. (2010)

$$CSY = (FSY - DSY) \times Brix \times 0.75$$
$$JY, 80\% \text{ extracted} = [FSY - (DSY - CSY)] \times 0.8$$
$$SY = JY \times Brix \times 0.75$$

where “CSY is conservative sugar yield (Mg ha^{-1}), FSY is fresh stalk yield (Mg ha^{-1}), DSY is dry stalk yield (Mg ha^{-1}), JY is juice yield (Mg ha^{-1}), and SY is sugar yield (Mg ha^{-1}). Sugar concentration of juice is 75% if Brix is expressed in g kg^{-1} sugar juice.” It was assumed that extraction efficiency was 80% using a three-roller press, 95% of sugar

yields are fermented to alcohol, and that the sugar to ethanol conversion is 665 L Mg^{-1}. Brix values used in the calculations were the average of three measurements per plot and are reported as g of soluble solids per kg of juice in Table B-1.

Ethanol yields for starch in grain were also estimated. Grain was conservatively assumed to be 600 kg starch Mg^{-1} grain (Clark et al., 2001) and this was multiplied by the total weight in Mg ha^{-1} of grain head material and then converted to theoretical ethanol yield using a conversion of 423 L Mg^{-1} of starch (Wortmann et al., 2010).

Whole-plant TEP yields were determined by summing TEP juice ethanol yield, TEP starch ethanol yield from grain heads, and TEP yields from structural carbohydrates of leaf, stem and grain head tissues.

Statistical Analysis

Data were analyzed using SAS software (SAS Inc., 1996). Analyses of variance for all biomass composition and yield, as well as TEP yield data, were performed for a randomized complete block design using the generalized linear mixed models procedure (PROC GLIMMIX) with tissue type, cultivar and site as fixed effects. Year and block were treated as random effects. The only exception to this was for total whole-plant TEP yields (juice, grain head, and structural yields combined), where only cultivar and site were independent variables since individual tissue TEP yields were combined to produce whole-plant yields. All treatment effects were considered significant at $P \leq 0.05$, and pairwise comparisons were made using the lsmeans statement with the Tukey method. Main effects for site, cultivar and tissue and their interactions were analyzed for all biomass composition and yield data, as well as for TEP yield data. Total whole-plant TEP yield was analyzed for main effects of site and cultivar, and their interaction. Additional analyses for structural component and

theoretical ethanol yields on a land-area basis were conducted as above on individual tissue types using cultivar and site as fixed effects to identify differences within tissues that were not identified in the main analyses.

Results

Biomass Yield and Partitioning

Grain head yield averaged 2.5 Mg ha^{-1} and did not differ for site or cultivar (Fig. 3-1). Grain head was approx. 14% of total dry biomass. Leaf yield was not affected by site, but was significantly higher for M-81E than Dale (3.5 vs 2.1 Mg ha^{-1}) (Fig. 3-1). Leaf dry weight was approx. 12% of total dry weight. Stem dry weight was higher for M-81E, averaging 18.9 Mg ha^{-1}, and lower for Dale at 12.7 Mg ha^{-1} (Fig. 3-1) Stem weight represented approx. 74% of total dry weight. Total dry biomass was thus significantly higher in M-81E than Dale (23.9 vs 17.6 Mg ha^{-1}), and was not affected by site (Fig. 3-1).

Theoretical Ethanol Potential Yields on a Dry Matter Basis

The theoretical ethanol potential from structural sugars per unit of dry biomass was highest for leaf tissue, followed by stem, and was lowest for grain heads at Ona and lowest for grain heads at Citra (422 vs. 331; 247 vs. 163 L Mg^{-1}) (Table 3-2). Thus, based on structural carbohydrate composition, structural TEP averaged 422 L Mg^{-1} across sites for leaves, which was greater than stems at 332 L Mg^{-1}, neither of which differed across site, but structural TEP for grain heads was greater at Ona than Citra (Table 3-2). Structural TEP across three tissue types, two sites, two years and four blocks (n=48) was greater for M-81E than Dale (330 vs 309 L Mg^{-1}).

Theoretical Ethanol Yields on a per-Hectare Basis

Juice TEP was affected by the site by cultivar interaction, ranging from 2510 to 3420 L ha^{-1}, and was highest in M-81E grown at Citra, lower in both M-81E and Dale at Ona, and lowest in Dale at Citra (Table 3-4). Juice extraction efficiencies were approx. 55% (juice weight/stem fresh weight) and were similar between cultivars and sites (Data not shown). Structural TEP (L ha^{-1}) from the stem was greater for M-81E than Dale (Table 3-5) and was not affected by site. Structural TEP for leaf tissue was greater for M-81E than Dale (1530 vs. 840 L ha^{-1}) (Table 3-5), and was not affected by site. Grain head structural TEP was not affected by site, but was higher in Dale than M-81E (Table 3-5). Grain starch TEP was higher in Dale than M-81E (570 vs 400 L ha^{-1}) (Table 3-5), and was not affected by site. Thus, whole plant TEP was higher for M-81E than Dale (12 000 vs. 8510 L ha^{-1}) (Table 3-5), and was not affected by site.

Biomass Composition

All biomass components are expressed per g of oven dry total biomass. Extractives (i.e., nonstructural soluble solids) concentration ranged from 378 mg g^{-1} for leaf tissue averaged across Citra and Ona to 741 mg g^{-1} in grain heads at Citra (Table 3-7). Extractives did not differ across sites for leaf and stem tissues, but were greater in grain heads at Citra compared to Ona. Additionally, extractives were higher in Dale compared to M-81E across all tissues and sites (Table 3-10).

Cellulose concentrations were lowest in grain heads and did not differ between leaf and stem at Ona, but were higher in leaves compared to stem at Citra (Table 3-7). Cellulose concentrations were greater in M-81E compared to Dale at Ona, but the cultivars did not differ at Citra (Table 3-8). Stem and leaf cellulose concentrations were similar in M-81E, but leaf cellulose was higher than stem in Dale (Table 3-7).

Hemicellulose concentrations were highest in leaves and lower in stems, with no difference between sites or cultivars. Grain head hemicellulose was significantly lower than either leaf or stem, but was affected by site and was lowest at Citra (Table 3-7).

Leaf lignin concentrations did not differ between cultivars, but stem and grain head concentrations were greater for Dale than M-81E (Table 3-9). Lignin concentrations were higher for Ona than Citra across all cultivars and tissue types (Table 3-11). Finally, concentrations of ash were higher in Dale than M-81E (Table 3-10), and at Ona than Citra (Table 3-11). Ash concentrations were greatest in leaf tissue and lowest in grain heads (Table 3-12).

Discussion

Biomass and Ethanol Yields

Average maximum TEP for M-81E in the present study was 12 000 L ha^{-1}, approximately 71% from structural biomass, 27% from juice sugars and 3% from grain starch. For TEP from structural biomass, ~78% is from stem, ~18% from leaves and ~3% from grain heads. Average maximum TEP for Dale in the present study was 8510 L ha^{-1}, approximately 62% from structural biomass, 32% from juice sugars and 7% from grain starch. For TEP from structural biomass, ~74% is from stem, ~16% from leaves and ~10% from grain heads.

Juice ethanol yields for M-81E were greater than in prior research by Wortmann et al. (2010), who reported theoretical juice ethanol yields of approximately 2100 L ha^{-1} for M-81E grown in Nebraska. However, this difference is due primarily to lower overall yields as Wortmann obtained sugar yields of only 3.1 to 3.2 Mg ha^{-1}, versus significantly higher extractives yields at both Citra and Ona. Yields may have differed between the studies for several reasons, including different fertilization rates (134 kg N ha^{-1} at Citra

and Ona vs. 0 to 90 kg N ha^{-1} for Wortmann), amount of water available, and/or phenological stage at harvest. Miller and Ottman (2010) reported average dry biomass yields of 25 Mg ha^{-1} and TEP yields from juice of 2600 to 2800 L ha^{-1} for M-81E grown under different levels of water availability. These yields were slightly lower than juice ethanol yields in the current study (3100 to 3400 L ha^{-1}), most likely due to their estimates based only on actual expressed juice compared to theoretical juice yields assuming complete extraction and conversion of all nonstructural sugars as used in the present study.

Due to relatively low levels of ethanol obtainable from grain starch compared to juice or lignocellulosic ethanol, coupled with the necessity of separate harvest procedures and processing technologies, it is unlikely that commercial production of ethanol would utilize the starch in grain. Grain starch will produce only 11 to 22% as much ethanol as juice, and provides only 3 to 7% of total theoretical whole plant ethanol based on these results. Propheter et al. (2010) supports this assumption and advocates the use of M-81E as a cellulosic conversion crop because relatively low grain yields allow for direct cellulosic conversion without separate grain harvesting and processing.

Deheading sorghum prior to grain fill may be a viable method for increasing juice ethanol yields while eliminating the need for starch processing capabilities. Since grain fill involves in part the translocation of soluble sugars in the stalk juice to the grain to form starch, the elimination of the grain head prior to filling may prevent this translocation. This would allow soluble sugars to continue to accumulate in the juice

without loss to grain, thereby increasing juice brix (Erickson et al., 2011). This increase in brix would result in increased ethanol yields from comparable amounts of juice.

Whole-plant ethanol yields for M-81E were reported by Propheter et al. (2010), and ranged from 9600 to 10 200 L ha^{-1}, slightly lower than theoretical yields of 12 000 L ha^{-1} obtained in this research. These differences are due to Propheter assuming a more conservative sugar extraction efficiency than assumed here, which was based on total extractives. Additionally, reported grain yields were slightly lower than in this research. While overall dry biomass yields were comparable between the studies, the assumption by Propheter that easily fermented but not extracted carbohydrates are converted to ethanol at the lower efficiency rate of lignocellulosic conversion is likely partially responsible for the differences in ethanol yields.

Zhao et al. (2009) reported total ethanol yields from M-81E sweet sorghum based on total soluble sugar, grain yields and cellulose and hemicellulose yields. Total dry matter yields and hemicellulose concentrations from Zhao et al. (2009) were comparable to yields obtained in this study, but stem cellulose and lignin contents were significantly higher than those obtained by Zhao et al (2009). Total calculated ethanol yields from their study are comparable to those than those obtained in this research, with slight differences due primarily to differences in structural ethanol yields. Juice ethanol yields are comparable (~3400 vs. ~3300 L ha^{-1}) between studies, while grain ethanol yields are slightly higher in their study (630 vs. 400 L ha^{-1}), most likely due to harvesting at grain maturity as opposed to harvesting intended to produce maximum juice sugar yields, which occurs prior to grain maturity.

Structural ethanol yields were influenced primarily by total dry biomass production and the ratios of cellulose, hemicellulose, lignin and ash. Higher ethanol yields in M-81E were due to higher total biomass production and a lower percentage of extractives, which resulted in more structural sugars available for fermentation.

Biomass Composition

Differences in extractives were due to differences in nonstructural material as a component of total plant biomass, and are consistent with prior research (Zhao et al., 2010). For sweet sorghum, extractives consist primarily of nonstructural storage sugars in the stalk and grain, typically predominantly sucrose in the stalk and starch in the grain, which are easily fermented to ethanol (Lingle, 1987; Murray et al., 2008a). For sweet sorghum, leaves were expected to have the highest proportion of structural (cellulose, hemicellulose and lignin) material, since they do not tend to store substantial amounts of non-structural carbohydrates like sucrose and starch (Lingle, 1987; Murray et al., 2008b). Typically 650 to 800 mg g^{-1} of sorghum grain is starch, a nonstructural sugar removed by alpha-amylase in the NDF process (Clark et al., 2001), which is consistent with obtained structural fiber concentrations of 324 mg g^{-1} for grain heads.

In the present study, higher concentrations of extractives were found in Dale compared to M-81E, but this was offset by greater biomass and juice yields in M-81E, resulting in higher sugar and ethanol yield per hectare from juice from M-81 E. Thus, if a juice-only ethanol production system is considered, Dale may be the more favorable cultivar because of higher extractives values. Energetic demands per unit of ethanol produced will be lower because less biomass will need to be transported to a mill to extract the same amount of sugar for fermentation.

Leaves are typically higher than stems, which are in turn higher than grain heads, in both cellulose and hemicellulose concentration and total structural fiber. These differences are due to lower extractive concentrations in leaves. Whole-plant lignin concentrations were lower in stems and grain heads for Dale compared to M-81E. More importantly, structural (i.e., extractives free) lignin concentrations of stem tissue for Dale were 29% less per unit of structural stem biomass (69 vs. 97 mg g^{-1}) (data not shown). Therefore, based on these results, Dale stems may be more amenable to cellulosic ethanol conversion as higher lignin levels negatively impact efficiency of conversion (Vermerris et al., 2007; Studer et al., 2011). It is unlikely though that higher lignin levels in M-81E would be sufficient to reduce conversion efficiency by the 35% necessary to make Dale competitive on a structural basis.

Higher ash concentration in biomass produced at Ona than Citra may have been due to a variety of factors, including greater availability of soil nutrients, slight differences in maturity at harvest or differences in rates and timing of leaf senescence. Higher ash levels in Dale compared with M-81E indicated higher mineral concentration. More efficient uptake may be advantageous for production on marginal lands, but greater nutrient demands may also decrease the efficiency of ethanol production. Ash levels are highest in leaves; therefore, a harvest strategy which returns leaves to the field, especially since they provide a significantly smaller contribution to ethanol yields than stems, may be beneficial to nutrient management and sustainable production.

Ethanol yields from a single crop of sorghum juice were competitive with current yields from corn grain but were only 50 to 65% of the ethanol yields obtained from sugarcane juice. Although Dale had a higher concentration of sugars in the juice,

potential ethanol yield from juice was greater for M-81E, because cultivar differences in biomass (i.e., juice yield) offset the differences in sugar concentration. It should be noted, however, that M-81E is a relatively late maturity cultivar. Additionally, differences in structural biomass may be also be important in processing facilities that incorporate structural material for cellulosic conversion or co-firing. Along with higher structural biomass yields, M-81E also had higher lignin concentrations that could have detrimental impacts on biomass conversion efficiencies relative to Dale. Based on whole-plant total ethanol yields, M-81E was the superior cultivar for ethanol production from both sorghum juice and structural biomass in the study.

Table 3-1. ANOVA table for structural biomass theoretical ethanol potential (TEP) per unit of total dry biomass.

Sources of variation	Structural TEP
Site	ns
Cultivar	*
Tissue	***
Site*Cultivar	ns
Site*Tissue	***
Cultivar*Tissue	ns
Site*Cultivar*Tissue	ns

ns denotes no significant effects

* Significant effect at P ≤ 0.05

*** Significant effect at P ≤ 0.001

Table 3-2. Theoretical ethanol potential (TEP) per unit of total dry biomass for sweet sorghum biomass as affected by the site x tissue type interaction. Data are means across 2 yr, two cultivars, and four replications (n = 16).

	Structural TEP	
Tissue	Citra	Ona
	-- L Mg^{-1} --	
Leaf	433a[†]	410a
Stem	332b	330b
Grain Head	163d	247d

[†] Means not followed by the same letter are different (*P* ≤ 0.05)

Table 3-3. ANOVA table for per hectare theoretical ethanol potential (TEP).

Sources of variation	Structural TEP			Juice TEP	Grain TEP	Whole Plant TEP
	Leaf	Grain Head	Stem			
Site	ns	ns	ns	ns	*	ns
Cultivar	***	**	***	**	ns	***
Site*Cultivar	ns	ns	ns	*	ns	ns

ns denotes no significant effects

* Significant effect at *P* ≤ 0.05

** Significant effect at *P* ≤ 0.01

*** Significant effect at *P* ≤ 0.001

Table 3-4. Juice theoretical ethanol potential (TEP) per land area for sweet sorghum biomass as affected by the site x cultivar interaction. Data are means across 2 yr and four replications (n = 8).

	Juice TEP	
Tissue	Citra	Ona
	-- L ha^{-1} --	
Dale	2510b†	3020ab
M-81E	3420a	3150ab

† Means not followed by the same letter are different ($P \leq 0.05$)

Table 3-5. Main effect of cultivar on the theoretical ethanol potential (TEP) per land area for sweet sorghum biomass components. Data represent means across 2 yr, 2 sites, and 4 replications (n = 16).

	Structural TEP			Grain TEP	Whole Plant TEP
Cultivar	Leaf	Grain Head	Stem		
	---------- L ha^{-1} ----------				
Dale	840b†	550a	3920b	570a	8510b‡
M-81E	1530a	370b	6630a	400b	12 000a

† Values within the same column not followed by the same letter are different ($P \leq 0.05$)

‡ Whole plant TEP is not the sum of structural, juice and grain head for Dale because outlying points due to grain loss were removed from whole plant calculations, but used in juice and structural calculations.

Table 3-6. ANOVA table for tissue composition on a dry matter basis.

Sources of variation	Extractives	Cellulose	Hemicellulose	Lignin	Ash
Site	*	ns	ns	***	*
Cultivar	***	***	*	***	***
Tissue	***	***	***	ns	***
Site*Cultivar	ns	*	ns	ns	ns
Site*Tissue	**	ns	***	ns	ns
Cultivar*Tissue	ns	*	ns	**	ns
Site*Cultivar*Tissue	ns	ns	ns	ns	ns

ns denotes no significant effects

* Significant effect at $P < 0.05$

** Significant effect at $P < 0.01$

*** Significant effect at $P < 0.001$

Table 3-7. Concentrations of extractives, cellulose, and hemicellulose of sweet sorghum biomass as affected by the site x tissue type interaction. Data are means across 2 yr, two cultivars, and four replications (n = 16).

	Extractives		Cellulose		Hemicellulose	
Tissue	Citra	Ona	Citra	Ona	Citra	Ona
			----- mg g^{-1} -----			
Leaf	370d†	385d	318a	313ab	277a	252a
Stem	510c	495c	286b	289b	171b	166b
Grain Head	741a	670b	131c	156c	94d	123c

† Means within extractives, cellulose, or hemicellulose not followed by the same letter are different ($P \leq 0.05$)

Table 3-8. Concentrations of cellulose of sweet sorghum biomass as affected by the site x cultivar interaction. Data are means across 2 yr, three tissues, and four replications (n = 24).

	Cellulose	
Cultivar	Citra	Ona
	--- mg g^{-1} ---	
Dale	238b†	234b
M-81E	251ab	272a

† Means not followed by the same letter are different ($P \leq 0.05$)

Table 3-9. Concentrations of cellulose and lignin of sweet sorghum biomass as affected by the tissue x cultivar interaction. Data are means across 2 yr, two sites, and four replications (n = 16).

	Cellulose		Lignin	
Tissue	Dale	M-81E	Dale	M-81E
	---------- mg g^{-1} ----------			
Leaf	302a†	328a	40.9bc	45.7ab
Stem	264b	310a	31.8d	52.2a
Grain Head	140c	146c	35.1cd	50.0a

† Means within cellulose or lignin not followed by the same letter are different ($P \leq 0.05$)

Table 3-10. Main effect of cultivar on the concentration of extractives and ash on a dry matter basis for sweet sorghum biomass. Data are means across 2 yr, two sites, three tissue types, and four replications (n = 48).

	Extractives	Ash
	----- mg g^{-1} -----	
Dale	555a†	33.8a
M-81E	502b	29.3b

† Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 3-11. Main effect of site on the concentration of lignin and ash on a dry matter basis for sweet sorghum biomass. Data are means across 2 yr, two cultivars, three tissue types, and four replications (n = 48).

	Lignin	Ash
	----- mg g^{-1} -----	
Citra	34.8b†	29.7b
Ona	50.5a	33.4a

† Means within a column not followed by the same letter are different ($P \leq 0.05$)

Table 3-12. Main effect of tissue type on the concentration of ash on a dry matter basis for sweet sorghum biomass. Data are means across 2 yr, two cultivars, two sites, and four replications (n = 32).

	Ash
	mg g^{-1}
Leaf	51.2a†
Stem	28.2b
Grain Head	15.1c

† Means within a column not followed by the same letter are different ($P \leq 0.05$)

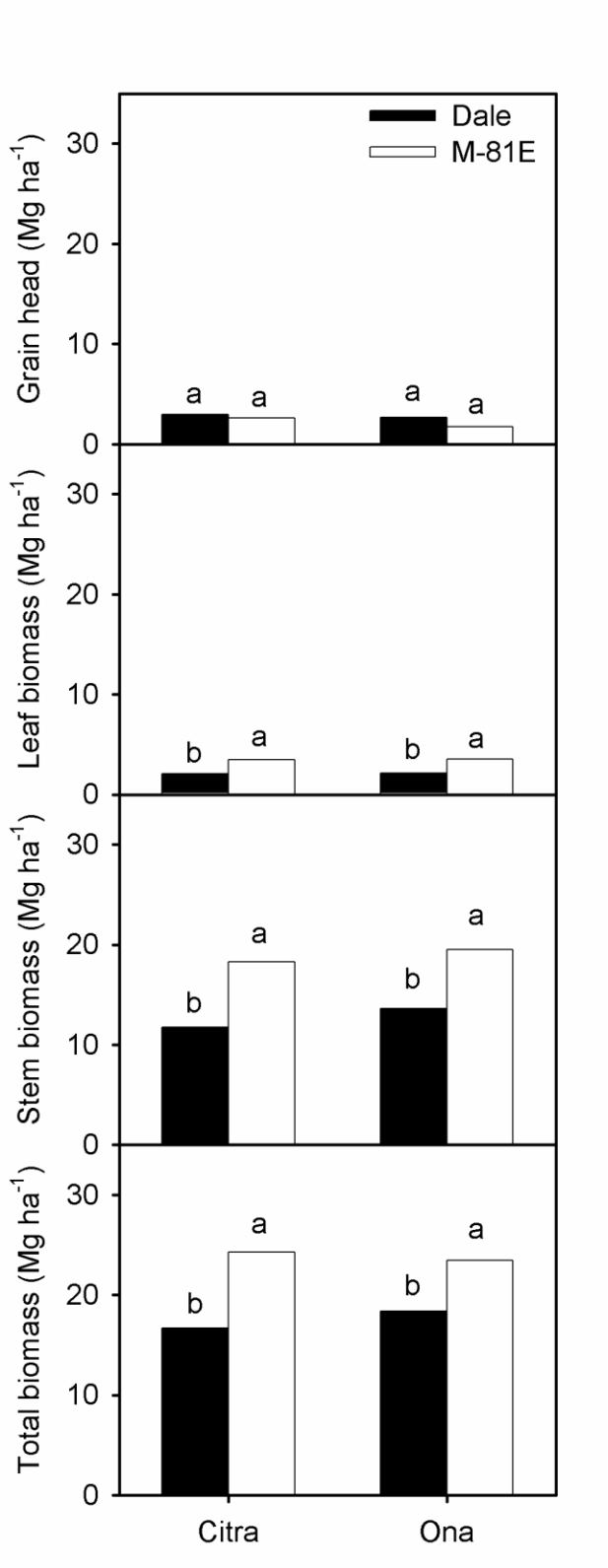

Figure 3-1. Tissue and whole plant dry biomass yields by cultivar and site. Bars in the same panel not accompanied by the same letter are different ($P \leq 0.05$).

CHAPTER 4
CONCLUSION

Based on this research, perennial grass crops such as sugarcane, energycane and elephantgrass are viable candidates for ethanol production depending on location and the production and conversion systems employed. Sugarcane is an ideal candidate for combined processing of nonstructural and structural biomass in locations with a favorable climate for production. Energycane and elephantgrass are excellent candidates for production in locations where sugarcane is not sufficiently cold tolerant, or where lower levels of extractives would be beneficial. Giant reed and sweetcane are less favorable due to higher lignin concentrations, as well as lower yields in the case of giant reed and difficulties with establishment sweetcane. Giant miscanthus had a potentially favorable tissue composition but is likely not the species of choice in Florida to low total biomass yields.

Sweet sorghum was a viable competitor with corn-based ethanol systems based on current technologies. In comparison to sugarcane, sweet sorghum produced relatively lower juice ethanol yields but can be produced in many more locations, and may be able to produce two crops per year in some locations, further increasing its appeal.

Research on nutrient and water requirements for both perennial species and sweet sorghum will help further identify species that are well suited for production and conversion. Additionally, research on harvest management (e.g., multiple cuttings) and storage of harvested biomass are needed to help determine the most sustainable bioenergy crops for the southeastern USA.

APPENDIX A
PERENNIAL DRY MATTER

Table A-1. Dry matter concentrations (g dry biomass per kg fresh weight) of perennial grasses by year and species, pooled across sites (n=12), as determined by drying at 50°C until a constant dry weight was achieved.

	2009	2010
Giant Reed	468a[†]	517b
Sugarcane	263c	288d
Sweetcane	342b	356c
Energycane	309b	329cd
Elephantgrass	336b	354c
Giant Miscanthus	506a	677a

[†] Means within a column not followed by the same letter are different ($P \leq 0.05$)

APPENDIX B
BRIX VALUES

Table B-1. Brix values (g of soluble solids per kg of juice) used in sorghum juice ethanol calculations in Chapter 3.

		2009		2010	
	Block	Citra	Ona	Citra	Ona
Dale	1	153	147	150	171
	2	155	170	154	179
	3	155	161	138	190
	4	159	150	158	188
M-81E	1	161	140	158	157
	2	154	148	155	160
	3	155	na‡	164	162
	4	140	129	154	158

‡ Data not available due to stand loss

LIST OF REFERENCES

Amaducci, S.A. Monti, and G. Venturi. 2004. Non-structural carbohydrates and fibre components in sweet and fibre sorghum as affected by low and normal input techniques. Ind. Crops and Products. 20:111-118.

Anderson, W.F., B.S. Dien, S.K. Brandon, and J.D. Peterson. 2008. Assessment of Bermudagrass and bunch grasses as feedstock for conversion to ethanol. Appl. Biochem. Biotechnol. 145:13-21.

Angelini L.G., L. Ceccarini, and E. Bonari. 2005. Biomass yield and energy balance of giant reed (*Arundo donax* L.) cropped in central Italy as related to different management practices. Europe. J. Agronomy 22:375-389.

Bandyopadhyay, R., D.E. Frederickson, N.W. McLaren, G.N. Odvody, and M.J. Ryley. 1998. Ergot: A new disease threat to sorghum in the Americas and Australia. Plant Dis. 82:356-367.

Bischoff, K.P., K.A. Gravois, T.E. Reagan, J.W. Hoy, C.A. Kimbeng, C.M. LaBorde, and G.L. Hawkins. 2008. Registration of 'L 79-1002' Sugarcane. J. of Plant Registrations. 2:211-217.

Broadhead, D.M. 1969. Sugar production from sweet sorghum as affected by planting date, after-ripe harvesting, and storage. Agron. J. 61:811-812.

Broadhead, D.M., K.C. Freeman, and N. Zummo. 1981. 'M 81E'---A new variety of sweet sorghum. Information Sheet 1309 [Online]. Available at http://msucares.com/crops/sorghum/m81e_description.pdf (verified at 13 June 2011). Mississippi Agricultural and Forestry Experiment Station. Mississippi State, MS.

Brown, L. R. 2006. Plan B 2.0: Rescuing a planet under stress and a civilization in trouble. W.W. Norton & Co. New York, NY.

Burner, D.M., T.J. Tew, J.H. Harvey, and D.P. Belesky. 2009. Dry matter partitioning and quality of *Misacanthus*, *Panicum*, and *Saccharum* genotypes in Arkansas, USA. Biomass and Bioenergy. 33:610-619.

Burton, G.W. 1989. Registration of 'Merkeron' napiergrass. Crop Sci. 29:1327.

Byrt, C.S., C.P.L. Grof, and R.T. Furbank. 2011. C_4 Plants as Biofuel feedstocks: Optimising biomass production and feedstock quality from a lignocellulosic perspective. J. of Integrative Plant Bio. 53:120-135.

Casler, M.D., H. Heaton, K.J. Shinners, H.G. Jung, P.J. Weimer, M.A. Liebig, R.B. Mitchell, and M.F. Digman. p. 205-219. *In* W.F. Wedin and S.L. Fales (ed.) Grassland: Quietness and strength for a new American agriculture. ASA/CSSA/SSSA, Madison, WI

Chen, F. and R.A. Dixon. 2007. Lignin modification improves fermentable sugar yields for biofuel production. Nat. Biotechnology 25:759-761.

Clark, J.H., D.K. Beede, R.A. Erdman, J.P. Goff, R.R. Grummer, J.G. Linn, A.N. Pell, C.G. Schwab, T. Tomkins, G.A. Varga, and W.P. Weiss. 2001. Carbohydrate chemistry and feed processing. p. 249-257. *In:* J.H. Clark et al. Nutrient requirements of dairy cattle. Natl. Academy Press. Washington, D.C.

Davison, B.H., S.R. Drescher, G.A. Tuskan, M.F. Davis, and N.P. Nghiem. 2006. Variation of S/G ratio and lignin content in *Populus* family influences the release of xylose by dilute acid hydrolysis. Appl. Biochem. and Biotechnology. 129-132:427-435.

Dien, B.S., S. Gautam, J.F. Pedersen, S.E. Sattler, H. Chen, D.L. Funnell-Harris, N.N. Nichols, and M.A. Cotta. 2009. Improved sugar conversion and ethanol yield for forage sorghum (Sorghum bicolor L. Moench) lines with reduced lignin contents. Bioenergy Res. 2:153-164.

Energy Independence and Security Act of 2007, Public Law 110-140, H.R. 6, 2007.

Erickson, J.E., C. Rainbolt, and Y. Newman. 2008. Production of biofuel crops in Florida: Miscanthus SS-AGR-292 [Online]. Available at http://edis.ifas.ufl.edu/ag297 (verified at 18 July 2011). Inst of Food and Agric Sci., Cooperative Extension Service, IFAS, Gainesville, FL.

Erickson, J.E., K.R. Woodard, and L.E. Sollenberger. 2011. Optimizing sweet sorghum production for biofuel in the southeastern USA through nitrogen fertilization and top removal. Bioenerg. Res. DOI 10.1007/s12155-011-9129-3.

Ferraris, R. 1981. Early assessment of sweet sorghum as an agro-industrial crop: I. Varietal evaluation. Aust. J. Exp. Agric. Anim. Husb. 21:75-82.

Fletcher Jr., R.J., B.A. Robertson, J. Evans, P.J. Doran, J.R.R. Alavalapati, and D.W. Schemske. 2011. Biodiversity conservation in the era of biofuels: risks and opportunities. Front. Ecol. Environ. 9:161-168.

Foyle, T., L. Jennings, and P. Mulcahy. 2007. Compositional analysis of lignocellulosic materials: Evaluation of methods used for sugar analysis of waste paper and straw. Bioresource Technol. 98:3026-3036.

Fu, C., J.R. Mielenz, X. Xiao, Y. Ge, C.Y. Hamilton, M. Rodriguez, Jr., F. Chen, M. Foston, A. Ragauskas, J. Bouton, R.A. Dixon, and Z. Wang. 2011. Genetic manipulation of lignin reduces recalcitrance and improes ethanol production from switchgrass. PNAS. 108: 3803-3808.

Geddes, C.C., I.U. Nieves, and L.O. Ingram. 2011. Advances in ethanol production. Curr. Opin. in Biotechnology. 22:312-319.

Gerbens-Leenes, W., A.Y. Hoekstra, and T.H. van der Meer. 2009. The water footprint of bioenergy. PNAS 106: 10219-10223.

Gilbert, R., J. Ferrell, and Z. Helsel. 2008. Production of giant reedgrass for biofuel SS-AGR-318 [Online]. Available at http://edis.ifas.ufl.edu/ag327 (verified at 7 July 2011). Inst. of Food and Agric. Sci., Cooperative Extension Service, IFAS, Gainesville, FL.

Glaz, B., and R.A. Gilbert. 2010. Sugarcane variety census: Florida 2005 SS-AGR-268 [Online]. Available at http://edis.ifas.ufl.edu/sc083 (verified at 18 July 2011). Inst. of Food and Agric. Sci., Cooperative Extension Service, IFAS, Gainesville, FL.

Goff, B.M., K.J. Moore, L. Fales, and A. Heaton. 2010. Double-cropping sorghum for biomass. Agron. J. 102:1586-1592.

Goldemberg, J. 2007. Ethanol for a sustainable energy future. Science. 315:808-810.

Groom, M.J., E.M. Gray, and P.A. Townsend. 2008. Biofuels and biodiversity: Principles for creating better policies for biofuel production. Conservation Bio. 22:602-609.

Heaton, E.A., J. Clifton-Brown, T.B. Voigt, M.B. Jones, and S.P. Long. 2003. *Miscanthus* for renewable energy generation: European Union experiences and projections for Illinois. Mitigation and Adaptation Strategies for Global Change. 9:433-451.

Himmel, M.E., S. Ding, D.K. Johnson, W.S. Adney, M.R. Nimlos, J.W. Brady, and T.D. Foust. 2007. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. Science. 315:804-807.

Iakovlev, M., and A. van Heiningen. 2011. SO2-Ethanol-Water (SEW) pulping: I. Lignin determination in pulps and liquors. J. of Wood Chem. and Technol. 31:233-249.

Ingram, L.O., H.C. Aldrich, A.C.C. Borges, T.B. Causey, A. Martinez, F. Morales, A. Saleh, S.A. Underwood, L.P. Yomano, S.W. York, J. Zaldivar, and S. Zhou. 1999. Enteric bacterial catalysts for fuel ethanol production. Biotechnol. Prog. 15:855-866.

Karp, A., and I. Shield. 2008. Bioenergy from plants and the sustainable yield challenge. New Phytol. 179:15-32.

Lee, E.A., and M. Tollenaar. 2007. Physiological basis of successful breeding strategies for maize grain yield. Crop Sci. 47: S202-S215.

Lingle, S.E. 1987. Sucrose metabolism in sweet sorghum. Crop Sci. 27:1214-1219.

Miller, A.N., and M.J. Ottman. 2010. Irrigation frequency effects on growth and ethanol yield in sweet sorghum. Agron. J. 102:60-70.

Mislevy, P., R.S. kalmbacher, A.J. Overman, and F.G. Martin. 1986. Effect of fertilizer and nematicide treatments on crops grown for biomass. Biomass. 11:243-253.

Mislevy, P., W.G. Blue, and C.E. Roessler. 1989. Productivity of clay tailings from phosphate mining: I. Biomass crops. J. Environ. Qual. 18:95-100.

Mislevy, P., F.G. Martin, M.B. Adjei, and J.D. Miller. 1997. Harvest management effects on quantity and quality of *Erianthus* plant morphological components. Biomass and Bioenergy. 13:51-58.

Murray, S.C., A. Sharma, W.L. Rooney, P.E. Klein, J.E. Mullet, S.E Mitchell, and S. Kresovich. 2008a. Genetic improvement of sorghum as a biofuel feedstock: I. QTL for stem sugar and grain nonstructural carbohydrates. Crop Sci. 48:2165-2179.

Murray, S.C., W.L. Rooney, S.E Mitchell, A. Sharma, P.E. Klein, J.E. Mullet, and S. Kresovich. 2008b. Genetic improvement of sorghum as a biofuel feedstock: II. QTL for stem and leaf structural carbohydrates. Crop Sci. 48:2180-2193.

Pohit, S., P.K. Biswas, R. Kumar, and J. Jha. 2011. International experiences of ethanol as transport fuel: Policy implications for India. Energy Policy. 37:4540-4548.

Propheter, J.L., S.A. Staggenborg, X. Wu, and D. Wang. 2010. Performance of annual and perennial biofuel crops: Yields during the first two years. Agron. J. 102:806-814.

Rainbolt, C., and R. Gilbert. 2008. Production of biofuel crops in Florida: Sugarcane/Energycane SS-AGR-298 [Online]. Available at http://edis.ifas.ufl.edu/ag303 (verified at 7 July 2011). Inst. of Food and Agric. Sci., Cooperative Extension Service, IFAS, Gainesville, FL.

RFA. 2011. Renewable Fuels Association. Available at http://www.ethanolrfa.org (verified 7 July 2011). RFA, Washington, D.C.

Rice, R.W., R.A. Gilbert, and J.M. McCray. 2010. Nutritional requirements for Florida sugarcane SS-AGR-228 [Online]. Available at http://edis.ifas.ufl.edu/sc028 (verified at 7 July 2011). Inst. of Food and Agric. Sci., Cooperative Extension Service, IFAS, Gainesville, FL.

Saha, B.C. 2003. Hemicellulose bioconversion. J. Ind. Microbiol. Biotechnol. 30:279-291.

Sami, M., K. Annamalai, and M. Wooldridge. 2001. Co-firing of coal and biomass fuel blends. Prog. in Energy and Combustion Sci. 27:171-214.

Sanderson, M.A., G.E. Brink, K.F. Higgins, and D.E. Naugle. 2004. Alternative uses of warm-season forage grasses. p. 389-416. *In* L. E. Moser et al. (ed.) Warm-season (C_4) grasses. ASA/CSSA/SSSA, Madison, WI.

Sanderson, M.A., and P.R. Adler. 2008. Perennial forages as second generation bioenergy crops. Int. J. Mol. Sci. 9:768-788.

SAS Institute. 1996. SAS statistics user's guide. Release version 6. SAS Inst., Cary, NC.

Sathitsuksanoh, N., Z. Zhu, S. Wi, and Y.H. Perceival-Zhao. 2011. Cellulose solvent-based biomass pretreatment breaks ordered hydrogen bonds in cellulose fibers of switchgrass. Biotechnology and Bioengineering. 108:521-529.

Schmer, M.R., K.P. Vogel, R.B. Mitchell, and R.K. Perrin. 2008. Net energy of cellulosic ethanol from switchgrass. PNAS. 105: 464-469.

Sims, R.E.H., A. Hastings, B. Schlamadinger, G. Taylor, and P. Smith. 2006. Energy crops: Current status and future prospects. Global Change Bio. 12:2054-2076.

Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker. 2008. Determination of structural carbohydrates and lignin in biomass. National Renewable Energy Laboratory.

Sommerville, C.,H. Youngs, C. Taylor, S.C. Davis and S.P. Long. 2010. Feedstocks for lignocellulosic biofuels. Science. 329:790-792.

Stricker, J.A., G.M. Prine, D.L. Anderson, D.B. Shibles, and T.C. Riddle. 2009. Energy from crops: Production and management of biomass/energy crops on phosphatic clay in Central Florida CIR-1084 [Online]. Available at http://edis.ifas.ufl.edu/eh213 (verified at 7 July 2011). Inst. of Food and Agric. Sci., Cooperative Extension Service, IFAS, Gainesville, FL.

Studer, M.H., J.D. DeMartini, M.F. Davis, R.W. Sykes, B. Davison, M. Keller, G.A. Tuskan, and C.E. Wyman. 2011. Lignin content in natural *Populus* variants affects sugar release. PNAS. 108:6300-6305.

Tamang, P.L., K.F. Bronson, A. Malapati, R. Schwartz, J. Johnson, and J. Moore-Kucera. 2011. Nitrogen requirements for ethanol production from sweet and photoperiod sensitive sorghums in the southern high plains. Agron. J. 103:431-440.

Tarpley, L., S.E. Lingle, D.M. Vietor, D.L. Andrews, and F.R. Miller. 1994. Enzymatic control of nonstructural carbohydrate concentrations in stems and panicles of sorghum. Crop Sci. 34:446-452.

Thai Hoa, D., T.D. Man, and N.G. Hau. 2008. Pretreatment of lignocellulosic biomass for enzymatic hydrolysis. ASEAN Journal on Science and Technology for Development. 25:341-346.

Thomason, W.E., W.R. Raun, G.V. Johnson, C.M. Taliaferro, K.W. Freeman, K.J. Wynn, and R.W. Mullen. 2004. Switchgrass Response to Harvest Frequency and Time and Rate of Applied Nitrogen. J. of Plant Nut. 27:1199-1226.

Varvel, G.E., K.P. Vogel., R.B. Mitchell, R.F. Follett, and J.M. Kimble. 2008. Comparison of corn and switchgrass on marginal soils for bioenergy. Biomass and Bioenergy. 32: 18-21.

Vermerris, W.A. Saballos, G. Ejeta, N.S. Mosier, M.R. Ladisch, and N.C. Carpita. 2007. Molecular breeding to enhance ethanol production from corn and sorghum stover. Crop Sci. 47: S142-S153.

Vermerris, W. 2008. Composition and biosynthesis of lignocellulosic biomass. p. 89-129. *In* W. Vermerris (ed.) Genetic improvement of bioenergy crops. Springer Science+Business Media, New York.

Walford, S.N. 2008. Sugarcane bagasse: How easy is it to measure its constituents?. Proc. S. Afr. Sug. Technol. Ass. 81:266-273.

Woodard, K.R., and G.M. Prine. 1991. Forage yield and nutritive value of elephantgrass as affected by harvest frequency and genotype. Agron. J. 83:541-546.

Woodard, K.R., and G.M. Prine. 1993. Dry matter accumulation of elephantgrass, energycane, and elephantmillet in a subtropical climate. Crop Sci. 33:818-824.

Woodard, K.R., and L.E. Sollenberger. 2011. Production of biofuel crops in Florida: Elephantgrass SS-AGR-297 [Online]. Available at http://edis.ifas.ufl.edu/ag302 (verified at 7 July 2011). Inst of Food and Agric Sci., Cooperative Extension Service, IFAS, Gainesville, FL.

Wortmann, C.S., A.J. Liska, R.B. Ferguson, D.J. Lyon, R.N. Klein, and I. Dweikat. 2010. Dryland performance of sweet sorghum and grain crops for biofuel in Nebraska. Agron. J. 102:319-326.

Wu, X., S. Staggenborg, J.L. Propheter, W.L. Rooney, J. Yu, and D. Wang. 2009. Features of sweet sorghum juice and their performance in ethanol fermentation. Industrial Crops and Products. 31:164-170

Zhao, Y.L., A. Dolat, Y. Steinberger, X. Wang, A. Osman, and G.H. Xie. 2009. Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. Field Crops Research. 111:55-64.

BIOGRAPHICAL SKETCH

Jeff Fedenko was born in 1986 in Clearwater, FL and earned his B.S. degrees in biology and psychology in 2009 from the University of Florida, Gainesville, FL. He joined the Agronomy Department at the University of Florida in summer 2009 in the lab of Dr. John Erickson and completed his master's degree program in summer 2011.