

HARVEST MANAGEMENT AND GROWTH PERIOD EFFECTS ON  
PERENNIAL BIOENERGY GRASS PRODUCTION AND  
COMPOSITION IN THE SOUTHEASTERN USA

By

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To my parents and wife

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## TABLE OF CONTENTS

	<u>page</u>
ACKNOWLEDGMENTS.....	4
LIST OF TABLES.....	9
LIST OF FIGURES.....	12
ABSTRACT .....	15
CHAPTER	
1 INTRODUCTION .....	17
2 LITERATURE REVIEW .....	22
Why Are Warm-Season Grasses Considered for Use as Bioenergy Feedstocks? .....	22
Physiological Characteristics of Warm-Season Grasses.....	22
Elephantgrass .....	23
Energycane .....	24
Composition of Bioenergy Grasses and Conversion to Ethanol .....	26
Composition of Perennial Grasses .....	26
Fiber Analysis of Biomass .....	27
Theoretical Ethanol Potential and Yield.....	28
Ash and Mineral Elements.....	28
Management Effects on Perennial Bioenergy Grasses .....	29
Logistical Issues and Field Management in the Biofuel Industry .....	29
Harvest Frequency and Seasonality Affect Biomass Yield.....	29
Harvest Frequency and Seasonality Affect Composition of Perennial Grasses.....	31
Fertilizer Management.....	32
Regional Adaptation .....	33
Nitrogen-Use Efficiency and Concentration in Biomass.....	33
Importance of N in Grass-based Systems.....	33
Seasonal Dynamics of N in Perennial Grasses .....	34
N-use Efficiency by Quantification of Isotopic N ( <sup>15</sup> N) .....	35
Morphology of Elephantgrass and Energycane .....	36
Importance of Morphology in Bioenergy Crops .....	36
Seasonal Dynamics of Perennial Grass Morphology .....	37
Carbon Sequestration of Perennial Grasses.....	38
Role of Perennial Grass Cropland for C Sequestration .....	38
Importance of Studying Warm-season Grassland C Sequestration in Florida..	39
3 BIOMASS HARVESTED AND PLANT-PART PROPORTION RESPONSES OF PERENNIAL BIOENERGY GRASSES TO HARVEST MANAGEMENT.....	41

Overview of Research.....	41
Materials and Methods.....	42
Experimental Site .....	42
Treatments and Experimental Design .....	43
Plot Establishment and Management.....	44
Response Variables .....	45
Statistical Analysis.....	46
Results and Discussion.....	46
Biomass Harvested .....	46
Leaf:stem Ratio .....	49
Biomass Dry Matter Concentration.....	51
Persistence.....	53
Implications of Research.....	54
4 BIOMASS COMPOSITION RESPONSES OF PERENNIAL BIOENERGY GRASSES TO HARVEST MANAGEMENT .....	63
Overview of Research.....	63
Materials and Methods.....	65
Experimental Site .....	65
Treatments and Experimental Design .....	66
Plot Establishment and Management.....	67
Biomass Fiber Analysis .....	68
Total Nitrogen, Phosphorus, and Ash.....	69
Statistical Analysis.....	70
Results and Discussion.....	71
Van Soest Fiber Analyses .....	71
Neutral detergent fiber .....	71
Acid detergent fiber.....	74
Acid detergent lignin .....	75
Cellulose .....	77
Hemicellulose.....	78
National Renewable Energy Laboratory Procedures.....	81
Extractives .....	81
Total soluble sugars.....	83
Structural hexose .....	84
Structural pentose.....	86
Lignin .....	87
Mineral Composition.....	88
Nitrogen .....	88
Phosphorus.....	90
Ash.....	91
Harvest Management by Year Interactions for Total Biomass Composition.....	92
Entry by Year Interactions for Total Biomass Composition.....	93
Implications of Research.....	94

5	SEASONAL CHANGES IN MORPHOLOGICAL CHARACTERISTICS OF ELEPHANTGRASS AND ENERGYCANE.....	117
	Overview of Research.....	117
	Materials and Methods.....	119
	Experimental Site .....	119
	Treatments and Experimental Design .....	119
	Plot Establishment and Management.....	120
	Response Variables .....	120
	Statistical Analysis.....	122
	Results and Discussion.....	122
	Tiller Density.....	122
	Tiller Mass.....	124
	Leaf Area Index .....	126
	Canopy Height.....	128
	Stem Proportion .....	129
	Biomass Dry Matter Concentration.....	131
	Implications of Research.....	133
6	SEASONAL CHANGES IN CHEMICAL COMPOSITION OF ELEPHANTGRASS AND ENERGYCANE.....	148
	Overview of Research.....	148
	Materials and Methods.....	150
	Treatments and Experimental Design .....	150
	Response Variables .....	151
	Statistical Analysis.....	152
	Results and Discussion.....	153
	Neutral Detergent Fiber.....	153
	Acid Detergent Fiber .....	154
	Acid Detergent Lignin .....	155
	Cellulose.....	157
	Hemicellulose .....	158
	Nitrogen.....	160
	Phosphorus .....	163
	Ash .....	163
	Implications of Research.....	166
7	TIME AFTER A FREEZE EVENT AFFECTS PERENNIAL GRASS BIOMASS HARVESTED AND CHEMICAL COMPOSITION .....	179
	Overview of Research.....	179
	Materials and Methods.....	180
	Experimental Site .....	180
	Plot Establishment and Management until a Freeze Event .....	181
	Treatments and Experimental Design .....	182
	Response Variables .....	182

Statistical Analysis.....	184
Results and Discussion.....	185
Harvested Biomass .....	185
Biomass Dry Matter Concentration.....	187
Leaf Proportion.....	189
Fiber Analysis.....	190
Nitrogen.....	193
Ash.....	194
Implications of Research.....	195
8 CONCLUSIONS .....	210
Effect of Harvest Management on Perennial Grasses – Chapters 3 and 4.....	210
Morphological and Chemical Changes of Perennial Grasses During the Growing Season – Chapters 5 and 6.....	212
Effects of Delaying Harvest After a Freeze Event on Biomass Harvested and Chemical Composition – Chapter 7 .....	214
Implications of the Research.....	215
Future Research Needs.....	216
LIST OF REFERENCES .....	218
BIOGRAPHICAL SKETCH.....	231

## LIST OF TABLES

<u>Table</u>	<u>page</u>
3-1 Harvest dates for 2X, 1X-Nov, and 1X-Dec harvest management treatments in 2010, 2011, and 2012.....	56
3-2 Sources of variation and levels of probability ( <i>P</i> ) for their effects on response variables reported in Chapter 3. ....	56
3-3 Grass entry × year interaction ( <i>P</i> < 0.001) effect on biomass harvested.....	57
3-4 Harvest management × year interaction ( <i>P</i> = 0.002) effect on biomass harvested of three perennial grasses. ....	57
3-5 Grass entry × year interaction ( <i>P</i> < 0.001) effect on biomass leaf:stem ratio. ....	58
3-6 Harvest management × year interaction ( <i>P</i> = 0.002) effect on leaf:stem ratio of three perennial grasses. ....	58
3-7 Grass entry × harvest management interaction ( <i>P</i> < 0.001) effect on biomass dry matter concentration at harvest. ....	59
3-8 Harvest management × year interaction ( <i>P</i> < 0.001) effect on biomass dry matter concentration of three perennial grasses.....	59
3-9 Harvest management × entry interaction ( <i>P</i> = 0.009) effect on proportion of row without viable tillers after 3 yr defoliation. ....	60
4-1 Composition and characteristics of components analyzed in Chapter 4.....	96
4-2 Sources of variation and levels of probability ( <i>P</i> ) for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations and their effects on response variables reported in Chapter 4...	97
4-3 Sources of variation and levels of probability ( <i>P</i> ) for concentration of cell wall components (cellulose and hemicellulose) from detergent fiber analysis and their effects on response variables reported in Chapter 4. ....	98
4-4 Effect of grass entry × harvest management interaction on neutral detergent fiber (NDF) concentration in leaf ( <i>P</i> = 0.272), stem ( <i>P</i> < 0.001), and total biomass ( <i>P</i> < 0.001). ....	99
4-5 Effect of grass entry × harvest management interaction on acid detergent fiber (ADF) concentration in leaf ( <i>P</i> = 0.090), stem ( <i>P</i> < 0.001), and total biomass ( <i>P</i> < 0.001). ....	100

4-6	Effect of grass entry x harvest management interaction on acid detergent lignin (ADL) concentration in leaf ( $P = 0.037$ ), stem ( $P = 0.006$ ), and total biomass ( $P < 0.001$ ). .....	101
4-7	Effect of grass entry x harvest management interaction on cellulose (detergent fiber analysis) concentration in leaf ( $P = 0.195$ ), stem ( $P < 0.001$ ), and total biomass ( $P < 0.001$ ).....	102
4-8	Effect of grass entry x harvest management interaction on hemicellulose (detergent fiber analysis) concentration in leaf ( $P = 0.029$ ), stem ( $P = 0.009$ ), and total biomass ( $P < 0.284$ ).....	103
4-9	Sources of variation and levels of probability ( $P$ ) for concentration of non-structural components (extractives and total soluble sugars) and their effects on response variables reported in Chapter 4.....	104
4-10	Sources of variation and levels of probability ( $P$ ) for concentration of structural components (hexose, pentose, and total lignin) and their effects on response variables reported in Chapter 4.....	105
4-11	Effect of grass entry x harvest management interaction on extractives in leaf ( $P = 0.044$ ), stem ( $P < 0.001$ ), and total biomass ( $P < 0.001$ ). .....	106
4-12	Effect of grass entry x harvest management interaction on total soluble sugars concentration in leaf ( $P = 0.044$ ), stem ( $P < 0.001$ ), and total biomass ( $P < 0.001$ ).....	107
4-13	Effect of grass entry x harvest management interaction on structural hexose concentration in leaf ( $P = 0.562$ ), stem ( $P = 0.001$ ), and total biomass ( $P = 0.001$ ). .....	108
4-14	Effect of grass entry x harvest management interaction on structural pentose concentration in leaf ( $P = 0.977$ ), stem ( $P = 0.003$ ), and total biomass ( $P = 0.139$ ). .....	109
4-15	Effect of grass entry x harvest management interaction on lignin concentration in leaf ( $P = 0.069$ ), stem ( $P = 0.002$ ), and total biomass ( $P = 0.002$ ). .....	110
4-16	Sources of variation and levels of probability ( $P$ ) for nitrogen, phosphorus, and ash concentrations and their effects on response variables reported in Chapter 4.....	111
4-17	Effect of grass entry x harvest management interaction on nitrogen concentration in leaf ( $P = 0.334$ ), stem ( $P = 0.942$ ), and total biomass ( $P = 0.955$ ). .....	112

4-18	Effect of grass entry by harvest management interaction on phosphorus concentration in leaf ( $P = 0.139$ ), stem ( $P = 0.360$ ), and total biomass ( $P = 0.348$ ). .....	113
4-19	Effect of grass entry x harvest management interaction on ash concentration in leaf ( $P = 0.359$ ), stem ( $P = 0.257$ ), and total biomass ( $P = 0.853$ ). .....	114
4-20	Effect of harvest management x year interaction on concentration of acid detergent lignin (ADL) ( $P = 0.002$ ), hemicellulose ( $P < 0.001$ ), extractives ( $P = 0.011$ ), total soluble sugars ( $P < 0.001$ ), structural hexose ( $P < 0.001$ ) and pentose ( $P < 0.001$ ), N ( $P = 0.005$ ), P ( $P = 0.013$ ), and ash ( $P = 0.002$ ) in total biomass. ....	115
4-21	Effect of entry by year on concentration of neutral detergent fiber (NDF) ( $P = 0.009$ ), acid detergent lignin (ADL) ( $P = 0.006$ ), hemicellulose ( $P < 0.001$ ), extractives ( $P < 0.016$ ), total soluble sugars ( $P = 0.001$ ), structural hexose ( $P = 0.034$ ) and pentose ( $P < 0.001$ ), and lignin ( $P = 0.002$ ) in total biomass. ....	116
5-1	Energycane and elephantgrass sampling dates for responses reported in Chapter 5.....	135
6-1	Sampling dates for energycane and elephantgrass for responses reported in Chapter 6.....	168
6-2	Effect of grass entry or sampling date main effects or their interaction on leaf proportion in total biomass and proportion of total N and ash in the leaf fraction in 2010. ....	169
6-3	Effect of grass entry or sampling date main effects or their interaction on leaf proportion in total biomass and proportion of total N and ash in the leaf fraction in 2011. ....	170
7-1	Sampling and freeze event dates for delayed harvest management in 2010-2011, 2011-2012, and 2012-2013. ....	197

## LIST OF FIGURES

<u>Figure</u>	<u>page</u>
3-1 Monthly average and monthly maximum and minimum air temperatures for 2010, 2011, and 2012 for the experimental location, and the 30-yr average for Gainesville, Florida.....	61
3-2 Monthly rainfall for 2010, 2011, and 2012 for the experimental location and the 30-yr average for Gainesville, Florida.....	62
5-1 Seasonal changes in tiller density of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower).....	136
5-2 Seasonal changes in tiller density of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower).....	137
5-3 Seasonal changes in tiller mass of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower).....	138
5-4 Seasonal changes in tiller mass of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower).....	139
5-5 Seasonal changes in leaf area index of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower).....	140
5-6 Seasonal changes in leaf area index of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower).....	141
5-7 Seasonal changes in canopy height of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower).....	142
5-8 Seasonal changes in canopy height of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower).....	143
5-9 Seasonal changes in stem proportion of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower).....	144
5-10 Seasonal changes in stem proportion of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower).....	145
5-11 Seasonal changes in dry matter concentration of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower).....	146
5-12 Seasonal changes in dry matter concentration of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower).....	147

6-1	Seasonal changes in NDF concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). .....	171
6-2	Seasonal changes in ADF concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). .....	172
6-3	Seasonal changes in ADL concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). .....	173
6-4	Seasonal changes in cellulose concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). .....	174
6-5	Seasonal changes in hemicellulose concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). .....	175
6-6	Seasonal changes in nitrogen concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). .....	176
6-7	Seasonal changes in phosphorus concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). .....	177
6-8	Seasonal changes in ash concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). .....	178
7-1	Weekly average air temperature (Avg.) and average of weekly maximum (Max.) and minimum (Min.) air temperatures for 2010-2011, 2011-2012, and 2012-2013 at the experimental location (Citra, FL).....	198
7-2	Weekly total rainfall for 2010-2011, 2011-2012, and 2012-2013 at the experimental location (Citra, FL).....	199
7-3	Effect of days after a freeze event on biomass harvested of two perennial grass entries in three years. ....	200
7-4	Effect of days after a freeze event on dry matter (DM) concentration of two perennial grass entries in three years.....	201
7-5	Effect of days after a freeze event on leaf proportion of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). ....	202
7-6	Effect of days after a freeze event on neutral detergent fiber (NDF) concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower).....	203
7-7	Effect of days after a freeze event on acid detergent fiber (ADF) concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower).....	204

7-8	Effect of days after a freeze event on acid detergent lignin (ADL) concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower).....	205
7-9	Effect of days after a freeze event on cellulose concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower).....	206
7-10	Effect of days after a freeze event on hemicellulose concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower).....	207
7-11	Effect of days after a freeze event on N concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). ....	208
7-12	Effect of days after a freeze event on ash concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower).....	209

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In the southeastern USA, warm-season grasses elephantgrass (*Pennisetum purpureum* Schum.) and energycane (*Saccharum* spp. hybrid) are recognized for their biomass production and therefore are candidates for cellulosic biofuel crops. Targeted to seasonality of biomass production, the research objectives were: 1) determine the effect of harvest management (multiple vs. single and fall vs. winter) of two grasses (two elephantgrasses, 'Merkeron' and a breeding line UF-1, and energycane 'L79-1002') on biomass yield and composition; 2) assess their morphological and chemical changes throughout the growing season; and 3) investigate effects of delayed harvest after a killing freeze. Delaying a single harvest from fall to winter or harvesting twice per year increased the effective harvest period of biomass for the refinery. However, multiple harvests per year compromised long-term biomass production. Energycane biomass decreased 41% from Year 2 to 3 due to damage from sugarcane smut (*Sporisorium scitamineum*). Delaying harvest from fall to winter reduced the leaf percentage and increased the biomass dry matter concentration. Harvest frequency affected

compositional quality of grass biomass. A single fall harvest of elephantgrass maximized the concentration of cellulose in total biomass. In contrast, energycane increased soluble sugar concentration late in the growing season resulting in a decrease in structural carbohydrate concentration. Later harvests were associated with lesser leaf percentage in total biomass and this caused N, P, and ash to decrease for single harvests. The elephantgrasses had more favorable morphological characteristics (greater height, tiller mass, and stem proportion) for biomass production than energycane. Cell wall constituents increased until late summer and then either remained relatively constant (UF-1) or increased slightly (Merkeron). In contrast, energycane cell wall constituents decreased after they peaked in summer. Delayed harvest after a freezing event increased the duration of the biomass harvesting period with varying consequences. Elephantgrass biomass yield decreased and composition changed to a greater degree than energycane when harvest was delayed, and composition was sensitive to weather conditions after the first freeze event. Decreasing leaf proportion in elephantgrass caused N and ash concentration to decrease during the delayed harvest period. Elephantgrass UF-1 showed excellent feedstock characteristics and is a candidate for cultivar release.

## CHAPTER 1 INTRODUCTION

Emissions of CO<sub>2</sub> associated with burning fossil fuels contribute to increased atmospheric CO<sub>2</sub> concentration (de la Mata et al., 2012). Greenhouse gas (GHG) emission from fossil fuel combustion is believed to be a major cause of climate change (Cheng, 2010). This association of fossil fuel use with climate change has stimulated research to identify other sources of energy. Moreover, environmental consequences of continued large-scale use of fossil fuels for energy provide impetus for research aimed at development of bioenergy technologies that mitigate GHG emissions and slow climate change (Wedin, 2004).

The justification for bioenergy research is compelling for economic, national security, and environmental conservation reasons. From a national energy resource perspective, turmoil in the Middle East and large fluctuations in energy prices illustrate long-term economic and security risks of dependence on imported oil. The high cost of petroleum imports appears “here to stay” as yearly average Brent Crude oil price has remained  $\geq$  \$60 per barrel since 2006 (U.S. Energy Information Administration, 2013). Bioenergy and its related products have potential for significant economic and environmental benefits to society including near-zero net emissions of GHG, improved soil and water quality, and increased enterprise opportunities for a depressed rural economy (McLaughlin et al., 2002).

Greater interest in dedicated bioenergy crops, however, has stimulated debate of food versus fuel. If demand for biofuel feedstock is such that crop land is converted to production of biofuels, there could be significant disruption of food supply and increasing food cost (Mathews, 2008). Mathews (2008) referred to this devastating

scenario as “a crime against humanity”, especially in developing countries. A single filling of a SUV’s fuel tank with ethanol requires enough grain (450 pound of corn) to feed a person for a year (The Economist, 2007). If due to demand for biofuels farmers were to plant bioenergy feedstocks instead of cereal crops, it would affect the cost of even rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (Tenenbaum, 2008). For instance, since 2010 more than 40% of domestic corn (*Zea mays* L.) use in the USA, up to one hundred sixty million Mg (five billion bushels) each year, has been diverted to fuel alcohol production (USDA Economic Research Service, 2012). The USDA Economic Research Service data also show that in 2010 domestic corn use for fuel alcohol exceeded animal feed and residual use. It is believed that converting corn to bio-ethanol is one of reasons for recent ‘Agflation’, i.e., inflation in cost of agricultural commodities.

To address both energy and food security, fuel production from non-food resources is rising. “Breaking the Biological Barriers to Cellulosic Ethanol”, the U.S. Department of Energy (DOE) publication, states that “fuels derived from cellulosic biomass – the fibrous, woody, and generally inedible parts of plant matter—offer an alternative to conventional energy sources that support national economic growth, national energy security, and environmental goals” (Department of Energy, 2006a). Cellulosic biomass is an attractive energy feedstock in the southeastern USA because supplies are abundant, it is renewable, and its use for energy does not add previously stored soil C to the atmosphere (Fargione et al., 2008). So-called 2nd generation biofuel crops (cellulosic biomass) are thought to offer less competition with food crops for land use as well as improved energy efficiency and lower GHG emissions (Erisman et al.,

2010). Non-arable or marginal land (2 Gha worldwide) is suitable for production of 2nd generation energy crops if management is such that the soils are protected from erosion (Strezov et al., 2008). In addition, perennial energy crop cultivation has been associated with significantly improved soil health and quality (Strezov et al., 2008).

Florida is well positioned to capitalize on the national need for biofuel crops. Florida ranks first in the USA in annual growth of plant biomass because its climate conveys unique advantages for bioenergy crop production. Specifically, the state experiences relatively few frost events during mild winters. In addition, precipitation is reasonably well-distributed throughout the year (Smith and Dowd, 1981). The development of production systems for high yielding energy crops that can be grown in Florida is considered essential for establishment of a sustainable biomass to energy industry.

Transportation cost is high for biomass relative to energy-dense materials like grain, so cellulosic biomass production must occur near the refinery to keep cost low (Brecht et al., 2011). Thus, long-term availability of sufficient amounts of reasonably priced biomass will be an important determinant of if, and where new biofuel and bioenergy facilities will be built.

Because of their C4 carbon fixation pathway and upright growth habit, warm-season grasses like elephantgrass (*Pennisetum purpureum* Schum.) and energycane (*Saccharum* sp.) are widely recognized for their biomass production (Woodard and Prine, 1991; Woodard and Prine, 1993a). Research initiatives to identify herbaceous plants with the highest biomass yields for renewable energy purposes have consistently found elephantgrass and energycane to have the highest or near the highest DM

production across the southeastern USA. Yields of 20 to 48 Mg ha<sup>-1</sup> yr<sup>-1</sup> have been reported in the region (Prine et al., 1984; Woodard and Prine, 1993a; Bouton, 2002; Woodard and Sollenberger, 2008). These species grow rapidly and establish canopy cover quickly so that less effort and fewer inputs are required to achieve appropriate weed control. Moreover, they can be propagated using internodes of above-ground stems, which requires relatively less labor than the rhizome-based vegetative propagation of *Miscanthus* (*Miscanthus giganteus*), and it also allows for propagation without genetic variation. Although they are regarded as important candidate species for bioenergy, only a few field-based experiments and morphological studies have been conducted on these species as bioenergy crops.

Plant biomass production is affected by soil nutrient quantity and availability. High-yielding bioenergy crops remove relatively large amounts of nutrients at harvest. Soil fertility of sandy soils in Florida is notoriously low, and replenishing nutrients removed at harvest of biomass crops is an important challenge to development of sustainable production systems. Nitrogen is poorly held by sandy soils in Florida and can be easily leached by rainfall (Obreza, 2003). Nutrient dynamics and particularly N dynamics have not yet been evaluated for many potential biofuel crops in Florida. It has been suggested that perennial crops have a large capacity for storage of nutrients, and harvest timing may impact N dynamics of bioenergy crops, particularly when harvest is delayed until after physiological maturity (Lewandowski and Heinz, 2003). There is currently no information available that describes these relationships for elephantgrass or energycane in Florida.

For most candidate bioenergy grasses being evaluated in Florida, there is limited information on chemical composition as related to their potential for use as a feedstock. Chemical composition such as structural and non-structural carbohydrates and lignin has a major effect on the utility of biomass for various purposes, thus more information is needed on composition characteristics of various species and the effect of harvest management.

A key to addressing the feedstock productivity and composition issues of the future is developing systems that combine the best plant species and cultivars with environmentally and ecologically sustainable production and harvest management practices. The proposed studies to be included in this dissertation will build on previous work conducted in the Southeast USA to provide additional information needed to identify such systems. More specifically, the research reported in this dissertation was conducted to address the following objectives: 1) determine the effect of harvest management of elephantgrass and energycane on biomass yield and composition when plants are managed as bioenergy feedstock; 2) assess morphological changes of elephantgrass and energycane throughout the growing season; and 3) determine the effect of delayed harvest after a freeze event on biomass harvested and composition.

## CHAPTER 2 LITERATURE REVIEW

### **Why Are Warm-Season Grasses Considered for Use as Bioenergy Feedstocks?**

#### **Physiological Characteristics of Warm-Season Grasses**

Many warm-season grasses have been considered for use as cellulosic biofuel feedstock due in part to their C4 photosynthetic pathway. In C4 plants, bundle sheath and mesophyll cells surround the vascular bundle and cause a wreath-like appearance (Kranz anatomy). This arrangement creates a physical barrier between initial carbon fixation in the mesophyll and the activity of the Calvin Cycle in the bundle sheath. In addition, there is a CO<sub>2</sub>-concentrating mechanism associated with C4 plants that results in a large CO<sub>2</sub>-concentration gradient between mesophyll cells and the binding site with Rubisco in the bundle sheath cell, resulting in C4 plants having up to two times greater water- and N-use efficiency than C3 plants (Jakob et al., 2009). Although this procedure requires two more ATPs to regenerate phosphoenolpyruvate (PEP) from pyruvate, the plant achieves a far greater advantage by minimizing or eliminating the occurrence of photorespiration. This allows these plants to synthesize sugars more efficiently than C3 plants, at least at current atmospheric CO<sub>2</sub> concentrations (380 ppm) (Langdale, 2011).

Harvested biomass of C4 grasses in the US Gulf Coast region can total 20 to 40 Mg ha<sup>-1</sup> yr<sup>-1</sup>, depending upon species, growing environment, and management (Woodard and Prine, 1993a; Anderson et al., 2008a). Among the perennial grasses that have been evaluated in the region, elephantgrass [*Pennisetum purpureum* (L.) Schum.] and energycane (*Saccharum* spp. hybrid) both take advantage of the C4 pathway and have been among the most productive species in regional trials (Prine et al., 1984; Woodard and Prine, 1993a; Woodard and Sollenberger, 2008).

## Elephantgrass

Elephantgrass or napiergrass is indigenous to equatorial Africa in areas of rainfall exceeding 1000 mm. It is in the tribe Paniceae of the Poaceae (Panicoideae) family (Hanna et al., 2004). Elephantgrass is a robust, creeping rhizomatous plant that perennates in the tropics and subtropics. It was introduced into the USA in 1913 (Thompson, 1919). The northern limit for survival and adequate yield under production-type management is where the lowest temperature does not drop below -7 to -9°C (Woodard and Sollenberger, 2008). In the USA, this is roughly a line across extreme southern Georgia, Alabama, and Mississippi, the southern half of Louisiana, and the southeastern portion of Texas (Woodard and Sollenberger, 2008). Plants produce many large tillers with 20 or more internodes ranging in length from 20 to 25 cm, a diameter of up to 3 cm, and a height of up to 7 m (Hanna et al., 2004). Because of its yield potential, it is considered a very promising grass for bioenergy. It has been reported that elephantgrass yield can reach 5 to 10 Mg ha<sup>-1</sup> yr<sup>-1</sup> when plants are unfertilized, 15 to 30 Mg ha<sup>-1</sup> yr<sup>-1</sup> in well-fertilized pastures (Bogdan, 1977), and 70 to 85 Mg ha<sup>-1</sup> yr<sup>-1</sup> in environments with year-round growing conditions and optimal management (Vicente-Chandler et al., 1959).

In the USA, breeding efforts with *Pennisetum* spp. have occurred primarily in Georgia and Florida, resulting in numerous breeding lines and cultivars. The elephantgrass cultivar 'Merkeron' is an F1 hybrid of a cross made in Georgia between a very leafy dwarf type and a tall selection (Burton, 1989). It is short-day sensitive and reaches a height of 4 to 5 m in autumn when it flowers. It is considered an excellent cultivar for bioenergy production. Merkeron has produced 172 Mg ha<sup>-1</sup> yr<sup>-1</sup> of green forage in Puerto Rico (Burton, 1989). In Florida field trials, DM yield of 16 to 32 Mg ha<sup>-1</sup>

yr<sup>-1</sup> has been reported (Woodard et al., 1991b). Other elephantgrass breeding lines from the Georgia program include N13, N43, and N51.

In Florida, evaluation of promising *Pennisetum* spp. plant introductions and breeding lines has occurred over several decades (Woodard and Prine, 1991; Woodard et al., 1991b; Spitaleri et al., 1994; Macoon et al., 2001; Macoon et al., 2002). During this period, S.C. Schank led breeding efforts in Florida with *Pennisetum* spp. including elephantgrass and its interspecific hybrids with pearl millet [*Pennisetum glaucum* (L.) R. Br.] (Schank and Diz, 1991). During this time and prior to his death in 1997, Dr. Schank also developed a number of breeding lines of elephantgrass. In 2000, Dr. Gordon M. Prine chose several outstanding elephantgrass lines at the Green Acres Agronomy Farm in Gainesville, FL from what had been the breeding nursery of Dr. Schank. These breeding lines were established by Dr. Prine in a grass nursery at the University of Florida Plant Science Research and Education Unit (PSREU) at Citra, FL. The parentage of these lines has not been identified, and there is no evidence in the literature that they were evaluated under production. One of the breeding lines, now called UF-1, was observed in the nursery in 2006 by Dr. K.R. Woodard and Dr. L.E. Sollenberger, and because of its vigorous appearance was selected for evaluation in subsequent experiments, including those reported in this dissertation. Currently, UF-1 is being considered for potential cultivar release.

## **Energycane**

Modern sugarcane (*Saccharum* spp.) is thought to originate from hybridization among four species; *S. officinarum*, *S. sinense*, *S. barberi*, and *S. spontaneum*. Cultivated sugarcane is predominately outcrossing, very heterozygous, and vegetatively

propagated (Bischoff et al., 2008). It is a tall perennial tropical grass, up to 4 m or more, with thick stems up to 5 cm in diameter (James, 2003).

At the USDA-ARS Sugarcane Field Station in Canal Point, FL, inter-specific crosses between sugarcane and other species are made with the goal of incorporating desirable traits such as ratooning ability, growth vigor, cold tolerance, and disease resistance from wild relatives into breeding lines of sugarcane as a part of a breeding program (Wang et al., 2008). These characteristics are associated with high biomass production, and when hybrids have a high concentration of cellulose instead of sucrose they are a potentially valuable feedstock resource for cellulosic ethanol production. This is why high cellulose *Saccharums* are called “energycanes” (León et al., 2012). To better understand *Saccharum* spp. as a biofuel crop, it is useful to note that there are three distinctive types. These include sugarcane (primarily sugar, conventional sugarcane), Type I energycane (sugar and fiber), and Type II energycane (primarily fiber).

Cultivar L79-1002 is a Type II energycane developed by Louisiana State University with USDA-ARS and American Sugar Cane League, Inc. (Tew and Cobill, 2008). The female parent was ‘CP52-68’ and the male parent was ‘Tainan’ (Bischoff et al., 2008). L79-1002 can be cropped in colder regions than the current sugarcane growing areas, particularly those areas where freezing of above-ground tissue is desired in order to achieve desiccation prior to harvest. This cultivar has high fiber and biomass yield, 18.0 Mg ha<sup>-1</sup> of dry biomass from fiber and 6.6 Mg ha<sup>-1</sup> from extractives for a total of 24.6 Mg ha<sup>-1</sup>, outstanding ratooning ability, and vigorous growth (Bischoff et al., 2008; Tew and Cobill, 2008)

## **Composition of Bioenergy Grasses and Conversion to Ethanol**

### **Composition of Perennial Grasses**

A unique feature of plant versus animal cells is the presence of a rigid cell wall. The primary constituents of plant cell walls are cellulose, hemicellulose, pectin, lignin, and proteins. Plant cell wall is composed of long crystalline cellulose microfibrils embedded in a matrix of other polysaccharides (Vermerris, 2008a). Cellulose is regarded as the most abundant biopolymer on Earth. Within the fibril of cellulose, two glucose units are aligned such that they are 180° rotated, i.e.,  $\beta$  (1,4)-linked dimers of D-glucoses called cellobiose (Perez et al., 2002; Vermerris, 2008a). Hemicellulose in grasses contains glucuronoarabinoxylans (GAXs) that have a xylose backbone and are the predominant hemicellulosic polysaccharide in cell walls of the grass family (Vermerris, 2008a). Lignin is an aromatic polymer synthesized from phenylpropanoid precursors (Perez et al., 2002), and lignin negatively affects the yield of fermentable sugars by shielding cellulose from degradation by providing a surface that cellulolytic enzymes adsorb to irreversibly (Akin, 2007; Vermerris, 2008a). Given the abundance of cellulose and hemicellulose in plant cell walls of perennial grasses, they represent a major source of carbohydrate for conversion to energy.

Cellulose and hemicellulose are polysaccharides that can be hydrolyzed to sugars and then fermented to ethanol, but lignin cannot be used in fermentation processes (Cherubini, 2010). For the conversion to ethanol, the cellulose and hemicellulose in biomass must be broken down into their correspondent monomers; hexoses and pentoses (Kumar et al., 2009). The cascade conversion procedures can be categorized as pretreatment, enzymatic hydrolysis (saccharification), and ethanol fermentation (Lu and Mosier, 2008). It is important to know compositional properties of

lignocellulosic biomass because feedstock response to pretreatment, saccharification, and fermentation are controlled by properties of the feedstock. Monomers from cellulose and hemicellulose can be analyzed by the Laboratory Analytical Procedures (LAP) established by the National Renewable Energy Laboratory (NREL). Those procedures quantify sugar monomers from extractives (non-structural carbohydrates) and structural carbohydrates, and they measure acid soluble and acid insoluble lignin (Sluiter, 2008a; Sluiter, 2008b).

### **Fiber Analysis of Biomass**

Analyses of chemical composition and ethanol yield, such as those included in the NREL procedures, are very laborious and time consuming (Han et al., 2012). This is why cost-effective methods are proposed for screening a large number of lines or seasonal changes in feedstock composition. Concentration of plant components resistant to acid detergent and neutral detergent have been used for decades to predict relative digestibility and intake of forages (Van Soest et al., 1991). These analyses have relevance to bioenergy research because biological degradation by the ruminant is somewhat similar to saccharification and fermentation in bioethanol production (Han et al., 2012). Lignin concentration is not able to be estimated correctly using the detergent fiber analysis because it measures only acid-insoluble lignin which usually underestimates actual lignin concentration (Jarchow et al., 2012). According to a recent study, 95% of actual ethanol yield can be explained by a regression model consisting of neutral detergent fiber (NDF) and NDF digestibility (NDFD) (Lorenz et al., 2009a). This research also showed that NDF alone is negatively correlated with actual ethanol yield. Cellulose (acid detergent fiber [ADF] minus acid detergent lignin [ADL]) and

hemicellulose (NDF-ADF) concentrations are also highly correlated with theoretical ethanol potential at 91 and 51%, respectively (Lorenz et al., 2009b).

### **Theoretical Ethanol Potential and Yield**

It is hard to estimate efficiency of overall fermentation procedures on a commercial scale because numerous hydrolysis methods or combinations are available, each with specific advantages and disadvantages and different reaction yields (Lu and Mosier, 2008). To estimate ethanol yield, a reasonable formula for ethanol yield potential is needed. For those reasons, theoretical ethanol potential (TEP) has been proposed to estimate maximum achievable ethanol potential from a feedstock. The ethanol potential is defined as the amount of ethanol that is able to be produced from the post-enzymatic hydrolysis broth, assuming 100% fermentation efficiency, as a percentage of the amount of ethanol that could theoretically be produced from the original feedstock (Department of Energy, 2006b). The TEP can be estimated by i) using NDF and ADF analysis with subsequent estimation of cellulose and hemicellulose concentration or ii) total structural carbohydrate concentration using the NREL procedure. After that, theoretical ethanol yield (TEY) can be estimated through combining TEP with biomass yield data.

### **Ash and Mineral Elements**

Grass maturity and soil nutrients affect not only biomass yield but N and ash concentrations (Adler et al., 2006). Desirable composition of biomass for bioenergy is dependent upon the post-harvest conversion processes used. Unlike forages for livestock, minimizing N and ash in biofuel feedstock is desirable (Waramit et al., 2011). This is why investigating ash and macro-elemental mineral concentration is essential for characterizing compositional quality of biomass feedstock.

## **Management Effects on Perennial Bioenergy Grasses**

### **Logistical Issues and Field Management in the Biofuel Industry**

In the biofuel industry, one barrier to utilization is cost of logistics (Rentizelas et al., 2009). Unlike petroleum, many types of biomass are characterized by seasonal variation in availability as they are harvested only at a specific optimal time to maximize yield, but the power plant must operate on a year-round basis to optimize efficiency (Rentizelas et al., 2009). Although this limitation can be partially solved by using storage strategies, any storage method involves additional logistics and cost. Harvest management is one option for achieving superior seasonal distribution of biomass without large increases in cost. If the harvest window can be widened, it would improve efficiency of operation of processing plants.

Delaying harvest until after aboveground shoots of miscanthus (*Miscanthus giganteus*) were killed by freezing reduced moisture and increased leaf senescence leading to decreases in ash and N concentrations (Jørgensen and Sander, 1997; Lewandowski and Kicherer, 1997). Harvest management of reed canarygrass (*Phalaris arundinacea* L.) in Iowa and Wisconsin affected biomass composition; more mature biomass had greater concentrations of fiber, reduced digestibility, and lower protein than less mature biomass. In that research, autumn-harvested biomass from a two harvests per year system had higher protein and digestibility and lower cell wall concentration than a single harvest in autumn. It allowed feedstock to remain in the field and improved feedstock quality by decreasing Cl, K, P, and Ca, but increasing Si.

### **Harvest Frequency and Seasonality Affect Biomass Yield**

Harvest frequency and timing affect grass performance. It has been shown, for example, that different harvest management practices can affect biomass yield and

quality. With delayed harvest in southern Germany, miscanthus biomass yield decreased by 14 to 15% from December to February and an additional 13% decrease occurred from February to March along with a significant decrease in water concentration, ash, N, Cl, and S (Lewandowski and Heinz, 2003). Delaying harvest from October to April of the following year decreased switchgrass (*Panicum virgatum* L.) yields 40% in Pennsylvania (Adler et al., 2006); however, only 10% of the yield reduction was caused by decreased tiller mass and 90% resulted from the inability of harvest equipment to pick up the biomass in the field.

Woodard et al. (1991) investigated the effect of harvest frequency on tall perennial grasses in Florida. They observed a significant decrease in overall biomass yield of elephantgrass and energycane by increasing harvest frequency. The magnitude of yield reduction differed by number of harvests. For instance, three harvests per year decreased yield 35% compared with a single harvest, but two harvests per year decreased yield only 12% compared with a single harvest. Two-year averages for yield of one, two, and three harvests per year treatments were 25.6, 22.5, and 14.2 Mg ha<sup>-1</sup>, respectively (Woodard et al., 1991b). Their study indicated that elephantgrass and energycane may tolerate two harvests per year with only modest decrease in yield, so this may be a useful strategy to increase the duration of the harvest period.

In addition to direct effects of defoliation on plant regrowth vigor and production, timing of defoliation can affect the degree to which perennial grasses mobilize mineral nutrients from shoots to roots or rhizomes for storage at the end of growing season (Somerville et al., 2010). This can be important because nutrient storage affects long-term survival of perennial grasses (Chaparro et al., 1995).

## Harvest Frequency and Seasonality Affect Composition of Perennial Grasses

Seasonality of harvest and harvest frequency affect plant maturity and cell wall concentration and composition. They affect leaf:stem ratio because stem accumulates as length of regrowth period increases and leaves senesce at the end of growing season (Chaparro et al., 1995; Macoon et al., 2001). As a result, delaying harvest can decrease N concentration and increase dry matter concentration and may be practical if the bioenergy crop is resistant to degradation. In this case, field-stored feedstock could be provided to the processing plant on a 'just-in-time' basis, saving on storage cost (Heaton et al., 2009). The effect of harvest frequency occurs in part because of its impact on leaf:stem ratio and the fact that structural composition of leaf and stem are very different and are affected differently by maturation.

For the C3 reed canarygrass, a single harvest in fall or winter resulted in NDF (706 mg g<sup>-1</sup> DW) and ADF (400 mg g<sup>-1</sup> DW) concentrations that were greater than a double harvest treatment (spring + fall; NDF of 591 mg g<sup>-1</sup> DW and ADF of 308 mg g<sup>-1</sup> DW) (Tahir et al., 2011). For sweet sorghum (*Sorghum bicolor* L. Moench) in northern coastal China, cellulose and hemicellulose concentrations ranged from 206 to 265 g kg<sup>-1</sup> DW and 159 to 191 g kg<sup>-1</sup> DW, respectively, in stems at anthesis, after which they decreased until grain maturity (40 d after anthesis) (Zhao et al., 2009). However, there was little information about the effect of harvest management on structural composition changes of warm-season grasses.

Seasonality of harvest of warm-season grasses was investigated in Iowa (Waramit et al., 2011). In this study, the concentration of cellulose in switchgrass and big bluestem (*Andropogon gerardii* Vitman) increased with increasing maturity; the average early- and late-season concentrations were 250 and 398 mg g<sup>-1</sup> DW,

respectively in a 2-yr study. There was a clear difference among species in cellulose concentration during the late season; concentrations for switchgrass were  $365 \text{ mg g}^{-1}$  DW while those for big bluestem were  $430 \text{ mg g}^{-1}$ .

Average NDF, ADF, and ADL concentrations in elephantgrass were relatively consistent in previous research. In a study with elephantgrass in Ethiopia, NDF, ADF, and ADL concentrations were 616, 326, and  $36 \text{ mg g}^{-1}$  DW, respectively (Tessema and Baars, 2004). This result was similar to NDF, ADF, and ADL concentrations of separated leaf and stem of 'Merkeron' elephantgrass where leaf concentrations were 694, 360, and  $30 \text{ mg g}^{-1}$ , respectively, and stem concentrations were 742, 481, and  $69 \text{ mg g}^{-1}$ , respectively (Anderson et al., 2008a).

### **Fertilizer Management**

Fertilizer management of bioenergy grasses is particularly important because in order for biomass to be a viable energy source production costs must be low. In Louisiana, L79-1002 energycane was fertilized with N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O levels of 0-0-0, 179-0-0, and 179-67-134 kg ha<sup>-1</sup>. Yield was affected by fertilization treatment with yield totals of 16, 25.4, and 25.2 Mg ha<sup>-1</sup>, respectively (Bischoff et al., 2008). In Florida, when applied N rate was almost double that of the Louisiana study (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O; 336-85-166 kg ha<sup>-1</sup>) the 2-yr average yield of L79-1002 ( $20.7 \text{ Mg ha}^{-1}$ ) from a single harvest each year was similar to that observed in Louisiana. In Georgia, L79-1002 energycane yield was above  $20 \text{ Mg ha}^{-1}$  during the first 3 yr without fertilizer application, but it declined to  $8.1 \text{ Mg ha}^{-1}$  in Year 4. In contrast, Merkeron elephantgrass yielded above  $30 \text{ Mg ha}^{-1}$  during first 2 yr and then declined to 11.2 and  $7.1 \text{ Mg ha}^{-1}$  in the third and fourth years (Knoll et al., 2012). These studies show that fertilizer inputs are an important yield determinant for energycane and elephantgrass. Lower-input systems are possible, but yields will be

lower in these systems and some level of fertilizer input is essential to maintain persistence and acceptable biomass yield.

### **Regional Adaptation**

Planting site significantly affects biomass yield of elephantgrass and energycane. Regional micro-climate and soil properties may influence biomass yield and compositional quality. From Ona, FL to Auburn, AL, 3-yr average yield of energycane averaged 33.9 to 24.2 Mg ha<sup>-1</sup>, respectively, however, elephantgrass showed a greater yield decline when it was planted at the cooler site in Alabama (46.7 at Ona to 18.6 Mg ha<sup>-1</sup> at Auburn) (Woodard and Prine, 1993b). At three locations within Florida, both elephantgrass and energycane yield was above 30 Mg ha<sup>-1</sup> without any difference by site in the first year (Fedenko, 2011). However, in the same study, site differences occurred in the second growing season with highest yields in high organic matter soils in Belle Glade, FL compared with Spodosols at Ona, FL and Entisols in Citra, FL. The author suggested that Florida is an ideal region for production of tall warm-season perennial grasses. However, even if sites are within a subtropical climate, performance of perennial grasses varies due to regional differences in climate and soil fertility.

### **Nitrogen-Use Efficiency and Concentration in Biomass**

#### **Importance of N in Grass-based Systems**

Nitrogen is often the essential mineral element that crops require in greatest amount, and it represents a major production cost and environmental concern in US agriculture. Nitrogen is a major constituent of enzymes including those involved in photosynthesis (Heaton et al., 2009). Sources of N for plant growth include N mineralization from soil organic matter, N fixation from legumes, N fertilizer, and a number of minor N inputs, such as lightning, to agricultural systems. Nitrogen fertilizers

are produced from industrial N fixation, specifically the Haber-Bosch process. Industrial N fixation alone accounts for almost 2% of all human energy uses (Carroll and Somerville, 2009). High N concentration is valued in feed for livestock, but in contrast, greater N concentration is associated with greater emission of environmentally harmful substances during combustion of biofuel crops (Lewandowski and Heinz, 2003). While not beneficial in fuel production, N is critical to crop growth and development (Heaton et al., 2009). Consequently, it is critical to minimize N input in biomass production while maintaining biomass yield.

### **Seasonal Dynamics of N in Perennial Grasses**

Rhizomatous perennial grasses can mobilize mineral nutrients from above-ground organs into roots and rhizomes at the end of growing season (Carroll and Somerville, 2009). It has been proposed that harvest management can be used as a tool to increase the degree to which N is recycled from above-ground growth to storage organs for use the next season (Lewandowski and Heinz, 2003). These authors proposed that delaying harvest to February decreased biomass harvest 14 to 15%, but the biomass had significantly less water and N than that harvested in December. In a study with switchgrass, it was observed that spring harvest of the previous season's growth decreased biomass yield compared with fall; however, biomass macro-elemental concentrations including N decreased with delayed harvest (Adler et al., 2006). Another switchgrass experiment showed that N concentration peaked in June ( $12.4 \text{ g kg}^{-1}$ ), significantly decreased by November ( $3.9 \text{ g kg}^{-1}$ ), and remained relative constant until April ( $3 \text{ g kg}^{-1}$ ) (Wilson et al., 2013a).

Changes in N concentration differ by plant part from early season to harvest period. Nitrogen is largely withdrawn from senescing leaves before abscission, and

used for the next growing season (Van Heerwaarden et al., 2003). Cellular constituents are systematically broken down and transported out of senescing organs, mostly leaves, which allow > 50% of leaf N to be reallocated by the plant (Van Heerwaarden et al., 2003). For instance, it has been shown that reallocable N content is decreased in miscanthus in early winter compared to late summer by decreasing the proportion of green and dead leaves and increasing leaf litter (Heaton et al., 2009). This research suggested that N in dropped leaves is contributed and may become incorporated into the soil organic matter pool, thus potentially improving N recycling during the next season. The N concentration of miscanthus and giant reed (*Arundo donax L.*) were studied in the United Kingdom (Smith and Slater, 2011). Although miscanthus leaf N concentration decreased rapidly during November before leaf abscission, miscanthus and giant reed leaf N concentration was higher than in their canes. If reallocable N from leaf and stem are measured separately over the season, this information may aid in determining the ideal harvest season for optimal N use efficiency.

#### N-use Efficiency by Quantification of Isotopic N ( $^{15}\text{N}$ )

Nitrogen-use efficiency in perennial grasses may be affected by applied N rate. For instance, apparent N uptake (there was no zero N control in the experiment) in energycane shoots was 72% when  $336 \text{ kg N ha}^{-1}$  was applied and 95% for  $168 \text{ kg N ha}^{-1}$  (Mislevy et al., 1995). This study also showed that the  $336 \text{ kg N ha}^{-1}$  rate did not result in a biomass yield increase but did increase N concentration in plant tissue compared with  $168 \text{ kg N ha}^{-1}$ . Greater N concentration is considered desirable in grasses used for forage, but in the case of biomass for bioenergy, greater N concentration either has no benefit (when biomass is fermented) or it can negatively affect the conversion process (when biomass is used for combustion). Although many studies have been conducted to

determine N recovery in forage grasses (Martha et al., 2004), there is almost no N-use efficiency or N cycling information available for bioenergy grasses. Such work is needed because N inputs to biomass production must be minimized in order to keep production costs low. It may be possible to investigate N fertilizer removal and carryover using isotopic N ( $^{15}\text{N}$ ) to separate applied N from various other sources such as carryover, atmospheric deposition, mineralization of soil organic matter, and biological fixation.

### **Morphology of Elephantgrass and Energycane**

#### **Importance of Morphology in Bioenergy Crops**

As was mentioned earlier, harvesting frequency and timing play key roles in determining biomass yield and composition. Basically, the principle of harvesting frequency and timing for biomass is similar to that for defoliation management in livestock-related grassland studies. For example, Chaparro et al. (1995) reported that defoliation frequency and height affected not only biomass but also morphological development in 'Mott' dwarf elephantgrass. This study suggested that infrequent harvests allowed the grass to restore leaf area and reserves, but these benefits were accompanied by lower leaf:stem ratio (Chaparro et al., 1995).

Although most defoliation studies have been conducted with perennial forage grasses, it is also important to understand physiological and morphological responses to harvest management of perennial grasses used for biofuel. Leaf area index (LAI), stalk number, canopy development, spatial distribution, and proportion of leaf are important responses to be assessed (Madakadze et al., 1998; Trócsányi et al., 2009; León et al., 2012). In a study with switchgrass in southern Quebec, Canada, LAI reached a maximum in August (6.1 to 8) and decreased during the remainder of the growing season (Madakadze et al., 1998). This pattern was also observed with elephantgrass

and energycane in Florida (Woodard et al., 1993). In elephantgrass, LAI increased from 1.4 at 30 d after staging to 7.1 at 105 d after staging before gradually decreasing to 3.4 at 245 d after staging. In energycane, LAI similarly increased to 6.9 by 161 d after mowing and then decreased to about 4. In Florida, there was a significant positive relationship between canopy height and dry biomass, with  $r^2$  values of 0.94 for elephantgrass and 0.89 for energycane (Woodard and Prine, 1993b).

### **Seasonal Dynamics of Perennial Grass Morphology**

Morphological change can be used as an indicator of timing of harvest in warm-season grasses. Stems are the most important organ for bio-ethanol production, and they generally constitute the highest proportion of total aboveground dry weight. For example, sweet sorghum stems composed 56 to 73% of aboveground biomass followed by leaf dry weight in the range of 19 to 33% (Zhao et al., 2009) and a very small proportion of inflorescence. Yield dynamics over the season are primarily associated with changes in proportion of the harvestable part (standing tiller) and non-harvestable part (residue); 21% of switchgrass biomass was left in the field after machine harvest in the fall but the amount left behind increased to 45% for a winter harvest (Adler et al., 2006). Interestingly, harvested leaf and panicle weight decreased but harvested stem weight increased in winter (Adler et al., 2006). Thus, leaf and stem proportions vary by season and harvest management (Zhao et al., 2009). Because cost of biomass transportation is a critical factor in the bioenergy industry, harvest timing is most effective when less valuable plant organs (leaves and inflorescence), which have low bulk density and high mineral element concentration, are left in the field. This goal can be achieved by investigating seasonal dynamics of morphological factors.

## **Carbon Sequestration of Perennial Grasses**

### **Role of Perennial Grass Cropland for C Sequestration**

One of the anticipated benefits to the use of biofuels is a reduction in GHG emissions relative to that associated with the use of petroleum. Bioenergy crops fix atmospheric CO<sub>2</sub> which is released when crops are burned (Vermerris, 2008b). However, there is still a net emission of GHG associated with the post-harvesting procedure, transportation, and field management (Vermerris, 2008b). One other component that needs to be accounted for in determining the net carbon emission of bioenergy crops is soil C sequestration. It is very important to understand that perennial grasses capture atmospheric CO<sub>2</sub> not only in harvested above-ground plant biomass but also in the below ground and the soil C pools.

The soil C pool is a function of the dynamic equilibrium between C gains and losses from the system under a specific land use (Lemus and Lal, 2005). Increased sequestration of soil organic C (SOC) is a potentially crucial strategy for offsetting CO<sub>2</sub> emission to the atmosphere (Lemus and Lal, 2005). The potential of perennial bioenergy grasses to offset CO<sub>2</sub> emission through soil C sequestration is dependent on the rate of soil C additions, long-term capacity of soil for C storage, and the stability of sequestered soil C overtime (McLaughlin et al., 2002). Candidate bioenergy grasses have advantages that favor soil C sequestration including perenniality, high biomass production, and a deep root system which can increase soil C compared with an annual crop (Ma et al., 2000b; Lemus and Lal, 2005). Moreover, bioenergy crops can be grown on marginal and non-arable land where soil erosion and degradation occur (Lemus and Lal, 2005). For instance, if forest and prairie grassland are converted to cropland for 1<sup>st</sup> generation biofuel crop production, the time required to restore C loss associated with

land conversion ranges from 17 to 423 yr; however, conversion of marginal and abandoned cropland to 2<sup>nd</sup> generation biofuel crop production does not result in large initial SOC loss nor require a long time to restore SOC to the pre-conversion level (Fargione et al., 2008). If we choose the right location (i.e., degraded cropland or marginal land) to produce 2<sup>nd</sup> generation biofuel crops, the criticism could be mitigated that using cropland for biofuels sacrifices the potential C benefit due to C emissions associated with the land-use change (Searchinger et al., 2008).

### **Importance of Studying Warm-season Grassland C Sequestration in Florida**

Soil degradation can be associated with disruptions such as erosion and runoff, which expose SOC to oxidative processes (Follett, 2001). In perennial grasses, the large active pool of root biomass is a major source of rhizosphere deposition and fine root turnover, sequestering C in the soil. In a switchgrass study where it was used in a Conservation Reserve Program (CRP) in South Dakota, there was no significant effect of harvest treatments (once every year vs. alternate years) on SOC but source of N fertilizer affected SOC deposition rate, with 2.4 and 4 Mg C ha<sup>-1</sup> yr<sup>-1</sup> deposited for NH<sub>4</sub>NO<sub>3</sub>-N and manure, respectively (Lee et al., 2007b). This study also indicated that there were no increases in SOC without N application.

In contrast, N application (0, 112, and 224 N kg ha<sup>-1</sup>) did not affect root weight density in switchgrass in Alabama (Ma et al., 2000a). The authors indicated that the majority of root biomass was distributed in the surface soil (0-15 cm), with proportions of 90 and 68% of total C in the top 15 cm for samples taken from intra-row and inter-row locations, respectively. A switchgrass study in the Dakotas and Nebraska showed SOC change varied considerably, ranging from -0.6 to 4.3 Mg C ha<sup>-1</sup> yr<sup>-1</sup> with average increase across sites of 1.0 Mg C ha<sup>-1</sup> yr<sup>-1</sup> for the 30-cm depth in a 5-yr study (Liebig et

al., 2008). This study showed that rate of change in SOC varied by site, indicating that it is important to assess changes of SOC associated with various management systems in Florida. Moreover, most SOC studies with bioenergy grasses have been done with switchgrass as the model crop. It is also necessary to investigate the change of SOC associated with use of tall sub-tropical perennial grasses.

It has been suggested that the potential mechanism responsible for SOC accrual is an N-induced increase in root mass and subsequent turnover of roots (Fornara and Tilman, 2012). Likewise, it has been argued that increases in C sequestration by perennial grass roots will be due to increased root biomass rather than increased C concentration in the root (Ma et al., 2000a). Research with grazed 'Tifton 85' bermudagrass (*Cynodon* spp.) pastures in Florida showed that soil C concentration increased linearly as the height of post-graze residual stubble increased from 8 to 24 cm over 2 yr of grazing (Liu et al., 2011). In the same pastures, greater Tifton 85 post-graze stubble height and rate of N fertilizer resulted in a linear increase in particulate organic C and total soil C and N in the < 53- $\mu$ m soil particle size fraction (Silveira et al., 2013). Thus, there are data indicating a role of management in C sequestration under forage grasses in Florida, but there are currently no published data with perennial bioenergy grasses. These data are needed to help assess the overall C budget associated with growth of perennial grasses for biofuel.

## CHAPTER 3 BIOMASS HARVESTED AND PLANT-PART PROPORTION RESPONSES OF PERENNIAL BIOENERGY GRASSES TO HARVEST MANAGEMENT

### **Overview of Research**

Greenhouse gas (GHG) emissions associated with fossil fuel combustion are believed to be a major cause of climate change (Cheng, 2010). This association of fossil fuel use with climate change has stimulated research to investigate alternative energy sources. From a national energy resource perspective, the USA is heavily dependent on imported petroleum, and the combination of political turmoil in major production regions and large fluctuations in oil prices illustrate long-term economic and security risks of this dependence.

To increase energy security while minimizing the impact on food supply, use of non-food resources for energy production is rising. The US Department of Energy (DOE) and the US Department of Agriculture (USDA) have estimated that 1.2 billion metric tons of biomass can be produced per year in the USA and about 30% of this production could be biomass from perennial grasses (342 million metric tons) (Perlack, 2008). Lignocellulosic biomass is believed to offer less competition with food crops for existing cropland as well as improved energy efficiency and low GHG emissions (Erisman et al., 2010). Cellulosic biomass from perennial grasses, especially those which utilize the C4 photosynthetic pathway, is an attractive biofuel feedstock in the Gulf Coast region of the USA because supplies are abundant, they are renewable, and these grasses have high photosynthetic efficiency (Knoll et al., 2012).

Because of their C4 carbon fixation pathway and upright growth habit, elephantgrass (*Pennisetum purpureum* Schum.) and energycane (*Saccharum* spp. hybrid) are widely recognized for their biomass production in the southeastern USA

(Woodard and Prine, 1991; Woodard and Prine, 1993a). Biomass yields of 20 to 48 Mg ha<sup>-1</sup> yr<sup>-1</sup> have been reported for these species in the region (Prine et al., 1984; Woodard and Prine, 1993a; Bouton, 2002; Woodard and Sollenberger, 2008).

Although regional biomass production is high, a logistical challenge facing processing plants that convert feedstock to fuel is seasonality of biomass production. This results in uneven supply of feedstock to the conversion facility and limits efficiency of operation. Storage of biomass is one alternative, but storage increases overall cost of energy production. A preferable option would be field management practices that address the seasonality of biomass supply. Harvest management affects biomass yield (Woodard and Prine, 1991) and could possibly be used to improve distribution of biomass to the refinery. Additionally, different grass species or taxa within species may be better adapted to flexible harvest management, but there is relatively little information available describing such differences for elephantgrass and energycane. The objectives of this study were to quantify the effects of elephantgrass and energycane harvest frequency and timing on biomass yield, leaf:stem ratio, and dry matter (DM) concentration, and to compare the potential for use in biomass production systems of an elephantgrass breeding line with that of the current predominant cultivar.

## **Materials and Methods**

### **Experimental Site**

The experiment was conducted during 2010, 2011, and 2012 at the Plant Science Research and Education Unit (PSREU) at Citra, FL (29.41° N, 82.17° W). The soil was a well-drained Candler sand (hyperthermic, uncoated Lamellic Quartzipsamments). Initial soil characterization of topsoil (0-20 cm) showed an average soil pH of 7.0, and Mehlich-1 extractable P, K, Mg, and Ca of 54, 20, 123, and 496 mg

kg-1, respectively. These concentrations are considered to be high for P, very low for K, and very high for Mg. Monthly average, maximum, and minimum temperatures (Figure 3-1) and monthly precipitation (Figure 3-2) are shown for the experimental period. Temperature and rainfall data during the study are from the PSREU weather station that is part of the Florida Automated Weather Network (<http://fawn.ifas.ufl.edu/>). The 30-yr average data are for Gainesville, Florida and were reported by the Florida Climate Center (<http://climatecenter.fsu.edu/>).

### **Treatments and Experimental Design**

The treatments were all factorial combinations of three grass entries and three harvest management practices. Each treatment was replicated four times in a split-plot arrangement of randomized complete block design. Harvest treatment was the main plot and grass entry was the sub-plot.

The three grass entries included two elephantgrasses, 'Merkeron' (Burton, 1989) and a breeding line referred to as UF-1, and 'L79-1002' energycane (Bischoff et al., 2008). The two grass species were chosen because earlier work with biomass feedstock identified them as having the greatest potential for use in this region (Woodard et al., 1991a; Woodard and Prine, 1993a; Bouton, 2002). Merkeron elephantgrass and L79-1002 energycane are also widely available cultivars of these two species. Breeding line UF-1 was included because preliminary research had demonstrated its potential (Sollenberger et al., 2011), and larger-scale plot work was needed to compare it with existing feedstock options and to provide data to guide decisions on potential cultivar release.

Three harvest management treatments were imposed that included different frequencies and timing of harvest. These were i) two harvests per year (2X; one in

summer with a ratoon harvest before first freeze in fall), ii) one harvest per year in fall (1X-Nov; before first freeze and at initiation of flowering of Merkeron, generally the first of the entries to flower), and iii) one harvest per year in winter (1X-Dec; within 1 wk after first freeze, with a freeze defined as a temperature of less than 0°C at 2 m above soil level). Harvest dates for the three treatments are shown in Table 3-1. The 1X-Nov treatment was considered to be a control because most data reported in the literature for these species are from experiments harvested once at the end of the growing season but before a freeze event. The 2X treatment was included to evaluate plant responses to more frequent harvest that would increase the period during which biomass could be supplied to the biorefinery. The winter (1X-Dec) treatment was imposed to evaluate timing of harvest, specifically the effect of delaying harvest until after a freeze event vs. harvest prior to freezing temperature (1X-Nov). If 1X-Dec treatment proves viable it would allow extension of the harvesting period into the winter season.

### **Plot Establishment and Management**

Plots contained six rows of 6-m length, with 1-m spacing between rows. Plots were established using mature above-ground stem pieces planted on 15 Dec. 2009. Thus, the 2010 data are from the establishment year of the crops, and 2011 and 2012 data are from established stands. In all 3 yr, N was applied as ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) at a rate of 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>, and K was applied as muriate of potash (KCl) at a rate of 90 kg K ha<sup>-1</sup> yr<sup>-1</sup>. Nutrients were split applied, with applications of 50 kg N and 45 kg K ha<sup>-1</sup> in mid-April and 100 kg N and 45 kg K ha<sup>-1</sup> in mid-May. No P was needed based on soil test. Elephantgrass is responsive to N at rates above those used in this study (Vicente-Chandler et al., 1959), but the intent was to select a rate that may

more nearly represent what will be considered practical within a production context. Limited irrigation was applied to the experiment only at sign of significant drought stress (leaf rolling). Water was applied using a traveling gun system. There were five irrigation events in 2010 totaling 60 mm, three irrigation events in 2011 totaling 50 mm, and there was no irrigation applied in 2012.

### **Response Variables**

At harvest, a 4-m portion of one of the two middle rows was cut using a brush cutter to a 12-cm stubble height to determine biomass harvested. To minimize border effects, the 1-m portion at the end of the harvested row was not part of the yield sample. The harvested area in each plot was thus considered to be 4 m<sup>2</sup>. All material from the harvested portion of row was weighed fresh in the field and then subsampled to determine DM concentration and to calculate DM harvested. In 2010 and 2011, four additional representative tillers from each plot were hand-separated into leaf (blade and sheath) and stem (including inflorescence, if present) components and dried to determine leaf:stem ratio. After the biomass sample was collected, the remaining area of the plot was clipped to the target stubble using a disk mower.

Persistence was characterized after the third year of defoliation. Plots were cleared after November and December 2012 harvests, but mild early-winter weather stimulated regrowth and provided opportunity to quantify persistence on 24 Jan. 2013. After 3 yr of growth it was not always possible to distinguish individual plants, so the measure of persistence chosen was percentage of row length per plot without viable tillers. A segment of row was included in the total length having no tillers only if it was longer than 30 cm. Areas devoid of tillers were measured within the middle four rows of each plot, i.e., a total of 24 m of row length.

## Statistical Analysis

Data were analyzed using mixed-model methods in PROC MIXED (SAS Institute, 2008). In all models, harvest treatment and grass entry were considered fixed effects. Year was considered a repeated measurement (fixed) for all responses except persistence because this response was quantified only at the end of the experiment. Because harvest treatment was the main plot in the randomized complete block design, block and block  $\times$  harvest treatment interaction were considered random effects. Means were compared using the pdiff test of LSMEANS. All means reported in the text are least squares means and were considered different if  $P \leq 0.05$ . If statistical analysis did not detect difference ( $P > 0.05$ ) but it showed meaningful information, it is defined as a trend.

## Results and Discussion

### Biomass Harvested

There were grass entry  $\times$  year and harvest management  $\times$  year interactions for total annual biomass harvested (Table 3-2). The entry  $\times$  year interaction occurred primarily because of poor performance of energycane in the third year. Elephantgrass UF-1 outyielded energycane by 34% in 2011 and by 70% in 2012, while in the establishment year of 2010 there was only a trend ( $P = 0.158$ ) toward greater biomass harvested by UF-1 (11.3%; Table 3-3). Merkeron yield was 28% lower than UF-1 in 2011, but it was not different in 2012 and only tended to be lower ( $P = 0.158$ ) in 2010. Removing energycane from the analysis, there was no entry  $\times$  year interaction for the two elephantgrasses, and across the 3-yr study period UF-1 biomass harvested averaged  $28.0 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  compared with 24.3 for Merkeron ( $P = 0.010$ ). Merkeron biomass harvested was not affected by year, while both energycane and UF-1

elephantgrass had lowest biomass in 2012 (Table 3-3). The third-year decrease in biomass harvested was more pronounced in energycane than for either of the elephantgrasses.

The harvest management  $\times$  year interaction occurred because there was no difference in biomass harvested due to harvest management in 2010 and 2011, but by 2012 the 1X-Dec treatment produced 47% more biomass than the 2X treatment (Table 3-4). Within a harvest management treatment, there was no effect of year for 1X-Dec, but both 2X and 1X-Nov treatments produced less biomass in the third year than in either of the first 2 yr. In a previous 3-yr study, Merkeron elephantgrass yielded an average of 26.8 Mg ha<sup>-1</sup> yr<sup>-1</sup> of dry biomass when harvested once yr<sup>-1</sup> (Woodard and Prine, 1991), relatively similar to the 3-yr average of 24.3 Mg ha<sup>-1</sup> in the current study. Woodard and Prine (1991) reported that Merkeron biomass harvested gradually decreased from 31.9 (Year 1) to 21.4 Mg ha<sup>-1</sup> yr<sup>-1</sup> (Year 3), unlike in the current research where biomass harvested decreased only from 24.8 (2010) to 22.8 Mg ha<sup>-1</sup> yr<sup>-1</sup> (2012). In the previous work, 3-yr average yields were reduced by 19 and 33% for two- and three-harvest yr<sup>-1</sup> treatments, respectively, when compared with a single harvest (Woodard and Prine, 1991). There were no differences among harvest frequency treatments in plant survival in their study, however, so it seems likely that the treatment effect on biomass harvested must have been due to reduction in average leaf area index and canopy light interception associated with multiple harvests yr<sup>-1</sup>. No known previous studies have evaluated the effect of timing of a single harvest, so the greater yield stability across years of the 1X-Dec vs. 1X-Nov treatment has not been reported before this experiment.

The large quantity of biomass harvested from perennial bunchgrasses has been attributed to their ability to continue biomass accumulation over an extended growing season (Woodard and Prine, 1993b). Although they found that LAI peaked 150 d after staging and then decreased over time, elephantgrass and energycane canopies continued to intercept more than 80% of photosynthetically active radiation (PAR) for 250 d after the initial mowing (Woodard et al., 1993). This is possible in subtropical regions where first freeze is delayed well into the fall (NOAA, 2013), extending the biomass accumulation period. A factor that may affect yield potential of UF-1 is that unlike Merkeron and L79-1002, which flowered in early November, UF-1 maintained vegetative growth until the time when winter freeze killed above ground biomass in each of the 3 yr of this study. Additionally, some tillers (~10%) of Merkeron flowered in April to May 2011 and 2012. This is thought to be due to an early cessation to freezing temperatures in those years resulting in early onset of plant growth which allowed some tillers to be of sufficient size to respond to short days and initiate flowering. No tillers of UF-1 flowered in either spring. This response of Merkeron may decrease net biomass yield by reducing tiller vegetative growth.

The 10 Mg ha<sup>-1</sup> decrease in energycane biomass harvested from 2011 to 2012 was associated with severe damage from the fungal disease 'sugarcane smut' (*Sporisorium scitamineum*). Although severity of sugarcane smut damage was not rated quantitatively, it appeared visually that there was much greater presence of smut by the third year (2012). Previously in Louisiana, L79-1002 was rated as 'moderately susceptible', however, that evaluation was during the establishment year when disease infection was relatively low (Bischoff et al., 2008). An energycane selection study

showed that L79-1002 had a greater number of stools (bunches) and whips (classic symptom of the disease is the production of a black whip-like structure from the central core of the meristematic tissue) in comparison with other energycane genotypes, indicative of high level of smut susceptibility (León et al., 2012). Affected plants may tiller profusely but with poor cane formation and small narrow leaves (Ramesh Sundar et al., 2012), thus a greater number of tillers is not an indicator of fungal resistance. It is believed that since apical meristems of tillers are infected by the disease (Ramesh Sundar et al., 2012), apical dominance is broken and the plant produces many small but often non-viable tillers. Further breeding effort for energycane smut disease resistance is needed.

### **Leaf:stem Ratio**

There were entry  $\times$  year ( $P < 0.001$ ) and harvest management  $\times$  year ( $P = 0.002$ ) interactions for leaf:stem ratio (Table 3-2). Leaf:stem ratio was measured only in 2010 and 2011, but in both years UF-1 elephantgrass had the lowest ratio (0.31-0.41; Table 3-5). The entry  $\times$  year interaction occurred because there was no difference between Merkeron and L79-1002 in 2010, but in 2011, L79-1002 energycane had greater leaf:stem ratio than either Merkeron or UF-1 elephantgrasses. For all grass entries, leaf:stem was greater in 2011 than in 2010, but the difference between years was greatest for energycane (Table 3-5).

Harvest management  $\times$  year interaction occurred because leaf:stem ratio was quite low in the July harvest of the 2X treatment in 2010 (0.37) vs. 2011 (0.63), while differences between years were less pronounced for the other harvest management treatments (Table 3-6). In both years the greatest leaf:stem occurred in the November harvest of the 2X treatment (0.60-0.70), while the least was observed with the 1X-Dec

treatment (0.24-0.33). This was because the growth period of the ratooning plants after summer harvest was only 100 to 107 d, so their lower leaves showed minimal senescence and stems were relatively young.

In both 2010 and 2011, leaf:stem ratio decreased over the growing season. This was caused by a significant decrease in LAI during fall (Chapter 5), associated with senescence of lower leaves and resultant loss in leaf biomass, and by increasing stem DM over the season. In a Florida study in which elephantgrass was harvested at 1.2, 2.5, 3.7, and 4.9 m of growth, green leaf percentage decreased from 50% (1.2-m tall) to 10% (4.9 m) (Mislevy et al., 1989). The current research showed a trend similar to that observed by Mislevy et al. (1989).

Many studies in which elephantgrass leaf:stem ratio have been quantified have targeted forage production and imposed multiple harvests per year (Mislevy et al., 1989; Chaparro et al., 1995; Williams and Hanna, 1995; Hanna et al., 2004). Experiments with 'Mott' dwarf elephantgrass showed that leaf:stem ratio was negatively correlated with plant DM production (Williams and Hanna, 1995). The same authors reported that low leaf:stem ratio for Merkeron was due to stem internode composing a greater proportion of the plant. In the current study, there was no apparent relationship between biomass yield and leaf:stem ratio (Tables 3-4, 3-6) suggesting that the pattern of morphological development of full-season bunchgrass growth does not vary greatly among entries (Chapter 5) but does differ from that of bunchgrasses cut frequently for forage.

The application of forage studies to biofuel production is limited because greater leaf:stem ratio, which is desired for forage production, is not preferred for biomass feedstock for several reasons. First, the physical form of biomass has the greatest

influence on the cost of transportation (Sokhansanj et al., 2009), and the greater bulk density of the stem fraction allows more mass of material to be transported per unit of transport volume. Higher proportion of leaf in L79-1002 means less bulk density, thus L79-1002 likely will require higher transportation cost than elephantgrass entries, if it is transported as raw material. Moreover, leaf:stem ratio affects biomass quality. Leaf is reported to have greater concentration than stem of components considered to be unfavorable (e.g., ash, Cl, Si, and N) in biofuel conversion especially for combustion (Lewandowski and Heinz, 2003).

### **Biomass Dry Matter Concentration**

Dry matter concentration was affected by harvest management  $\times$  entry ( $P < 0.001$ ) and harvest management  $\times$  year ( $P < 0.001$ ) interactions. Harvest management  $\times$  entry interaction occurred because there were no differences among entries in dry matter concentration for 1X-Nov and 1X-Dec harvest treatments, but there were differences among entries for the 2X treatment at both harvests (Table 3-7). In July for the 2X treatment, elephantgrasses had or tended to have greater dry matter concentration than energycane, while in 2X for November energycane had greater dry matter concentration than either of the elephantgrass entries (Table 3-7).

The harvest management  $\times$  year interaction occurred because in 2010 the 1X-Dec management resulted in greater biomass dry matter concentration than 1X-Nov, and 1X-Nov had greater dry matter concentration than either of the 2X harvests (Table 3-8). In contrast, 1X-Dec and 1X-Nov were not different in 2011 and 2012, but in both of those years 1X-Dec and 1X-Nov harvest treatments had greater biomass dry matter concentration than either harvest of the 2X treatment. In 2 of 3 yr, the July harvest of the 2X management had the lowest dry matter concentration (Table 3-8).

There are two primary factors that appeared to affect dry matter concentration in the current study, plant maturity and rainfall pattern. The effect of maturity is supported in the literature. For example, when five C4 grasses were harvested at either 8 or 12 wk following seedling emergence (Costa and Gomide, 1991), dry matter concentration of 12-wk herbage averaged  $36 \text{ g kg}^{-1}$  greater than that of 8-wk herbage. In Puerto Rico, dry matter concentration of five C4 grasses increased from  $\sim 200 \text{ g kg}^{-1}$  after 35 d of regrowth to  $240 \text{ g kg}^{-1}$  after 45 d to  $270 \text{ g kg}^{-1}$  after 55 d (Mendez-Cruz et al., 1988). Spitaleri et al. (1995) reported that dry matter concentration of *Pennisetum* hybrids increased from  $187$  to  $228 \text{ g kg}^{-1}$  as regrowth interval increased from 6 to 12 wk. This response to maturity likely explains greater dry matter concentration of single vs. multiple harvest treatments in current experiment. Additionally, in the current study elephantgrass began growth earlier in the spring than energycane leading to earlier physiological maturation of elephantgrass plants and greater elephantgrass dry matter concentration than energycane for the summer harvest of the 2X treatment. This pattern of response did not carry over to the fall harvest of the 2X treatment or to 1X-Nov and 1X-Dec treatments (table 3-7).

Greatest dry matter concentration of the 1X-Nov and 1X-Dec treatment and the November harvest date of the 2X treatment occurred in 2012. These concentrations were associated with no measureable rainfall during November 2012 (Figure 3-1). Greatest November rainfall during the experiment occurred in 2011 (47 mm), approaching the 30-yr average of 52 mm, and this was associated with lowest dry matter concentration of the 1X-Dec treatment in 2011. These data suggest that amount of rainfall during the fall affects dry matter concentration of standing tillers. Dry matter

concentration is particularly important for biofuel feedstock because lesser moisture concentration reduces transportation cost and cost of additional drying that may be required during post-harvest processing.

### **Persistence**

Starting in 2011, portions of row devoid of tillers began to appear in some plots, so proportion of planted row without tillers was quantified after the 2012 growing season. There was harvest management x entry interaction for persistence ( $P = 0.009$ ; Table 3-2). In the 2X harvest management, L79-1002 had the greatest proportion of row without tillers present (32%; Table 3-9), while 1X-Nov and 1X-Dec harvest management of the same entry had gaps of 19 and 17%, respectively. Greater loss in the 2X treatment suggests either that more frequent harvest makes L79-1002 more susceptible to smut or simply that twice per year harvesting negatively affects energycane persistence. The response of elephantgrass entry UF-1 contrasted with that of energycane in that it had loss of only 9% of row with the 2X treatment but losses were 18 to 19% for the 1X-Nov and 1X-Dec treatments. Unlike other entries, Merkeron persistence was unaffected by harvest treatment and losses were not greater than 6%.

Some elephantgrasses, including PI 300086, are susceptible to winter kill especially under multiple harvest management (Prine et al., 1988). Following 2 yr of defoliation, PI 300086 had 59, 8, and 8% stand survival for defoliation frequencies of 1, 2, and 3 harvests  $\text{yr}^{-1}$ , respectively; comparable percentages for Merkeron were 84, 75, and 81, respectively (Woodard and Prine, 1991). Thus our observation of excellent persistence of Merkeron is supported by previous results. Although UF-1 had greater gaps between tillers than Merkeron for treatments harvested once per  $\text{yr}^{-1}$ , average biomass harvested of UF-1 across the 3 yr was greater than Merkeron and in 2012

there was no difference in biomass harvested between elephantgrasses. Thus the degree of importance for UF-1 of the greater proportion of row without viable tillers than Merkeron is not totally clear at this point. It may be that UF-1 has greater ability for tiller size-density compensation, allowing it to overcome the apparent disadvantage of greater gaps without tillers. Based on results of the current study, it is not possible to totally separate the impact of smut disease and defoliation frequency on energycane persistence, but it appears that both contributed significantly.

### **Implications of Research**

Delaying a single harvest until after a freeze event or harvesting twice per year increased the effective harvest period of biomass and would therefore likely improve seasonal distribution of biomass to the biorefinery. Under the conditions of this experiment, however, there was a 42% reduction in biomass harvested for the 2X treatment in the third year (averaged across entries) suggesting that increasing harvest frequency may compromise long-term biomass production. This reduction was associated with a loss of stand for L79-1002 energycane, but stands of UF-1 and Merkeron harvested twice per year remained at greater than 90% through the third year. Energycane biomass decreased 41% from Year 2 to Year 3, due in significant measure to infestation with sugarcane smut disease. Smut-resistant energycane cultivars will be required if this species is to provide a sustainable biomass supply. Delaying harvest until after a freeze event reduced the leaf percentage in harvested biomass and increased the biomass dry matter concentration. These factors can contribute to reduced transportation cost of biomass to the biorefinery, but it is not yet known how long after a freeze event the unharvested biomass can remain in the field without incurring major dry matter losses. Breeding line UF-1 elephantgrass has excellent yield

potential and possesses characteristics that make it an effective alternative to currently used grasses for biomass production. Evaluation under a broader range of management practices is warranted to further assess its long-term persistence under defoliation.

Table 3-1. Harvest dates for 2X (summer and fall ratoon), 1X-Nov, and 1X-Dec harvest management treatments in 2010, 2011, and 2012.

Year	Harvest management		
	2X-July (summer)	1X-Nov and 2XNov (ratoon)	1X-Dec
2010	30 July	10 November	9 December
2011	21 July	8 November	15 December
2012	24 July	7 November	28 November

Table 3-2. Sources of variation and levels of probability (*P*) for their effects on response variables reported in Chapter 3.

Sources of variation	Total biomass harvested	Leaf:stem ratio	Dry matter concentration	Persistence <sup>†</sup>
Harvest management (HM)	0.635	< 0.001	< 0.001	0.577
Entry (E)	< 0.001	< 0.001	0.421	< 0.001
Year (Y)	< 0.001	< 0.001	< 0.001	----
HM × E	0.749	0.296	< 0.001	0.009
HM × Y	0.002	0.002	< 0.001	----
E × Y	< 0.001	< 0.001	0.171	----
HM × E × Y	0.380	0.110	0.394	----

<sup>†</sup> Persistence measured only at the end of the experiment, so year was not part of the model.

Table 3-3. Grass entry × year interaction ( $P < 0.001$ ) effect on biomass harvested. Data are means across four replicates and three harvest treatments ( $n = 12$ ).

Entry	Year		
	2010	2011	2012
	-----Mg ha <sup>-1</sup> -----		
L79-1002	24.8 a <sup>†</sup> A <sup>‡</sup>	24.2 aB	14.2 bB
Merkeron	24.8 aA	25.3 aB	22.8 aA
UF-1	27.6 bA	32.5 aA	24.1 cA
SE		1.98	

<sup>†</sup> Year means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>‡</sup> Entry means within a year not followed by the same upper case letter are different ( $P < 0.05$ )

Table 3-4. Harvest management × year interaction ( $P = 0.002$ ) effect on biomass harvested of three perennial grasses. Data are means across four replicates and three species ( $n = 12$ ).

Year	Harvest management <sup>†</sup>		
	2X	1X-Nov	1X-Dec
	-----Mg ha <sup>-1</sup> -----		
2010	27.0 a <sup>‡</sup> A <sup>§</sup>	24.3 aA	25.9 aA
2011	29.0 aA	25.2 aA	27.8 aA
2012	16.9 bB	19.3 abB	24.8 aA
SE		1.69	

<sup>†</sup> Harvest management treatments were twice per year (July and November; 2X), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within a year not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Year means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 3-5. Grass entry × year interaction ( $P < 0.001$ ) effect on biomass leaf:stem ratio. Data are means across four replicates and three harvest treatments ( $n = 12$ ).

Entry	Year		P value
	2010	2011	
-----Leaf:stem ratio-----			
L79-1002	0.41 a <sup>†</sup>	0.70 a	< 0.001 <sup>‡</sup>
Merkeron	0.43 a	0.51 b	0.012
UF-1	0.31 b	0.41 c	< 0.001
SE	0.034		

<sup>†</sup> Grass entry means within a year not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>‡</sup> P value for year effect within a grass entry

Table 3-6. Harvest management × year interaction ( $P = 0.002$ ) effect on leaf:stem ratio of three perennial grasses. Data are means across four replicates and three species ( $n = 12$ ).

Year	Harvest management <sup>†</sup>			
	2X-July	2X-Nov	1X-Nov	1X-Dec
-----Leaf:stem ratio-----				
2010	0.37 b <sup>‡</sup>	0.60 a	0.32 bc	0.24 c
2011	0.63 a	0.70 a	0.50 b	0.33 c
P value	<0.001 <sup>§</sup>	0.004	<0.001	0.010
SE	0.034			

<sup>†</sup> Harvest management treatments were twice per year (July and November; 2X), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within a year not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> P value for year effect within a harvest management

Table 3-7. Grass entry × harvest management interaction ( $P < 0.001$ ) effect on biomass dry matter concentration at harvest. Data are means across four replicates and three years ( $n = 12$ ).

Entry	Harvest management <sup>†</sup>			
	2X-July	2X-Nov	1X-Nov	1X-Dec
	-----g dry matter kg <sup>-1</sup> fresh weight-----			
L79-1002	209 b <sup>‡</sup> B <sup>§</sup>	304 aA	318 aA	331 aA
Merkeron	236 bA	260 bB	338 aA	355 aA
UF-1	233 bAB	255 bB	326 aA	345 aA
SE		12.8		

<sup>†</sup> Harvest management treatments were twice per year (July and November; 2X), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ ).

<sup>§</sup> Entry means within a harvest treatment not followed by the same upper case letter are different ( $P < 0.05$ )

Table 3-8. Harvest management × year interaction ( $P < 0.001$ ) effect on biomass dry matter concentration of three perennial grasses. Data are means across four replicates and three species ( $n = 12$ ).

Year	Harvest management <sup>†</sup>			
	2X-July	2X-Nov	1X-Nov	1X-Dec
	-----g dry matter kg <sup>-1</sup> fresh weight-----			
2010	214 d <sup>‡</sup> B <sup>§</sup>	252 cB	315 bB	343 aB
2011	240 bA	256 bB	298 aB	316 aC
2012	225 cAB	311 bA	368 aA	373 aA
SE		8.4		

<sup>†</sup> Harvest management treatments were twice per year (July and November; 2X), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within a year not followed by the same lower case letter are different ( $P < 0.05$ ).

<sup>§</sup> Year means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 3-9. Harvest management x entry interaction ( $P = 0.009$ ) effect on proportion of row without viable tillers after 3 yr defoliation. Data are means across four replicates ( $n = 4$ )

Entry	Harvest management		
	2X	1X-Nov	1X-Dec
	-----%-----		
L79-1002	32 a <sup>†</sup> A <sup>‡</sup>	19 bA	17 bA
Merkeron	6 aB	5 aB	3 aB
UF-1	9 bB	19 aA	18 aA
SE		4.35	

<sup>†</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>‡</sup> Year means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

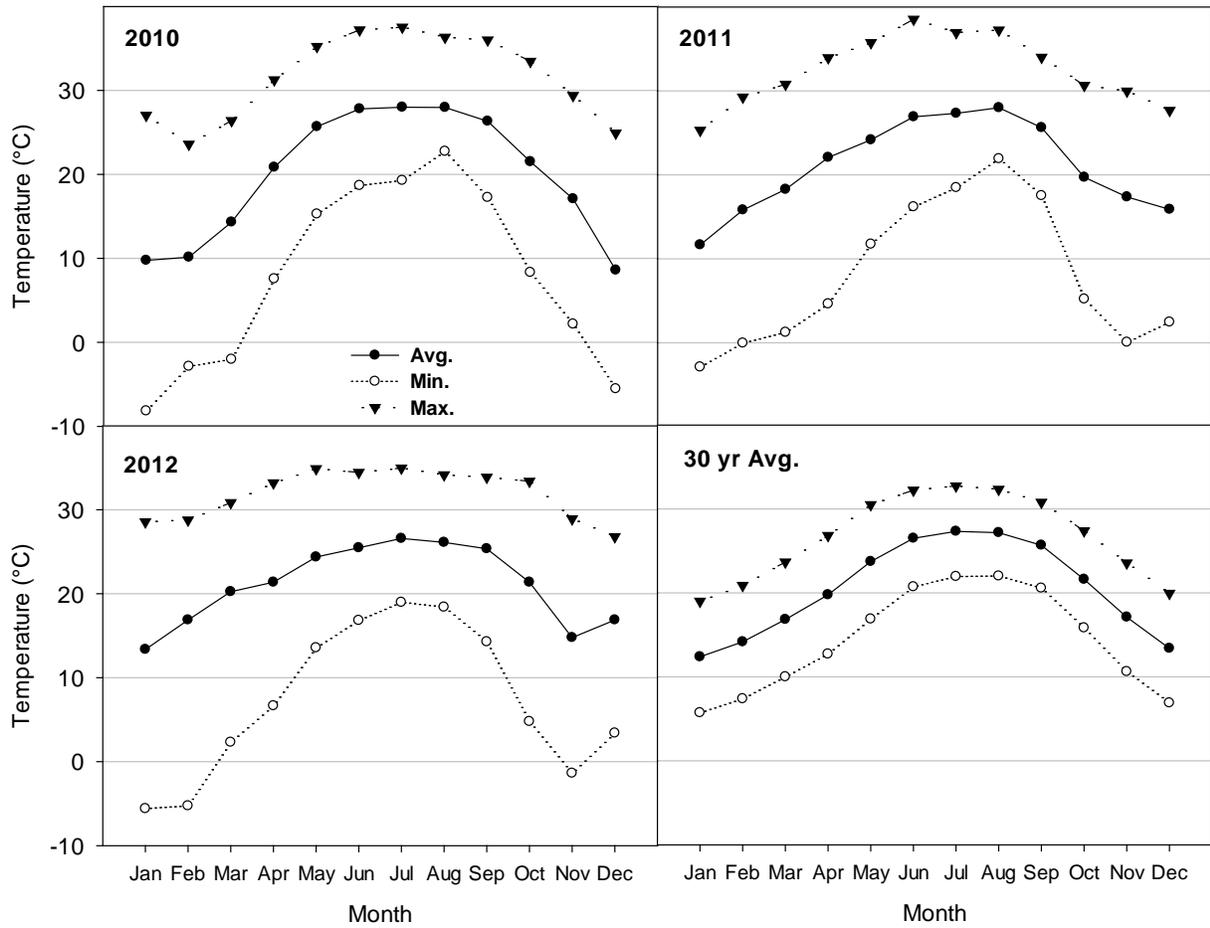


Figure 3-1. Monthly average and monthly maximum and minimum air temperatures for 2010, 2011, and 2012 for the experimental location, and the 30-yr average for Gainesville, Florida.

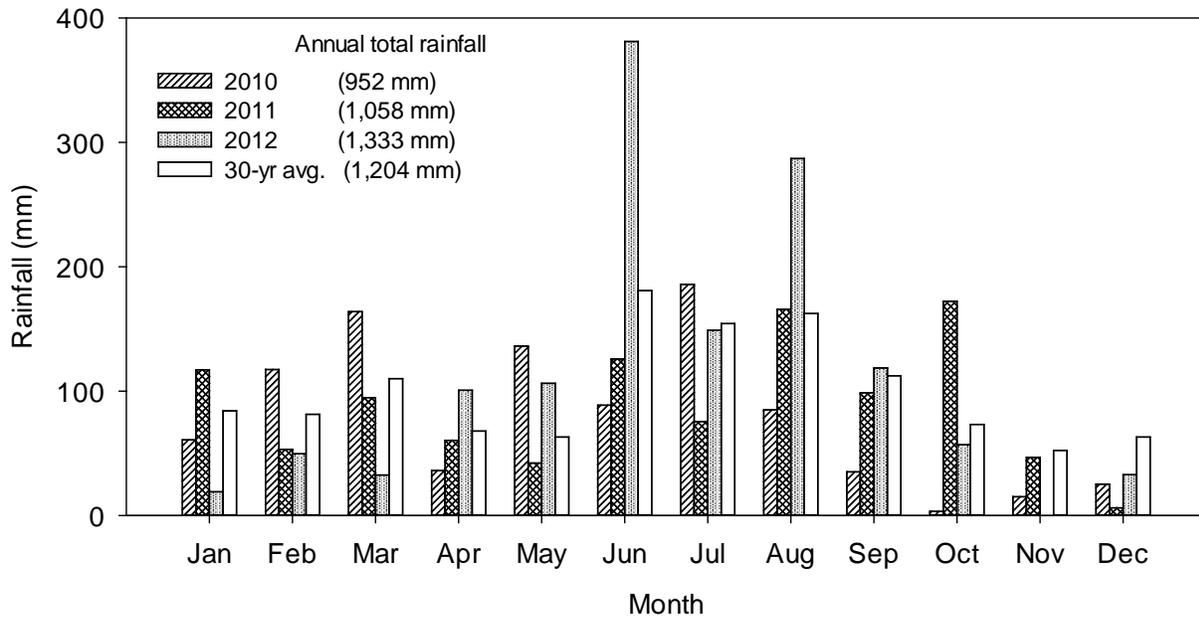


Figure 3-2. Monthly rainfall for 2010, 2011, and 2012 for the experimental location and the 30-yr average for Gainesville, Florida.

## CHAPTER 4 BIOMASS COMPOSITION RESPONSES OF PERENNIAL BIOENERGY GRASSES TO HARVEST MANAGEMENT

### **Overview of Research**

Chemical composition of cellulosic biomass feedstocks plays a key role in their conversion to biofuel and the efficiency of biofuel production (Lee et al., 2007a). Genotype x environment interaction exists and influences structural and chemical composition of lignocellulosic feedstocks (Lee et al., 2007a). Harvest management is known to be an important determinant of chemical composition of perennial grasses used for forage (Chaparro and Sollenberger, 1997), and it is reasonable to hypothesize that it affects composition of bioenergy feedstocks as well.

Plant biomass consists of three different types of polymers including cellulose, hemicellulose, and lignin, that are strongly bonded by non-covalent forces and by covalent cross-linkages (Perez et al., 2002). Cell wall is composed of long crystalline cellulose microfibrils embedded in a matrix of other polysaccharides (Perez et al., 2002). Grasses contain glucuronoarabinoxylans (GAXs) that have a xylose backbone and are the predominant hemicellulosic polysaccharide in cell walls of the grass family (Vermerris, 2008a). Given the abundance of cellulose and hemicellulose in cell walls of perennial grasses, they represent a major source of structural carbohydrate for conversion to energy. Lignin is a complex, amorphous, three-dimensional phenolic polymer that negatively affects the yield of fermentable sugars from cellulosic biomass. This occurs as a result of shielding cellulose from microbial degradation by providing a surface that cellulolytic enzymes adsorb to irreversibly (Akin, 2007; Vermerris, 2008a).

The proportions of cellulose and lignin in biomass affect yield of biochemical conversion processes. For instance, due to high lignin concentration in wood, 36% more

ethanol can be produced from switchgrass (*Panicum virgatum* L.) than from same weight of wood biomass (McKendry, 2002). Neutral and acid detergent fiber (NDF and ADF, respectively) and acid detergent lignin (ADL) procedures (Van Soest et al., 1991) are used frequently to characterize forage cell wall composition, and those methods can be applied to investigation of the compositional characteristics of cellulosic biomass to be used for biofuel. Limitations of the procedures include underestimation of total lignin and ADF may be contaminated by hemicellulose which causes an overestimation of cellulose and underestimation of hemicellulose (Morrison, 1980; Hatfield et al., 1994; Lowry et al., 1994; Jung et al., 1999; Jung and Lamb, 2004). Actual concentration of monomers from cellulose and hemicellulose can be analyzed by the Laboratory Analytical Procedures established by the National Renewable Energy Laboratory (NREL). Those procedures quantify sugar monomers from extractives (non-structural carbohydrates; soluble sugars) and structural carbohydrates, and they measure acid soluble and acid insoluble lignin (Sluiter, 2008a; Sluiter, 2008b).

Desirable composition of biomass for bioenergy is dependent upon the post-harvest conversion process used, and unlike the situation with forage, minimizing N and ash in biofuel feedstock is preferred (Lewandowski and Heinz, 2003; Waramit et al., 2011). High levels of N and/or ash can reduce outputs of thermochemical conversion (Shahandeh et al., 2011). Warm-season perennial grasses have up to two times greater N-use efficiency than C3 plants (Jakob et al., 2009), and their high production of biomass with low N concentration makes them important candidate bioenergy grasses.

Harvest management affects not only biomass yield but also its composition (Casler and Boe, 2003; Lewandowski and Heinz, 2003; Adler et al., 2006). Miscanthus

(*Miscanthus giganteus*) and giant reed (*Arundo donax L.*) showed a gradual decline in stem N concentration with increasing maturity in the UK (Smith and Slater, 2011). The decline in plant N with increasing maturity also has been associated with decreasing leaf proportion and much lower N concentration in stem than in leaf. Average switchgrass leaf N concentration was 13.5 mg g<sup>-1</sup> compared with 5.7 mg g<sup>-1</sup> for stem (Shahandeh et al., 2011). Similarly, giant reed and switchgrass leaf contained two-fold or greater N compared to stem, and a decline in N and P concentration occurred from October to December (Kering et al., 2012)

There are very limited data describing harvest management effects on chemical composition of grasses used as biofuel feedstock. The objectives of this research were to determine the effect of harvest management and grass entry on i) concentration of the Van Soest fiber fractions NDF, ADF, and acid detergent lignin (ADL), cellulose, and hemicellulose in biomass, ii) concentration of structural and non-structural carbohydrates and lignin in biomass determined using NREL methodology, iii) mineral and ash concentration in biomass, and iv) the relationship between ADL using the Van Soest technique and lignin concentration using the NREL method.

## **Materials and Methods**

### **Experimental Site**

The experiment was conducted during 2010 and 2011 at the Plant Science Research and Education Unit (PSREU) at Citra, FL (29.41° N, 82.17° W). The soil was a well-drained Candler sand (hyperthermic, uncoated Lamellic Quartzipsamments). Initial soil characterization of topsoil (0-20 cm) showed an average soil pH of 7.0, and Mehlich-1 extractable P, K, Mg, and Ca of 54, 20, 123, and 496 mg kg<sup>-1</sup>, respectively.

These concentrations are considered to be high for P, very low for K, and very high for Mg.

### **Treatments and Experimental Design**

Treatments were all the factorial combinations of three grass entries and three harvest management practices. Each treatment was replicated four times in a split-plot arrangement of a randomized complete block design. Harvest treatment was the main plot and grass entry was the sub-plot. The three grass entries included two elephantgrasses (*Pennisetum purpureum* Schum.) 'Merkeron' (Burton, 1989) and a breeding line referred to as UF-1, and 'L79-1002' energycane (*Saccharum* spp. hybrid) (Bischoff et al., 2008). These species were chosen because earlier work with biomass feedstocks identified them as having the greatest potential for use in this region.

Merkeron elephantgrass and L79-1002 energycane were the available cultivars of these two species. Breeding line UF-1 was included because preliminary research had demonstrated its potential (Sollenberger et al., 2011), and larger-scale plot work was needed to compare it with existing feedstock options and to provide data to guide potential cultivar release.

Three harvest management treatments were imposed that included differences in frequency and timing of harvest. These included i) two harvests per year (2X; first harvest in July, ratoon harvest in November before first freeze), ii) one harvest per year in fall (1X-Nov; before first freeze with timing based on flowering of Merkeron, generally the first entry to flower), and iii) one harvest per year in winter (1X-Dec; within 1 wk after first freeze, with a freeze defined as occurrence of temperature below 0°C at 2 m above soil level). The 1X-Nov treatment was considered a control because most data reported in the literature for these species are from experiments harvested once at the end of the

growing season but before a freeze event. The 2X treatment was included to evaluate plant responses to harvest frequency. The 1X-Dec treatment was imposed to evaluate timing of harvest, specifically the effect of delaying harvest until after a freeze event vs. harvest prior to freezing temperature (1X-Nov).

In 2010, harvests occurred on 30 July for 2X-July, 10 November for 1X-Nov and 2X-November, and 9 December for 1X-Dec. In 2011, harvest dates were 21 July for 2X-July, 8 November for 1X-Nov and 2X-Nov, and 9 December for 1X-Dec.

### **Plot Establishment and Management**

Plots contained six rows of 6-m length, with 1-m spacing between rows and were established using above-ground stem pieces planted on 15 Dec. 2009. Thus, the 2010 data are from the establishment year. In both years, N was applied as ammonium sulfate  $[(\text{NH}_4)_2\text{SO}_4]$  at a rate of  $150 \text{ kg ha}^{-1}$ , and K was applied as muriate of potash (KCl) at a rate of  $90 \text{ K ha}^{-1}$ . Nutrients were split applied, with applications of  $50 \text{ kg N}$  and  $45 \text{ kg K ha}^{-1}$  in mid-April and  $100 \text{ kg N}$  and  $45 \text{ kg K ha}^{-1}$  in mid-May. No P was needed based on soil test.

Limited irrigation was applied to the experiment only at sign of drought stress (leaf rolling). Water was applied using a traveling gun system. There were five irrigation events in 2010 totaling 60 mm and three irrigation events in 2011 totaling 50 mm.

At harvest, a 4-m portion of one of the two middle rows was cut to 12-cm stubble height using a brush cutter. Four representative tillers were selected from the harvested biomass for determination of plant-part proportion and biomass composition. Those tillers were hand-separated into leaf (blade and sheath) and stem (including inflorescence, if present) components. Samples were dried at  $60^\circ\text{C}$  until constant weight. Stem samples were initially ground through a hammer mill to reduce particle

size. Stem and leaf samples were ground to pass a 1-mm stainless steel screen in a Wiley mill (Model 4 Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedeboro, NJ).

### **Biomass Fiber Analysis**

Each component of dried biomass was analyzed for chemical composition. Two different procedures were used for compositional analysis; detergent fiber analysis (Van Soest et al., 1991) and modified NREL procedures (Sluiter, 2008a; Sluiter, 2008b).

For the detergent fiber procedure, the samples were sequentially analyzed for NDF, ADF, and ADL (Van Soest et al., 1991), with the exception that sodium sulfite and alpha-amylase were excluded from the NDF analysis (Casler and Boe, 2003). The ANKOM fiber analyzer (ANKOM 2000 Fiber Analyzer, ANKOM Technology Corporation, Fairport, NY) was used for NDF and ADF determinations followed by the procedure “Method for Determining Acid Detergent Lignin in Beakers” (proposed by ANKOM Technology Corporation) which was used for ADL determination. Cellulose concentration was calculated as the difference between ADF and ADL and Hemicellulose concentration was calculated as the difference between NDF and ADF concentrations (Jung and Lamb, 2004; Waramit et al., 2011).

In the modified NREL procedure, dried biomass was analyzed for non-structural extractives, structural carbohydrates, and total lignin (Fedenko, 2011). For nonstructural extractives, 100 ml of deionized (D.I.) water was added to 1 g of dried sample and autoclaved in sealed pressure tubes (ACE Glass, Inc., Vineland, NJ) at 121°C for 1 h. Filtered extractives were collected for high performance liquid chromatography (HPLC) analysis. The captured structural biomass from extraction was dried then weighed. A 0.3-g dry subsample was then used for further two-stage hydrolysis; the first stage was a 1-h incubation with concentrated sulfuric acid (HPLC grade 72%, Fluka Alalytical,

Sigma-Aldrich, St. Louis, MO). In the next stage, sulfuric acid was diluted to 4% by adding D.I. water then autoclaved for 1 h. Supernatant was collected and then neutralized to pH 7 for HPLC analysis. From the supernatant, acid soluble lignin determination was done at a wavelength of 240 nm using a UV-Vis Spectrophotometer (StellarNet, Inc., Tampa, FL). Insoluble lignin was determined gravimetrically as total solids remaining in the crucible after vacuum filtration of hydrolyzed biomass. Total lignin was calculated as the sum of acid soluble and insoluble lignin. Extractives and hydrolyzed samples were analyzed by HPLC (Perkin-Elmer Flexar system, Waltham, MA) using a refractive index detector and a Biorad Aminex HPX-87H column (300 x 7.8 mm) maintained at 50°C. Sulfuric acid (HPLC grade, 4mM) was used as the mobile phase at a flow-rate of 0.3 mL min<sup>-1</sup> with a 10 µL injection and 60 min run time. Perkin-Elmer's Chromera software was used to identify peaks. Linear regressions between peak areas of standard sugar concentrations were determined and used to calculate unknown sugar concentrations from the samples. A brief explanation of each component analyzed is in Table 4-1.

### **Total Nitrogen, Phosphorus, and Ash**

For N and P analyses, the method used was a modification of the standard Kjeldahl procedure and it was conducted at the Forage Evaluation Support Laboratory of the University of Florida. Samples were digested using a modification of the aluminum block digestion procedure (Gallaher et al., 1975). Sample weight was 0.25 g, catalyst used was 1.5 g of 9:1 K<sub>2</sub>SO<sub>4</sub>:CuSO<sub>4</sub>, and digestion was conducted for at least 4 h at 375°C using 6 ml of H<sub>2</sub>SO<sub>4</sub> and 2 ml H<sub>2</sub>O<sub>2</sub>. Analysis of digestate was carried out using the Technicon Autoanalyzer and semiautomated colorimetry to determine N and

P in the digestate (Hambleton, 1977). Samples were ashed at 500°C for 6 h to determine ash concentration.

### **Statistical Analysis**

Data were analyzed using mixed-model methods in PROC MIXED (SAS Institute, 2008). In all models, harvest treatment and grass entry were considered fixed effects. Year was considered a repeated measurement (fixed). Block and all interactions with block were considered random effects. Because there were two harvests per year of the 2X treatment, samples from both harvests were analyzed separately in the laboratory and the model effectively contains four levels of harvest management, i.e., 2X-July, 2X-Nov, 1X-Nov, and 1X-Dec. Means were compared using the pdiff test of LSMEANS. All means reported in the text are least squares means and were considered different if  $P \leq 0.05$ .

Because the effects of greatest interest were harvest management, entry, and their interaction, and because the harvest management x entry interaction was significant in most cases, data in tables will show the means for this interaction. If the interaction was not significant, only main effect means will be compared in the tables. If there were harvest management x year, entry x year, or harvest management x entry x year interactions for composition of leaf and stem components, interaction means are not presented nor discussed in the main body of the chapter in order to reduce the volume of data presented and to simplify its presentation. For responses in which harvest management x year and entry x year interactions were significant, means for measures of total biomass (not leaf or stem components) composition are presented briefly at the end of the chapter but are not discussed in detail. This was done because upon close inspection of these interactions the ranking of harvest management

treatments or entry treatments was similar across years, thus the interaction was primarily caused by changes in magnitude and not direction of the response.

## **Results and Discussion**

### **Van Soest Fiber Analyses**

Sources of variation (harvest management, entry, year, and their interactions) and levels of probability for their effects on NDF, ADF, ADL, cellulose, and hemicellulose of leaf, stem, and total biomass are shown in Tables 4-2 and 4-3.

#### **Neutral detergent fiber**

Leaf NDF concentration was affected by harvest management and entry ( $P < 0.001$  for both, Table 4-2). Entry L79-1002 had greater leaf NDF concentration than elephantgrasses (Table 4-4). Averaged across the three grass entries, the single harvest treatments (780 and 789 mg g<sup>-1</sup> for 1X-Nov and 1X-Dec, respectively) had greater leaf NDF concentration than the 2X harvest treatments (748 and 747 mg g<sup>-1</sup> for 2X-July and 2X-Nov, respectively).

In stem, there was a harvest management x entry interaction ( $P < 0.001$ , table 4-2). This was because L79-1002 had lesser NDF concentration in single harvest treatments (1X-Nov and 1X-Dec) than in 2X harvests, but elephantgrass NDF was at least as great or greater for the single harvests compared with 2X. Within a harvest management, NDF concentration of the two elephantgrasses was greater than that of energycane for all but the November harvest of the 2X treatment.

In total biomass, harvest management x entry interaction occurred ( $P < 0.001$ ). L79-1002 had similar or greater NDF concentration than elephantgrasses in the 2X harvest treatment but lower NDF concentration than elephantgrasses in 1X-Nov and 1X-Dec. Within an entry, L79-1002 NDF concentration was least in 1X-Dec and greatest

in July and November harvests of the 2X treatment, but Merkeron and UF-1 had least NDF concentration in 2X harvests.

The overall range of NDF in total elephantgrass biomass was similar to that reported in previous research (Van Man and Wiktorsson, 2003). Most energycane fiber data reported in the literature were from analyses conducted after juice extraction, so viable comparisons with previous energycane research are few. In the current study, elephantgrass and energycane NDF concentration responded differently to harvest frequency. Specifically, full-season growth of energycane stem and total biomass had lesser NDF concentration than stem harvested twice per year, while the pattern of response was the opposite for elephantgrass.

Harvest frequency studies with reed canarygrass (*Phalaris arundinacea* L.) in Iowa showed that NDF concentration of biomass harvested twice a year (595 and 587 mg g<sup>-1</sup> for summer and ratoon, respectively) was less than that of a single fall harvest (650 mg g<sup>-1</sup>) (Tahir et al., 2011). A harvest frequency and timing study in Oklahoma with switchgrass showed that a single harvest treatment had greater NDF concentration than twice a year harvests (Guretzky et al., 2011). In Eastern Canada, switchgrass NDF increased from 750 mg g<sup>-1</sup> in June to 850 mg g<sup>-1</sup> in July and remained constant for the remainder of the season (Madakadze et al., 1999). In the current research, NDF in biomass from single harvest treatments was greater than the 2X harvests for elephantgrass. This response is supported by previous forage studies with elephantgrass showing that as defoliation interval increased NDF concentration increased (Manyawu et al., 2003; Van Man and Wiktorsson, 2003). Observed increases

in grass NDF concentration with increasing maturity have been related to changes in leaf:stem ratio (Madakadze et al., 1999).

Although elephantgrass NDF response in the current study was similar to that observed for other grasses in previous research, energycane NDF response was different. Research with energycane has shown that it produces stem sap with relatively low Brix (range of 100 to 120 mg g<sup>-1</sup> in juice) (Bischoff et al., 2008; Kim and Day, 2011; Tew et al., 2011). Although the Brix is low compared with commercial sugarcane, it composes a substantial portion of the biomass, and sucrose accumulation in late season is likely why energycane NDF concentration decreases as it matures.

Relative to plant-part NDF concentration, most research has shown that perennial grass NDF concentration in leaf was less than in stem (Griffin and Jung, 1983; Anderson et al., 2008b). For perennial C4 grasses big bluestem (*Andropogon gerardi* Vitman) and switchgrass, leaf NDF varied little with increasing maturity and ranged from 642 to 663 mg g<sup>-1</sup>; in contrast, stem NDF concentration of both grasses increased rapidly with maturation and averaged 8% greater than in leaves (Griffin and Jung, 1983). In the current study, leaf NDF concentration of elephantgrass was likely greater than typically observed because most previous studies involved use of the grass as forage and harvests were more frequent and did not involve the accumulation of large amounts of dead leaf as occurred in the single harvest treatments (Chapter 5). Greater NDF in leaf than stem has been observed previously in a study with elephantgrass conducted in China (Hsu et al., 1990). The difference between NDF in leaf and stem fractions was particularly pronounced for energycane, and this relates to the

accumulation of non-structural sugars in energycane stem with increasing maturity, something that did not happen in leaf.

### **Acid detergent fiber**

Similar to NDF, leaf ADF concentration was affected by harvest management and entry ( $P < 0.001$ , Table 4-2). Energycane leaf had approximately 13% greater ADF concentration than Merkeron and UF-1 (Table 4-5). Across entries, leaf ADF was approximately 10% greater for the single harvest treatments (1X-Nov and 1X-Dec) than either harvest of the 2X treatment.

For the stem component, there was harvest management x entry interaction ( $P < 0.001$ , Table 4-2). Interaction occurred because ADF concentration in L79-1002 stem did not differ and ranged only from 442 to 455 mg g<sup>-1</sup> across harvest management treatments, but the range was much greater for elephantgrass and the 1X-Nov and 1X-Dec treatments always had greater ADF than the 2X treatments (Table 4-5).

For total biomass there also was harvest management x entry interaction ( $P < 0.001$ , Table 4-2), Interaction occurred because elephantgrass ADF concentration was greater than energycane with the exception of the 2X treatment harvested in November when there was no difference among entries (Table 4-5). For all three grass entries, the 2X treatment had lesser ADF concentration than the single harvest treatments, and for elephantgrass the lowest ADF was observed for the 2X harvest in November.

A harvest frequency study with reed canarygrass in Iowa showed that plants harvested in June had slightly greater ADF concentration (314 mg g<sup>-1</sup>) than the ratoon harvest (302 mg g<sup>-1</sup>), but ADF was greatest for a single harvest in fall (356 mg g<sup>-1</sup>) (Tahir et al., 2011). In Oklahoma, a single harvest of switchgrass had greater ADF concentration than twice per year harvests (Guretzky et al., 2011). Defoliation frequency

studies with elephantgrass showed that increasing interval between grazing events increased ADF concentration from 360 to 398 mg g<sup>-1</sup> in Zimbabwe, 358 to 455 mg g<sup>-1</sup> in Vietnam, and 360 to 410 mg g<sup>-1</sup> in Ethiopia (Manyawu et al., 2003; Van Man and Wiktorsson, 2003; Tessema et al., 2010). In eastern Canada, switchgrass ADF concentration increased from 500 mg g<sup>-1</sup> in June to 650 mg g<sup>-1</sup> in July and remained relatively constant thereafter (Madakadze et al., 1999). This study showed that a large decrease in leaf:stem ratio occurred as plants matured, thus it was primarily changes in stem composition which affected ADF concentration in total biomass. Similar to the response observed for NDF in the current study, elephantgrass ADF concentration increased with single vs. 2X harvest treatments.

Relative to plant-part ADF concentration, in a previous elephantgrass cutting height study in Taiwan, stem had greater ADF than leaf across cutting heights (Hsu et al., 1990). Similarly, mature Merkeron stem had greater ADF concentration than leaf (481 and 360 mg g<sup>-1</sup>, respectively) (Anderson et al., 2008b). These results follow similar patterns to those observed for elephantgrass plant parts in the current study. Because of the substantial amount of extractable sugars that energycane stem accumulates late in the growing season, decreasing stem ADF, energycane leaf and stem ADF concentrations were more nearly the same than observed for elephantgrass.

### **Acid detergent lignin**

Harvest management x entry interaction occurred for leaf ADL concentration ( $P = 0.037$ , Table 4-2). Entry L79-1002 showed greater leaf ADL concentration than elephantgrass entries for all harvest management treatments (Table 4-6). Interaction occurred because UF-1 leaf ADL was similar for all harvest treatments except the

November harvest of the 2X treatment, whereas the other entries showed greater leaf ADL for both 1X-Nov and 1X-Dec treatments than for either harvest of the 2X treatment.

There also was harvest management x entry interaction ( $P < 0.001$ ) for stem ADL (Table 4-2). Unlike leaf ADL concentration, elephantgrass showed greater stem ADL concentration than L79-1002 for each harvest treatment, and Merkeron had greater ADL than UF-1 for all but the November harvest of the 2X treatment (Table 4-6). Stem biomass in the 1X-Nov harvest treatment had greater ADL than either of the 2X harvests for all three entries, and the November harvest of the 2X treatment had lower ADL than the July harvest for all entries except UF-1 (Table 4-6).

For ADL in total biomass, there was harvest management x entry interaction ( $P < 0.001$ ), and the pattern of response was very similar to that of stem (Table 4-6). Specifically, ADL was greater for elephantgrass than energycane and greater for Merkeron than UF-1 elephantgrass for all but the 2X treatment in November.

Reed canarygrass in Iowa had slightly lesser ADL concentration for a first harvest in June ( $25 \text{ mg g}^{-1}$ ) than a ratoon harvest in fall ( $29 \text{ mg g}^{-1}$ ), but both were less than observed for a single harvest in fall ( $39 \text{ mg g}^{-1}$ ) (Tahir et al., 2011). Several experiments have shown that increasing the interval between harvests is associated with increasing lignin concentration in elephantgrass (Dien et al., 2006; Tessema et al., 2010; Rengsirikul et al., 2011). Seasonal changes in lignin concentration of perennial grasses have been studied with results showing that lignin concentration increased during summer, but the magnitude of the change varied by species (Waramit et al., 2011). In terms of plant-part composition, stem was reported to have greater ADL than leaf in elephantgrass (Anderson et al., 2008b). This corresponds to results of the current

study, where because of maturity differences single harvest treatments (1X-Nov and 1X-Dec) had greater total ADL concentration than 2X treatments. Similar to the response of ADF, the difference between leaf and stem ADL concentration was less pronounced for energycane than for elephantgrass.

## **Cellulose**

Cellulose concentration was calculated as the difference between ADF and ADL (Jung and Lamb, 2004; Waramit et al., 2011). In leaves, cellulose concentration was affected by harvest management and entry ( $P < 0.001$  for both, Table 4-3). Energycane had approximately 10% greater cellulose concentration than elephantgrass entries (Table 4-7). The single harvest treatments (1X-Nov and 1X-Dec) showed greater leaf cellulose than 2X July and November harvests. This trend is very similar to those observed for leaf NDF and ADF concentrations (Tables 4-4 and 4-5).

In the stem, there was harvest management x entry for cellulose concentration ( $P < 0.001$ ). This was because L79-1002 had relatively constant cellulose concentration across harvest treatments, but elephantgrass cellulose concentration was generally greater with single vs. 2X harvests (Table 4-7). Elephantgrass stem cellulose concentration was always greater than for energycane, and the two elephantgrasses generally had similar cellulose concentrations (Table 4-7).

Cellulose concentration in total biomass was affected by the harvest management x entry interaction ( $P < 0.001$ , Table 4-3). This response was nearly identical to that for stem, with the only effect of harvest treatment on energycane cellulose concentration observed for the 2X July treatment which was lower than 1X-Nov and 1X-Dec (Table 4-7). Merkeron showed greatest cellulose concentration for 1X-Dec but there was no difference between Merkeron and UF-1 for the other harvest

treatments, although both were greater than energycane for all but the 2X November treatment.

Similar to the results for elephantgrass in the current study, reed canarygrass in Iowa had lesser cellulose concentrations in twice per year harvest treatments than single harvests with means of 273, 289, and 317 mg g<sup>-1</sup> for first harvest, ratoon, and single harvest, respectively (Tahir et al., 2011). In Iowa, cellulose concentration of several native, warm-season perennial grasses increased with increasing maturity, with differences existing among species (Waramit et al., 2011). Less frequent grazing of elephantgrass for forage caused increased cellulose concentration (Tessema et al., 2010; Rengsirikul et al., 2011). In general, delaying a single harvest until fall is likely to maximize the concentration of cellulose in perennial grasses; however, the magnitude of this response is species dependent and the availability of that cellulose for conversion to energy is likely to be affected by increasing lignin concentration.

Similar to the results observed in this study for elephantgrass, stem cellulose concentration (412 mg g<sup>-1</sup>) was greater than leaf (357 mg g<sup>-1</sup>) in mature Merkeron (Anderson et al., 2008b). In sweet sorghum (*Sorghum bicolor* L. Moench), cellulose concentration was greater in leaves than stem (316 vs. 288 mg g<sup>-1</sup>) (Fedenko, 2011), probably due to accumulation of non-structural sugars in stem, similar to the response observed for energycane in the current study.

## **Hemicellulose**

Hemicellulose concentration was calculated as the difference between NDF and ADF concentrations (Jung and Lamb, 2004; Waramit et al., 2011). There was interaction of harvest management x entry for leaf hemicellulose concentration ( $P =$

0.029, Table 4-3). The range in response was only 339 to 355 mg g<sup>-1</sup>, thus the impact of treatment was minimal and the direction of the response was not consistent (Table 4-8).

Unlike cellulose concentration, which was in the same general range for both leaf and stem (Table 4-7), hemicellulose concentration in leaves was greater than stem with an average of 346 and 249 mg g<sup>-1</sup>, respectively, Table 4-8). The harvest management x entry interaction ( $P = 0.009$ ) was caused by greater hemicellulose concentration in L79-1002 stem vs. the elephantgrasses for the 2X-July treatment harvest, but there were no differences among entries for 1X-Nov and 1X-Dec harvest treatments. For all three grasses, greatest stem hemicellulose concentration occurred in the 2X-Nov treatment.

In total biomass, hemicellulose concentration was affected by harvest management and entry ( $P < 0.001$  for both) but not their interaction ( $P = 0.284$ , Table 4-3). In total biomass, hemicellulose concentration was greatest in L79-1002 followed by Merkeron and then UF-1. Similar to stem hemicellulose concentration, 1X-Nov and 1X-Dec harvest management showed lowest hemicellulose concentration in total biomass.

Hemicellulose concentration of reed canarygrass in Iowa was relatively unaffected by harvest management with biomass from the two harvest per year treatment in June and fall and the single fall harvest treatment having concentrations of 281, 281, and 294 mg g<sup>-1</sup>, respectively (Tahir et al., 2011). Other studies have shown that increasing rest periods between defoliation events resulted in greater elephantgrass hemicellulose concentrations (Tessema et al., 2010; Rengsirikul et al., 2011). Different patterns of hemicellulose concentration were observed in several native warm-season grasses in Iowa (Waramit et al., 2011). Data from the current experiment do not support the observation of greater hemicellulose concentration with increasing

maturity observed in some studies, and it seems likely that hemicellulose response is less consistent across species and treatments than are the other cell wall fractions.

Similar to results in the current research, it was reported that stem hemicellulose concentration ( $261 \text{ mg g}^{-1}$ ) was less than in leaf ( $334 \text{ mg g}^{-1}$ ) of mature Merkeron elephantgrass (Anderson et al., 2008b). In China, elephantgrass leaf had greater hemicellulose concentration than stem across several cutting height treatments (Hsu et al., 1990). Sweet sorghum showed a similar response with hemicellulose concentration of  $265$  and  $169 \text{ mg g}^{-1}$  in leaf and stem, respectively (Fedenko, 2011)

Considering the Van Soest fiber components as a group, concentration responses in total biomass usually paralleled those in the stem component for the 1X-Nov and 1X-Dec treatments. This is because stem proportion of perennial grasses increases as they mature (Griffin and Jung, 1983; Woodard and Prine, 1993b; Woodard et al., 1993), and this has been shown to be true for energycane and elephantgrass as well (Chapter 5). There were important differences in fiber component responses among species (elephantgrass vs. energycane). This is not thought to be due to difference in the composition of cell wall per se but to differences in the proportion of structural (i.e., cell wall) vs. non-structural (i.e., extractives) components, particularly as maturity increased in the 1X-Nov and 1X-Dec treatments. Energycane accumulated non-structural sugars late in the growing season and as a result the proportion of most structural components was less at this time than in elephantgrass. Delayed harvest (1X-Dec) generally did not affect concentration of cell wall constituents vs. that observed in 1X-Nov. Thus from a compositional standpoint, it would be a viable option to extend the window of harvest to at least the occurrence of first freeze in both elephantgrass and

energycane. Extending the harvest period by using multiple harvests has greater impact on biomass cell wall composition. In general, biomass from early harvests or subsequent ratoon harvests has lesser concentrations of cell wall constituents compared with single harvests at season end. Depending on the conversion process, this may or may not be advantageous. More frequent harvest does result in greater leaf proportion (Chapters 3 and 5) and associated greater concentrations of N and ash (Chapter 6) which can be antiquality factors in biomass conversion.

### **National Renewable Energy Laboratory Procedures**

Extractives are the sum of all non-structural components of plant tissue removed during extraction (Sluiter, 2008a). Soluble sugars and other soluble substrates compose extractives. Hexoses and pentoses are the sum of six-carbon (6C; glucose and mannose, but mannose was not detected) and five-carbon (5C; xylose and arabinose) structural carbohydrates, respectively. Total lignin is the sum of acid soluble and insoluble lignin.

### **Extractives**

Harvest management x entry interaction occurred ( $P = 0.044$ ) in the leaf because UF-1 showed greater extractives concentration than other entries in 1X-Dec but there were no differences between elephantgrass entries for the other harvest managements (Table 4-9 and 4-11). Single harvest (1X-Nov and 1X-Dec) leaf extractives concentration was lower than either 2X-July or 2X-Nov. This was because leaf senescence occurred during late season (Woodard and Prine, 1993b), and green leaf has a relatively greater concentration of extractives than senesced leaf.

Harvest management x entry interaction occurred for stem extractives ( $P < 0.001$ , Table 4-9). Entry L79-1002 stem extractives was greatest in 1X-Dec ( $338 \text{ mg g}^{-1}$ )

followed by 1X-Nov (313 mg g<sup>-1</sup>), however, Merkeron and UF-1 had greater stem extractives in 2X-July and 2X-Nov treatments (275 and 268 mg g<sup>-1</sup>, respectively) than 1X-Nov and 1X-Dec (256 and 252 mg g<sup>-1</sup>, respectively). In 1X-Dec, L79-1002 (338 mg g<sup>-1</sup>) showed greatest stem extractives concentration followed by UF-1 (263 mg g<sup>-1</sup>) and then Merkeron (241 mg g<sup>-1</sup>).

In total biomass, harvest management x entry interaction occurred ( $P < 0.001$ ). This was because there was no difference among entries within the 2X-Nov harvest, but L79-1002 had greater extractives concentration than Merkeron for all other harvest treatments and greater than UF-1 in both 1X-Nov and 1X-Dec. Elephantgrasses generally had greater extractives concentration in the 2X than single harvest treatments, but the pattern was generally reversed for energycane.

There are very limited data available for extractives concentration in grasses. Elephantgrass in Vietnam had 182 mg g<sup>-1</sup> of extractives measured using hot water extraction (Hoa et al., 2008). Stem had greater extractives concentration than leaf in sweet sorghum in Florida (502 vs. 378 mg g<sup>-1</sup>) (Fedenko, 2011). This author also evaluated total extractives of several perennial grass species, and concentrations occurred over a similar range as in the current study. He also found that extractives concentration was greater in energycane than elephantgrass (273 vs. 238 mg g<sup>-1</sup>) as observed in the current study. Sugar accumulation in energycane results in increased extractives concentration when using a single harvest management. Corn (*Zea mays* L.) stover water soluble extractives ranged from 142 to 203 mg g<sup>-1</sup>, with total sugars composing 30 to 48% of extractives (Chen et al., 2007). In Colorado, extractives concentration varied among entries including 164 mg g<sup>-1</sup> for switchgrass, 254 mg g<sup>-1</sup> for

fescue (*Festuca arundinacea* L.), and 172 mg g<sup>-1</sup> for corn stover (Thammasouk et al., 1997).

### **Total soluble sugars**

Harvest management x entry interaction occurred ( $P = 0.044$ ) because there were no differences among entries within the 2X-July management, but differences among entries occurred within other harvest managements and one of the elephantgrasses always had the greatest concentration (Table 4-9 and 4-12). However, overall concentration of total soluble sugars in the leaves was many times smaller than in stem (27 vs. 164 mg total sugars g<sup>-1</sup>). Thus, although differences occurred in leaf, it contributed only marginally to differences in total biomass.

In the stem, harvest management x entry interaction occurred ( $P < 0.001$ ) for concentration of total soluble sugars (Table 4-9). For all three entries, 2X-July total soluble sugar concentration was less than 1X-Dec. Magnitude of the difference varied among entries (84% increase in L79-1002, 51% in Merkeron, and 36% in UF-1, respectively). The large difference in energycane is related to sucrose accumulation. As mentioned previously, although energycane has relatively low sugar concentration compared with sugarcane, its brix value shows measurable levels of sugar accumulation in late season (Bischoff et al., 2008; Kim and Day, 2011; Tew et al., 2011).

In total biomass, there was harvest management x entry interaction ( $P < 0.001$ ). As mentioned before, because the concentration of leaf soluble sugar was very small and because leaf composed a small percentage of harvested biomass, particularly for 1X-Nov and 1X-Dec R, total soluble sugar concentration generally followed the same pattern as in stem. Concentration of total soluble sugars varied most among entries for

single vs. 2x harvest treatments. Interestingly, among elephantgrass entries, UF-1 showed 44% greater soluble sugar concentration in total biomass harvested compared with Merkeron. Further investigation of UF-1 soluble sugar concentration is needed to determine the specific sugars present.

Unlike cell wall polysaccharides, non-cell wall carbohydrates are directly fermentable; however, they are susceptible to microbial degradation during storage (Dien et al., 2006). Moreover, they contain cellulosic fermentation inhibitors including furfural (Tran and Chambers, 1986; Zhang et al., 2011). If they cannot be used for conversion to biofuel, they will decrease feedstock quality. Fedenko (2011) reported that L79-1002 had up to two-fold greater soluble sugar concentration than Merkeron, a similar result to the current study. Within species differences in sucrose concentration were shown among four different sorghum cultivars in Texas, ranging from 2 (forage) to 239 mg g<sup>-1</sup> (sweet) (Stefaniak et al., 2012). Thus, intra-species differences have occurred for grasses in addition to those observed between the two elephantgrasses included in the current experiment.

### **Structural hexose**

In the leaves, structural hexose concentration was affected by harvest management and entry ( $P < 0.001$  for both) (Table 4-10). Entry L79-1002 showed greater hexose concentration than elephantgrass entries by 7% (Table 4-13). Structural hexose concentration was greater in 1X-Nov and 1X-Dec followed by 2X-July and then 2X-Nov.

In stem, there was a harvest management x entry interaction ( $P = 0.001$ ). Elephantgrass entries had greater structural hexose concentration than L79-1002 for all harvest treatments (Table 4-13). Hexose concentration in L79-1002 was similar for 2X

and 1X-Nov treatments, but it was least for 1X-Dec. Merkeron and UF-1 had lowest structural hexose concentration in 2X-Nov, while UF-1 had greatest hexose concentration in 1X-Nov and for Merkeron 1X-Nov and 1X-Dec were not different.

In total biomass, harvest management x entry occurred ( $P = 0.001$ ). For 2X-Nov, there was no difference among grass entries; however, entry L79-1002 had lower hexose concentration in total biomass than the elephantgrasses in 2X-July, 1X-Nov, and 1X-Dec (Table 4-13). Comparing harvest managements within an entry, both Merkeron and UF-1 had greatest hexose concentration in 1X-Nov and 1X-Dec and least in 2X-Nov, while energycane hexose concentration varied relatively little and was greater in 1X-Nov than in 2X-Nov and 1X-Dec.

A large amount of glucose is advantageous for ethanol production because glucose can be converted more efficiently to ethanol than most other sugars, especially, pentoses. Similar to values observed in the current study, structural hexose concentrations of  $347 \text{ mg g}^{-1}$  have been reported with mixtures of C4 grasses in Minnesota (Gillitzer et al., 2013). Likewise the range in six-carbon structural sugars of four different varieties of sorghum in Texas (Stefaniak et al., 2012) was similar to concentrations reported in the current experiment. In a switchgrass study, chemical composition of six-carbon structural sugars was 300 to  $337 \text{ mg g}^{-1}$  (Xu et al., 2011). Similar to the results in the current study, structural glucose concentration in elephantgrass and energycane was 374 vs.  $366 \text{ mg g}^{-1}$  in the first year and 472 vs.  $416 \text{ mg g}^{-1}$ , respectively, in the second year (Fedenko, 2011). Late harvests and increasing maturity have been observed to increase glucose and non-glucose structural sugars in reed canarygrass (233 to  $286 \text{ mg g}^{-1}$ ) and switchgrass (294 to  $340 \text{ mg g}^{-1}$ ) (Dien et al.,

2006). This corresponds to what was observed for elephantgrass in the current study, but is not similar to the response of energycane.

Interestingly, structural hexose concentration using the NREL procedures was similar to Van Soest cellulose concentration in the current study. Further investigation of this relationship is needed.

### **Structural pentose**

In leaves, pentose concentration was affected by harvest management ( $P = 0.001$  Table 4-10). The 2X-July treatment had the lowest pentose concentration compared with other treatments, but there were no differences among the other three.

There was harvest management x entry interaction for stem pentose concentration ( $P = 0.003$ ). The most pronounced difference was that 2X-Nov had greatest pentose concentration for all entries. For both 2X treatments, L79-1002 had greatest pentose concentration compared with the two elephantgrasses (Table 4-14). There was no difference among entries in 1X-Nov, while in 1X-Dec, Merkeron had greater pentose concentration than L79-1002.

In total biomass, structural pentose concentration was affected by harvest management and entry ( $P = 0.001$ ,  $P < 0.001$ , respectively, Table 4-10). Entry L79-1002 had greater pentose concentration than the elephantgrasses; however, the difference among entries was relatively small (4-5 mg of pentose  $g^{-1}$ ). The 2X-Nov harvest management had greater pentose concentration than the other defoliation treatments.

In switchgrass, concentration of five carbon structural sugars ranged from 183 to 196 mg  $g^{-1}$  (Xu et al., 2011). Structural xylose concentration in season-long growth of elephantgrass in Florida was similar to energycane (135 and 145 mg  $g^{-1}$ , respectively) (Fedenko, 2011). For both reed canarygrass and switchgrass, structural five carbon

sugars increased with increasing maturity (147 to 191 mg g<sup>-1</sup> in reed canarygrass; 210 to 253 mg g<sup>-1</sup> in switchgrass) (Dien et al., 2006). That was not observed in the current study. Pentose concentration from structural carbohydrates of 198 mg g<sup>-1</sup> has been reported for a mixture of C4 grass and 187 mg g<sup>-1</sup> for a mixture of C3 grasses in Minnesota (Gillitzer et al., 2013). Although there were statistical differences in pentose concentration in the current study, unlike hexose concentration, pentose was relatively constant across harvest managements and entries (ranging only from 218-253 mg g<sup>-1</sup>).

### **Lignin**

Leaf lignin concentration was affected by harvest management ( $P = 0.005$ ), and entry ( $P < 0.001$ ) (Table 4-10). Entry L79-1002 leaf lignin concentration was greater than elephantgrass entries (Merkeron and UF-1) by 8% (218, 204, and 201 mg lignin g<sup>-1</sup>, respectively, Table 4-15). Single harvest management (1X-Nov and 1X-Dec; 214 and 212 mg lignin g<sup>-1</sup>, respectively) had greater leaf lignin concentration than 2X harvest managements (2X-July and 2X-Nov; 204 and 202 mg lignin g<sup>-1</sup>, respectively).

For stem lignin concentration, there was harvest management x entry interaction ( $P = 0.002$ , table 4-10). For all entries, 1X-Nov and 1X-Dec biomass had greater stem lignin concentration than either of the 2X harvest managements (Table 4-15). The interaction occurred because there was no difference between Merkeron and UF-1 for 1X-Nov; in contrast, within the other harvest management treatments Merkeron had greater stem lignin concentration than UF-1 which had a greater lignin concentration than L79-1002.

In total biomass, harvest management x entry interaction occurred ( $P = 0.002$ ). Similar to stem lignin concentration, both of the 2X harvest managements generally had

lesser lignin concentration compared with 1X-Nov and 1X-Dec. Merkeron had greater lignin concentration than energycane for all but the 2X-Nov treatment.

Total lignin concentration in the current study is similar to data reported in a previous study with full-season growth of elephantgrass and energycane (Fedenko, 2011), and the results of both studies agree that elephantgrass generally has greater lignin concentration than energycane. There have been concerns raised by Jung and his colleagues about the accuracy of various analyses of lignin concentration (Jung et al., 1997; Jung et al., 1999; Jung and Lamb, 2004). Similar to challenges associated with quantifying structural carbohydrates, it is difficult to determine the actual amount of lignin in the plant cell wall. Because the NREL method is sub-step of a series of structural component analyses (Sluiter, 2008a; Sluiter, 2008b), it is very hard to find previous research that reports this approach, although there are data in the literature using the Klason lignin analysis procedure which does not account for acid soluble lignin. In a study with three switchgrass entries, lignin concentration was determined using the NREL procedure and ranged from 214 to 230 mg g<sup>-1</sup> for full-season growth (Xu et al., 2011), a range that is only slightly greater than that observed for total biomass in the current study.

## **Mineral Composition**

### **Nitrogen**

Nitrogen concentration in the leaves was affected by harvest management and entry ( $P < 0.001$  for both, Table 4-16). Energycane leaf had a lower N concentration (9.9 mg g<sup>-1</sup>) than elephantgrass entries (average of 11.5 mg g<sup>-1</sup>) (Table 4-17). Leaf N concentration averaged approximately 37% lower for the single harvest treatments (1X-Nov and 1X-Dec) compared with the 2X treatments.

In stem, N concentration was affected by harvest management ( $P = 0.002$ ), and entry ( $P = 0.011$ ) (Table 4-16). Merkeron ( $5.1 \text{ mg g}^{-1}$ ) N concentration was greater than other entries ( $4.3 \text{ mg g}^{-1}$  in UF-1 and  $4.0 \text{ mg g}^{-1}$  in L79-1002, Table 4-17). The 2X-July treatment, which was harvested about 2 mo after the final fertilizer application, had greatest N concentration, while 1X-Nov and 1X-Dec harvest management were lowest in N.

Similar to stem N, total biomass N concentration was affected by harvest management ( $P < 0.001$ ) and entry ( $P = 0.004$ ) (Table 4-16). Merkeron had greatest N concentration. Nitrogen concentration in total biomass decreased 39% from 2X-July and 2X-Nov to 1X-Nov and 46% to 1X-Dec.

Similar to responses in the current study, harvest frequency of reed canarygrass in Iowa affected N concentration. When biomass was harvested twice per year (June and fall ratoon), N concentration was  $13.4$  and  $8.8 \text{ mg g}^{-1}$  compared with  $8.3 \text{ mg g}^{-1}$  for a single harvest in fall (Tahir et al., 2011). Elephantgrass plant height at cutting in Japan affected N concentration, with taller plants having lesser N concentration in both leaf and stem (Hsu et al., 1990). In the same study, the difference between leaf and stem N concentration increased as height increased (from  $10.8 \text{ mg g}^{-1}$  in leaf vs.  $5.0 \text{ mg g}^{-1}$  in stem at a 1-m cutting height;  $8.6 \text{ mg g}^{-1}$  in leaf vs.  $2.6 \text{ mg g}^{-1}$  in stem at a 2-m cutting height). Switchgrass biomass N concentration decreased over the season (Madakadze et al., 1999); however, N concentration was relatively constant after September at about  $5 \text{ mg g}^{-1}$ . Miscanthus N concentration decreased until October and then remained constant for the rest of the season (Heaton et al., 2009). Similarly, delaying harvest from 1X-Nov to 1X-Dec in the current study did not result in a significant decrease in either

leaf or stem N, but 1X-Dec N concentration of total biomass was less than 1X-Nov (Fig. 4-17) because of the decrease in leaf percentage in 1X-Dec relative to 1X-Nov (Chapter 3). In Georgia, Merkeron had greater overall N concentration than L79-1002 (3.8 vs. 2.7 mg g<sup>-1</sup>) (Knoll et al., 2012), similar to the pattern of response in the current study.

## **Phosphorus**

Phosphorus concentration in leaf, stem, and total biomass showed very similar trends. Phosphorus concentration was affected by harvest management and entry in leaf ( $P = 0.002$  and  $P = 0.001$ , respectively), stem ( $P < 0.001$  for both), and total biomass ( $P = 0.001$  and  $P < 0.001$ , respectively) (Table 4-16). In both part parts and in total biomass, Merkeron had greatest overall P concentration (Table 4-18). In leaf, stem, and total biomass, P concentration was greater for the 2X treatments compared with 1X-Nov and 1X-Dec. For example, in total biomass, P concentration in 2X-Nov was approximately twice as great as the single harvest treatment.

Reed canarygrass P concentration in Iowa was greater for two harvests per year than for a single harvest (Tahir et al., 2011), similar to the response observed in the current study. Switchgrass P concentration also was affected by harvest management, and it decreased slightly from first harvest of a two harvests per year treatment to ratoon harvest in fall to fall harvest of a single harvest per year treatment (1.3, 1.1, and 0.8 mg g<sup>-1</sup>, respectively) (Guretzky et al., 2011). Giant reed and switchgrass P concentration decreased from October to December in Oklahoma (Kering et al., 2012). It is clear that plant part N and P concentration are affected differently by increasing maturity. Switchgrass leaf N was much greater compared to stem, but leaf and stem P concentration varied only slightly (Shahandeh et al., 2011). The responses were similar with elephantgrass and energycane in the current study.

## Ash

There were harvest management and entry effects ( $P = 0.012$  and  $P < 0.001$ , respectively, Table 4-16) on leaf ash concentration. Entry UF-1 had greatest leaf ash concentration followed by Merkeron and then L79-1002 (Table 4-19). 1X-Nov and 1X-Dec harvested biomass had lesser ash concentrations (48 and 44 mg g<sup>-1</sup>, respectively) than 2X-July and 2X-Nov treatments (54 and 54 mg g<sup>-1</sup>, respectively).

In stem, ash concentration was affected by harvest management ( $P < 0.001$ ). The average of the single harvest treatments (1X-Nov and 1X-Dec) was 41% less than the average of 2X-July and 2X-Nov (Table 4-19).

In total biomass, ash concentration was affected by harvest management ( $P < 0.001$ ) and entry ( $P < 0.012$ ). Elephantgrass entries had greater (up to 11%) ash concentration than energycane (Table 4-19). Because of generally greater ash concentration in leaves than stem and greater leaf proportion in the 2X treatments (Chapter 3), total ash concentration in 1X-Nov and 1X-Dec was less than in either of the 2X treatments.

The results of the current study are similar to those reported previously for Merkeron and L79-1002. Four-year data were averaged, and Merkeron had greater ash concentration than L79-1002 (45.9 vs. 34.4 mg g<sup>-1</sup>, respectively) (Knoll et al., 2012). Reed canarygrass responded differently than elephantgrass and energycane, as ash concentration increased slightly over the season (96, 106, and 107 mg g<sup>-1</sup> for two harvest per year in June and October, and for one harvest in October, respectively) (Tahir et al., 2011). In switchgrass, ash concentration in Iowa peaked in July (71 mg g<sup>-1</sup>) and decreased until fall after which it remained relatively constant between 4.3 and 4.5 mg g<sup>-1</sup> (Wilson et al., 2013a). Delayed harvest after fall is not expected to affect ash

concentration greatly. First, most biomass is stem by the end of the growing season and stem has lesser ash concentration than leaves. Secondly, it is much more difficult for nutrients to be leached out of stem than leaf. There are limited data for plant part ash concentration, with leaf was found to have greater ash than stem because of large K removal in rice (Summers et al., 2001; Bakker and Elbersen, 2005).

### **Harvest Management by Year Interactions for Total Biomass Composition**

As indicated in the Materials and Methods, in order to present a manageable volume of data, harvest management x year interactions were not discussed in the main body of the chapter. There was harvest management x year interaction for total biomass ADL, hemicellulose, extractives, total soluble sugars, hexose, pentose, N, P, and ash concentrations, and the interaction means are presented (Table 4-20).

For total biomass, ADL concentration in 2010 was greater than in 2011 only for the 1X-Dec harvest management, and the ranking of harvest management treatments was similar in both years with lesser ADL for the 2X treatments (Table 4-20). Hemicellulose concentration was greater in 2011 for all harvest managements except 2X-Nov, but in both years lowest hemicellulose concentrations occurred in 1X-Nov and 1X-Dec treatments. Extractives concentration was greater in 2010 than in 2011 for all harvest treatments, and in both years there was very little variation among harvest treatments ( $15 \text{ mg g}^{-1}$  in 2010 and  $17 \text{ mg g}^{-1}$ ) in 2011. For total soluble sugars, concentrations were less in 2011 than 2010 for 1X-Nov and 1X-Dec only, but in both years greatest soluble sugar concentration occurred in 1X-Dec. Structural hexose concentration was greater in 2011 than 2010 with the exception of 1X-Nov, and 1X-Nov had greatest hexose concentration in both years. Pentose concentration in total biomass was greater in 2011 than 2010 for all harvest management treatments and was

greatest for 2X-Nov in both years. Nitrogen concentration was greater in 2010 than 2011 for 2X-Nov only, and in both years the greatest N concentration was observed for the 2X treatments. Phosphorus concentration was greater in 2010 than 2011 for all but the 2X-Nov treatment, and in both years it was greater for the 2X treatments than for 1X-Nov and 1X-Dec. Ash concentration was greater in 2011 than 2010 for only the 2X-July treatment, but as with N and P the ranking of harvest treatments was similar in both years with greatest ash concentration in biomass from the 2X treatments.

The differences among harvest management treatments were already discussed earlier in the chapter. Although there were interactions of harvest management and year for quite a few responses, the general ranking of defoliation management treatments was similar in both years in all cases and thus the interaction was primarily due to changes in magnitude instead of direction of the response. This provides additional justification for minimizing consideration of these data.

### **Entry by Year Interactions for Total Biomass Composition**

There was entry x year interaction for total biomass NDF, ADL, hemicellulose, extractives, total soluble sugars, hexose, pentose, and lignin concentrations (Table 4-21). For total biomass NDF concentration, L79-1002 and Merkeron had greater NDF in the second year, but in both years NDF concentration in Merkeron was greater than for energycane. For ADL concentration, differences between years were no greater than 5 mg g<sup>-1</sup>, and only UF-1 ADL concentration differed between years. Energycane had the lowest ADL in both years. Hemicellulose concentration was greater in 2011 for all entries, and in both years it was lowest in UF-1. Extractives concentration was greater in 2010 than 2011 for all three entries, and energycane had greater concentration than Merkeron in both years. Total soluble sugars concentration was greater in 2010 than

2011 for all entries and like for extractives Merkeron had less soluble sugars than energycane. Structural hexose concentration was greater in 2011 than 2010 for all three entries, and energycane had least structural hexose in both years. Structural pentose concentration was also greater in 2011 compared with 2010. Range in the response was narrow in both years, but in 2011 energycane had greater structural pentose than either of the elephantgrasses. Lignin concentration was greater in the second year for all entries, and in both years Merkeron had greater lignin concentration than energycane.

Although there were interactions of entry and year for eight responses, the general ranking of entries was similar in both years in all cases and thus the interaction was primarily due to changes in magnitude instead of direction of the response. One pattern did emerge in these interactions; most composition responses associated with greater structural constituents (e.g., NDF, structural hexose, structural pentose, and lignin) were greater in 2011, while those associated with lesser structural constituents (e.g., extractives and total soluble sugars) were greater in 2010 than 2011. This is consistent with the fact that 2010 was the establishment year and growth was slower to initiate while in 2011 shoot growth began earlier and more vigorously resulting in greater effective maturity at a given 2011 calendar date. This issue is discussed in more detail in Chapter 6.

### **Implications of Research**

Harvest frequency (2X vs. single) significantly affects compositional quality of perennial grasses. Delaying a single harvest until fall appears to maximize the concentration of cellulose in total biomass, however, delaying harvest from 1X-Nov to 1X-Dec had very little impact on most responses with the exception of extractives and

soluble sugars in energycane which increased significantly between 1X-Nov and 1X-Dec. Relatively greater concentration of soluble sugars in energycane reduced the concentration of numerous structural components. A major factor affecting concentration differences due to harvest management was differences in leaf:stem ratio because leaves generally had greater N, P, and ash than stem. Later harvests were associated with lesser leaf percentage in total biomass and this caused N, P, and ash to decrease in 1X-Nov and 1X-Dec relative to 2X treatments. Total biomass N concentration in Merkeron decreased to a greater extent than the other entries because of greater leaf abscission. The 1X-Nov and 1X-Dec treatments of all entries had lesser concentrations of N and ash, component which can negatively affect some conversion processes, but they had greater lignin concentration than for 2X which may reduce accessibility of enzymes to structural hexoses.

Table 4-1. Analyses conducted and compositional characteristics of components analyzed in Chapter 4.

Procedure	Analysis	Composition
Van Soest	NDF	Cell wall structural constituents primarily composed of cellulose, hemicellulose, and lignin
	ADF	Primarily composed of cellulose and lignin
	ADL	Lignin (acid insoluble)
	Cellulose	Estimated by ADF minus ADL
	Hemicellulose	Estimated by NDF minus ADF
NREL	Extractives	Sum of nonstructural components; non-chemically bound components (soluble sugars + organic acids)
	Total Soluble sugars	Nonstructural carbohydrates primarily composed of glucose, fructose, and sucrose
	Structural hexose	6-C structural polymeric carbohydrates (glucose + mannose)
	Structural pentose	5-C structural polymeric carbohydrates from (xylose + arabinose)
	Total lignin	Sum of acid soluble and insoluble lignin

Table 4-2. Sources of variation and levels of probability (*P*) for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentrations and their effects on response variables reported in Chapter 4.

Source of variation	Leaf	Stem	Total
NDF			
Harvest management (HM)	< 0.001	0.716	0.332
Entry (E)	< 0.001	< 0.001	< 0.001
Year (Y)	0.011	0.003	< 0.001
HM × E	0.272	< 0.001	< 0.001
HM × Y	< 0.001	0.186	0.086
E × Y	0.078	0.309	0.009
HM × E × Y	0.087	0.233	0.089
ADF			
Harvest management (HM)	< 0.001	0.007	< 0.001
Entry (E)	< 0.001	< 0.001	< 0.001
Year (Y)	0.591	0.056	0.141
HM × E	0.090	< 0.001	< 0.001
HM × Y	< 0.001	0.112	0.210
E × Y	0.215	0.233	0.263
HM × E × Y	0.317	0.330	0.458
ADL			
Harvest management (HM)	< 0.001	0.001	< 0.001
Entry (E)	< 0.001	< 0.001	< 0.001
Year (Y)	0.278	0.287	0.012
HM × E	0.037	0.006	< 0.001
HM × Y	< 0.001	0.013	0.002
E × Y	0.893	0.014	0.006
HM × E × Y	0.007	0.540	0.699

Table 4-3. Sources of variation and levels of probability (*P*) for concentration of cell wall components (cellulose and hemicellulose) from detergent fiber analysis and their effects on response variables reported in Chapter 4.

Source of variation	Leaf	Stem	Total
		Cellulose	
Harvest management (HM)	< 0.001	0.046	0.001
Entry (E)	< 0.001	< 0.001	< 0.001
Year (Y)	0.375	0.006	0.008
HM × E	0.195	< 0.001	< 0.001
HM × Y	0.001	0.142	0.252
E × Y	0.167	0.560	0.500
HM × E × Y	0.689	0.235	0.357
		Hemicellulose	
Harvest management (HM)	0.026	< 0.001	< 0.001
Entry (E)	0.312	0.001	< 0.001
Year (Y)	< 0.001	0.002	< 0.001
HM × E	0.029	0.009	0.284
HM × Y	0.001	< 0.001	< 0.001
E × Y	0.038	0.026	< 0.001
HM × E × Y	0.354	0.629	0.198

Table 4-4. Effect of grass entry x harvest management interaction on neutral detergent fiber (NDF) concentration in leaf ( $P = 0.272$ ), stem ( $P < 0.001$ ), and total biomass ( $P < 0.001$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg NDF g <sup>-1</sup> dry matter -----					
Leaf					
L79-1002	782	784	822	823	803 A <sup>§</sup>
Merkeron	732	726	763	783	751 B
UF-1	731	733	757	761	745 B
Mean	748 b <sup>‡</sup>	747 b	780 a	789 a	
Stem					
L79-1002	710 abC	728 aB	694 bB	675 cC	
Merkeron	747 bA	752 bA	756 abA	775 aA	
UF-1	727 bB	734 abB	749 aA	740 abB	
SE			8.0		
Total biomass					
L79-1002	738 abA	752 aA	735bC	718 cC	
Merkeron	743 cA	742 cB	759bA	778 aA	
UF-1	730 cB	734 bcC	751aB	744 abB	
SE			5.4		

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-5. Effect of grass entry x harvest management interaction on acid detergent fiber (ADF) concentration in leaf ( $P = 0.090$ ), stem ( $P < 0.001$ ), and total biomass ( $P < 0.001$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg ADF g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	435	429	477	479	455 A <sup>§</sup>
Merkeron	388	379	417	431	404 B
UF-1	392	384	413	412	400 B
Mean	405 b <sup>‡</sup>	397 b	436 a	441 a	
Stem					
L79-1002	442 aC	445 aB	455 aB	442 aC	
Merkeron	501 bA	477 cA	526 aA	537 aA	
UF-1	484 bB	467 bA	514 aA	507 aB	
SE			10.0		
Total biomass					
L79-1002	441 bB	439 bA	462 aB	453 abC	
Merkeron	466 cA	438 dA	498 bA	515 aA	
UF-1	458 bA	437 cA	495 aA	494 aB	
SE			7.6		

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-6. Effect of grass entry x harvest management interaction on acid detergent lignin (ADL) concentration in leaf ( $P = 0.037$ ), stem ( $P = 0.006$ ), and total biomass ( $P < 0.001$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>			
	2X-July	2X-Nov	1X-Nov	1X-Dec
-----mg ADL g <sup>-1</sup> dry matter-----				
Leaf				
L79-1002	57 b <sup>‡</sup> A <sup>§</sup>	51 cA	66 aA	64 aA
Merkeron	47 bB	37 cB	53 aB	54aB
UF-1	48 aB	41 bB	51 aB	48 aC
SE			1.5	
Stem				
L79-1002	63 bcC	62 cB	73 aC	69 abC
Merkeron	86 bA	76 cA	99 aA	98 aA
UF-1	77 bB	72 bA	90 aB	87 aB
SE			3.1	
Total biomass				
L79-1002	61 bC	58 bA	71aC	68 aC
Merkeron	74 bA	61 cA	87 aA	89 aA
UF-1	69 bB	61 cA	82 aB	82 aB
SE			2.5	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-7. Effect of grass entry x harvest management interaction on cellulose (detergent fiber analysis) concentration in leaf ( $P = 0.195$ ), stem ( $P < 0.001$ ), and total biomass ( $P < 0.001$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg cellulose g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	378	378	411	414	395 A <sup>§</sup>
Merkeron	341	342	365	377	356 B
UF-1	344	343	362	364	353 B
Mean	354 b <sup>‡</sup>	354 b	379 a	385 a	
Stem					
L79-1002	379 aB	383 aB	383 aB	373 aC	
Merkeron	415 bcA	401 cA	426 abA	439 aA	
UF-1	407 bcA	395 cA	425 aA	420 abB	
SE			7.1		
Total biomass					
L79-1002	380 bB	381 abA	391 aB	386 abC	
Merkeron	392 cA	378 dA	411 bA	426 aA	
UF-1	389 bA	377 cA	413 aA	412 aB	
SE			5.2		

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-8. Effect of grass entry x harvest management interaction on hemicellulose (detergent fiber analysis) concentration in leaf ( $P = 0.029$ ), stem ( $P = 0.009$ ), and total biomass ( $P < 0.284$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg hemicellulose g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	347 b <sup>‡</sup> A <sup>§</sup>	355 aA	345 bA	345 bB	
Merkeron	344 bAB	346 abB	346 abA	352 aA	
UF-1	339 bB	349 aAB	344 abA	350 aAB	
SE			2.6		
Stem					
L79-1002	269 bA	283 aA	239 cA	233 cA	
Merkeron	246 bB	275 aAB	231 cA	238 bcA	
UF-1	243 bB	267 aB	235 bA	234 bA	
SE			4.3		
Total biomass					
L79-1002	297	313	272	265	287 A
Merkeron	277	303	261	262	276 B
UF-1	271	297	256	250	269 C
Mean	282 b	304 a	263 c	259 c	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-9. Sources of variation and levels of probability (*P*) for concentration of non-structural components (extractives and total soluble sugars) and their effects on response variables reported in Chapter 4.

Source of variation	Leaf	Stem	Total
Extractives			
Harvest management (HM)	< 0.001	0.609	0.367
Entry (E)	< 0.001	< 0.001	< 0.001
Year (Y)	< 0.001	< 0.001	< 0.001
HM × E	0.044	< 0.001	< 0.001
HM × Y	0.200	0.046	0.011
E × Y	0.207	0.210	0.016
HM × E × Y	0.002	0.103	0.002
Total soluble sugars			
Harvest management (HM)	0.004	0.001	< 0.001
Entry (E)	0.001	< 0.001	< 0.001
Year (Y)	< 0.001	0.001	< 0.001
HM × E	0.044	< 0.001	< 0.001
HM × Y	0.003	< 0.001	< 0.001
E × Y	0.259	0.031	0.001
HM × E × Y	0.009	0.769	0.036

Table 4-10. Sources of variation and levels of probability (*P*) for concentration of structural components (hexose, pentose, and total lignin) and their effects on response variables reported in Chapter 4.

Source of variation	Leaf	Stem	Total
		Hexose	
Harvest management (HM)	< 0.001	0.013	0.001
Entry (E)	< 0.001	< 0.001	< 0.001
Year (Y)	< 0.001	< 0.001	< 0.001
HM × E	0.562	0.001	0.001
HM × Y	< 0.001	< 0.001	< 0.001
E × Y	0.048	0.075	0.034
HM × E × Y	0.401	0.123	0.252
		Pentose	
Harvest management (HM)	0.001	< 0.001	< 0.001
Entry (E)	0.141	0.132	0.017
Year (Y)	< 0.001	< 0.001	< 0.001
HM × E	0.977	0.003	0.139
HM × Y	0.001	< 0.001	< 0.001
E × Y	0.138	0.034	0.004
HM × E × Y	0.439	0.649	0.153
		Lignin	
Harvest management (HM)	0.005	< 0.001	0.001
Entry (E)	< 0.001	< 0.001	< 0.001
Year (Y)	< 0.001	< 0.001	< 0.001
HM × E	0.069	0.002	0.002
HM × Y	0.021	0.388	0.303
E × Y	0.054	0.197	0.002
HM × E × Y	0.108	0.034	0.005

Table 4-11. Effect of grass entry x harvest management interaction on extractives in leaf ( $P = 0.044$ ), stem ( $P < 0.001$ ), and total biomass ( $P < 0.001$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>			
	2X-July	2X-Nov	1X-Nov	1X-Dec
-----mg extractives g <sup>-1</sup> dry matter-----				
Leaf				
L79-1002	199 a <sup>‡</sup> B <sup>§</sup>	196 aB	164 bB	173 bC
Merkeron	221 aA	226 aA	194 bA	187 bB
UF-1	216 aA	225 aA	197 bA	201 bA
SE			4.0	
Stem				
L79-1002	302 bcA	289 cA	313 bA	338 aA
Merkeron	265 aC	262 aC	250 abC	241 bC
UF-1	285 aB	274 abB	262 bB	263 bB
SE			7.9	
Total biomass				
L79-1002	267 bA	251 cA	269 bA	289 aA
Merkeron	251 aB	248 abA	234 bcB	229 cC
UF-1	262 aA	256 abA	244 bB	254 abB
SE			6.0	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-12. Effect of grass entry x harvest management interaction on total soluble sugars concentration in leaf ( $P = 0.044$ ), stem ( $P < 0.001$ ), and total biomass ( $P < 0.001$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>			
	2X-July	2X-Nov	1X-Nov	1X-Dec
-----mg total soluble sugars g <sup>-1</sup> dry matter -----				
Leaf				
L79-1002	23 bA	32 aB	14 cB	22 bB
Merkeron	26 bA	40 aA	24 bA	22 bB
UF-1	26 bA	35 aAB	29 abA	33 abA
SE			2.9	
Stem				
L79-1002	152 cA	165 cA	228 bA	280 aA
Merkeron	97 bB	134 aB	138 aC	142 aC
UF-1	138 bA	147 bB	161 bB	189 aB
SE			11.2	
Total biomass				
L79-1002	109 cA	110 cA	163 bA	205 aA
Merkeron	74 cB	96 bB	106 abC	116 aC
UF-1	102 cA	106 cAB	126 bB	167 aB
SE			6.9	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-13. Effect of grass entry x harvest management interaction on structural hexose concentration in leaf ( $P = 0.562$ ), stem ( $P = 0.001$ ), and total biomass ( $P = 0.001$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg hexose g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	371	356	404	395	381 A <sup>§</sup>
Merkeron	346	334	370	369	355 B
UF-1	342	333	369	357	350 B
Mean	353 b <sup>‡</sup>	341 c	381 a	374 a	
Stem					
L79-1002	363 aB	361 aB	361 aB	342 bB	
Merkeron	400 bA	385 cA	415 aA	406 abA	
UF-1	397 bA	378 cA	413 aA	394 bA	
SE			7.5		
Total biomass					
L79-1002	367 abB	360 bA	374 aB	357 bB	
Merkeron	382 bA	365 cA	403 aA	399 aA	
UF-1	381 bA	362 cA	402 aA	389 bA	
SE			6.2		

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-14. Effect of grass entry x harvest management interaction on structural pentose concentration in leaf ( $P = 0.977$ ), stem ( $P = 0.003$ ), and total biomass ( $P = 0.139$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg pentose g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	252	266	273	272	
Merkeron	251	266	271	272	
UF-1	254	273	279	274	
Mean	252 b <sup>‡</sup>	268 a	274 a	273 a	
Stem					
L79-1002	217 bA <sup>§</sup>	244 aA	209 bcA	202 cB	
Merkeron	206 bB	233 aB	209 bA	213 bA	
UF-1	206 bB	230 aB	209 bA	209 bAB	
SE			3.1		
Total biomass					
L79-1002	229	253	228	222	233 A
Merkeron	221	246	225	225	229 B
UF-1	222	246	228	218	228 B
Mean	224 b	248 a	227 b	222 b	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-15. Effect of grass entry x harvest management interaction on lignin concentration in leaf ( $P = 0.069$ ), stem ( $P = 0.002$ ), and total biomass ( $P = 0.002$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg lignin g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	213	213	226	221	218 A <sup>§</sup>
Merkeron	199	197	210	211	204 B
UF-1	200	197	204	202	201 C
Mean	204 b <sup>‡</sup>	202 b	214 a	212 a	
Stem					
L79-1002	167 bC	167 bC	182 aB	177 aC	
Merkeron	184 bA	182 bA	203 aA	206 aA	
UF-1	176 bB	174 bB	199 aA	199 aB	
SE			3.5		
Total biomass					
L79-1002	183 cB	186 bcAB	195 aB	190 abC	
Merkeron	189 bA	188 bA	204 aA	207 aA	
UF-1	183 bB	183 bB	201 aA	200 aB	
SE			2.9		

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-16. Sources of variation and levels of probability (*P*) for nitrogen, phosphorus, and ash concentrations and their effects on response variables reported in Chapter 4.

Source of variation	Leaf	Stem	Total
Nitrogen			
Harvest management (HM)	< 0.001	0.002	< 0.001
Entry (E)	< 0.001	0.011	0.004
Year (Y)	< 0.001	0.002	< 0.001
HM × E	0.334	0.942	0.955
HM × Y	< 0.001	0.048	0.005
E × Y	0.395	0.646	0.381
HM × E × Y	0.824	0.418	0.688
Phosphorus			
Harvest management (HM)	0.002	< 0.001	0.001
Entry (E)	0.001	< 0.001	< 0.001
Year (Y)	< 0.001	0.023	< 0.001
HM × E	0.139	0.360	0.348
HM × Y	0.030	< 0.001	0.013
E × Y	0.205	0.496	0.365
HM × E × Y	0.179	0.084	0.183
Ash			
Harvest management (HM)	0.012	< 0.001	< 0.001
Entry (E)	< 0.001	0.904	0.012
Year (Y)	0.804	0.014	0.022
HM × E	0.359	0.257	0.853
HM × Y	0.418	0.004	0.002
E × Y	0.071	0.300	0.740
HM × E × Y	0.987	0.672	0.858

Table 4-17. Effect of grass entry x harvest management interaction on nitrogen concentration in leaf ( $P = 0.334$ ), stem ( $P = 0.942$ ), and total biomass ( $P = 0.955$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg nitrogen g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	12.5	12.9	7.4	6.6	9.9 B <sup>§</sup>
Merkeron	14.1	14.2	9.5	8.2	11.5 A
UF-1	13.0	13.7	9.5	9.5	11.4 A
Mean	13.2 a <sup>‡</sup>	13.6 a	8.8 b	8.1 b	
Stem					
L79-1002	5.3	4.4	3.3	3.0	4.0 B
Merkeron	7.0	5.2	4.4	3.9	5.1 A
UF-1	5.5	4.9	3.4	3.4	4.3 B
Mean	5.9 a	4.9 b	3.7 c	3.5 c	
Total					
L79-1002	7.8	7.9	4.8	4.1	6.1 B
Merkeron	9.1	8.7	5.8	4.8	7.1 A
UF-1	7.6	8.0	4.6	4.2	6.1 B
Mean	8.2 a	8.2 a	5.0 b	4.4 b	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-18. Effect of grass entry by harvest management interaction on phosphorus concentration in leaf ( $P = 0.139$ ), stem ( $P = 0.360$ ), and total biomass ( $P = 0.348$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg phosphorus g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	1.40	1.94	0.72	0.57	1.16 B <sup>§</sup>
Merkeron	1.95	2.13	1.14	1.03	1.56 A
UF-1	1.32	1.51	0.88	1.00	1.17 B
Mean	1.56 a <sup>‡</sup>	1.86 a	0.91 b	0.87 b	
Stem					
L79-1002	1.30	1.44	0.70	0.63	1.02 B
Merkeron	1.64	1.99	1.21	1.09	1.48 A
UF-1	1.07	1.56	0.78	0.94	1.09 B
Mean	1.34 b	1.66 a	0.89 c	0.89 c	
Total					
L79-1002	1.31	1.66	0.73	0.62	1.08 B
Merkeron	1.71	2.04	1.19	1.07	1.50 A
UF-1	1.13	1.53	0.80	0.95	1.10 B
Mean	1.38 b	1.74 a	0.90 c	0.88 c	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-19. Effect of grass entry x harvest management interaction on ash concentration in leaf ( $P = 0.359$ ), stem ( $P = 0.257$ ), and total biomass ( $P = 0.853$ ). Data are means across four replicates and two years ( $n = 8$ ).

Entry	Harvest management <sup>†</sup>				Mean
	2X-July	2X-Nov	1X-Nov	1X-Dec	
-----mg ash g <sup>-1</sup> dry matter-----					
Leaf					
L79-1002	44	45	33	32	38 C <sup>§</sup>
Merkeron	59	57	50	43	52 B
UF-1	60	60	60	56	59 A
Mean	54 a <sup>‡</sup>	54 ab	48 bc	44 c	
Stem					
L79-1002	44	43	28	22	
Merkeron	40	43	26	25	
UF-1	39	46	25	27	
Mean	41 a	44 a	26 b	24 b	
Total					
L79-1002	44	44	29	24	35 B
Merkeron	46	48	32	29	39 A
UF-1	45	51	32	31	40 A
Mean	45 a	48 a	31 b	28 b	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within an entry not followed by the same lower case letter are different ( $P < 0.05$ )

<sup>§</sup> Entry means within a harvest management not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-20. Effect of harvest management x year interaction on concentration of acid detergent lignin (ADL) ( $P = 0.002$ ), hemicellulose ( $P < 0.001$ ), extractives ( $P = 0.011$ ), total soluble sugars ( $P < 0.001$ ), structural hexose ( $P < 0.001$ ) and pentose ( $P < 0.001$ ), N ( $P = 0.005$ ), P ( $P = 0.013$ ), and ash ( $P = 0.002$ ) in total biomass. Data are means across four replicates and 2 yr ( $n = 8$ ).

Harvest management <sup>†</sup>	2010	2011	<i>P</i>	2010	2011	<i>P</i>	2010	2011	<i>P</i>
	-----mg hemicellulose g <sup>-1</sup>			-----mg hemicellulose g <sup>-1</sup>			-----mg extractives g <sup>-1</sup>		
	--mg ADL g <sup>-1</sup> dry matter--			dry matter-----			dry matter-----		
2X-July	69 B <sup>‡</sup>	67 C	0.190	270 B	294 B	< 0.001	276 A	245 A	< 0.001
2X-Nov	59 C	60 D	0.583	305 A	303 A	0.414	261 A	242 AB	0.001
1X-Nov	80 A	80 A	0.869	254 C	273 C	< 0.001	270 A	228 B	< 0.001
1X-Dec	83 A	76 B	< 0.001	256 C	262 D	0.015	276 A	239 AB	< 0.001
SE		2.4			3.5			5.8	
	-----mg total soluble sugars								
	g <sup>-1</sup> dry matter-----			-mg hexose g <sup>-1</sup> dry matter-			-mg pentose g <sup>-1</sup> dry matter-		
2X-July	100 C	90 C	0.074	370 B	383 B	0.006	205 B	243 BC	< 0.001
2X-Nov	105 C	103 BC	0.771	340 C	384 B	< 0.001	243 A	253 A	0.001
1X-Nov	147 B	116 B	< 0.001	391 A	395 A	0.403	208 B	246 B	< 0.001
1X-Dec	185 A	140 A	< 0.001	375 B	388 AB	0.008	207 B	237 C	< 0.001
SE		6.3			5.8			2.6	
	-----mg N g <sup>-1</sup> dry matter-----			-----mg P g <sup>-1</sup> dry matter-----			---mg ash g <sup>-1</sup> dry matter---		
2X-July	8.2 A	8.1 A	0.868	1.48 A	1.28 B	0.050	41 B	49 A	0.001
2X-Nov	9.5 A	7.0 A	<0.001	1.71 A	1.77 A	0.578	49 A	46 A	0.112
1X-Nov	5.3 B	4.7 B	0.241	1.05 B	0.76 C	0.005	30 C	32 B	0.174
1X-Dec	4.6 B	4.1 B	0.297	1.09 B	0.67 C	0.001	27 C	29 B	0.379
SE		0.508			0.126			1.85	

<sup>†</sup> Harvest management treatments were harvested twice per year (2X-July, and 2X-Nov), once per year in November (1X-Nov), and once per year after first freeze (1X-Dec).

<sup>‡</sup> Harvest management means within a year not followed by the same upper case letter are different ( $P < 0.05$ )

Table 4-21. Effect of entry by year on concentration of neutral detergent fiber (NDF) ( $P = 0.009$ ), acid detergent lignin (ADL) ( $P = 0.006$ ), hemicellulose ( $P < 0.001$ ), extractives ( $P < 0.016$ ), total soluble sugars ( $P = 0.001$ ), structural hexose ( $P = 0.034$ ) and pentose ( $P < 0.001$ ), and lignin ( $P = 0.002$ ) in total biomass. Data are means across four replicates and two years ( $n = 8$ ).

Entry	2010	2011	<i>P</i>	2010	2011	<i>P</i>	2010	2011	<i>P</i>
	---mg NDF g <sup>-1</sup> dry matter---			---mg ADL g <sup>-1</sup> dry matter---			-----mg hemicellulose g <sup>-1</sup> dry matter-----		
L79-1002	722 C <sup>†</sup>	749 B	< 0.001	65 B	63 C	0.074	276 A	298 A	< 0.001
Merkeron	748 A	762 A	0.006	77 A	78 A	0.319	273 A	279 B	0.004
UF-1	737 B	742 B	0.257	76 A	71 B	0.001	265 B	273 C	0.001
SE		4.2			2.2			3.2	
	-----mg extractives g <sup>-1</sup> dry matter-----			---mg total soluble sugars g <sup>-1</sup> dry matter----			-mg hexose g <sup>-1</sup> dry matter-		
L79-1002	290 A	248 A	< 0.001	166 A	128 A	< 0.001	352 B	377 C	< 0.001
Merkeron	256 C	225 B	< 0.001	107 C	89 B	0.001	377 A	397 A	< 0.001
UF-1	266 B	242 A	< 0.001	130 B	120 A	0.038	378 A	389 B	0.013
SE		4.2			4.5			5.3	
	mg pentose g <sup>-1</sup> dry matter			--mg lignin g <sup>-1</sup> dry matter--					
L79-1002	215 A	251 A	< 0.001	181 B	196 B	< 0.001			
Merkeron	215 A	243 B	< 0.001	192 A	202 A	< 0.001			
UF-1	217 A	240 B	< 0.001	189 A	194 B	0.007			
SE		1.9			2.3				

<sup>†</sup> Entry means within a year not followed by the same upper case letter are different ( $P < 0.05$ ).

CHAPTER 5  
SEASONAL CHANGES IN MORPHOLOGICAL CHARACTERISTICS OF  
ELEPHANTGRASS AND ENERGYCANE

**Overview of Research**

Due in part to their C4 carbon fixation pathway, tropical grasses like elephantgrass (*Pennisetum purpureum* Schum.) and energycane (*Saccharum* spp. hybrids) are widely recognized for their biomass production potential (Woodard and Prine, 1991; 1993a). Research efforts to identify herbaceous plants with the highest biomass yields for renewable energy purposes have consistently found elephantgrass and energycane to be attractive candidate species across the USA Gulf Coast Region. Yields of 20 to 48 Mg ha<sup>-1</sup> yr<sup>-1</sup> have been reported in the region (Prine et al., 1984; Woodard and Prine, 1991; Bouton, 2002; Woodard and Sollenberger, 2008).

Morphological characteristics are important determinants of perennial grass biomass yield potential. Elephantgrass and energycane tillers grew vegetatively for 30 to 35 wk, and their greater yield than other grasses was associated with a longer linear growth phase (Woodard et al., 1991b). It has been reported that the long growth phase of elephantgrass was achieved in part because plants maintained a favorable light environment by altering structure of the canopy from more planophile early in the growing season to increasingly erectophile as the season progressed (Kubota et al., 1994).

Morphological characteristics of candidate bioenergy species may also affect optimum harvest management. Elephantgrass leaf area index (LAI) increased more rapidly than energycane during early-season growth in north Florida (Woodard et al., 1993). Elephantgrass LAI increased from 1.4 at 30 d after mowing to a maximum of 7.1 at 105 d after mowing. In contrast, energycane LAI was 0.5 at 30 d after mowing and

reached a maximum of 6.9 at 161 d after mowing (Woodard et al., 1993). This was associated with differences in light interception, with elephantgrass reaching 90% light interception by 49 d after mowing compared with 91 d after mowing for energycane. It is reasonable to anticipate that different patterns of leaf area accumulation and light interception would be associated with differences in optimal harvest interval. Light environment may also affect tiller dynamics, as tiller senescence was observed for sugarcane (*Saccharum* spp.) after canopy light interception exceeded 70% of photosynthetic active radiation (Inman-Bamber, 1994).

Changes in plant-part proportion may also affect a range of biomass traits including dry matter concentration, nutrient removal in harvested biomass, and conversion of biomass to energy. Stems are typically considered the most important organ for bio-ethanol production, and they generally constitute the highest proportion of total aboveground dry weight. Elephantgrass and energycane green leaf mass reached a maximum approximately 100 d after mowing, but stem mass and total biomass (stem and green plus dead leaves) increased linearly over 250 d resulting in much greater stem proportion with longer intervals following mowing (Woodard et al., 1993). Sweet sorghum [*Sorghum bicolor* (L.) Moench] stems were found to compose 56 to 73% of aboveground biomass, and leaf and stem proportions varied with season and harvest management (Zhao et al., 2009).

Seasonal changes in a wide range of morphological traits have not been studied comprehensively during first and ratoon growth periods and over more than one growing season for elephantgrass and energycane. It has been suggested that LAI, tiller number and mass, and plant-part proportion are important responses to be assessed

because of their effect on biomass accumulation and responses to defoliation (Madakadze et al., 1998; Trócsányi et al., 2009; León et al., 2012). The objective of this study was to quantify monthly changes in a range of morphological characteristics during first and ratoon growth of elephantgrass and energycane and relate these findings to differences in plant responses to defoliation (Chapter 3).

## **Materials and Methods**

### **Experimental Site**

The experiment was conducted during 2010 and 2011 at the Plant Science Research and Education Unit (PSREU) at Citra, FL (29.41° N, 82.17° W). The soil was a well-drained Candler sand (hyperthermic, uncoated Lamellic Quartzipsamments). Initial soil characterization of topsoil (0-20 cm) showed an average soil pH of 7.0 and Mehlich-1 extractable P, K, Mg, and Ca of 54, 20, 123, and 496 mg kg<sup>-1</sup>, respectively. Monthly average, maximum, and minimum temperatures (Figure 3-1) and monthly precipitation (Figure 3-2) were shown previously for the experimental period. In 2010, last freeze event in spring was 7 March (-1.4°C) and first freeze event was 2 December (-2°C). In 2011, the last freeze event in spring was 14 February (-0.9°C), but the first freeze did not occur until last sampling date, 13 December.

### **Treatments and Experimental Design**

The three grass entries included two elephantgrasses, 'Merkeron' (Burton, 1989) and a breeding line referred to as UF-1, and 'L79-1002' energycane (Bischoff et al., 2008). As mentioned previously (Chapter 3), these species have potential as biofuel feedstock in this region. Merkeron elephantgrass and L79-1002 energycane were chosen because they are the most available cultivars of these two species. In addition, breeding line UF-1 elephantgrass was included because in preliminary research it

demonstrated outstanding potential for use in bioenergy feedstock production (Sollenberger et al., 2011) and potential exists for it to be released as a cultivar.

Plots used were a subset of those from the experiment described in Chapter 3. Full-season growth sampling occurred on plots that were harvested once per year within 1 wk following the first freeze event in fall (treatment referred to as 1X-Dec in Chapter 3), and first growth/ratoon growth sampling occurred on the plots that were harvested twice per year with first harvest on 30 July 2010 and 21 July 2011 (treatment referred to as 2X in Chapter 3). Data from full-season growth plots were analyzed separately from first growth/ratoon growth data, so treatments were grass entries replicated four times in a randomized complete block design.

### **Plot Establishment and Management**

Plots contained six rows of 6-m length, with 1-m spacing between rows. Plots were established using above-ground stem pieces planted on 15 Dec. 2009. Thus, the 2010 data are from the establishment year and 2011 data are from well-established stands. In both years, N was applied as ammonium sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ) at a rate of 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>, and K was applied as muriate of potash (KCl) at a rate of 90 kg K ha<sup>-1</sup> yr<sup>-1</sup>. Nutrients were split applied, with applications of 50 kg N and 45 kg K ha<sup>-1</sup> in mid-April and 100 kg N and 45 kg K ha<sup>-1</sup> in mid-May. No P was needed based on soil test. Limited irrigation was applied to the experiment only at sign of significant drought stress (leaf rolling). Water was applied using a traveling gun system. There were five irrigation events in 2010 totaling 60 mm and three irrigation events in 2011 totaling 50 mm.

### **Response Variables**

Data collection occurred at approximately 4-wk intervals (Table 5-1). In 2011, the fully established plants began growth earlier and more vigorously than the newly

established plants in 2010, so first data collection started 4 wk earlier in 2011 than in 2010. At each collection date, LAI, canopy height, tiller density, and tiller mass were measured.

The SS1-SunScan canopy analysis system (Delta-T Devices Ltd, Cambridge, United Kingdom) was used to obtain non-destructive estimates of LAI. Attenuation of photosynthetically active radiation (PPFD) was measured with a linear photo-sensor array probe. External canopy beam fraction sensor (BF-3, located in a nearby plot alley) provided the simultaneous beam fraction data for SunScan system and six under canopy measurements were averaged to obtain the plot LAI estimates. The probe was placed about a 45° angle across the four center rows (near the center of the plot) from four azimuth angles of each plot and a reading taken. To obtain consistent LAI estimates, LAI data were collected at high sun angles from 10 am to 2 pm (Eastern Standard Time) under generally sunny or partly cloudy condition. All readings were collected following canopy closure using a default ellipsoidal leaf angle distribution parameter (ELADP) value of 1.0. When leaf senescence occurred in the late summer, the probe was placed above dead leaves because this system could not distinguish between green vs. dead leaves.

Canopy height was the average undisturbed height of plants quantified by taking 10 measurements in each plot. Height was measured to the nearest 0.1 m using a tool made from graduated interlocking sections of PVC pipe.

To measure tiller density, number of tillers was quantified in inner rows by counting in two permanently marked 0.5-m sections of row per plot. To quantify tiller mass, plant-part proportion, and tiller dry matter (DM) concentration, four representative

tillers per plot were collected at each sampling event and hand-separated into leaf (blade plus sheath) and stem (including inflorescence, if present) components. Fresh weight of each component was recorded. The samples were dried at 60°C until constant weight. Sum of leaf and stem dry weight was divided by the sum of leaf and stem fresh weight to determine DM concentration. For plant-part data, only stem proportion is presented, and it was calculated as stem mass divided by total tiller mass and multiplied by 100 to be expressed as a percentage.

### **Statistical Analysis**

Data were analyzed using mixed-model methods in PROC MIXED (SAS Institute, 2008). Data for full-season growth and first growth/ratoon growth were analyzed separately because comparisons among entries was of primary interest and comparisons between full-season growth and ratoon growth after mid-season harvest were not deemed to be of biological importance. Years were analyzed separately because number of sampling events differed between years and sampling began earlier in 2011 than 2010 because 2010 was the year of establishment. In all models, grass entry was a fixed effect and sampling date (Table 5-1) was considered a repeated measurement (fixed). Block was considered a random effect. Means were compared using the pdiff test of LSMEANS. All means reported in the text are least squares means and were considered different if  $P \leq 0.05$ . Trends are discussed if  $P \leq 0.10 > 0.05$ .

## **Results and Discussion**

### **Tiller Density**

For the full-season growth treatment, there were entry x sampling date interactions ( $P < 0.001$ ) in 2010 and 2011. In June 2010, energycane tiller density was

greater than Merkeron and UF-1 (34, 20, and 20 tillers m<sup>-2</sup>), and although this difference narrowed throughout the year, energycane had greatest tiller density at each sampling date (Fig. 5-1). In May 2011, energycane again had greater tiller density than Merkeron or UF-1 (54, 37, and 32 tillers m<sup>-2</sup>). Unlike 2010, this difference lasted only through the end of June and thereafter there were no differences among treatments at any sampling date. In both years, energycane tiller number was greatest early in the season and decreased later, but tiller number of elephantgrass entries did not vary widely throughout the year. In both years, tiller density of all entries changed very little after mid-August. Previous research in Japan has shown that regardless of fertilizer level, elephantgrass tiller density increased from time of spring planting through July and decreased thereafter (Wadi et al., 2003). Working with sugarcane, Inman-Bamber (1994) reported that tiller density was a function of growing degree days, peaking about 500 degree (°C) days after ratooning and then declining until about 1200 degree (°C) days after which it stabilized.

For the first growth/ratoon growth treatment, tiller density of first growth was affected by entry x sampling date interactions ( $P < 0.001$ ) in both years. Energycane had greater tiller density throughout the first growth in 2010 but only at the start of 2011 (Fig. 5-2). Tiller number was generally greater for ratoon than first growth, but variability was larger for tiller number in ratoon growth than first growth. This response is likely due to repeated cutting, as previous studies have shown that elephantgrass harvested three times per year had tiller density ranging from 45 to 122 m<sup>-2</sup>, while the range for plants harvested twice per year was 37 to 42 m<sup>-2</sup> (Mukhtar et al., 2003). The latter is similar to ratoon tiller number for elephantgrass in the current study. Energycane had more tillers

than elephantgrass at three of four sampling dates in 2010, but no entry differences were detected in 2011 due to the large standard errors (Fig. 5-2). In 2011 ratoon growth, only the sampling date effect was significant ( $P < 0.001$ ). Especially for elephantgrass there was a much larger decrease in tiller number from beginning to end of the ratoon growth compared with the first growth. This was related to poor viability of emerging tillers in the ratoon growth.

### **Tiller Mass**

For full-season growth, there was entry  $\times$  season interaction ( $P < 0.001$ ) in 2010, but in 2011 there were entry and season effects ( $P < 0.001$  for both) and only a trend toward entry  $\times$  season interaction ( $P = 0.097$ ). At all 2010 sampling dates except the first of the season, energycane tiller mass was less than that of the two elephantgrass (Fig. 5-3). At four sampling dates during 2010 there were differences between elephantgrasses and in each case UF-1 had greater tiller mass than Merkeron. Entry UF-1 continued to accumulate tiller mass until 23 Oct. 2010 while Merkeron and energycane tiller mass did not increase beyond 22 September. The greater period of mass accumulation for UF-1 tillers may be a function of its later flowering date (~ 10 December vs. 10 November for Merkeron) (Sollenberger et al., 2011). Previous comparisons have shown that UF-1 had greater tiller mass than Merkeron during 2 yr in two locations in Florida, and this was associated with greater stem diameter and individual leaf area for UF-1 relative to Merkeron (Sollenberger et al., 2011). The decrease in tiller mass between November and December was associated with a freeze event on 2 Dec. 2010 ( $-2^{\circ}\text{C}$ ). In 2011, the elephantgrasses had greater tiller mass than energycane throughout the entire season, but the elephantgrasses did not differ

consistently (Fig. 5-3). There was no clear difference among entries when tiller mass accumulation ceased in 2011.

First-growth tiller mass was affected by entry x sampling date interaction ( $P < 0.001$ ) in both 2010 and 2011, while ratoon-growth tiller mass was affected only by entry ( $P = 0.009$ ) and sampling date ( $P < 0.001$ ) main effects in 2010 and entry x sampling date interaction ( $P < 0.007$ ) in 2011. First-growth tiller mass was greater for UF-1 than Merkeron and greater for Merkeron than energycane in June and July 2010 and July 2011 (Fig. 5-4). Ratoon growth tiller mass was consistently greater for UF-1 than energycane in 2010 and was greater for the elephantgrasses than energycane at three of four sampling dates in 2011 (Fig. 5-4). In both years tiller mass was consistently greater for first-growth than ratoon-growth.

Previous research has shown that increasing cutting frequency may decrease average tiller mass. For example, Mukhtar et al. (2003) reported that tiller mass of Merkeron elephantgrass was 8.9 g following a single harvest but only 1.8 g following a third harvest at a 60-d cutting interval. Moreover, there was a negative correlation between elephantgrass tiller density and tiller mass (Mukhtar et al., 2003). This pattern was observed in the current study as UF-1 generally had greatest tiller mass but least tiller density, and energycane often had the most tillers but their mass was least. In another study, the range in elephantgrass tiller mass was 39 to 51 g with a 90-d cutting interval, but it was 11 to 16 g with a 60-d cutting interval (Wadi et al., 2004). This research also showed that although rapid ratoon growth is possible with elephantgrass, a longer regrowth period may aid in maximizing crop growth rate (CGR); the range in

CGR was 10.6 to 13.4 g m<sup>-2</sup> d<sup>-1</sup> with a 90-d cutting interval vs. 6.7 to 8.6 g m<sup>-2</sup> d<sup>-1</sup> with a 60-d interval.

### **Leaf Area Index**

Leaf area index in full-season plots was greater early in the season for Merkeron and UF-1 elephantgrasses than for energycane; however, as the season progressed, differences between species became smaller and disappeared by August in both years (Fig. 5-5). This response is similar to that observed in previous research; elephantgrass showed greater LAI than energycane in early season but by 125 d after mowing on 28 March there were no species differences (Woodard et al., 1993). Maximum LAI was between 6 and 7 for elephantgrasses each year, and this level was reached by the end of June 2010 and mid-July 2011. Similarly, maximum LAI for energycane and elephantgrass was 6.9 and 7.1, respectively (Woodard et al., 1993). In current research, energycane reached its maximum approximately 30 d after elephantgrass in each year. Energycane reached its maximum 28 to 56 d later than elephantgrass in previous Florida research (Woodard et al., 1993).

Other studies have reported elephantgrass LAI of 10 or greater (Ito and Inanaga, 1988; Ishii et al., 2005), but it appears that these measurements of light intensity were made at soil level. This leads to an overestimation of green leaf LAI because dead leaf mass begins to accumulate approximately 100 d after the previous defoliation and increases thereafter (Woodard et al., 1993). In the current study, LAI decreased due to leaf senescence starting in September 2010 and August 2011. This is comparable to the time when dead leaf number per tiller exceeded live leaf number (~125-150 d after mowing on 28 March) in a study conducted in northern Florida (Woodard et al., 1993). The decline in LAI was earlier and more greatly accentuated for elephantgrass in 2011

due to a severe thunderstorm with locally high wind gusts that caused significant plant damage on 12 Aug. 2011. Energycane was less susceptible to storm damage, likely due to markedly lower tiller mass at the time of the storm than for the two elephantgrass entries (Fig. 5-3), and the decline in LAI for energycane began later in the 2011 season. At the end of the 2010 growing season, the first freeze was severe and killed most leaves and caused a large decline in LAI from November to December sampling dates.

In southern Japan, elephantgrass live leaf number decreased from September forward and ratio of live leaf number to whole leaf number decreased from June forward at a latitude (32° N) comparable to southern Georgia (Wadi et al., 2003). In Florida, live leaf number of both elephantgrass and energycane remained nearly constant throughout the season, but dead leaf number increased starting in June and exceeded live leaf number by the end of July for elephantgrass and end of August for energycane. These combined effects associated with leaf senescence likely resulted in the seasonal decline in LAI observed in the current study.

Initial growth of the first growth/ratoon treatment followed a similar pattern as full-season growth, with elephantgrass entries achieving greater LAI. This difference was sustained during the entire period leading up to the 21 July defoliation event in 2011 and to within less than 28 d of the 30 July defoliation in 2010 (Fig. 5-6). Defoliation reset LAI to similar levels for all entries and there were no subsequent entry effects in 2010 ( $P = 0.185$ ) (Fig.5-6); however, in 2011, there were differences among entries ( $P = 0.007$ ) with Merkeron generally having greatest LAI. It is possible that the cumulative effects of defoliation resulted in the more pronounced first-growth entry effect in 2011 vs. 2010 and in the presence of an entry effect in ratoon growth in 2011 vs. no effect in 2010.

Refoliation of Entry UF-1 in ratoon-growth of 2011 also appeared to be slowed by cumulative effects of multiple harvests in 2 yr.

### **Canopy Height**

In both 2010 and 2011, there was entry × sampling date interaction ( $P < 0.001$ ) for the full-season treatment. In 2010, UF-1 reached a height greater than 4 m and was taller than Merkeron at all sampling dates, but it was only occasionally taller than energycane (Fig. 5-7). In 2011, maximum canopy height was slightly less than in 2010, but UF-1 was taller than at least one of the other entries from beginning of sampling in May through September (Fig. 5-8). Unlike 2010 when height continued to increase for all entries until late September to early November, in 2011 UF-1 height remained relatively constant after the 17 July sampling. This was due to the effects of the severe thunderstorm described earlier. Three out of four replicates of Merkeron and UF-1 were seriously affected by the 12 August storm that preceded sampling on 19 August. Specifically, tillers lodged due to high winds and were laid over on each other. None of the L79-1002 plots were damaged by the storm, likely due in part to lesser tiller mass for energycane than for the two elephantgrasses (Fig. 5-3). Tiller number of the elephantgrasses remained essentially constant through the remainder of the season following the storm (Fig. 5-1), so although laid over to some extent by the wind they recovered sufficiently to remain viable until the end of the growing season.

In an earlier study in Florida (Woodard and Prine, 1993a), the shape of the canopy height response curve to days after mowing was similar for elephantgrass plant introduction (PI) 300086 and breeding line N51 to those observed for Merkeron and UF-1 in this study. Those authors also reported maximum elephantgrass height of between 4 and 5 m and that energycane height was generally slightly shorter than the

elephantgrasses. Sollenberger et al. (2011) reported UF-1 was 0.46 to 0.63 m taller in September than Merkeron at two locations in Florida during 2 yr of evaluation, but energycane was not included in that experiment.

For the first growth/ratoon growth treatment, first growth height of UF-1 reached 3 m or taller by harvest in July and was taller than one or both of the other entries for all sampling dates in 2010 and three of four sampling dates in 2011 (Fig. 5-8). Height of ratoon growth was greater for UF-1 across sampling dates in 2010, but in 2011 there were no difference among grass entries. Height of ratoon growth generally was 2 to 2.5 m, approximately the height of first growth in late June. Unlike LAI, for which UF-1 and energycane showed lessened regrowth response following July harvest, ratoon canopy height response was quite similar for UF-1 and energycane in both years (Fig. 5-8).

### **Stem Proportion**

Stem proportion of full-season total tiller mass was affected by entry and sampling date ( $P < 0.001$  for both) in 2010 and entry x sampling date interaction in 2011 ( $P < 0.001$ ). In 2010, stem proportion increased until September and changed relatively little thereafter; throughout the season it was greater for UF-1 than Merkeron or energycane (Fig. 5-9). Stem proportion increased throughout the entire 2011 season for all entries, and energycane stem proportion was least among entries at all sampling dates (Fig. 5-9). Interaction occurred because Merkeron tended to have greatest stem proportion early in the year, but UF-1 showed greatest stem proportion in late season.

Plant-part proportion is important because it affects chemical composition of biomass. The reduction in switchgrass (*Panicum virgatum* L.) ash and mineral concentration was attributed to increasing proportions of stem relative to leaf as harvest was delayed into the winter (Sokhansanj et al., 2009). With elephantgrass in Florida,

increasing plant maturity in fall resulted not only in decreased LAI but also increased stem proportion (Woodard et al., 1993). High stem proportion can be achieved due to stem growth or it also occurs due to leaf senescence and associated decrease in leaf biomass. In Japan, elephantgrass leaf dry weight increased until September after which it decreased, but stem dry weight increased linearly throughout the season (Wadi et al., 2003). In Florida, green leaf biomass of several tall elephantgrasses was nearly constant at 5 Mg ha<sup>-1</sup> throughout the growing season while dead leaf biomass increased from mid-June to September and was relatively constant thereafter at the same mass as green leaf (Woodard et al., 1993). In the same study, stem biomass increased linearly throughout the growing season and stem accumulation was the main driver in sustained biomass accumulation of both elephantgrass and energycane (Woodard et al., 1993). This pattern of response was observed in 2011 in the current study and until October in 2010.

First-growth stem proportion was affected by entry and sampling dates ( $P < 0.001$  for both) in both years, but there was no interaction. Elephantgrass UF-1 showed the highest overall stem proportion for first-growth (Fig. 5-10). Ratoon-growth was affected by entry and sampling date ( $P < 0.001$  for both) in 2010, but there was a trend toward entry x sampling interaction ( $P = 0.076$ ). In 2011, there was entry x sampling date interaction ( $P < 0.001$ ). The trend toward interaction in 2010 and the interaction in 2011 occurred because energycane was slow to refoliate following July defoliation and thus in August had greater stem proportion than the elephantgrasses. Thereafter in both years, either UF-1 or Merkeron elephantgrass generally had greater stem proportion

than energycane, and stem proportion in ratoon growth was approximately 50% of total biomass compared with 70 to 80% for full-season growth.

### **Biomass Dry Matter Concentration**

Biomass DM concentration is an important determinant of feedstock quality because it affects transportation cost and may affect conversion processes (Adler et al., 2006; Sokhansanj et al., 2009). There was entry x sampling date interaction for DM concentration of full-season growth in both 2010 ( $P = 0.047$ ) and 2011 ( $P = 0.002$ ) (Fig. 5-11). In early June 2010, energycane had greater DM concentration than the elephantgrass entries, but there were no differences during the remainder of the 2010 growing season. In 2011, there were differences in DM concentration starting in late June and continuing through the remainder of the season, with DM concentrations greater in Merkeron than energycane during this entire period and UF-1 generally was intermediate. Regardless of entry, DM concentration increased throughout the growing period to approximately 300 to 350 mg g<sup>-1</sup> by the end of the season.

In Louisiana (Legendre and Burner, 1995), energycane DM concentration averaged 368 mg g<sup>-1</sup> with a range of 248 to 447 mg g<sup>-1</sup> by December. In the current study, DM concentration did not increase to a great degree after mid-October, so delaying harvest to after first freeze in December may not improve biomass quality from the perspective of DM concentration. This response, however, is likely to be plant species and environment dependent (Chapters 3 and 7). Greater DM concentration or a trend toward this response by energycane early in the season likely reflects delayed early-season growth of energycane relative to elephantgrass during this period of time (Fig. 5-5) (Woodard et al., 1993). Greater DM concentration in elephantgrass in 2011 may be associated with the generally greater proportion of stem in elephantgrass than

energycane in that year (Fig. 5-9). Differences in stem anatomy between elephantgrass and energycane may also affect DM concentration differences in late season. Previous work has shown that elephantgrass stem has aerenchyma tissue which makes the stem more hollow (Jennewein et al., 2012) and likely faster to dry, whereas in energycane the stem is not hollow (Griffie, 2000).

For first-growth, there was entry x sampling date interaction for DM concentration in both years ( $P < 0.001$ ) (Fig. 5-12). For ratoon-regrowth in 2010 there was only a sampling date ( $P < 0.001$ ) effect, but in 2011 there was an entry x sampling date interaction ( $P = 0.033$ ). Similar to that described above for full-season growth, energycane biomass had greater DM concentration at the first sampling date (June 2010 and May 2011) in both years and at the first sampling date for the ratoon in one year (August 2011). Dry matter concentration of first- and ratoon-growth within the context of a two-harvest per year system reached a maximum of approximately 200 to 250 mg g<sup>-1</sup>, or approximately 100 mg g<sup>-1</sup> less than end of year full-season growth. Previous research has identified harvest frequency as an important determinant of biomass DM concentration. When elephantgrass was harvested every 30, 40, and 60 d in Puerto Rico, DM concentration increased from 144 to 145 and then to 180 mg g<sup>-1</sup>, respectively (Velez-Santiago and Arroyo-Aguilu, 1981). In Japan, harvesting elephantgrass at 60- vs. 90-d intervals resulted in DM concentrations of 143 and 183 mg g<sup>-1</sup> (Wadi et al., 2004). Hybrids between elephantgrass and pearl millet [*Pennisetum glaucum* (L.) R.Br.] were harvested every 6 and 12 wk in Florida, and DM concentration was 187 and 228 mg g<sup>-1</sup>, respectively (Spitaleri et al., 1994). For several *Pennisetum* species harvested one, two, or three times per year in Florida, DM concentrations were

reported to range from 327 to 372, 249 to 263, and 189 to 202 mg g<sup>-1</sup>, respectively (Woodard et al., 1991a). Thus data from the current experiment fit within the context of previously reported results.

### **Implications of Research**

Merkeron and UF-1 elephantgrasses generally showed similar seasonal patterns in morphological characteristics. There were much more pronounced differences in seasonal morphological characteristics between elephantgrass and energycane. Relative to energycane, the elephantgrasses generally had 1) consistent tiller number throughout the season vs. greater tiller number early in the season for energycane, 2) greater tiller mass and earlier development of LAI than energycane, and 3) greater stem proportion and biomass dry matter concentration than energycane. Elephantgrass UF-1 showed desirable characteristics related to biomass yield. Specifically, tiller mass and canopy height increased until late season, likely associated with later initiation of flowering, and UF-1 generally had the greatest proportion of stem in biomass, an advantage in terms of transportation costs, and also lower concentrations of N and ash that can negatively affect some conversion processes at the biorefinery. Lack of difference in biomass yield due to harvest treatment in the first 2 yr of the experiment (Chapter 3), i.e., the years when morphological characteristics were being quantified, may be due to ratoon growth quickly achieving similar LAI following harvest to that of non-defoliated, full-season growth. This occurred because as ratoon growth was accumulating leaf area, LAI of full-season growth was decreasing markedly. There was evidence of less rapid refoliation during ratoon growth in Year 2 than Year 1, and this may be an early indicator of the negative effects of the 2X treatment on plant vigor that were not apparent in terms of biomass harvested until Year 3 (Chapter 3). These data

provide evidence of the value of detailed characterization of morphological responses in understanding plant biomass accumulation and responses to defoliation.

Table 5-1. Energycane and elephantgrass sampling dates for responses reported in Chapter 5. Full-season and ratoon growth were sampled on the same day.

Year	Sampling dates								
2010	---	6	30	28	23	22	23	8	8
		June	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
2011	3	1	30	17	19	16	11	10	13
	May	June	June	July	Aug.	Sep.	Oct.	Nov.	Dec.

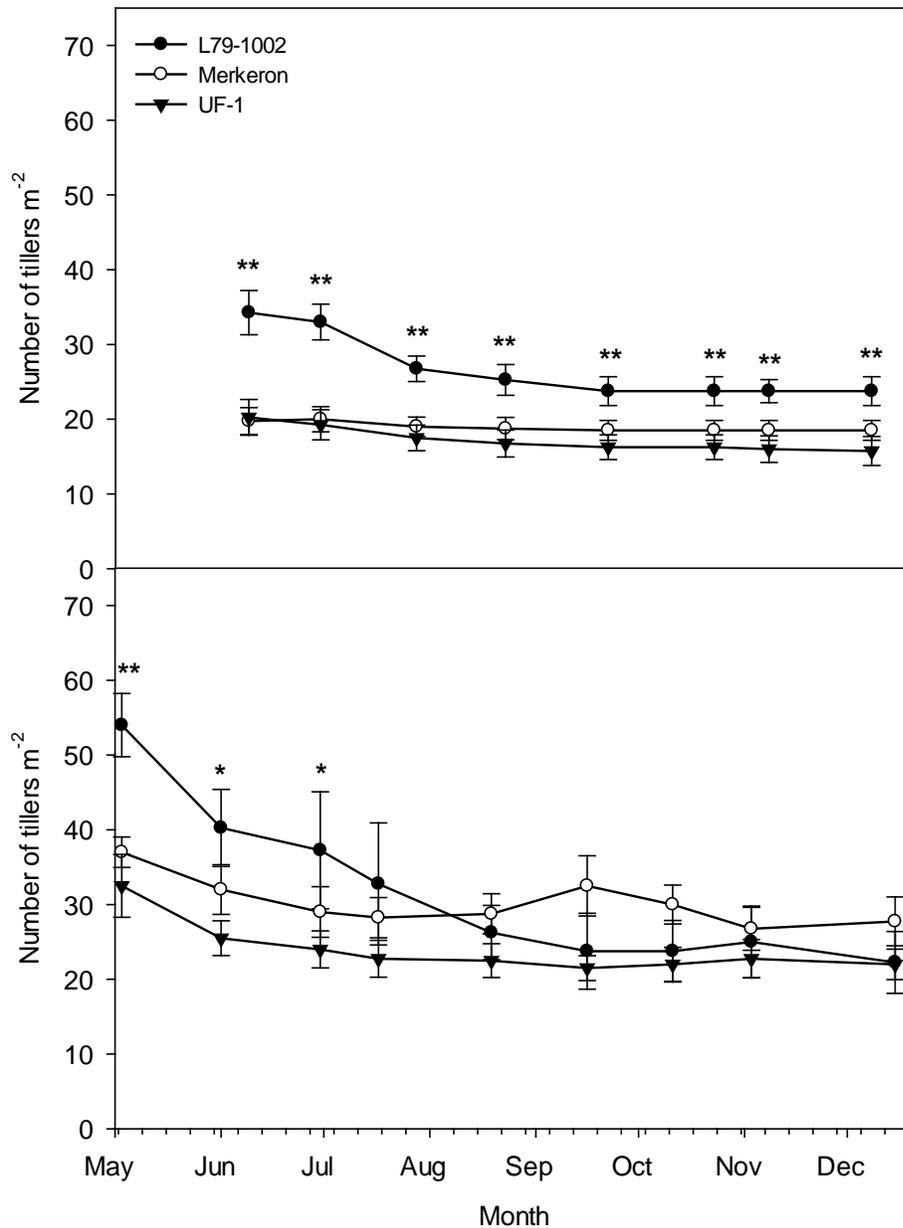


Figure 5-1. Seasonal changes in tiller density of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction *P* values were < 0.001 for all in 2010; and 0.177, < 0.001, and < 0.001, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ .

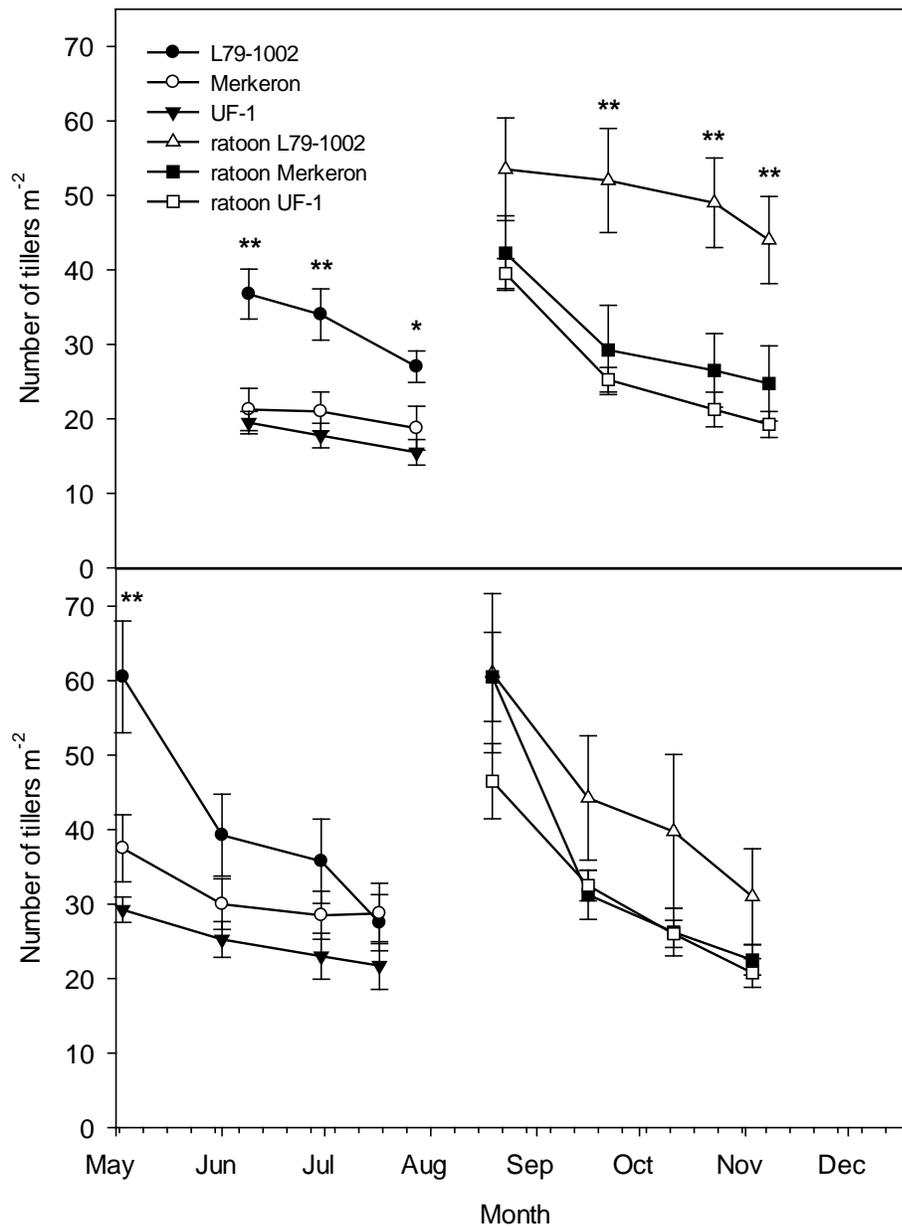


Figure 5-2. Seasonal changes in tiller density of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values for first-growth were 0.001, < 0.001, and 0.001, respectively, in 2010; and 0.017, < 0.001, and < 0.001, respectively, in 2011.  $P$  values for ratoon-growth were 0.004, < 0.001, and 0.001, respectively, in 2010; and 0.199, < 0.001, and 0.448, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ .

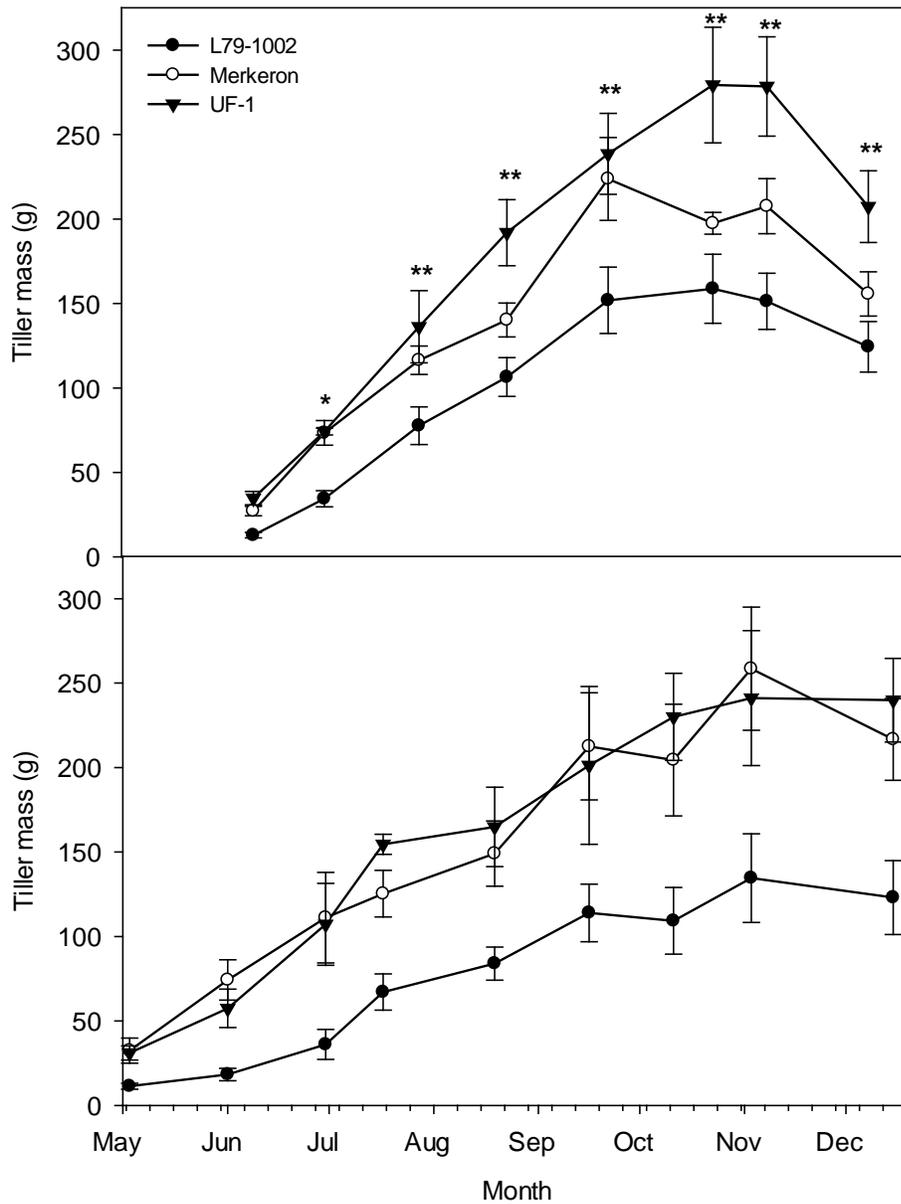


Figure 5-3. Seasonal changes in tiller mass of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values were  $< 0.001$  for all effects in 2010; and  $< 0.001$ ,  $< 0.001$ , and  $0.097$ , respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ .

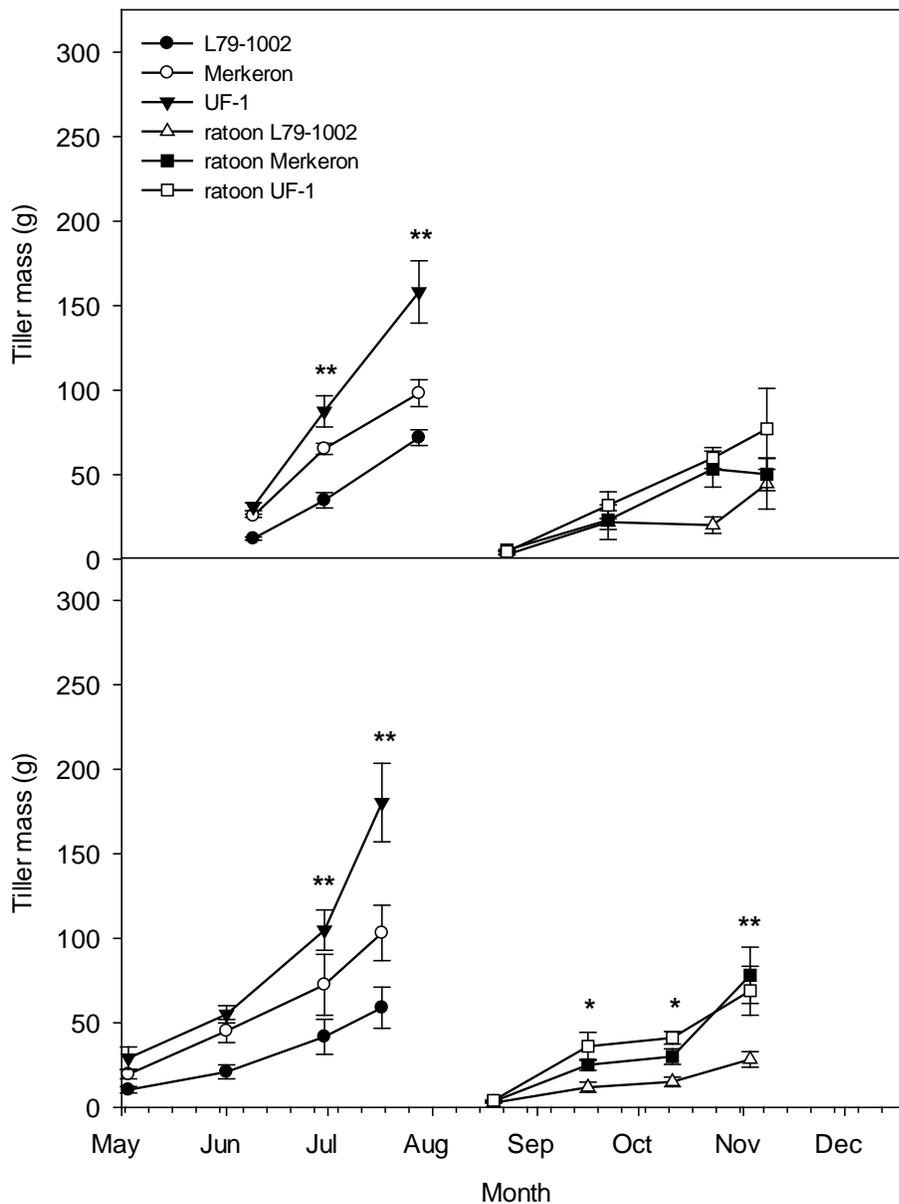


Figure 5-4. Seasonal changes in tiller mass of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values for first-growth were  $< 0.001$  for all effects in 2010; and  $< 0.001$ ,  $< 0.001$ , and  $0.001$ , respectively, in 2011.  $P$  values for ratoon-growth were  $0.009$ ,  $< 0.001$ , and  $0.225$ , respectively, in 2010; and  $< 0.001$ ,  $< 0.001$ , and  $0.007$ , respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ .

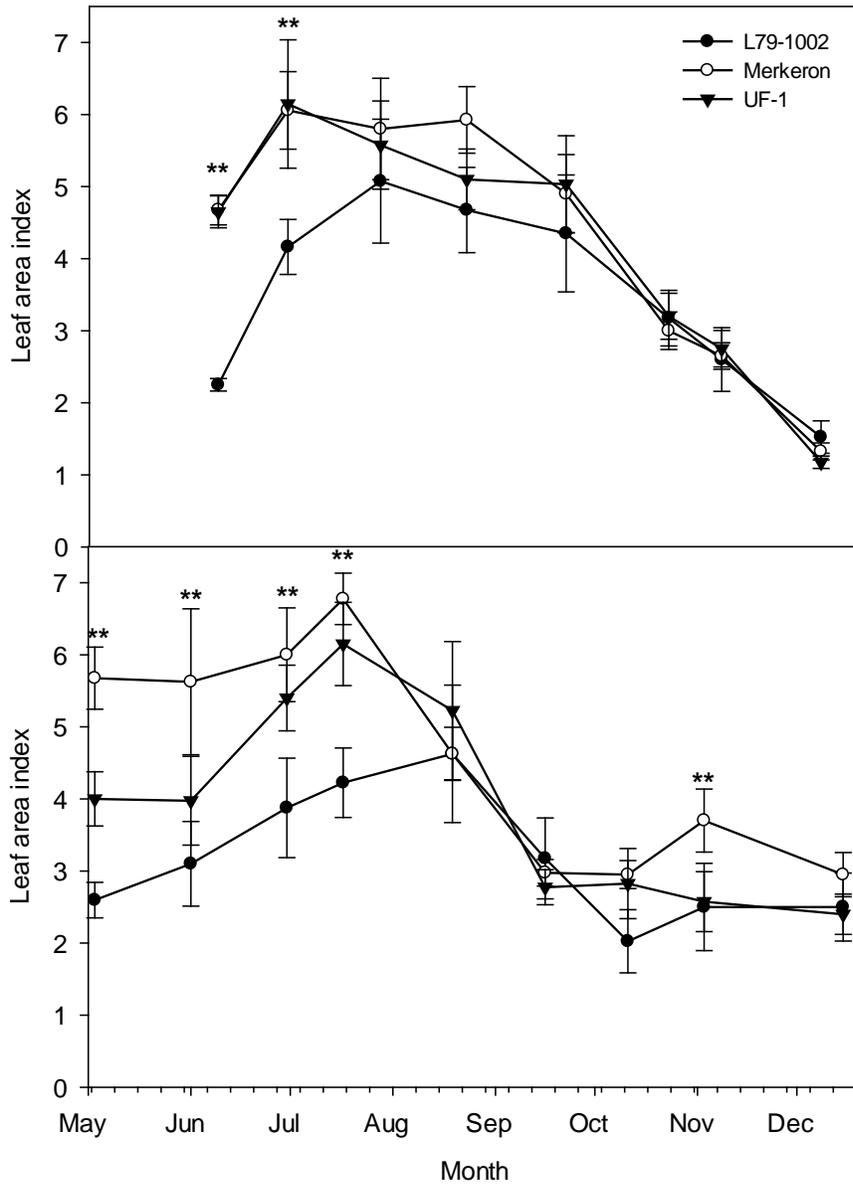


Figure 5-5. Seasonal changes in leaf area index of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values were 0.021,  $< 0.001$ , and  $< 0.001$ , respectively, in 2010; and  $< 0.001$  for all effects in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*\*,  $P \leq 0.01$

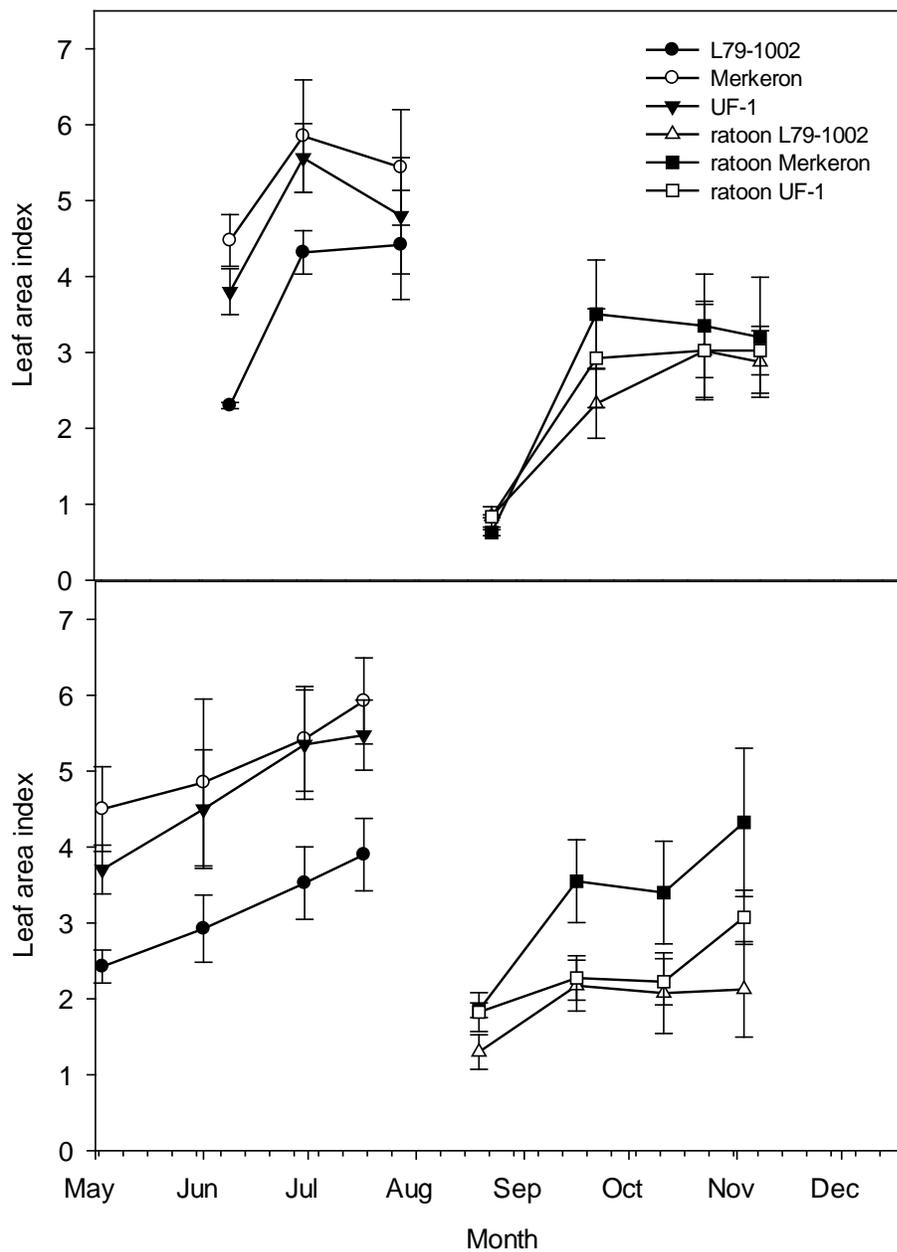


Figure 5-6. Seasonal changes in leaf area index of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction *P* values for first-growth were < 0.001, < 0.001, and 0.325, respectively, in 2010; and < 0.001, < 0.001, and 0.929, respectively, in 2011. The *P* values for ratoon-growth were 0.185, < 0.001, and 0.629, respectively, in 2010; and 0.007, < 0.001, and 0.116, respectively, in 2011. Bars show mean  $\pm$  one standard error.

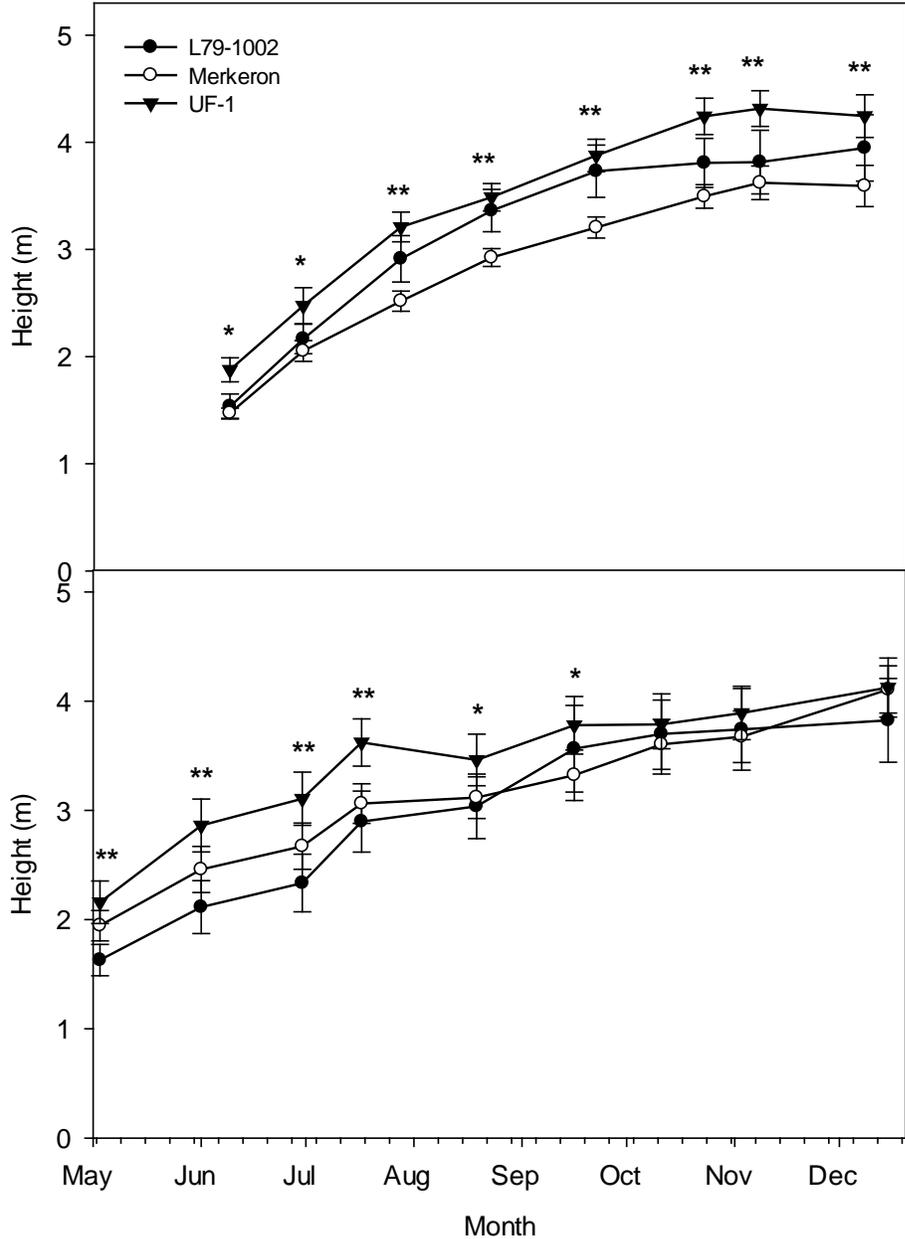


Figure 5-7. Seasonal changes in canopy height of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values were  $< 0.001$  for all effects in 2010; and 0.005,  $< 0.001$ , and  $< 0.001$ , respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ .

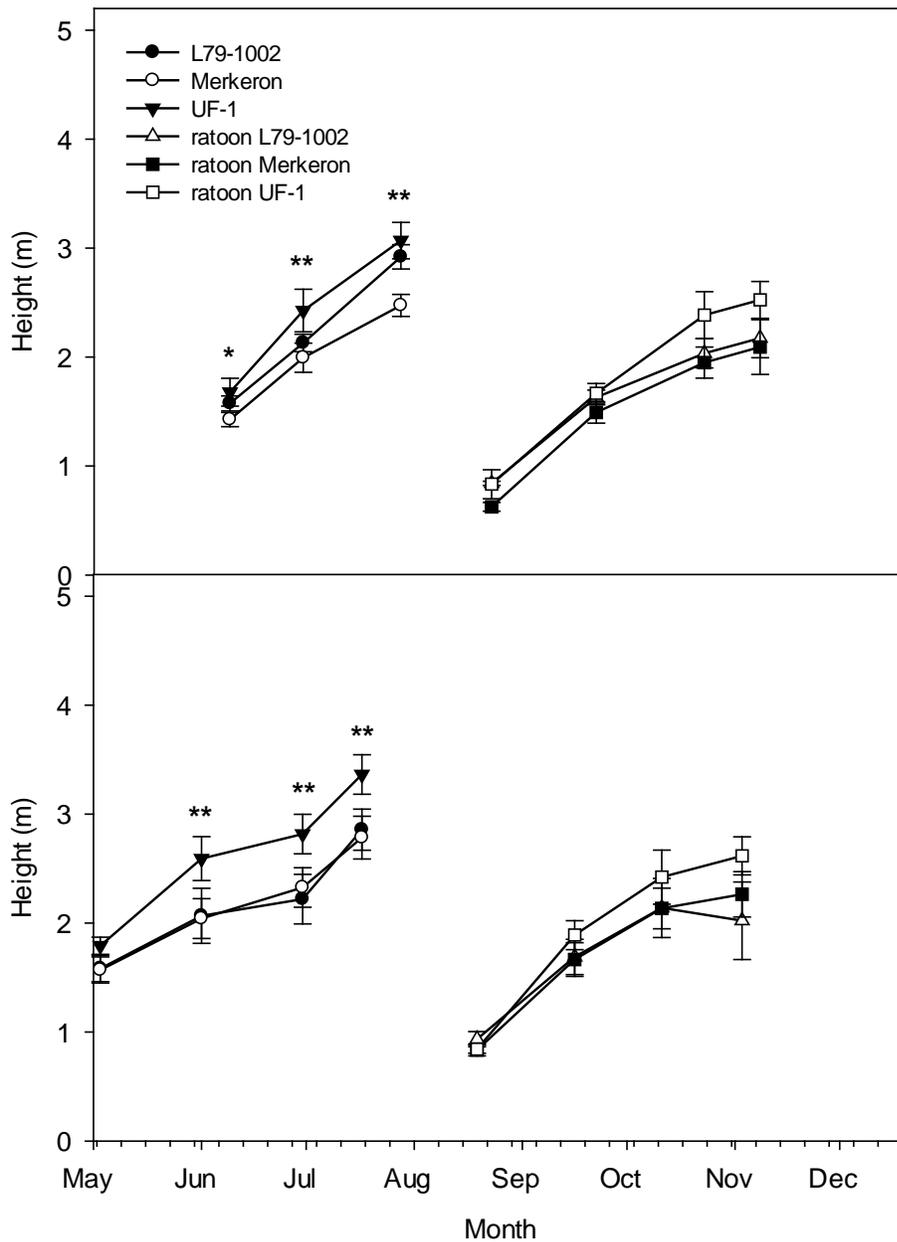


Figure 5-8. Seasonal changes in canopy height of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values for first-growth were  $< 0.001$  for all effects in 2010; and 0.002,  $< 0.001$ , and 0.027, respectively, in 2011. The  $P$  values for ratoon-growth were 0.001,  $< 0.001$ , and 0.418, respectively, in 2010; and 0.221,  $< 0.001$ , and 0.107, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ .

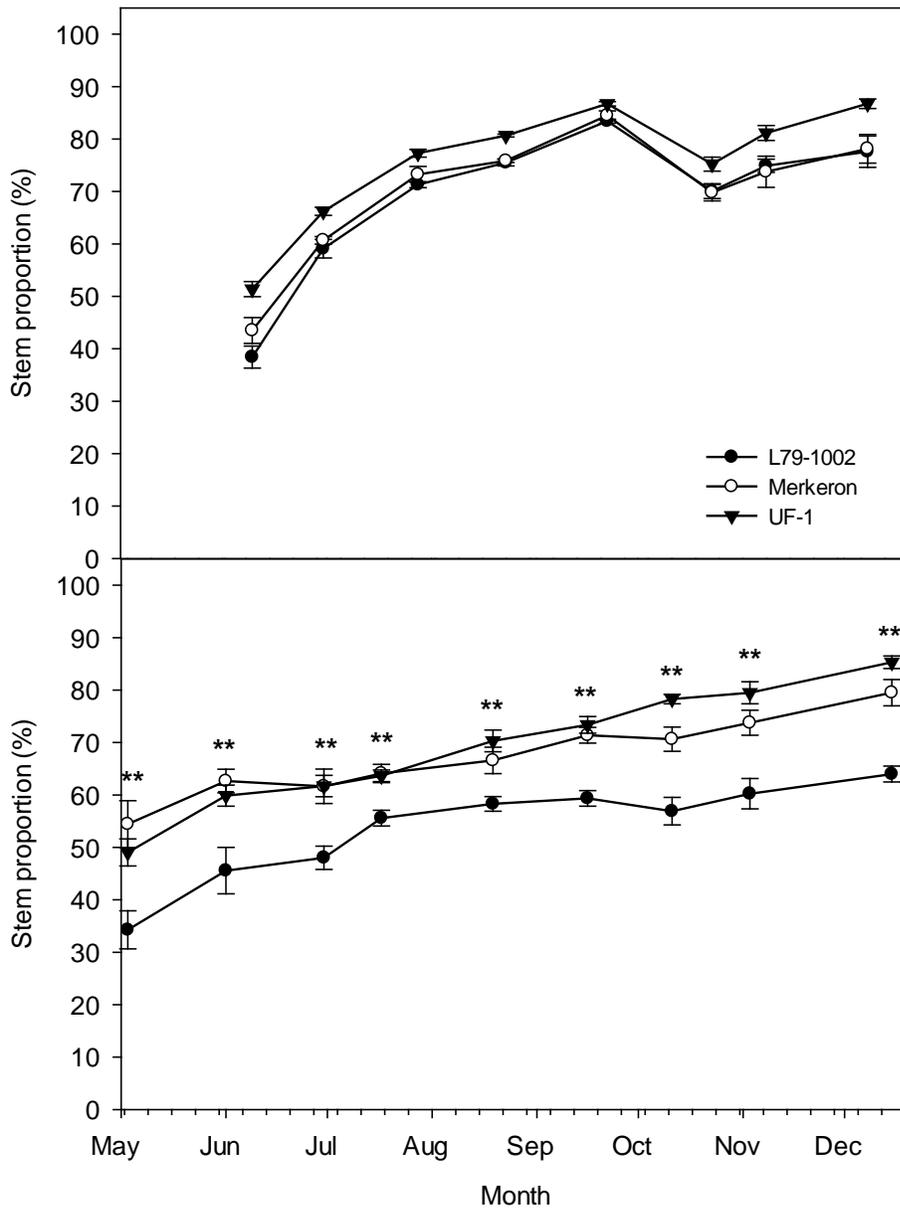


Figure 5-9. Seasonal changes in stem proportion of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values were  $< 0.001$ ,  $< 0.001$ , and  $0.075$ , respectively, in 2010; and  $< 0.001$  for all effects in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*\*,  $P \leq 0.01$ .

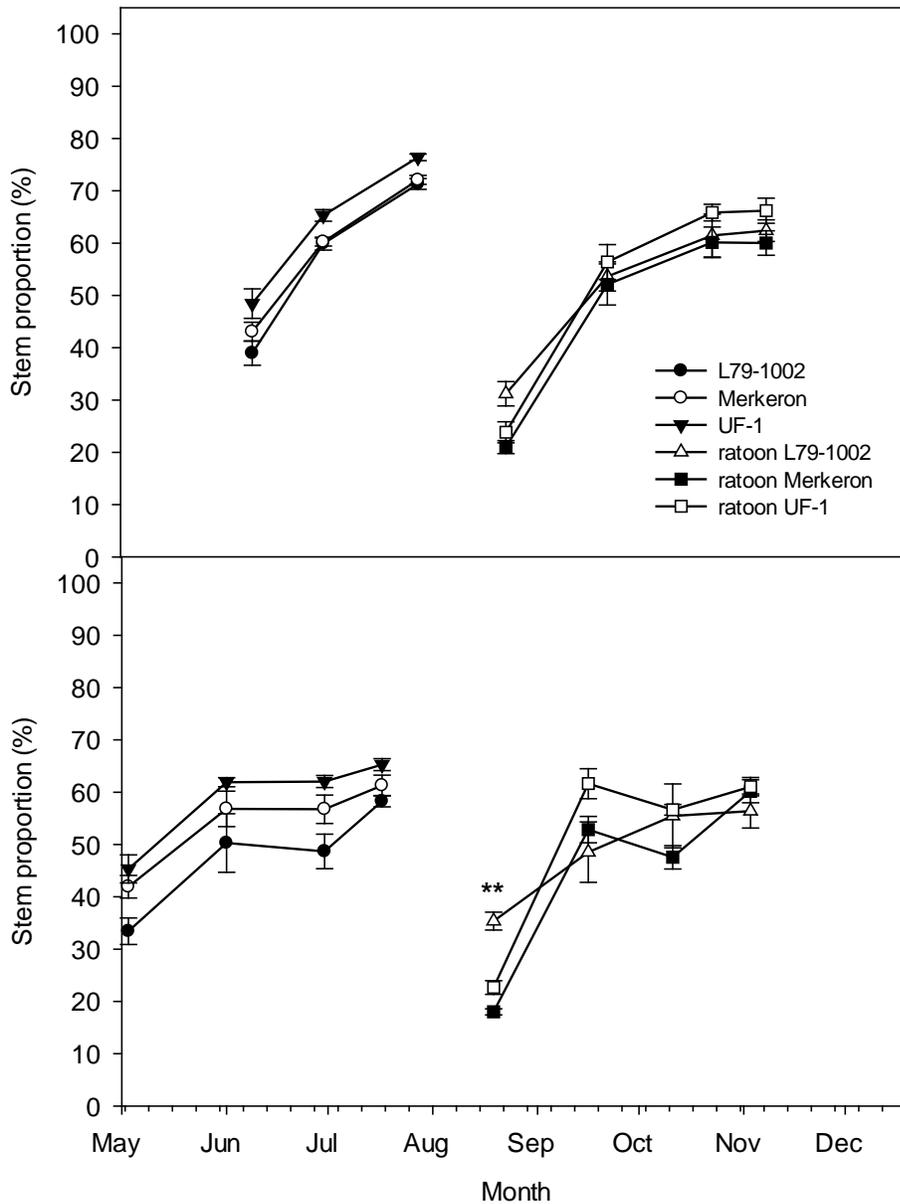


Figure 5-10. Seasonal changes in stem proportion of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values for first-growth were  $< 0.001$ ,  $< 0.001$ , and  $0.567$ , respectively, in 2010; and  $< 0.001$ ,  $< 0.001$ , and  $0.723$ , respectively, in 2011. The  $P$  values for ratoon-growth were  $< 0.001$ ,  $< 0.001$ , and  $0.076$ , respectively, in 2010; and  $< 0.001$  for all effects in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*\*,  $P \leq 0.01$ .

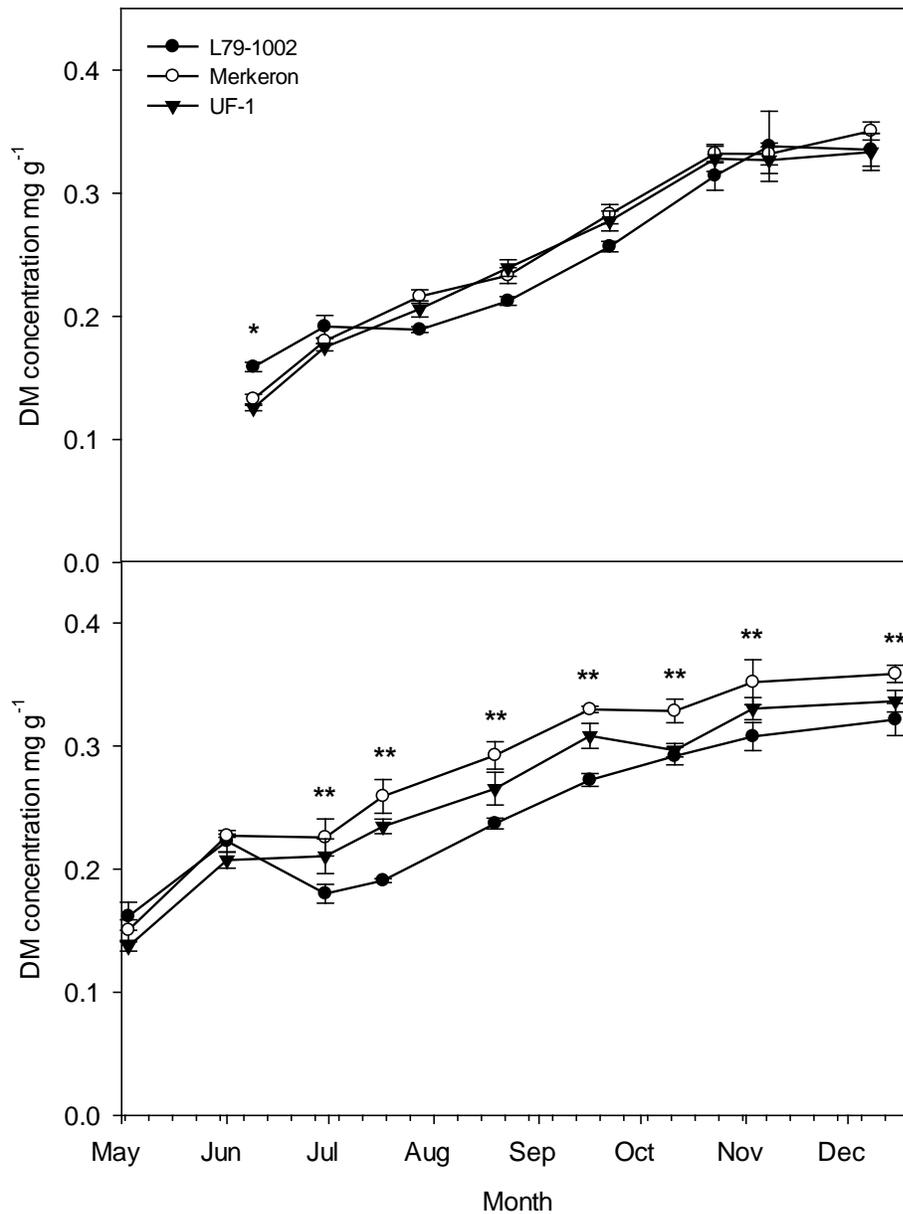


Figure 5-11. Seasonal changes in dry matter concentration of full-season growth of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction *P* values were 0.370, <0.001, and 0.047, respectively, in 2010; and < 0.001, < 0.001, and 0.002, respectively, in 2011. Bars show mean ± one standard error. Entries within a date: \*, *P* ≤ 0.05, \*\*, *P* ≤ 0.01.

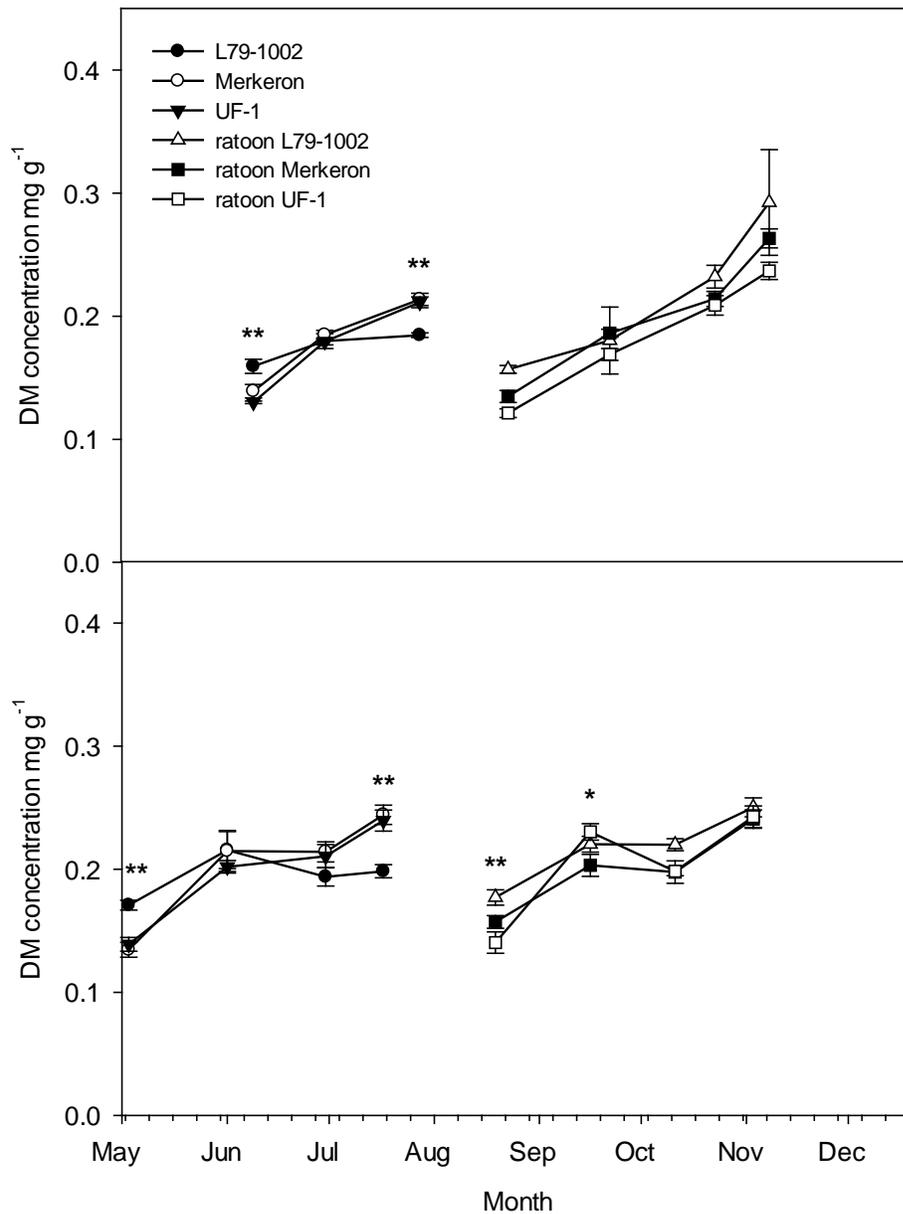


Figure 5-12. Seasonal changes in dry matter concentration of first- and ratoon-growth for three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values for first-growth were 0.670, < 0.001, and < 0.001, respectively, in 2010; and 0.676, < 0.001, and 0.001, respectively, in 2011. The  $P$  values for ratoon-growth were 0.130, < 0.001, and 0.669, respectively, in 2010; and 0.015, < 0.001, and 0.033, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date: \*,  $P \leq 0.05$ ; \*\*,  $P \leq 0.01$ .

## CHAPTER 6 SEASONAL CHANGES IN CHEMICAL COMPOSITION OF ELEPHANTGRASS AND ENERGYCANE

### Overview of Research

Dependence on imported fossil fuels has resulted in political and economic challenges for the USA and is associated with significant environmental concerns (Parrish and Fike, 2005; Kim and Day, 2011). Lignocellulose from grasses represents a potential cellulose-to-liquid fuel bioenergy production system (Anderson and Akin, 2008; Carroll and Somerville, 2009). Warm-season perennial grasses (C4) are a promising source of lignocellulose for conversion to biofuel in the Southeast USA (Knoll et al., 2012). Several candidate grasses have been evaluated and shown to have merit. In humid regions of the subtropics and tropics, elephantgrass (*Pennisetum purpureum* Schum.), or napiergrass, is known for its high biomass production (Woodard and Prine, 1993a; Morais et al., 2012). Energycane (*Saccharum* spp. hybrid) is another candidate bioenergy grass that is characterized by high fiber concentration and biomass yield, drought tolerance, and ratooning ability (Woodard and Prine, 1993b; Bischoff et al., 2008; Viator and Richard, 2012).

While high levels of lignocellulose are desirable for biofuel production, high N and/or ash concentrations in biomass may reduce the efficiency of thermochemical conversion to fuel (Shahandeh et al., 2011). Thus, examination of chemical properties of biomass is important. In evaluating the compositional characteristics of grasses, there exists a considerable body of knowledge relative to their use as animal feed (Jung and Lamb, 2004; Anderson and Akin, 2008). For example, the detergent fiber analyses, which were originally proposed for forages (Van Soest et al., 1991), provide estimates of cell wall constituents including cellulose, hemicellulose, and lignin and can be useful

as indicators of quality of cellulosic biomass (Jung and Lamb, 2004; Guretzky et al., 2011). This is because plant cell wall is the primary energy source for ruminant microorganisms and in the ruminant animal these microbes face various barriers limiting access to structural carbohydrates that are similar to obstacles that occur in bioconversion to ethanol (Lorenz et al., 2009a). Further, it has been shown that cellulose and hemicellulose concentrations are correlated with theoretical ethanol yield potential ( $r = 0.91$  and  $0.51$ , respectively) (Lorenz et al., 2009b).

For a low value crop such as perennial grasses for biofuel, it is essential to minimize N input and increase efficiency of N cycling (Erisman et al., 2010; Erickson et al., 2012). Previous research has shown that concentrations of N, P, and K in harvested plant material are dependent on harvest date in part because perennial grasses remobilize nutrients from above to belowground across the growing season (Adler et al., 2006; Heaton et al., 2009; Kering et al., 2012). Changes in plant-part proportion as the season progresses, specifically increasing amount of stem (Chapter 5), also contribute to changes in nutrient concentration in harvested biomass. Because N is considered an anti-quality component for some biomass conversion platforms, understanding these changes in plant-part composition can be quite important in terms of harvest timing or whether leaf should be included in harvested biomass. In addition, leaf may be considered of lesser value than stem in biofuel production system because its bulk density is lower than stem, increasing transportation cost (Sokhansanj et al., 2009; Brechbill et al., 2011).

Most biomass composition data for perennial grasses are from experiments harvested once at the end of the growing season. Thus, there are limited data available

that describe the compositional changes of perennial grasses throughout the growing season. This information, along with seasonal patterns of biomass accumulation, is valuable for identifying optimum harvest dates and frequencies. Thus, the objectives of this experiment were to quantify seasonal changes in i) fiber and nutrient composition of elephantgrass and energycane biomass and ii) leaf proportion and the effect of inclusion of leaf on composition of harvested biomass.

### **Materials and Methods**

Data reported in this chapter came from the experiment that was described in Chapter 5. Thus, detailed information about the experimental site, weather conditions, plot establishment and management, and statistical analyses will not be reiterated here. In addition, composition analysis methodology was described previously in Chapter 4.

#### **Treatments and Experimental Design**

The experiment was conducted during 2010 and 2011 at the Plant Science Research and Education Unit (PSREU) at Citra, FL (29.41° N, 82.17° W). The soil was a well-drained Candler sand (hyperthermic, uncoated Lamellic Quartzipsamments).

Treatments were three grass entries replicated four times in randomized complete block design. The grass entries included two elephantgrasses, 'Merkeron' (Burton, 1989) and a breeding line referred to as UF-1, and 'L79-1002' energycane (Bischoff et al., 2008). Merkeron elephantgrass and L79-1002 energycane were chosen because they are the most available cultivars of these two species. In addition, breeding line UF-1 elephantgrass was included because in preliminary research it had demonstrated outstanding potential for use in bioenergy feedstock production (Sollenberger et al., 2011) and potential exists for it to be released as a cultivar.

Plots used in this study were a subset of those from the experiment described in Chapter 3. Sampling occurred on plots that were harvested once per year within 1 wk following the first freeze event in fall (this treatment was referred to as 1X-Dec in Chapter 3). Unlike Chapter 5, defoliation treatment (full season vs. first growth/ratoon growth) was not compared in this chapter.

### **Response Variables**

Tiller sampling occurred at approximately 8-wk intervals with the exception of the final collection (Table 6-1). Final sampling date varied between years because it was targeted for the end of the growing season, defined as within 1 wk of the first freeze event in fall (freeze was defined as occurrence of 0°C recorded at a 2-m height above soil level). The final sampling occurred 4 wk (2010) and 5 wk (2011) after the previous collection date.

At each sampling date, plant material for compositional analysis was collected. Four representative tillers from each plot were cut at a 12-cm stubble height and hand-separated into leaf (blade plus sheath) and stem (including inflorescence, if present) components. The samples were dried at 60°C until constant weight and leaf and stem data were used to calculate leaf proportion in total biomass. Dried samples were ground first in a hammer mill to reduce particle size and subsamples were ground through a Wiley mill (1-mm screen) before compositional analyses were conducted.

Leaf and stem components of dried biomass were analyzed for composition. The modified method of detergent fiber analysis (Van Soest et al., 1991) was used for sequential neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) analyses. The samples were sequentially analyzed for NDF and then ADF using the ANKOM fiber analyzer (ANKOM 2000 Fiber Analyzer, ANKOM Technology

Corporation, Fairport, NY). The ADF residue was used for analysis of ADL following the procedure “Method for Determining Acid Detergent Lignin in Beakers” (proposed by ANKOM Technology Corporation). Cellulose was estimated as the difference between ADF and ADL concentrations, and hemicellulose was estimated as the difference between NDF and ADF concentrations (Jung and Lamb, 2004; Waramit et al., 2011). Data reported are for total harvested biomass and were calculated using leaf and stem composition data and weighting the calculation based on the relative proportion of each plant part in total biomass.

As described in Chapter 4, total N and P concentrations in the leaf and stem were determined using a standard-Kjeldahl method, a modification of the aluminum block digestion procedure (Gallaher et al., 1975) followed by semi-automated colorimetric determination (Hambleton, 1977). Digestions were conducted at the Forage Evaluation Support Laboratory of the University of Florida. Samples were ashed using a muffle furnace for a minimum of 6 hr at 500°C.

### **Statistical Analysis**

Data were analyzed using mixed-model methods (SAS Institute, 2008). In all models, grass entry was considered a fixed effect. Sampling date (Table 6-1) was considered a repeated measurement (fixed) within each year, and data were analyzed by year because 2010 was the establishment year and growth was delayed relative to 2011 resulting in later onset of sampling in 2010. Block was considered a random effect. Means were compared using the pdiff test of LSMEANS. All means reported in the text are least squares means and were considered different if  $P \leq 0.05$ . Trends were noted when  $P \leq 0.10$  and  $> 0.05$ .

## Results and Discussion

### Neutral Detergent Fiber

There were entry x sampling date interactions for NDF concentration in both years ( $P < 0.001$  in 2010 and  $P = 0.009$  in 2011; Fig. 6-1). The seasonal pattern of responses differed for the two grass species in 2010. In June 2010, L79-1002 had greater NDF concentration than Merkeron and UF-1 (709, 656, and 659 mg g<sup>-1</sup>, respectively, Fig. 6-1). However, NDF concentration of L79-1002 was lowest at the last two sampling dates of the same year. Energycane approached its peak NDF concentration in July and decreased through the remainder of the 2010 season. Unlike energycane, elephantgrass NDF increased throughout the growing season, although the rate of increase was slow starting in September. In 2011, energycane NDF concentration peaked later in the year than in 2010, September vs. July, and then declined. Merkeron NDF concentration was greater than the others at the beginning (June) of the season, increased throughout the year, and was greatest in December as well (Fig. 6-1). Neither elephantgrass had a large increase in NDF after September. Seasonal trends of NDF concentration in switchgrass (*Panicum virgatum* L.) were similar; NDF initially increased and then it remained nearly constant at around 850 mg g<sup>-1</sup> from August through the rest of the season (Madakadze et al., 1999).

For Merkeron, increasing NDF concentration with increasing maturity was similar to the response observed in previous forage harvest frequency studies (Manyawu et al., 2003; Van Man and Wiktorsson, 2003); by increasing interval between cutting events in those studies, NDF concentration increased linearly from 704 to 785 mg g<sup>-1</sup> and from 636 to 753 mg g<sup>-1</sup>, respectively. Unlike Merkeron, NDF in L79-1002 peaked in July 2010 and September 2011 and declined thereafter. This response has been associated with

relatively greater nonstructural sugar concentration in energycane than in Merkeron at late sampling dates (Chapter 4). Illustrating this response, Bischoff et al. (2008) reported that L79-1002 accumulated substantial amounts of soluble solids (104 mg g<sup>-1</sup> Brix with 80 mg g<sup>-1</sup> sucrose concentration) in fresh cane at Louisiana during the harvest period. Similarly, energycane at 6 mo of growth in March in Brazil had Brix of 160 mg g<sup>-1</sup>, and Brix increased linearly until it peaked at 220 mg g<sup>-1</sup> in September (Waclawovsky et al., 2010). In Florida at the end of growing season, L79-1002 energycane had 13 to 63 mg g<sup>-1</sup> greater extractives concentration than Merkeron elephantgrass (Fedenko, 2011). Water soluble carbohydrate (WSC) concentration was 2.2-fold greater for L79-1002 than Merkeron (Woodard et al., 1991b). These results were observed even though energycane has less sucrose than commercial sugarcane; the amount of sucrose is sufficiently high, however, to result in relatively high concentration of extractives and low cell wall fiber concentration when compared with elephantgrass, especially late in the growing season.

### **Acid Detergent Fiber**

There was entry x sampling date interaction in 2010 ( $P < 0.001$ ; Fig. 6-2). In 2011 there was no interaction ( $P = 0.146$ ), but there were entry and sampling date main effects ( $P < 0.001$  for both). In June 2010, L79-1002 had the greatest ADF; however, ADF of the two elephantgrasses was greater by September and the difference between them and energycane increased as the season progressed (Fig. 6-2). In 2011, Merkeron showed greatest ADF concentration followed by UF-1 and then L79-1002 (seasonal average ADF of 480, 464, and 442 mg g<sup>-1</sup>, respectively; Fig. 6-2). All three grasses had increasing ADF concentration through the September sampling date in

both years; thereafter elephantgrass ADF remain relatively constant or increased while energycane ADF decreased slightly.

The ADF analysis was developed as a pre-treatment step prior to ADL measurement (Van Soest et al., 1991), thus cellulose and lignin (an indication of lignified fiber) are the primary components of ADF (Jung and Lamb, 2004). Similar to the response of NDF, elephantgrass ADF increased at the fastest rate during the early part of the season. This response has been reported previously in harvest frequency studies (Manyawu et al., 2003; Van Man and Wiktorsson, 2003), whereby ADF concentration increased linearly with increasing interval between harvests (360 to 398 mg g<sup>-1</sup> and 358 to 450 mg g<sup>-1</sup>, respectively). The seasonal pattern of response observed in the current research, i.e. increasing ADF early in the season with little change thereafter, was also observed for switchgrass in eastern Canada. Switchgrass ADF concentration increased linearly until September and then remained nearly constant during the remainder of season (Madakadze et al., 1999). The overall seasonal pattern of ADF responses in the current study follows that observed for NDF concentration.

### **Acid Detergent Lignin**

There was entry x sampling date interaction for ADL in total biomass in 2010 ( $P < 0.001$ ), but in 2011, there were only entry and sampling date main effects ( $P < 0.001$  for both, Fig. 6-3). The seasonal pattern of ADL in 2010 was very similar to ADF.

Energycane L79-1002 showed greater ADL concentration in June; however, the two elephantgrass entries had greater ADL concentration in July (Fig. 6-3). Thereafter, the magnitude of the difference between energycane and elephantgrass increased (11 mg g<sup>-1</sup> in July vs. 19 mg g<sup>-1</sup> in December, Fig. 6-3), and ADL of both elephantgrasses was greater than energycane throughout the remainder of the season. During the second

year, the pattern of response of the three grass entries was similar across sampling dates, and Merkeron had greatest ADL followed by UF-1 and L79-1002 (seasonal average ADL of 81, 71, and 61 mg g<sup>-1</sup>, respectively, Fig. 6-3). Averaged across the three grasses, ADL increased in 2011 from 56 to 80 mg g<sup>-1</sup> in June and November and then decreased slightly to 76 mg g<sup>-1</sup> in December. These data suggest that once structural lignin reaches a maximum in fall, further change in lignin concentration is likely to be small and if it occurs it is likely to be a function of increasing concentration of other constituents, e.g., nonstructural sugars in energycane, rather than change in lignin content.

It is known that ADL underestimates true lignin concentration (Jung et al., 1999; Jung and Lamb, 2004; Dien et al., 2006), however, it has been reported that ADL has a similar pattern of response as Klason lignin, the analysis thought to one of best representations of true lignin concentration in legumes and grasses (Jung et al., 1997; Jung and Lamb, 2004). Using ADL for relative comparisons of lignin concentration is thus considered valid for bioenergy research. Lignin is considered to be an anti-quality component in forage, and ADL is strongly negatively correlated with in vitro dry matter digestibility and dry matter digestibility ( $r = -0.96$  for both) (Jung et al., 1997). In biomass for bioenergy, the situation is less clear cut. Recovered lignin can provide heat during a thermochemical conversion process, but on the other hand, since it binds to structural carbohydrates in plant cell wall, it reduces enzyme accessibility to cellulose and subsequent ethanol yield (Adler et al., 2006; Guretzky et al., 2011).

Klason lignin concentration in switchgrass in Pennsylvania was 10 to 33% greater in the fall compared with the spring; however, the difference narrowed in the

second year (182 vs. 165 mg g<sup>-1</sup>) compared with the first year (173 vs. 130 mg g<sup>-1</sup>) (Adler et al., 2006). Similarly, there was relatively less change from June through December in Year 2 of the current study (Fig. 6-3), but that was likely due to the fact that growth initiated earlier in Year 2 than in Year 1 (establishment year). As a result the effective maturity of plants was greater in June of Year 2 than Year 1. An important determinant of increasing lignin concentration with increasing maturity is leaf:stem ratio. As plants mature, stem proportion decreases (Chapter 5) and stem typically has greater lignin concentration than leaf. Measurement of switchgrass cell wall components in Texas showed that stem lignin concentration was greater than in leaf (Shahandeh et al., 2011). Average lignin concentration in big bluestem (*Andropogon gerardi* Vitman) and switchgrass plant parts (leaf and stem) was 48 vs. 64 mg g<sup>-1</sup> and 42 vs. 56 mg g<sup>-1</sup>, respectively, in West Virginia (Griffin and Jung, 1983).

### **Cellulose**

Cellulose concentration was affected by entry x sampling date interaction ( $P < 0.001$ ) in 2010, but in 2011 only entry and sampling date effects were significant ( $P = 0.002$ , and  $P < 0.001$ , respectively; Fig. 6-4). During 2010, interaction was caused by relatively greater cellulose concentration in energycane in June but lesser concentration than elephantgrasses from September through the rest of the season (Fig. 6-4). Only Merkeron continued to increase in cellulose concentration through December 2010, while cellulose in energycane decreased after September. In UF-1, the responses remained essentially unchanged from November to December. Unlike the first year, entry by sampling date interaction did not occur in 2011; however, there was a trend toward interaction ( $P = 0.061$ ). The pattern of response in 2011 was quite similar to that in 2010 whereby Merkeron cellulose concentration continued to increase until the end of

season, but for the other entries it remained constant (UF-1) or decreased slightly (L79-1002) at late season (Fig. 6-4). Averaged across sampling dates, Merkeron and UF-1 had greater cellulose concentration than L79-1002 (399, 393, and 380 mg g<sup>-1</sup>, respectively).

For elephantgrass, the observation of increasing cellulose concentration with maturity was similar to previous defoliation frequency studies (Tessema et al., 2010) in which increasing defoliation interval from 60 to 120 d increased cellulose concentration from 297 to 339 mg g<sup>-1</sup>, respectively. Lesser concentration of cellulose in late-season energycane is likely due to increasing concentrations of nonstructural sugars (Chapter 4). In comparison with previous work, greater cellulose concentration than observed in the current study was reported in full-season growth of energycane (433 mg g<sup>-1</sup>) in Louisiana (Kim and Day, 2011), however, it was from bagasse after nonstructural sugars were removed. Another study reported that energycane had two-fold greater sugars from extractives than elephantgrass had (Fedenko, 2011). A larger amount of glucose monomers, which compose cellulose, is advantageous for ethanol production because glucose can be converted at greater efficiency to ethanol than most other sugars, especially compared to pentose (Dien et al., 2006). Greater cellulose concentration and high yields of biomass (Chapter 3) during late season suggest that delayed harvest results in greater cellulose yields, but the concurrent increase in lignin concentration as plants mature may interfere with conversion of the cellulose to energy.

### **Hemicellulose**

There was entry x sampling date interaction for hemicellulose concentration in total biomass in 2010 ( $P < 0.001$ ), but in 2011 there were entry and sampling date main effects only ( $P = 0.002$ , and  $P < 0.001$ , respectively; Fig. 6-5). In June and July 2010,

L79-1002 had greater hemicellulose concentration than the two elephantgrasses, but thereafter hemicellulose did not differ from either elephantgrass (Fig. 6-5). In 2011, L79-1002 had average hemicellulose concentration of 299 mg g<sup>-1</sup> with a range of 276 (December) to 313 mg g<sup>-1</sup> (June). The energycane seasonal mean was greater than that of Merkeron and UF-1 (277 and 271 mg g<sup>-1</sup>, respectively; Fig. 6-5). Unlike cellulose concentration, in both years hemicellulose concentration decreased over the season although the shapes of the curve were different between years. It is likely that part of the reason for the different shape of curves is that biomass was more mature in June 2011 than in 2010, due to earlier initiation of growth in 2011. Thus hemicellulose in this more mature material had already declined from its maximum when sampling began in June 2011.

Previous research with elephantgrass had shown that hemicellulose concentration decreased from 337 to 280 mg g<sup>-1</sup> as regrowth interval increased from 60 d to 120 d, respectively (Tessema et al., 2010). There are no seasonal response data reported for energycane, but bagasse from season-long growth had a hemicellulose concentration of 238 mg g<sup>-1</sup> in Louisiana (Kim and Day, 2011), not greatly different than season-end concentrations of 154 to 276 mg g<sup>-1</sup> in the current study.

Reported trends in hemicellulose concentration with increasing plant maturity are not consistent, likely due in part to methodological differences. Hemicellulose can be determined as either sum of structural carbohydrates except glucose or as NDF minus ADF (as done in current research). Methodological differences in hemicellulose concentration have been reported in switchgrass research in Nebraska (Dien et al., 2006). There was no clear explanation in the literature why hemicellulose concentration

determined using the two methods shows opposite trends. Hemicellulose concentration determined by the Uppsala dietary fiber procedure increased from 235 to 279 mg g<sup>-1</sup> as maturity increased from pre-boot stage to a post-frost sampling, whereas the Van Soest technique showed that hemicellulose decreased slightly from 318 to 311 mg g<sup>-1</sup> (Dien et al., 2006)

## **Nitrogen**

Total biomass N concentration was affected by sampling date ( $P < 0.001$ ) in both years and also by entry in 2011 ( $P < 0.001$ , Fig. 6-6). Nitrogen concentration in biomass declined as the season progressed in both years, although the extent of the decline was much greater in 2010 (Fig. 6-6). In that year (2010), N concentration averaged across entries was 20.5 mg g<sup>-1</sup> in June and it decreased to 8.2 mg g<sup>-1</sup> in July. During the remainder of the season, N concentration varied little and was range of 4.6 to 5.5 mg g<sup>-1</sup>. In 2011, overall N concentration in Merkeron was greater than L79-1002 and UF-1 (7.5, 6.6, and 6.4 mg g<sup>-1</sup>, respectively, Fig. 6-6). With the exception of the June sampling date, N concentration was very similar to 2010. The difference between years in June again is likely attributed to the earlier regrowth in 2011 such that the major decrease in biomass N concentration had already occurred by the time sampling began. Similar to results in the current research, switchgrass N in Iowa decreased from 12.4 mg g<sup>-1</sup> in June to 0.39 mg g<sup>-1</sup> in November (Wilson et al., 2013a). Energycane N concentration in Florida also was a function of plant height (maturity) and decreased quadratically as plant height increased (Mislevy et al., 1995). Similar to current research, once N concentration of sweet sorghum approached a minimum threshold in Florida, biomass N remained relatively constant (Erickson et al., 2012). Once switchgrass N concentration reached a minimum in Pennsylvania, delayed harvest

management resulted in only minor (5 to 15%) variation between fall and spring harvests (Adler et al., 2006).

Because N is considered to be of no added value for conversion to energy and leaf N concentration is typically much greater than stem N, there may be value in returning leaf biomass to the production field. This may be advantageous both in terms of biomass quality and N recycling back to soil. To explore these relationships, leaf percentage in biomass and proportion of total biomass N in the leaf fraction were evaluated. Leaf percentage in total biomass was affected by entry and sampling date main effects in 2010 ( $P < 0.001$ ) and the interaction of entry and sampling date in 2012 ( $P < 0.001$ ). In 2010, leaf percentage decreased from 56% in June to 19% in December and average leaf percentage over the season was greatest in L79-1002 and Merkeron (34 and 33%, respectively) and least (26%) in UF-1 (Table 6-2). In 2011, L79-1002 had the greatest leaf percentage at each sampling date and leaf percentage decreased 18.5 units from June through December compared with 17 units for Merkeron and 25 units for UF-1 (Table 6-3). Leaf percentage in UF-1 reached 13 in December 2010 and 15 in December 2011 (Tables 6-2 and 6-3). This result indicates that reduced N concentration in total biomass in late season was a function of both an overall decrease of biomass N concentration over the season and reduced leaf proportion, the plant part which has greater N concentration than stem.

Proportion of total N in leaf was affected only by sampling date in 2010 ( $P < 0.001$ ) and by entry ( $P < 0.001$ ) and sampling date ( $P = 0.013$ ) in 2011. In June 2010, approximately 63% of total N was in the leaf fraction and it decreased to 32% by December. Leaf proportion in total biomass averaged 56 and 19% for those two dates

(Table 6-2). By late in the season, UF-1 leaf proportion was only 19 (November) and 13% (December) but 37 (November) and 27% (December) of N in harvested biomass was in leaf. Thus the proportion of N in leaf was approximately twice as great as the proportion of biomass that was leaf. This result indicates that returning elephantgrass leaf to the production field during a single fall or early-winter harvest would reduce total biomass yield by 13 to 22%, but it would reduce the amount of N in harvested biomass by 28 to 41%. Similar to the previous year, in 2011 approximately 54% of total N was in leaf in June and that number decreased only to 46% by December (Table 6-3). In December, proportion of total N in leaf for L79-1002, Merkeron, and UF-1 was 66, 104, and 147% greater than the percentage leaf in total biomass. While grasses had 15 to 40% leaf during November and December, 36 to 64% of N was in the leaf (Table 6-3).

Previous work with energycane in Florida showed that N concentration in stem decreased 81% across growing season, while the decrease in leaf was 51% (Mislevy et al., 1995). As result, the magnitude of the difference in N concentration between leaf and stem increased over season (Mislevy et al., 1995). Relatively low N concentration in stem compared to leaf was observed in a sorghum study in Florida (12 mg g<sup>-1</sup> in leaf vs. 4.4 mg g<sup>-1</sup> in stem) (Erickson et al., 2012). Previous research with miscanthus showed significant decreases in both leaf and stem N across the growing season. Live leaf N concentration was not significantly different than stem in June but the difference between fractions increased over season (Beale and Long, 1997). This study also showed increasing stem and decreasing leaf proportion over time; however, the proportion of total N in leaf was relatively constant at about one third of aboveground N

in both miscanthus and cordgrass [*Spartina cynosuroides* (L.) Roth] (Beale and Long, 1997). This result is within the range observed in the current research.

## **Phosphorus**

There was entry x sampling date interaction in both years ( $P = 0.006$  in 2010 and  $P < 0.001$  in 2011). In 2010, Merkeron biomass had greatest P concentration throughout the season until the final sampling date (Fig. 6-7). In 2011, Merkeron again had greatest or tended to have greatest P concentration except for the June sampling data. Average P concentration across the year was  $1.2 \text{ mg g}^{-1}$  for Merkeron and 1.0 and  $0.9 \text{ mg g}^{-1}$ , for L79-1002 and UF-1. Extent of seasonal change in P concentration was greater in 2010 than 2011. As with other response variables, this was likely due in large part to later tiller emergence in the establishment year of 2010. As a result the rapid early season decline in P concentration observed in 2010 had likely already occurred by the time sampling was initiated in 2011. Overall leaf and stem P concentrations averaged  $1.20$  and  $1.30 \text{ mg g}^{-1}$ , respectively.

Previous research with switchgrass showed an average 49% decrease in biomass P concentration over winter (Adler et al., 2006). Miscanthus P concentration decreased from June to August in both green leaf and stem (Beale and Long, 1997). In that study, there was a large difference in P concentration among plant parts; green leaf P concentration was two-fold greater than stem; however, dead leaf P concentration was lower than stem and likely offset overall differences between total leaf vs. stem as dead leaf accumulated late in the season (Chapter 3).

## **Ash**

There was entry x sampling date interaction for biomass ash concentration in 2010 ( $P < 0.001$ ) but only a sampling date effect in 2011 ( $P < 0.001$ ). In June 2010, ash

concentration in elephantgrasses was greater than energycane; no differences among elephantgrass entries occurred (Fig. 6-8). Ash concentration was greatest at the beginning of the season (average across entries of 77 mg g<sup>-1</sup> in June), but it decreased significantly through September (29 mg g<sup>-1</sup>). Thereafter it remained relatively constant in 2010 (Fig. 6-8). In 2011, the seasonal trend in concentration was similar to the previous year, absent the large decrease in ash early in the season that likely was not measured in 2011 because of earlier tiller emergence than in the first year. In 2011, ash concentration decreased from 54 mg g<sup>-1</sup> in June to 35 mg g<sup>-1</sup> in September (Fig. 6-8).

Inorganic constituents, commonly referred to “ash”, cannot be converted to energy, and greater ash concentration is considered to negatively affect biomass quality for conversion (Wilson et al., 2013a). However, inorganic constituents such as Si, K, Ca, S, and Cl play an essential role in plant growth and development (Bakker and Elbersen, 2005). If ash can be removed from harvested biomass there may be value to both conversion and soil nutrient composition in production fields.

With this in mind, data for seasonal changes in proportion of ash in leaf biomass will be presented. In both years, there was entry x sampling date interaction for proportion of ash in leaf biomass ( $P = 0.012$  and  $P < 0.001$ , respectively; Tables 6-2 and 6-3). There were no differences among entries in proportion of total ash that was in leaf biomass in June and July 2010, but UF-1 had the lowest proportion (24%) by December (Table 6-2). In June 2010, the proportion of leaf in total biomass ranged from 49 to 62% across entries, but leaf contained only 41 to 47% of ash. By December, much had changed as the leaf percentage ranged from 13 to 22% but the percentage of total ash in leaf biomass was 24 to 34%. Thus, by the end of the season including leaf in

harvested biomass had a greater impact on the amount of ash harvested than on the amount of biomass harvested, and one third to one fourth of ash harvested could be returned to the field if leaf was not harvested. In 2011, there were no differences among entries in percentage of total ash that was present in leaf biomass from June through November, but in December there was a lesser proportion of ash in elephantgrass than energycane leaf (Table 6-3). Early in 2011, the percentage of total biomass that was leaf and the proportion of total ash that occurred in leaf were very similar for all entries, but by December leaf percentage in total biomass ranged only from 15 to 36% while percentage of total ash in leaf ranged from 28 to 43% (Table 6-3). Results show that generally UF-1 leaf proportion in total biomass was lowest in both years, and proportion of ash in leaf was lower in UF-1 (2010) or as low as (2011) that of Merkeron.

Data for both N and ash indicate that returning leaf to the production field is a much more attractive option for elephantgrass than for energycane because elephantgrass leaf contains significant proportions of N and ash but elephantgrass leaf contributes less to total biomass harvested than energycane leaf. Likewise, among elephantgrasses, this practice appears to have greater potential for UF-1 because leaf proportion, especially at the end of the growing season, was less than for Merkeron while the proportion of ash or N in UF-1 leaf was still in the general range of 25 to 33%.

Similar to current research, Summer et al. (2001) investigated total ash concentration in different plant parts of rice straw (leaf, stem, node, and panicle). They found that leaves contained 18 to 19% total ash, whereas, stem contained only 12% of total ash. It has been suggested that C4 grass species have lower ash concentration compared with C3 plants (Bakker and Elbersen, 2005). Further, it was suggested that

amount of ash in herbaceous biomass can be controlled by the timing of harvest (Bakker and Elbersen, 2005), with longer harvest intervals associated with lower ash concentration due to senescence and translocation of constituents. These results support those of the current study.

### **Implications of Research**

Seasonal changes in chemical composition were characterized for three candidate bioenergy grasses in Florida by mineral analyses and quantifying cell wall constituents by Van Soest fiber analyses. Grass entries included Merkeron and UF-1 elephantgrasses and L79-1002 energycane. Different responses were detected in composition between elephantgrass and energycane. With the exception of hemicellulose, elephantgrass cell wall constituents increased from early in the growing season until late summer and either remained relatively constant (UF-1) or slightly increased (Merkeron) during the remainder of the growing season. In contrast, once energycane cell wall constituents peaked in late summer, concentration of cell wall constituents tended to decrease thereafter. Greater nonstructural sugar concentration in energycane than in Merkeron at late sampling dates (Chapter 4) was associated with the decrease in concentration of fiber components in energycane.

Nitrogen, P, and ash concentrations decreased with increasing maturity for all grass entries. When considering individual plant parts, leaf N and ash concentrations were much greater than in stem. Additionally, leaf percentage in total biomass decreased more rapidly over the growing season than did the percentage of total N and ash that occurred in the leaf fraction. Because N and ash are considered to be anti-quality components in biomass feedstocks, there may be value in returning leaf biomass to the production field or delaying harvest into winter to allow a greater natural return of

leaf biomass to the field by abscission. These approaches may be advantageous both in terms of biomass quality and recycling of nutrients back to soil. Data for both N and ash indicate that returning leaf to the production field is a much more attractive option for elephantgrass than for energycane. This is because elephantgrass leaf represents a lesser proportion of total biomass relative to the proportion of total N and ash contained in the leaf fraction, whereas with energycane leaf percentage and percentage of total N and ash are more nearly the same. In particular, through a large late-season decrease in leaf proportion (Chapter 5), UF-1 appears to reduce leaf percentage in biomass harvested and this may contribute to its potential value as a biomass biofuel crop.

Table 6-1. Sampling dates for energycane and elephantgrass for responses reported in Chapter 6.

Year	Sampling dates				
2010	6 June	28 July	22 September	8 November	8 December
2011	1 June	17 July	16 September	10 November	13 December

Table 6-2. Effect of grass entry or sampling date main effects or their interaction on leaf proportion in total biomass and proportion of total N and ash in the leaf fraction in 2010. Data are means across four replicates (n = 4).

Entry	Sampling date <sup>†</sup>				Mean
	6 June	28 July	8 Nov.	8 Dec.	
-----Leaf proportion in total biomass (%)-----					
L79-1002	62	29	25	22	34 A
Merkeron	57	27	26	22	33 A
UF-1	49	23	19	13	26 B
mean	56 a	26 b	23 b	19 c	
-----Proportion of total harvested N in leaf fraction (%)-----					
L79-1002	68	51	38	35	48
Merkeron	63	50	41	33	47
UF-1	7	48	37	28	42
Mean	63 a <sup>‡</sup>	50 b	39 c	32 d	
-----Proportion of total harvested ash in leaf fraction (%)-----					
L79-1002	46 aA <sup>§</sup>	33 bA	31 bB	33 bA	
Merkeron	47 aA	37 bcA	41 bA	34 cA	
UF-1	41 aA	32 bA	36 abAB	24 cB	
SE			2.5		

<sup>†</sup> Sampling dates were repeated measurements (6 June, 28 July, 8 Nov., and 8 Dec.). Data not available for sampling date 22 September.

<sup>‡</sup> Sampling date means within an entry and not followed by the same lower case letter are different ( $P < 0.05$ ).

<sup>§</sup> Entry means within a sampling date and not followed by the same upper case letter are different ( $P < 0.05$ ).

Table 6-3. Effect of grass entry or sampling date main effects or their interaction on leaf proportion in total biomass and proportion of total N and ash in the leaf fraction in 2011. Data are means across four replicates (n = 4).

Entry	Sampling date <sup>†</sup>					Mean
	1 June	17 July	16 Sep.	10 Nov.	13 Dec.	
-----Leaf proportion in total biomass (%)-----						
L79-1002	55 aA	45 bA	41 bcA	40 cdA	36 dA	
Merkeron	37 aB	36 aB	29 bB	26 bB	21 cB	
UF-1	40 aB	36 aB	27 bB	21 cC	15 dC	
SE	2.2					
-----Proportion of total harvested N in leaf fraction (%)-----						
L79-1002	63	62	60	64	60	63 A
Merkeron	48	48	45	47	42	48 B
UF-1	51	55	45	44	36	51 B
mean	54 ab	55 a	50 bc	51 ab	46 c	
-----Proportion of total harvested ash in leaf fraction (%)-----						
L79-1002	49 aA	41 bA	45 bA	42 bA	43 bA	
Merkeron	44 aA	43 aA	42 aA	40 aA	30 bB	
UF-1	45 aA	46 aA	42 aA	36 bA	28 cB	
SE	2.6					

<sup>†</sup> Sampling dates were repeated measurements (1 June, 17 July, 16 Sep., 10 Nov., and 13 Dec.).

<sup>‡</sup> Sampling date means within an entry and not followed by the same lower case letter are different ( $P < 0.05$ ).

<sup>§</sup> Entry means within a sampling date and not followed by the same upper case letter are different ( $P < 0.05$ ).

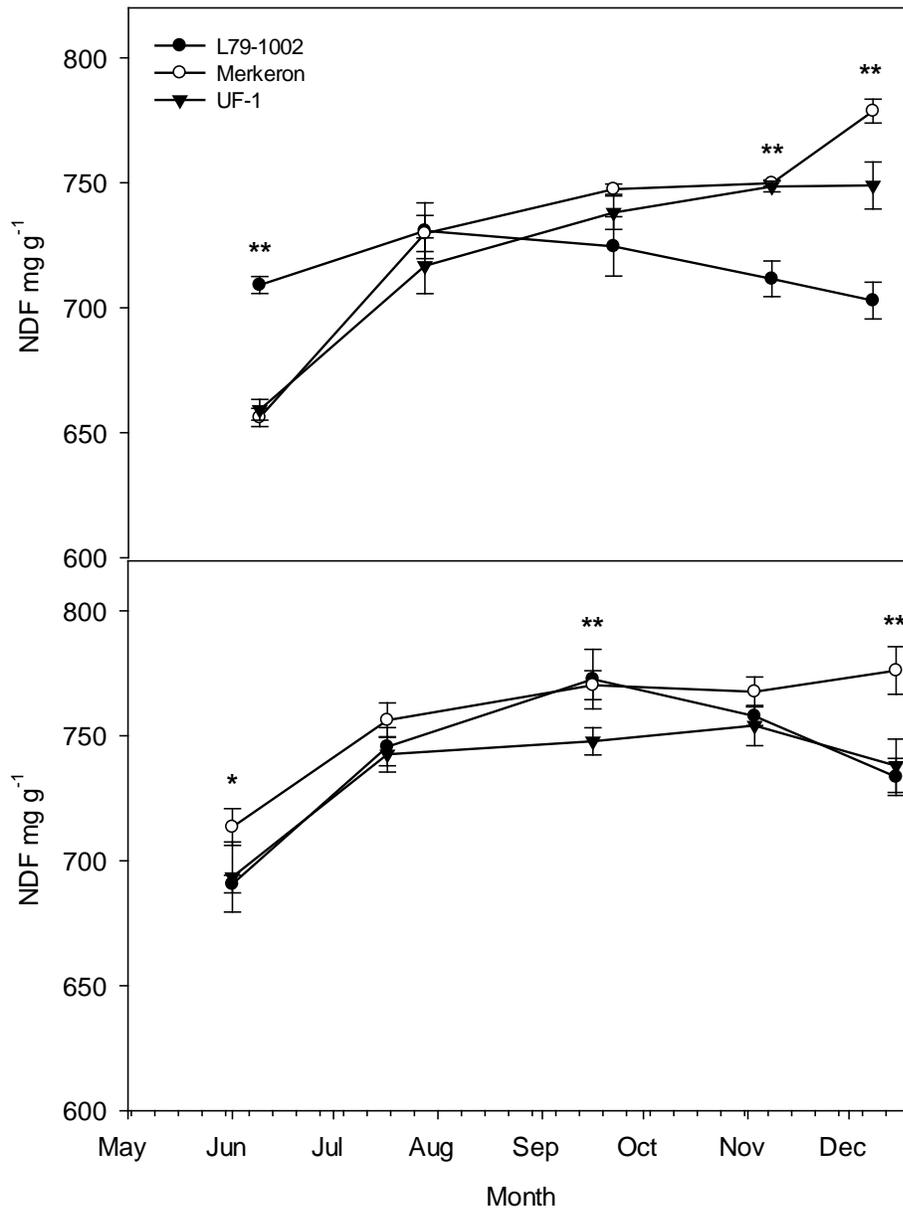


Figure 6-1. Seasonal changes in NDF concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction *P* values were < 0.001 for all in 2010; and < 0.001, < 0.001, and 0.009, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date, \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ .

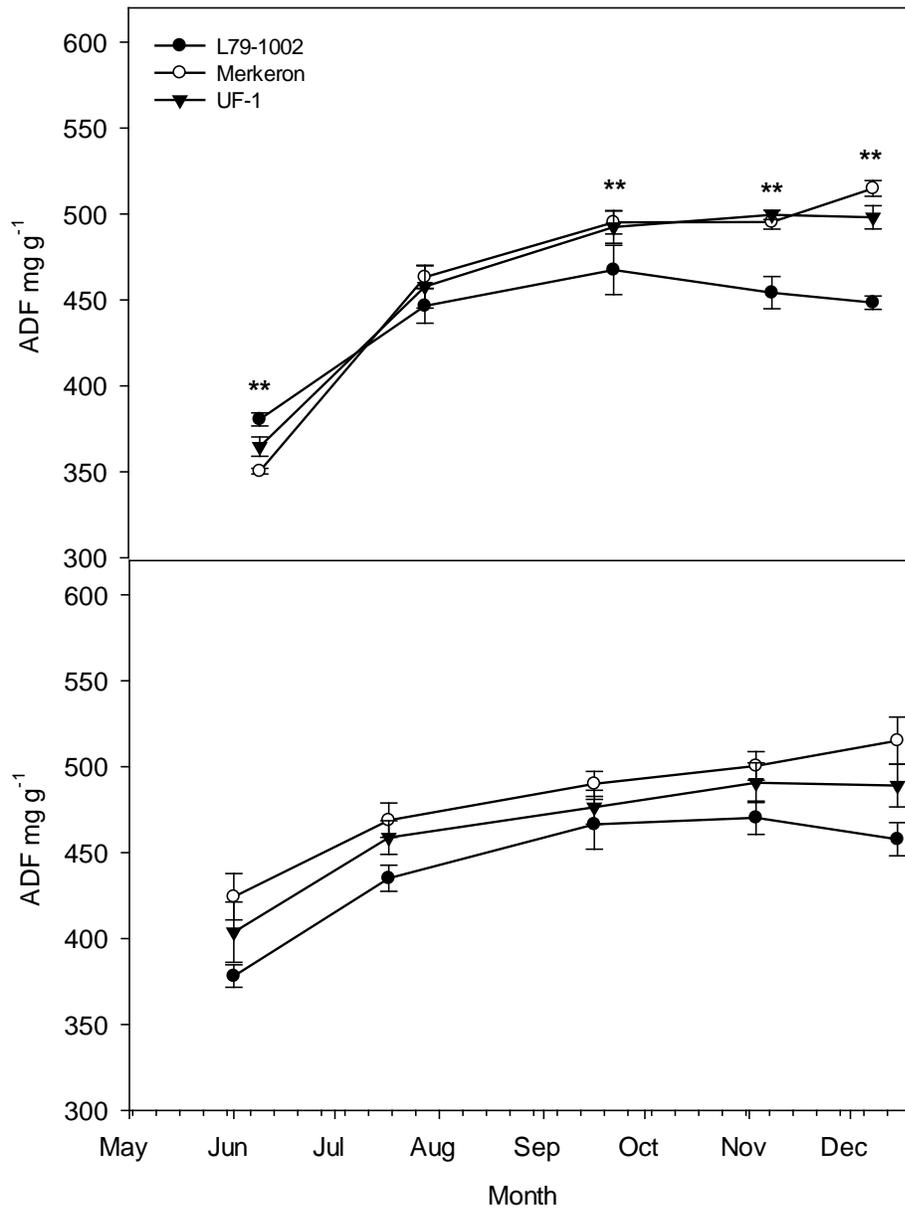


Figure 6-2. Seasonal changes in ADF concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction *P* values were < 0.001 for all in 2010; and < 0.001, < 0.001, and 0.146, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date, \*\* *P*  $\leq$  0.01.

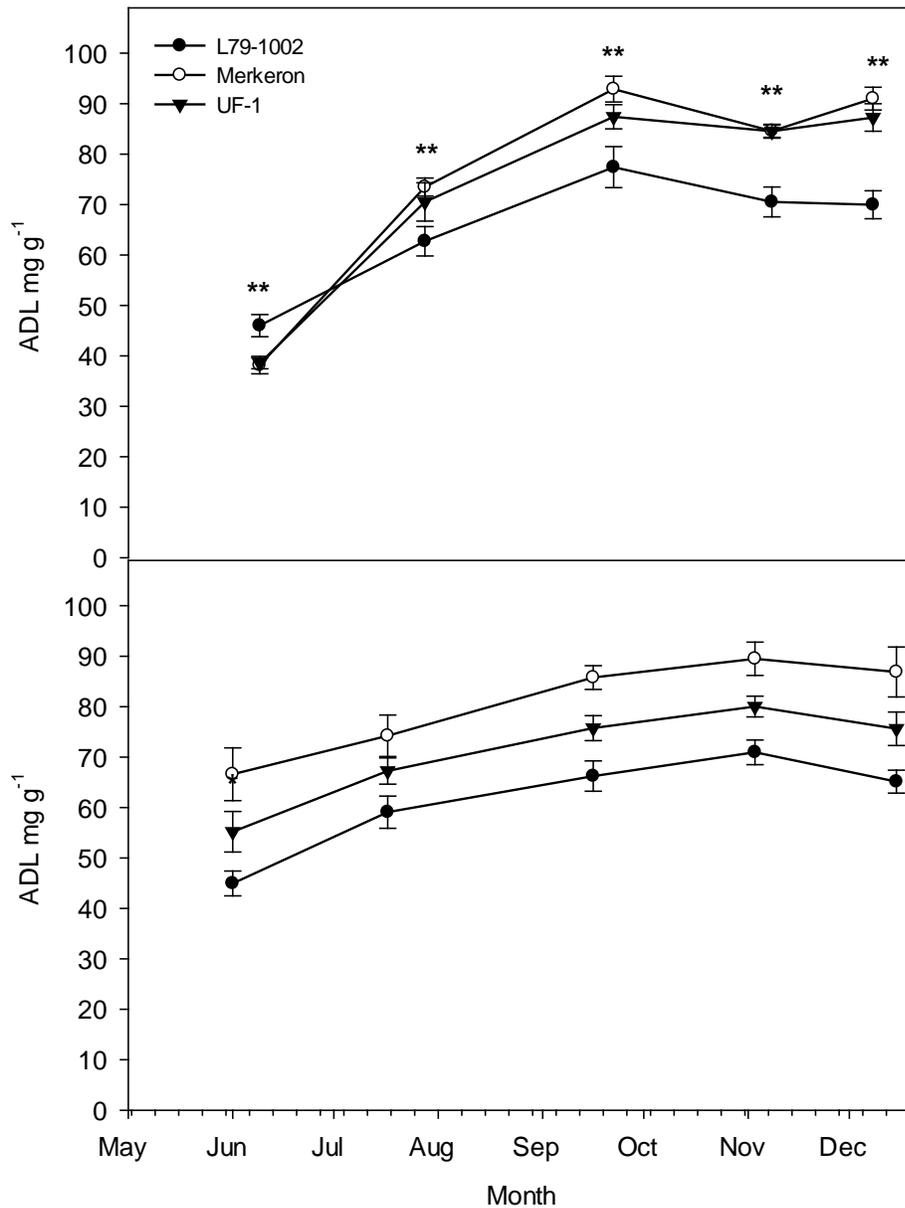


Figure 6-3. Seasonal changes in ADL concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction P values were < 0.001 for all in 2010; and < 0.001, < 0.001, and 0.720, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date, \*\*  $P \leq 0.01$ .

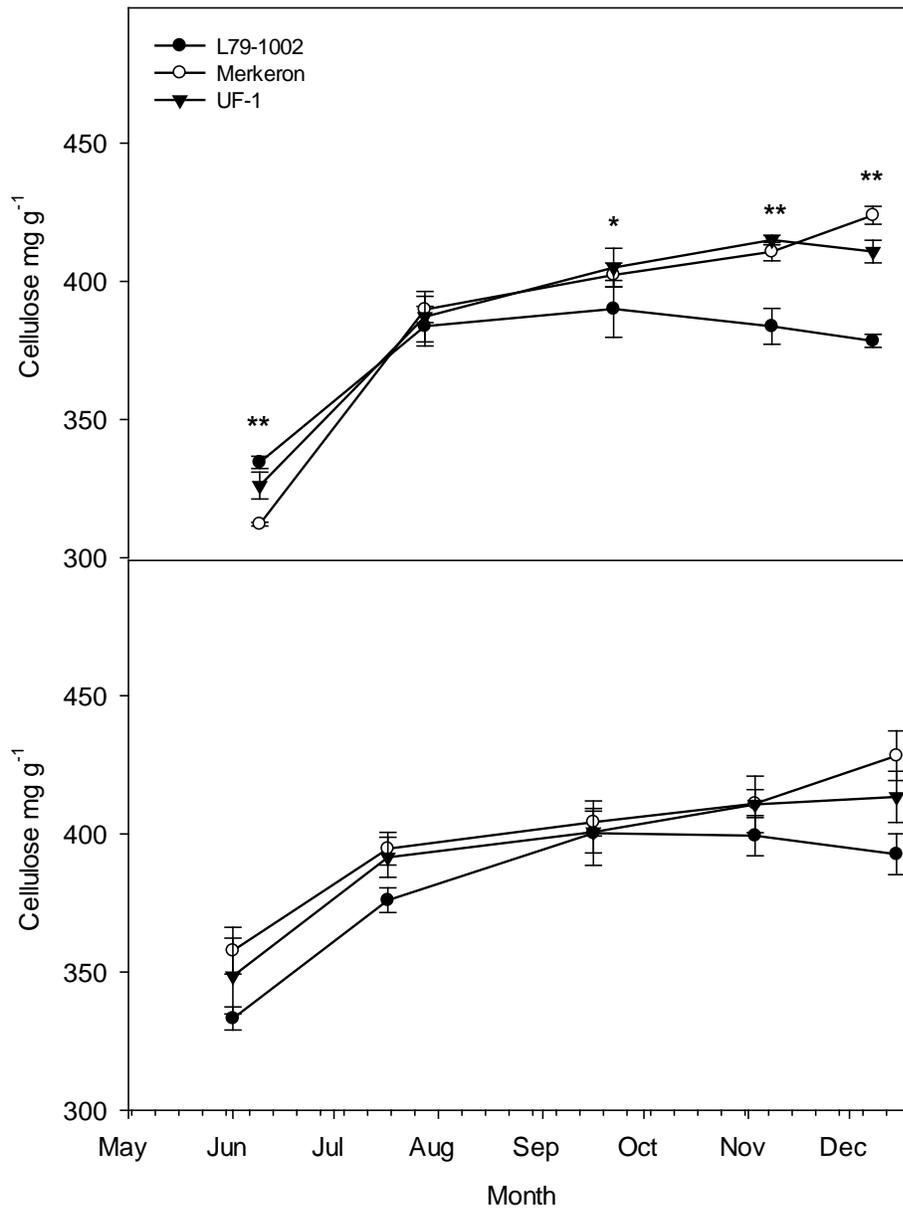


Figure 6-4. Seasonal changes in cellulose concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values were  $< 0.001$  for all in 2010; and 0.002,  $< 0.001$ , and 0.061, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date, \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ .

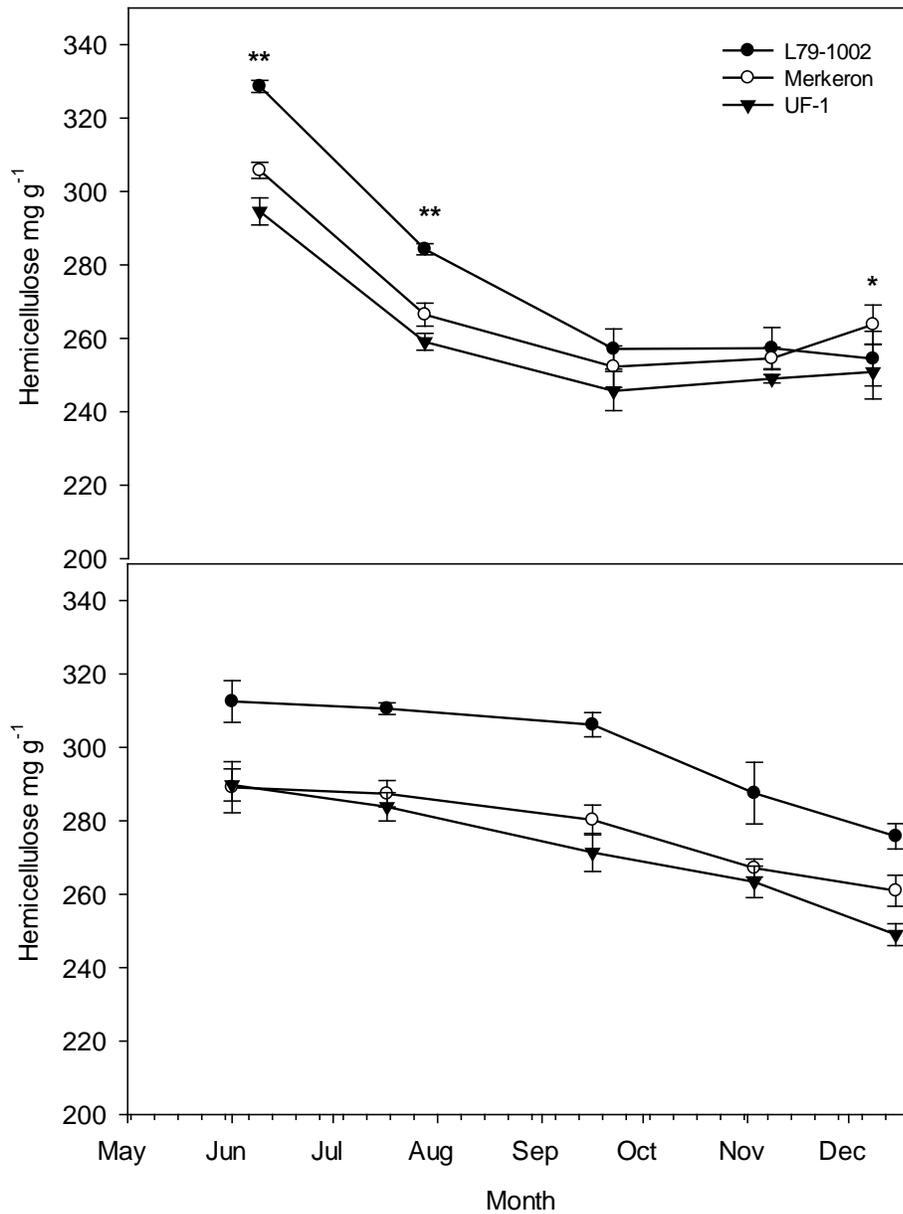


Figure 6-5. Seasonal changes in hemicellulose concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  values were  $< 0.001$ ,  $< 0.001$ , and  $0.001$ , respectively, in 2010; and  $0.002$ ,  $< 0.001$ , and  $0.437$ , respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date, \*  $P \leq 0.05$ , \*\*  $P \leq 0.01$ .

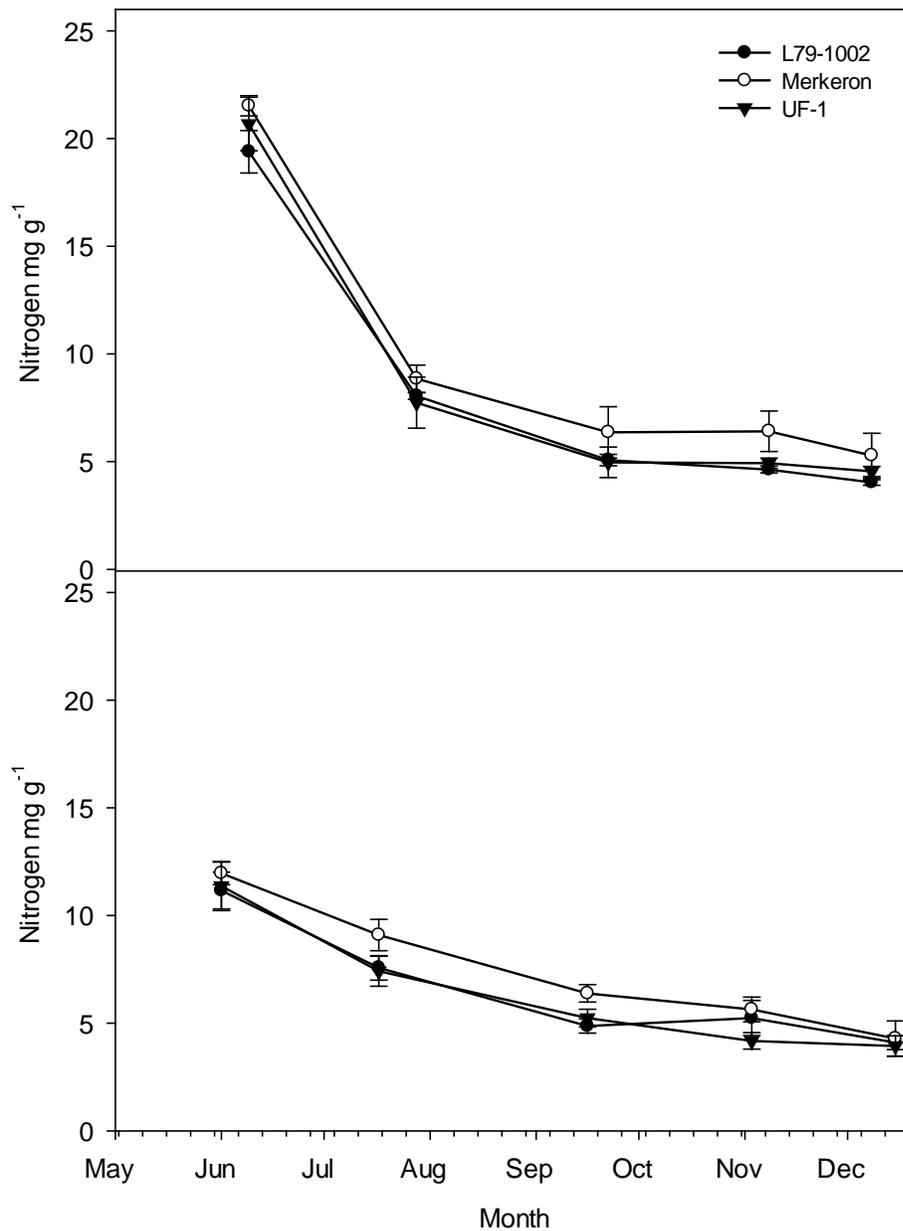


Figure 6-6. Seasonal changes in nitrogen concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction *P* values were 0.176, < 0.001, and 0.923 respectively, in 2010; and < 0.001, < 0.001, and 0.654, respectively, in 2011. Bars show mean  $\pm$  one standard error.

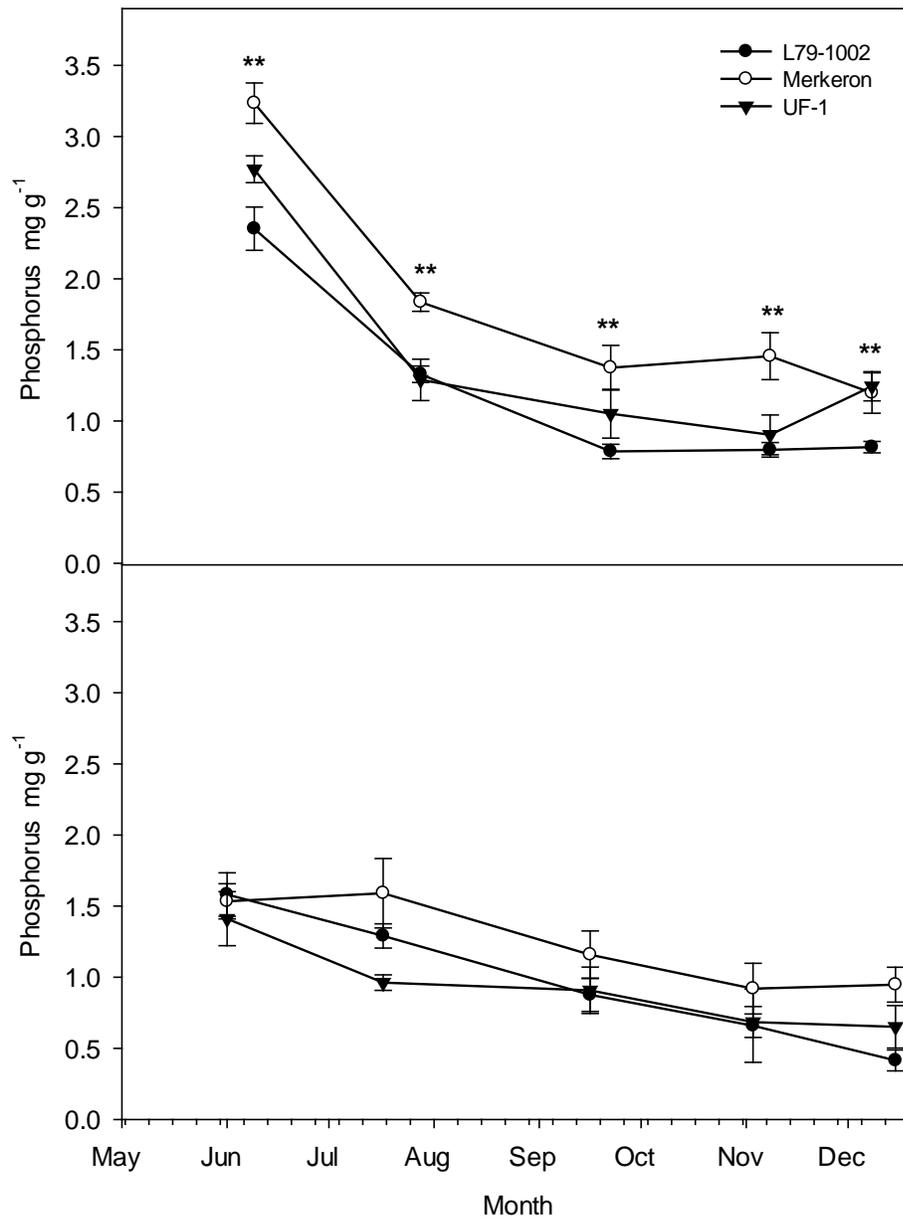


Figure 6-7. Seasonal changes in phosphorus concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction *P* values were 0.176, < 0.001, and 0.006 respectively, in 2010; and < 0.001, < 0.001, and 0.084, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date, \*\* *P*  $\leq$  0.01.

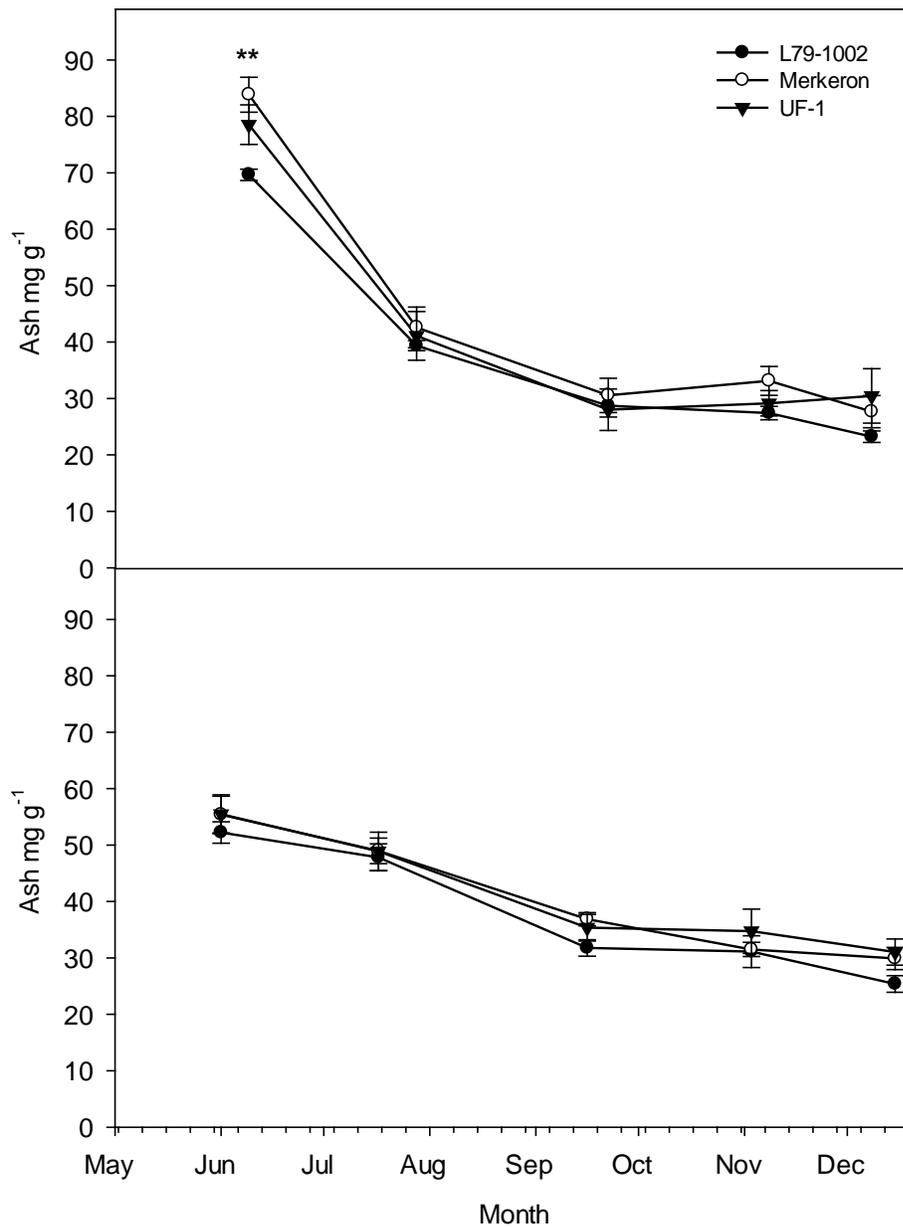


Figure 6-8. Seasonal changes in ash concentration of total harvested biomass of three perennial grass entries in 2010 (upper) and 2011 (lower). Entry, sampling date, and their interaction  $P$  value were 0.314,  $< 0.001$ , and  $< 0.001$ , respectively, in 2010; and 0.341,  $< 0.001$ , and 0.852, respectively, in 2011. Bars show mean  $\pm$  one standard error. Entries within a date, \*\*  $P \leq 0.01$ .

CHAPTER 7  
TIME AFTER A FREEZE EVENT AFFECTS PERENNIAL GRASS BIOMASS  
HARVESTED AND CHEMICAL COMPOSITION

**Overview of Research**

Increasing demand for energy within the context of finite oil reserves has affected energy security of petroleum-importing nations including the USA (Sticklen, 2008). Currently, only biomass-derived fuels offer renewable, liquid-type alternatives to petroleum-based transportation fuels (Rollin et al., 2011). Warm-season grasses have gained attention as bioenergy feedstocks for ethanol production in recent years because they can reduce reliance on imported fossil fuel, reduce greenhouse gas emissions, and help rural economies (McLaughlin et al., 2002; Waramit et al., 2011). In previous research (Woodard and Prine, 1993a; Kering et al., 2012; Knoll et al., 2012; Viator and Richard, 2012), perennial grasses with the C4 photosynthetic system have demonstrated outstanding growth potential and biomass production.

One challenge to the use of warm-season perennial grasses as bioenergy feedstocks is the non-uniform availability of biomass throughout the year. The failure to match supply of biomass to the biorefinery with the goal of year-round biofuel production may result in additional storage and handling costs (Rentizelas et al., 2009). Harvest practices are needed that provide biomass of high quality to the biorefinery during non-peak periods of plant growth. One such option is delaying harvest until winter. By delaying harvest, it may be possible to extend the time period when biomass is available (Finell et al., 2011) and delayed harvest may improve feedstock quality for direct combustion by reducing moisture concentration and leaching undesirable components such as Cl, K, and ash (Jørgensen and Sander, 1997; Lewandowski and Kicherer, 1997; Lewandowski and Heinz, 2003).

Delayed harvest until winter has been reported to negatively affect perennial grass biomass harvested (Lewandowski and Heinz, 2003). For instance, switchgrass (*Panicum virgatum* L.) biomass harvested decreased throughout winter (10.0 Mg ha<sup>-1</sup> in October vs. 5.6 Mg ha<sup>-1</sup> in April) (Wilson et al., 2013b), and the reduction was affected by winter weather, especially snow in the Midwest USA. Other authors have reported a reduction in both switchgrass biomass yield and moisture concentration during winter (Adler et al., 2006). These authors attributed 90% of the yield reduction due to lodging and subsequent difficulties in gathering biomass during baling.

Unlike the Midwest, Florida has a mild winter (Smith and Dowd, 1981) that may affect biomass yield and chemical composition differently than in temperate regions. Regional differences in severity and duration of winter may affect senescence, biomass yield, and biomass quality of perennial grasses resulting in locally specific recommendations relative to delayed harvest. Due to the extensive research conducted on switchgrass, best management practices for harvest management have been developed (Mitchell and Schmer, 2012). However, little is known about the effect of delayed harvest after first freeze on tall-growing, warm-season perennial grasses, particularly in a subtropical environment where freeze events occur only occasionally during winter. The objective of this experiment was to quantify the effect of delayed harvest following first freeze on biomass harvested and composition of elephantgrass (*Pennisetum purpureum* Schum.) and energycane (*Saccharum* spp. hybrid) in Florida.

## **Materials and Methods**

### **Experimental Site**

The experiment was conducted during the winter seasons of 2010-2011 (Year 1), 2011-2012 (Year 2), and 2012-2013 (Year 3) at the Plant Science Research and

Education Unit (PSREU) at Citra, FL (29.41° N, 82.17° W) at a site adjacent to the experiments reported in Chapters 3 through 6. The soil was a well-drained Candler sand (hyperthermic, uncoated Lamellic Quartzipsamments). Initial characterization of topsoil (0-20 cm) showed an average soil pH of 7.0, and Mehlich-1 extractable P, K, Mg, and Ca of 54, 20, 123, and 496 mg kg<sup>-1</sup>, respectively. Monthly weather conditions for the growing season (before the first freeze event occurred) were described previously (Chapter 3, Figs. 3-1 and 3-2). For each winter season, weather data are reported for the period of delayed harvest starting with the date of first freeze event (Table 7-1). A freeze was defined as a temperature of 0°C or less at 2 m above soil level that resulted in complete kill of the leaf canopy. Weather data reported are the weekly mean daily temperature and the weekly mean maximum and minimum temperatures (Fig. 7-1) as well as weekly total precipitation (Figure 7-2). To summarize the occurrences of freezes, there were six events between the pre-freeze harvest and first harvest after freeze, six events between the first and second harvest, and two events between the second and third harvests 2010. In 2011, there were six freeze events between the pre-freeze harvest and first harvest after freeze and two freezes between the first and second harvests. In 2012 there was only one freeze between the pre-freeze harvest and first harvest after freeze and five freezes between the first and second harvests.

### **Plot Establishment and Management until a Freeze Event**

Plots contained six rows of 6-m length, with 1-m spacing between rows. Plots were established using above-ground stem pieces planted on 15 Dec. 2009. Thus, the Year 1 data (2010-2011) are from the establishment year. In all 3 yr, N was applied as ammonium sulfate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) at a rate of 150 kg N ha<sup>-1</sup> yr<sup>-1</sup>, and K was applied as muriate of potash (KCl) at a rate of 90 kg K ha<sup>-1</sup> yr<sup>-1</sup>. Nutrients were split applied, with

applications of 50 kg N and 45 kg K ha<sup>-1</sup> in mid-April and 100 kg N and 45 kg K ha<sup>-1</sup> in mid-May. No P was needed based on soil test. Limited irrigation was applied to the experiment only at sign of significant drought stress (leaf rolling). There were five irrigation events during the growing season of 2010 totaling 60 mm, three irrigation events in 2011 totaling 50 mm, and no irrigation was applied in 2012.

### **Treatments and Experimental Design**

The two grass entries included 'Merkeron' elephantgrass (Burton, 1989) and 'L79-1002' energycane (Bischoff et al., 2008). As mentioned previously (Chapter 3), these two cultivars have documented potential as biofuel feedstocks in this region. Experimental design was completely randomized with four replications of the grass entries. In Year 3, stands of L79-1002 had become sufficiently depleted that they could no longer be used in the experiment, so data are reported for Merkeron only in that year.

### **Response Variables**

The initial biomass harvest occurred at times ranging from the date of the first freeze event (2010) to 15 (2011) to 25 d (2012) prior to first freeze (Table 7-1). In Years 2 and 3, the initial harvest occurred in advance of first freeze due to actual overnight temperatures being greater than forecast low temperatures. For presentation of results, data from the "at-freeze harvest" is shown as being collected on Day "0", but the counting of time after a freeze event did not start until the actual date of the freeze (Table 7-1).

At harvest, a 2-m (Year 1) or 3-m (Years 2 and 3) length of row from a middle row of each plot was clipped. The grass was cut to 12-cm stubble height using a brush cutter. To minimize border effects, the 1-m portion at the end of the harvested row was

not part of the yield sample. The harvested area in each plot was thus considered to be 2 m<sup>2</sup> (Year 1) or 3 m<sup>2</sup> (Years 2 and 3). There was one fewer harvest event in Years 2 and 3 than Year 1, allowing for the larger row length to be sampled in those years (Table 7-1). All material from the harvested portion of row was weighed fresh in the field and subsampled to determine DM concentration and to calculate DM harvested. The subsamples were dried at 60<sup>0</sup>C until constant weight. In 2010 and 2011, the dried subsamples for DM concentration were hand-separated into leaf (blade and sheath) and stem (including inflorescence, if present) components to determine leaf and stem proportion in the dry matter. After the biomass samples were collected at a given date, the remaining plot area remained undisturbed until the next sampling date. After the final harvest date, the remaining area of the plot was clipped to the target stubble (12 cm) using a disk mower.

To obtain representative whole-plant samples, stem and leaf samples were ground separately and a whole-plant composite for each plot was made based on weighted proportions of leaf and stem. Stem samples were initially ground through a hammer mill to reduce particle size. Stem and leaf samples were ground to pass a 1-mm stainless steel screen in a Wiley mill (Model 4 Thomas-Wiley Laboratory Mill, Thomas Scientific, Swedeboro, NJ).

Each component of dried biomass from Years 1 and 2 was analyzed for biomass composition. The modified method of detergent fiber analysis (Van Soest et al., 1991) was used for sequential neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) analysis. The samples were analyzed for NDF and ADF using the ANKOM fiber analyzer (ANKOM 2000 Fiber Analyzer, ANKOM Technology

Corporation, Fairport, NY). The procedure “Method for Determining Acid Detergent Lignin in Beakers” (proposed by ANKOM Technology Corporation) was followed for ADL determinations. Cellulose was estimated as the difference between ADF and ADL concentration and hemicellulose was estimated as the difference between NDF and ADF concentration (Jung and Lamb, 2004; Waramit et al., 2011).

As described in Chapter 4, total N and P concentrations in the leaf and stem were determined using a standard-Kjeldahl method, a modification of the aluminum block digestion procedure (Gallaher et al., 1975) followed by semi-automated colorimetric determination (Hambleton, 1977). Digestions were conducted at the Forage Evaluation Support Laboratory of the University of Florida. Samples were ashed using a muffle furnace at a temperature of 500°C for a minimum of 6 h.

### **Statistical Analysis**

Data were analyzed using mixed-model methods (SAS Institute, 2008). In all models, grass entry was considered a fixed effect. Sampling date (Table 7-1) was considered a repeated measurement (fixed). Means were compared using the pdiff test of LSMEANS. All means reported in the text are least squares means and were considered different if  $P \leq 0.05$ . Only Merkeron was sampled for biomass harvested and DM concentration in Year 3, so grass entry was not included in the model in that year. Sampling date was considered to be fixed and a repeated measurement for Year 3. Data were analyzed by year because of difference in number of harvest events among years and because energycane was not part of the experiment in Year 3. A subsequent analysis was conducted with Merkeron only in which year was included as a random effect and sampling date was considered a repeated measure. A trend was referred to if  $P \leq 0.10$  and  $> 0.05$ .

## Results and Discussion

During the years of study, there were large variations in weather patterns that followed the first freeze of winter. Average temperature following first freeze of Year 1 was much lower than for Years 2 and 3 (9.8, 15.4, and 15.4°C, respectively) (Fig. 7-1). Although average temperature of Years 2 and 3 were similar, Year 2 had additional freezing events at 2 (-1.9 °C) and 6 wk (-5.3°C) after the first freeze, but there was only one additional freeze event (-5.3°C) in Year 3, on Day 57 after first freeze which was the day before final biomass harvest.

### Harvested Biomass

Entry, sampling date, and their interaction had no effect on biomass yield in Years 1 or 2 ( $P = 0.773$ ,  $0.198$ , and  $0.320$  respectively, in Year 1;  $P = 0.153$ ,  $0.178$ , and  $0.184$ , respectively, in Year 2, Fig. 7-3). In Year 3, there was a strong trend ( $P = 0.067$ , Fig. 7-3) for biomass harvested to decrease from time of freeze until approximately 8 wk after freeze. Biomass harvested was within the range reported previously for biomass crops harvested once per year in this region (Woodard et al., 1991b; Woodard and Prine, 1993b; Knoll et al., 2012). In Year 1, average harvested biomass was 26.7 and 27.6 Mg ha<sup>-1</sup> for L79-1002 and Merkeron, respectively. Although it was not significant ( $P = 0.153$ ), Merkeron (avg. 29.2 Mg ha<sup>-1</sup>) tended to have greater biomass yield than L79-1002 (avg. 23.5 Mg ha<sup>-1</sup>) in Year 2. In Year 3, it was not possible to obtain meaningful biomass harvested data from L79-1002 plots. They had sustained severe damage from the fungal disease 'sugarcane smut' (*Sporisorium scitamineum*), an important pest of L79-1002 which was reported in previous energycane studies (Bischoff et al., 2008; León et al., 2012; Ramesh Sundar et al., 2012; Viator and Richard, 2012) and in the other experiment at this location (Chapter 3). Although energycane L79-1002 was

reported to be only 'moderately susceptible' in the previous study (Bischoff et al., 2008), at this location in the current study it was quite susceptible. The severity of smut on L79-1002 caused yield loss (Chapter 3), and susceptibility to smut may decrease the vigor and stand life of energycane.

If L79-1002 was removed from the analysis and data were analyzed with year in the model as a random effect, Merkeron biomass harvested decreased ( $P = 0.029$ ) from time of first freeze until the end of the delayed harvest period. The average decrease over 3 yr was from 30.7 to 18.9 Mg ha<sup>-1</sup> over a period after first freeze of 50 to 59 d. Although there was a wide range in weather conditions after the first freeze during the years of study, biomass harvested did not seem to be impacted to a greater or lesser degree by the colder Year 1 vs. the warmer Years 2 and 3.

Delayed cutting has been reported to negatively affect miscanthus biomass harvested (Lewandowski and Heinz, 2003), and switchgrass biomass decreased from 10.0 Mg ha<sup>-1</sup> in October to 5.6 Mg ha<sup>-1</sup> in April in Iowa (Wilson et al., 2013b). Other authors have reported a reduction in both switchgrass biomass during winter (Adler et al., 2006), with magnitude of reduction reaching 90% due to lodging and difficulties in gathering biomass during baling. In the current study, Merkeron biomass harvested decreased during the post-freeze period, but the variability was large. In addition, the harvest methodology used in the current study was quite thorough in capturing biomass, so although delayed harvest increased lodging (visual observation) the problem of gathering biomass for delayed harvest that was noted by Adler et al. (2006) did not arise. It may well be a problem in commercial production, however. Overall, yield reduction in the current study was less proportionally than the very large decreases

reported in previous studies (Adler et al., 2006; Tahir et al., 2011; Wilson et al., 2013a) in much more severe winter climates than Florida.

### **Biomass Dry Matter Concentration**

Effect of delayed harvest on biomass dry matter (DM) concentration was evaluated in all 3 yr (Fig. 7-4). There were effects of entry and sampling date ( $P = 0.025$ , and  $P < 0.001$ , respectively) in Year 1 (Fig. 7-4). In the second year, there was entry x sampling date interaction ( $P < 0.001$ ), and in the third year there was an effect of sampling date ( $P = 0.013$ ). In the first year, Merkeron had greater DM concentration than L79-1002 (average of 377 vs. 356 mg g<sup>-1</sup>, Fig. 7-4). Slightly, but not significantly, greater DM concentration was observed for Merkeron vs. L79-1002 in a single fall harvest treatment in Florida, but the range of responses was only from 311 to 338 mg g<sup>-1</sup> (Woodard et al., 1991b). Biomass DM concentration increased in Year 1 of the current study until 34 d after freeze (386 mg g<sup>-1</sup>) and then decreased slightly at final harvest on Day 50 (366 mg g<sup>-1</sup>). The decrease in biomass DM concentration at the final harvest is likely due to a 33-mm rainfall event that occurred the day before final harvest.

In Year 2, entry x sampling date interaction occurred because Merkeron DM concentration increased almost two-fold throughout the delayed-harvest period (351, 519, and 676 mg g<sup>-1</sup> at 0, 28, and 59 d after freeze, respectively), whereas energycane increased only from 329 mg g<sup>-1</sup> at first freeze to 401 mg g<sup>-1</sup> at final harvest on Day 51. In contrast, in Year 3 Merkeron DM concentration changed relatively little and was 355, 314, and 367 mg g<sup>-1</sup> before freeze and at Days 27 and 58 after freeze, respectively.

The very large change in Year 2 DM concentration was unique for Merkeron among years and also was very different than the responses of energycane. It is proposed that weather conditions affected the response. After the killing freeze in Year

1, the weather remained cold throughout the delayed harvest period. There were a total of 15 freeze events between first freeze and the final biomass harvest in Year 1, and the longest period between freeze events was 14 d. This type of environment apparently minimized DM loss. In both Years 2 and 3, there was a killing freeze followed by much warmer winter weather (avg. temp. in both years was 15.4°C vs. 9.8°C in Year 1). Exploring ways that Years 2 and 3 were different, in Year 2 the temperature fell to -5.6°C the day after first freeze and during the delayed harvest period there was a 26-d period without a freeze event. In Year 3, there was a period of 29 d following first freeze without occurrence of additional freezes, and there were no temperature below -2°C until the day before the final harvest. This resulted in regrowth of new tillers in Year 3 which caused DM concentration to decline between first freeze and the first harvest date after first freeze in Year 3 (Fig. 7-4). Although there is not a definitive cause and effect, it may be that the very low temperature that occurred early in the delayed harvest period of Year 2 affected the integrity of elephantgrass stems, opening them up to drying and the freeze-free period that followed provided excellent drying conditions. Previous research has shown differences in perennial grass DM concentration among years during a delayed harvest period in UK (Smith and Slater, 2011).

There also was a large difference between entries in Year 2. This may be due to differences in stem anatomy. The surfaces of plants are covered by several layers of lipophilic material (mostly epicuticular wax), and the primary function of ubiquitous presence of epicuticular wax is as a waterproof barrier (Purcell et al., 2005). Scanning electron micrographs showed that the outermost layer of sugarcane stem, i.e., comrind, is composed of epidermal cells that contain a waxy later, moreover, its rind fiber was

very thick (Han and Wu, 2004). The authors also indicated that the innermost layer of comrind is wrapped with pith cells. Those anatomical characteristics of energycane (sugarcane) stem may result in less seasonal change in stem DM concentration than in elephantgrass. Additionally, there is a well-developed aerenchyma-like tissue in the stem of elephantgrass (Jennewein et al., 2012), which if the outer stem layers are disrupted may accelerate field drying during periods of warm weather. Unlike elephantgrass, energycane has a relatively high concentration of water soluble carbohydrate and extractives (Woodard et al., 1991b; Fedenko, 2011) and that along with its anatomical characteristics may have reduced rate of stem dry down during delayed harvest.

### **Leaf Proportion**

Leaf proportion was quantified in Years 1 and 2 only. There was a sampling date effect on leaf proportion in Year 1 ( $P < 0.001$ , Fig. 7-5), and in Year 2 there were effects of entry and sampling date ( $P = 0.001$  and  $P < 0.001$ , respectively). During Year 1, leaf proportion increased to 18 d after first freeze and decreased until the final harvest (Fig. 7-5). There was no entry effect ( $P = 0.120$ ) but elephantgrass tended to have lower leaf proportion than energycane in Year 1 (23.2 vs. 27.0%, respectively). In the second year, L79-1002 leaf proportion across sampling dates (avg. 27.6%) was 2.6-fold greater than Merkeron (avg. 10.5%, Fig. 7-5). Leaf proportion across entries decreased from 26.6 prior to first freeze to 18.2 at Day 28 and 12.4% at 59.

These data show that in general delayed harvest after first freeze decreased leaf proportion of these perennial grass. Leaf is considered to be least valuable part of biomass harvested because of greater concentrations of N and ash (Adler et al., 2006; Sokhansanj et al., 2009), so delayed harvest may increase biomass quality without the

additional cost of removing leaves at harvest. However, it has been shown that the amount of abscised leaves varied with post-freeze weather conditions (Wilson et al., 2013a). In this research, extreme cold at first freeze in Year 2 followed by warm weather after freeze may have accelerated leaf abscission, and elephantgrass had only 5.3% leaf in total biomass by 59 d after freeze. This suggests that part of the decrease in elephantgrass biomass harvested and increase in dry matter concentration with delayed harvest is caused by leaf fall.

### **Fiber Analysis**

Biomass NDF concentration was affected by entry and sampling date ( $P < 0.001$  for both) in Year 1 (Fig. 7-6) and entry x sampling date interaction in Year 2 ( $P = 0.026$ ). There was a similar range of NDF concentration as reported in previous research (Woodard and Prine, 1991; Van Man and Wiktorsson, 2003). Across all sampling dates, average NDF concentration in elephantgrass was greater than energycane (784 and 702 mg g<sup>-1</sup>, respectively, Fig. 7-6). The NDF concentration increased with delayed harvest (709, 739, 754, and 771 mg g<sup>-1</sup> for 0, 18, 34, and 50 d after first freeze, respectively). In the second year, Merkeron had greater NDF concentration than L79-1002 and interaction occurred because the magnitude of the difference between entries increased as harvest was delayed (7, 10, and 12% difference at 0, 28, and 59 d after freezing, Fig. 7-6). When winter was cool in Year 1, the increase in NDF of Merkeron and energycane was similar (9% increase from pre-freeze to 50 d after first freeze for both), however, during the warmer winter in Year 2 the difference increased with time.

There were entry and sampling date effects on ADF concentration in Year 1 ( $P < 0.001$ , Fig. 7-7) and in Year 2 ( $P = 0.001$ ). Similar ADF concentration was reported in previous research with elephantgrass (Van Man and Wiktorsson, 2003). During the first

year, overall ADF concentration in Merkeron was greater than L79-1002 (avg. 528 vs. 451 mg g<sup>-1</sup>). Across entries, ADF concentration increased 12% throughout the season. In Year 2, entry x sampling date interaction occurred because the increase in Merkeron ADF during the delayed harvest period (19%) was greater than in L79-1002 (10%). The trend in ADF concentration was very similar to that of NDF.

Biomass ADL was affected by entry and sampling date ( $P < 0.001$  for both, Fig. 7-8) in Year 1 and entry x sampling date interaction in Year 2 ( $P = 0.019$ ). The range in elephantgrass ADL concentration in the current study was similar to that reported in previous research (12.0-12.5 mg g<sup>-1</sup>) (Rengsirikul et al., 2011). During the first year, the last sampling date (50 d after freeze) showed greatest ADL concentration in both grasses (77 and 106 mg g<sup>-1</sup>, Fig 7-8). Average ADL concentration of Merkeron (97 mg g<sup>-1</sup>) was greater than for L79-1002 (72 mg g<sup>-1</sup>) across sampling dates. During the second year, entry x sampling date interaction occurred because elephantgrass ADL concentration increased to a greater degree with delayed harvest than did energycane (31 and 18% increase for Merkeron and L79-1002, respectively). This pattern of response was the same as described for NDF and ADF. Previous research has reported an increase in lignin concentration of warm-season perennial grasses late in the season in Iowa (Waramit et al., 2011).

Cellulose was affected by entry and sampling date during the first year ( $P < 0.001$  for both, Fig. 7-9) and entry x sampling date interaction in Year 2 ( $P = 0.001$ ). Concentration of elephantgrass cellulose in the current study is comparable to that (447-462 mg g<sup>-1</sup>) reported previously (Rengsirikul et al., 2011). Average cellulose concentration in Merkeron (431 mg g<sup>-1</sup>) was greater than in L79-1002 (379 mg g<sup>-1</sup>) in

Year 1. Cellulose concentration across entries increased from 384 mg g<sup>-1</sup> in pre-freeze biomass to 425 mg g<sup>-1</sup> by 50 d after first freezing (Fig. 7-9). In the second year, entry x sampling date interaction occurred because the increase in Merkeron during the delayed harvest period was greater than in L79-1002 (16 and 8%, respectively, from pre-freeze to 59 d after first freeze). A similar overall trend for cellulose concentration during late season was reported in Iowa with perennial grasses (Waramit et al., 2011).

The pattern of hemicellulose concentration response was different from the other fiber components. There was entry x sampling date interaction in the first year ( $P = 0.040$ , Fig. 7-10) and an effect of entry in Year 2 ( $P = 0.009$ ). The range in elephantgrass hemicellulose concentration was similar to that reported previously (232-236 mg g<sup>-1</sup>) (Rengsirikul et al., 2011). From pre-freeze to 18 d after first freeze in Year 1, Merkeron had greater hemicellulose concentration than L79-1002 (254 vs. 243 mg g<sup>-1</sup> at pre-freeze, 265 vs. 255 mg g<sup>-1</sup> at 18 d after first freeze, Fig. 7-10), but thereafter no differences were detected and the average was 253 mg g<sup>-1</sup>. Unlike the first year, L79-1002 showed greater hemicellulose concentration (avg. 271 mg g<sup>-1</sup>) than Merkeron (avg. 254 mg g<sup>-1</sup>) throughout Year 2. Hemicellulose concentration remained relatively constant across the delayed harvest period. Similar late-season trends of hemicellulose concentration were reported in previous research (Waramit et al., 2011).

Considering the fiber components as a group and with the exception of hemicellulose, the pattern of change in concentration during the delayed harvest period was similar. Merkeron had greater structural component concentration than energycane. When temperatures were warm during the delayed harvest period in Year 2, the magnitude of change in structural components was greater in elephantgrass than

energycane. An overall increase in structural component concentration and a decrease in soluble carbohydrate was reported in switchgrass during the winter (Adler et al., 2006). An increase in NDF, ADF, and ADL were reported over winter in a study with reed canarygrass (*Phalaris arundinacea* L.) (Tahir et al., 2011), and similar seasonal patterns in NDF, ADL, cellulose, and hemicellulose was reported in switchgrass (Dien et al., 2006).

In a previous study, L79-1002 ( $94.6 \text{ mg g}^{-1}$ ) had greater water soluble concentration than Merkeron ( $43.5 \text{ mg g}^{-1}$ ) in a single fall harvest in Florida (Woodard et al., 1991b) and a similar pattern of extractives ( $256$  and  $209 \text{ mg g}^{-1}$ ; energycane and elephantgrass, respectively) (Fedenko, 2011). Greater concentration of extractives (mostly water soluble carbohydrates) in energycane likely caused the difference in fiber components among entries. As previously described for DM concentration, elephantgrass might be more sensitive to post-freeze weather conditions because of its anatomical characteristics. If elephantgrass is exposed to warm temperature after a severe killing freeze, contents of the stem appear likely to lose a greater concentration of non-structural constituents, perhaps by microbial activity.

## **Nitrogen**

There was no effect of entry, sampling date, or their interaction ( $P = 0.666$ ,  $0.285$ , and  $0.832$ , respectively) in the first year (Fig. 7-11), but in Year 2 there were entry and sampling date effects ( $P = 0.032$  and  $0.011$ , respectively). During first year, overall average N concentration was  $4.65 \text{ mg g}^{-1}$ . In the second year, elephantgrass biomass had greater N concentration ( $4.2 \text{ mg g}^{-1}$ ) than energycane ( $2.4 \text{ mg g}^{-1}$ ) when averaged across the delayed harvest period (Fig. 7-11). Average N concentration across entries

decreased with delayed harvest and was 4.0, 3.4, and 2.5 mg g<sup>-1</sup> in 0, 28, and 59 d after the freezing event, respectively, in Year 2.

Nitrogen concentration was stable in Year 1 when the delayed harvest period temperature was cool, perhaps due to less structural breakdown and leaching of N from plant tissue or to greater retention of leaves (Fig. 7-5). When temperatures were warm following a very severe freeze event in Year 2, N concentration decreased over the period. Previous research has reported mixed results relative to change in N concentration over winter with biomass N increasing in some experiments (Burvall, 1997; Lewandowski and Heinz, 2003; Dien et al., 2006) or remaining the same in others (Adler et al., 2006; Wilson et al., 2013a). It is believed that temperature and rainfall may significantly affect N concentration during delayed harvest. One difference between Years 1 and 2 in the current study was the amount of leaf drop (Fig. 7-5) which was much greater in Year 2, the year in which N concentration decreased markedly. Previous perennial grass studies have shown that although there were seasonal changes, leaf is greater in N concentration than stem in general (Griffin and Jung, 1983; Mislevy et al., 1995; Beale and Long, 1997; Smith and Slater, 2011). Thus, when perennial grasses lose leaves N concentration in biomass decreases.

### **Ash**

There were entry and sampling date effects ( $P = 0.042$  and  $0.040$ , respectively) in Year 1 (Fig. 7-12) and a sampling date effect in Year 2 ( $P = 0.017$ ). Average pre-freeze ash concentration in Year 1 was greater than at the end of delayed harvest period (30 vs. 25 mg g<sup>-1</sup>, respectively) across entries. Decreasing ash concentration during winter was reported in previous studies (Adler et al., 2006; Kludze et al., 2013; Wilson et al., 2013a). Merkeron had greater ash (29 mg g<sup>-1</sup>) than L79-1002 (24 mg g<sup>-1</sup>)

throughout the period in Year 1, with biomass from the latest harvest (59 d after freeze) having lesser ash concentration ( $21 \text{ mg g}^{-1}$ ) compared to pre-freeze biomass ( $27 \text{ mg g}^{-1}$ ). Ash is the inorganic residue remaining after ignition of biomass, and herbaceous crops produce meaningful amounts of ash (Kludze et al., 2013). There are five primary components (Si, K, Ca, S, and Cl) that have the greatest effect on ash concentration (Bakker and Elbersen, 2005). However, Si, which composes the greatest proportion of ash, is relatively stable so removal of other elements such as K is more likely to affect overall ash concentration (Tahir et al., 2011; Kludze et al., 2013). Further study is needed with ash, including which elements change over time and the extent to which change in ash concentration is related to leaf fall.

### **Implications of Research**

Delayed harvest until after occurrence of freezing temperatures can increase the duration of the harvest period, but it may impact quality and quantity of herbaceous biomass. Harvest of elephantgrass and energycane was delayed up to 2 mo after the first killing freeze event in this experiment. Energycane L79-1002 biomass yield was unaffected by delayed harvest, but elephantgrass biomass decreased with increasing number of days in the delayed harvest period. Leaf proportion in biomass decreased with increasing time between freeze and harvest, especially with elephantgrass. Abscission of leaf may decrease biomass yield and concentration of N and ash in biomass. Biomass DM concentration generally increased with delayed harvest with large year and species variation, especially in elephantgrass which is more sensitive to weather conditions after a freeze event.

Elephantgrass generally had greater concentration of cell wall constituents than energycane. Fiber component concentration generally increased after a freeze event

with the exception of hemicellulose. Increasing fiber component concentration was likely associated with loss of non-structural constituents, perhaps by leaching or microbial activity. Biomass N and ash concentration tended to decrease overwinter, but not to a large degree.

These data suggest that in terms of biomass harvested energycane is better suited for delayed harvest following a freeze because its biomass loss is minimal. Elephantgrass appears to be more susceptible to loss of biomass and non-structural constituents following a freeze event, particularly when warm weather follows the occurrence of freezing temperatures. Thus, post-freeze weather conditions will likely determine the viability of delaying harvest, especially with elephantgrass, with colder temperatures following a freeze event likely having less negative impact on biomass harvested and composition.

Table 7-1. Sampling and freeze event dates for delayed harvest management in 2010-2011, 2011-2012, and 2012-2013.

Sampling dates and freeze events					
Season	Harvest date before freeze	Date of first freeze	1 <sup>st</sup> harvest after freeze	2 <sup>nd</sup> harvest after freeze	3 <sup>rd</sup> harvest after freeze
2010- 2011	2 Dec. 2010	2 Dec. 2010	20 Dec. 2010 (18 DAF <sup>†</sup> )	5 Jan. 2011 (34 DAF)	21 Jan. 2011 (50 DAF)
2011- 2012	19 Dec. 2011	3 Jan. 2012	31 Jan. 2012 (28 DAF)	2 Mar. 2012 (59 DAF)	---
2012- 2013	28 Nov. 2012	23 Dec. 2012	19 Jan. 2013 (27 DAF)	19 Feb. 2013 (58 DAF)	---

<sup>†</sup>DAF; days after the first freeze event occurred.

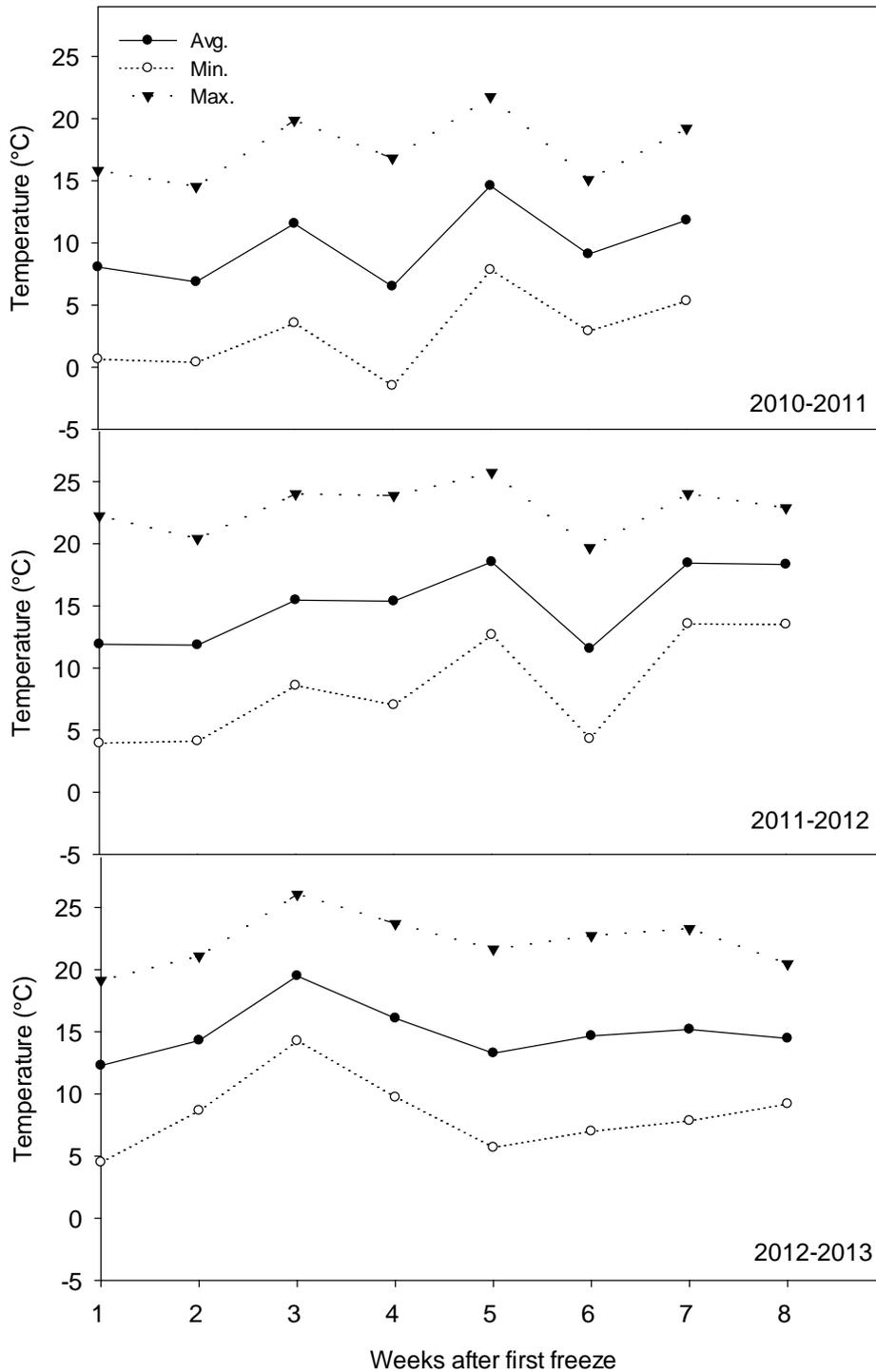


Figure 7-1. Weekly average air temperature (Avg.) and average of weekly maximum (Max.) and minimum (Min.) air temperatures for 2010-2011, 2011-2012, and 2012-2013 at the experimental location (Citra, FL)

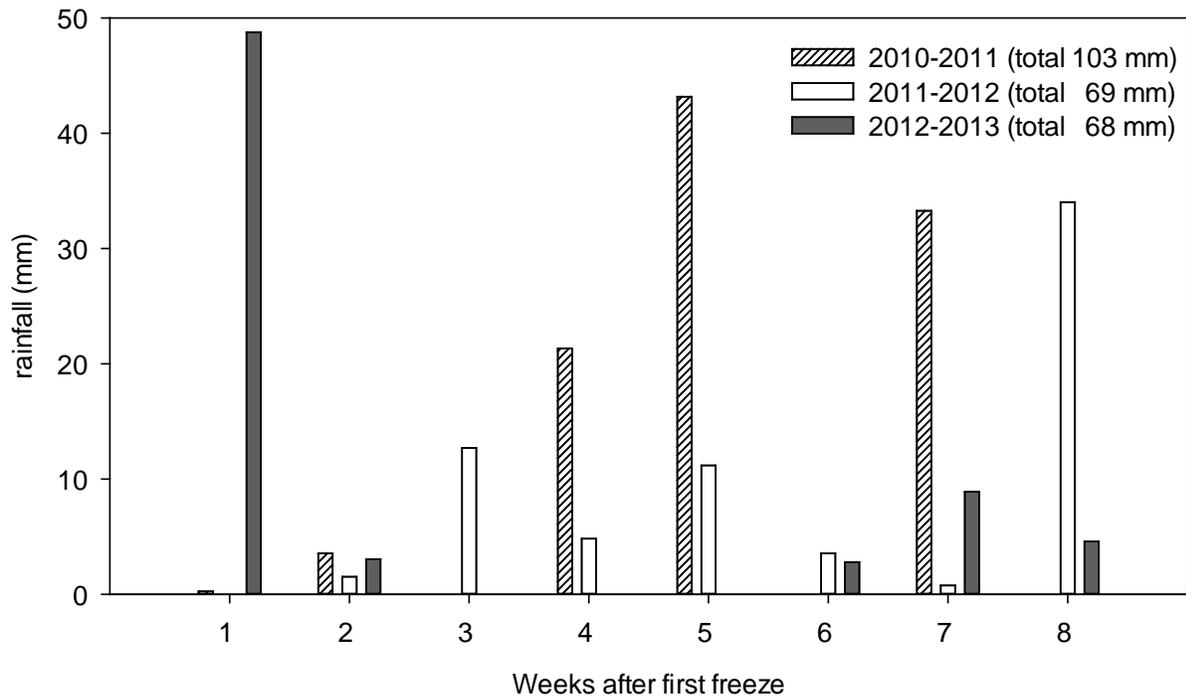


Figure 7-2. Weekly total rainfall for 2010-2011, 2011-2012, and 2012-2013 at the experimental location (Citra, FL)

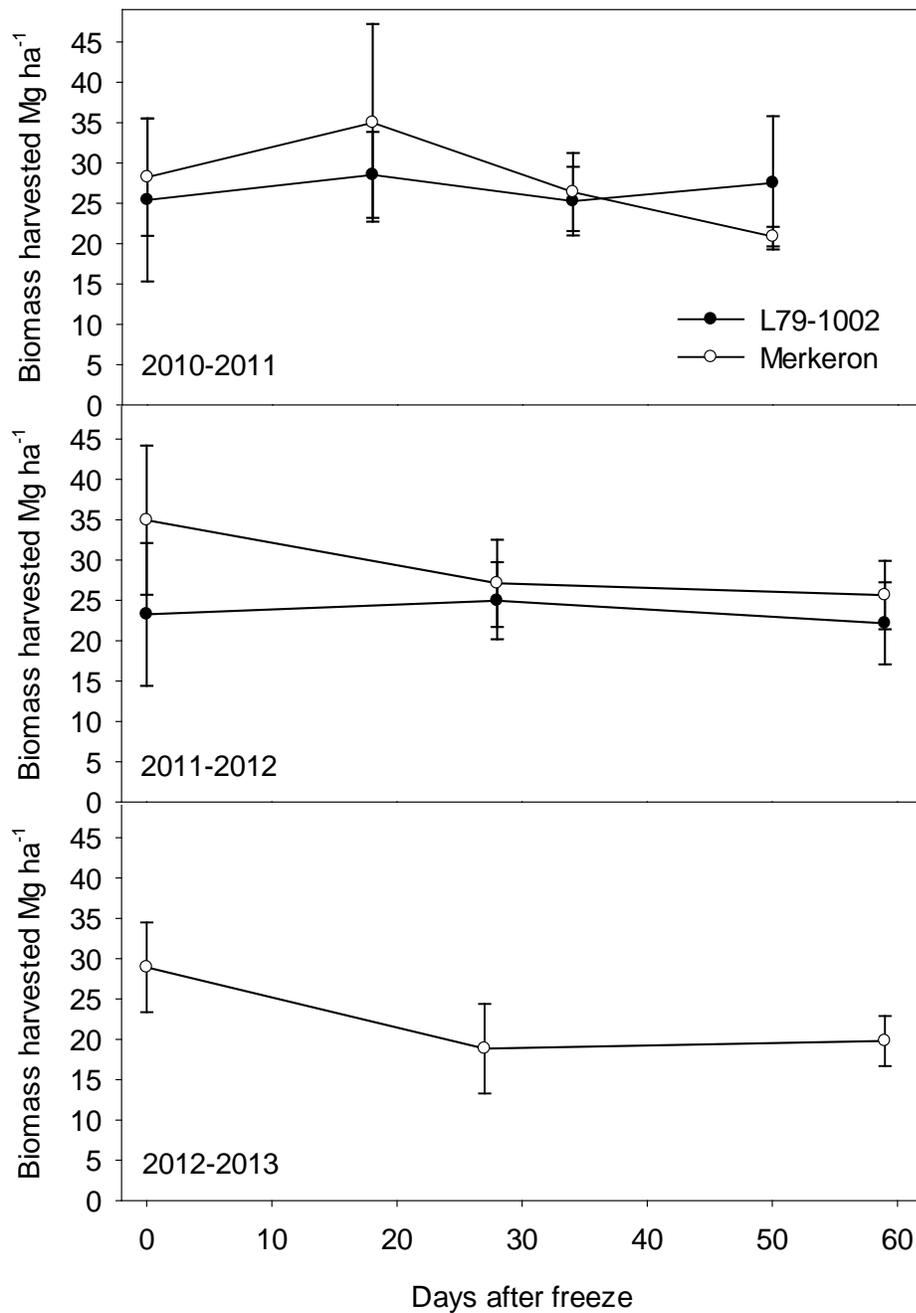


Figure 7-3. Effect of days after a freeze event on biomass harvested of two perennial grass entries in three years. Entry, sampling date, and their interaction  $P$  values were 0.773, 0.198, and 0.320, respectively, in 2010-2011; 0.153, 0.178, and 0.184, respectively, in 2011-2012; and 0.067 (sampling date) in 2012-2013. Bars show mean  $\pm$  one standard error.

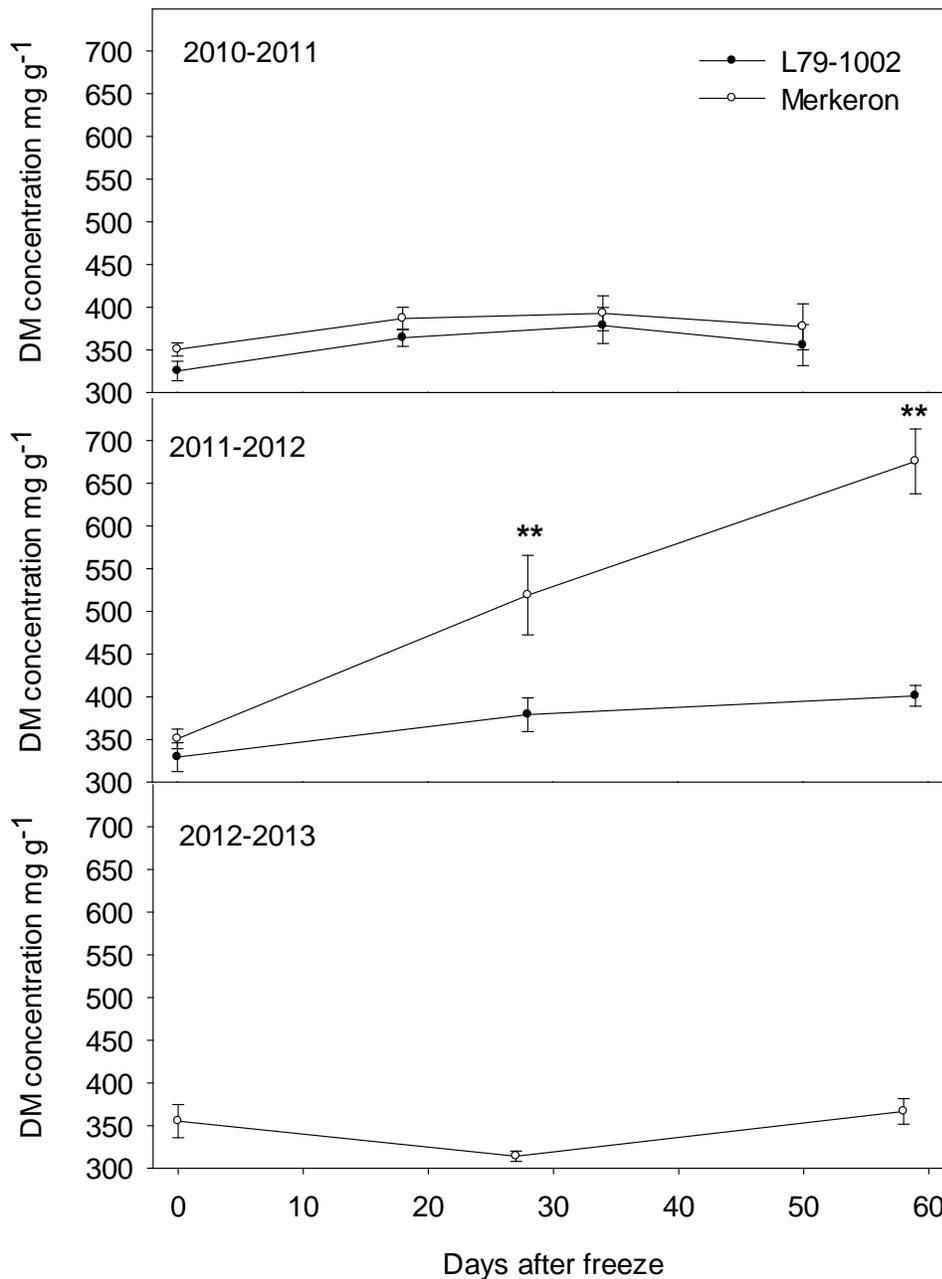


Figure 7-4. Effect of days after a freeze event on dry matter (DM) concentration of two perennial grass entries in three years. Entry, sampling date, and their interaction *P* values were 0.025, <0.001, and 0.928, respectively, in 2010-2011; < 0.001 for all in 2011-2012; and 0.013 (sampling date) in 2012-2013. Bars show mean  $\pm$  one standard error. Entries within a date, \*\* *P*  $\leq$  0.01.

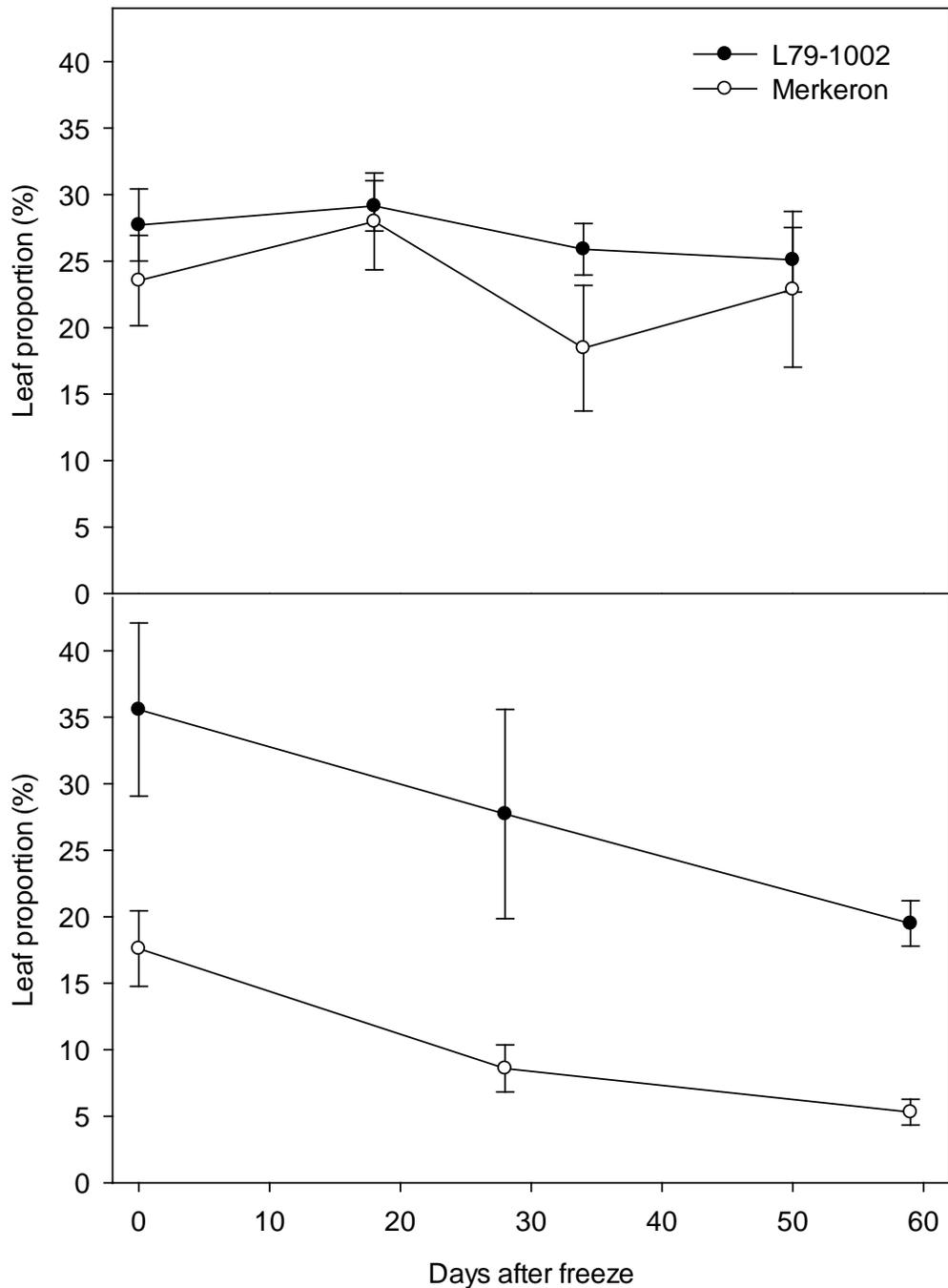


Figure 7-5. Effect of days after a freeze event on leaf proportion of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). Entry, sampling date, and their interaction *P* values were 0.120, < 0.001, and 0.069, respectively, in 2010-2011; and 0.001, < 0.001, and 0.309, respectively, in 2011-2012. Bars show mean  $\pm$  one standard error.

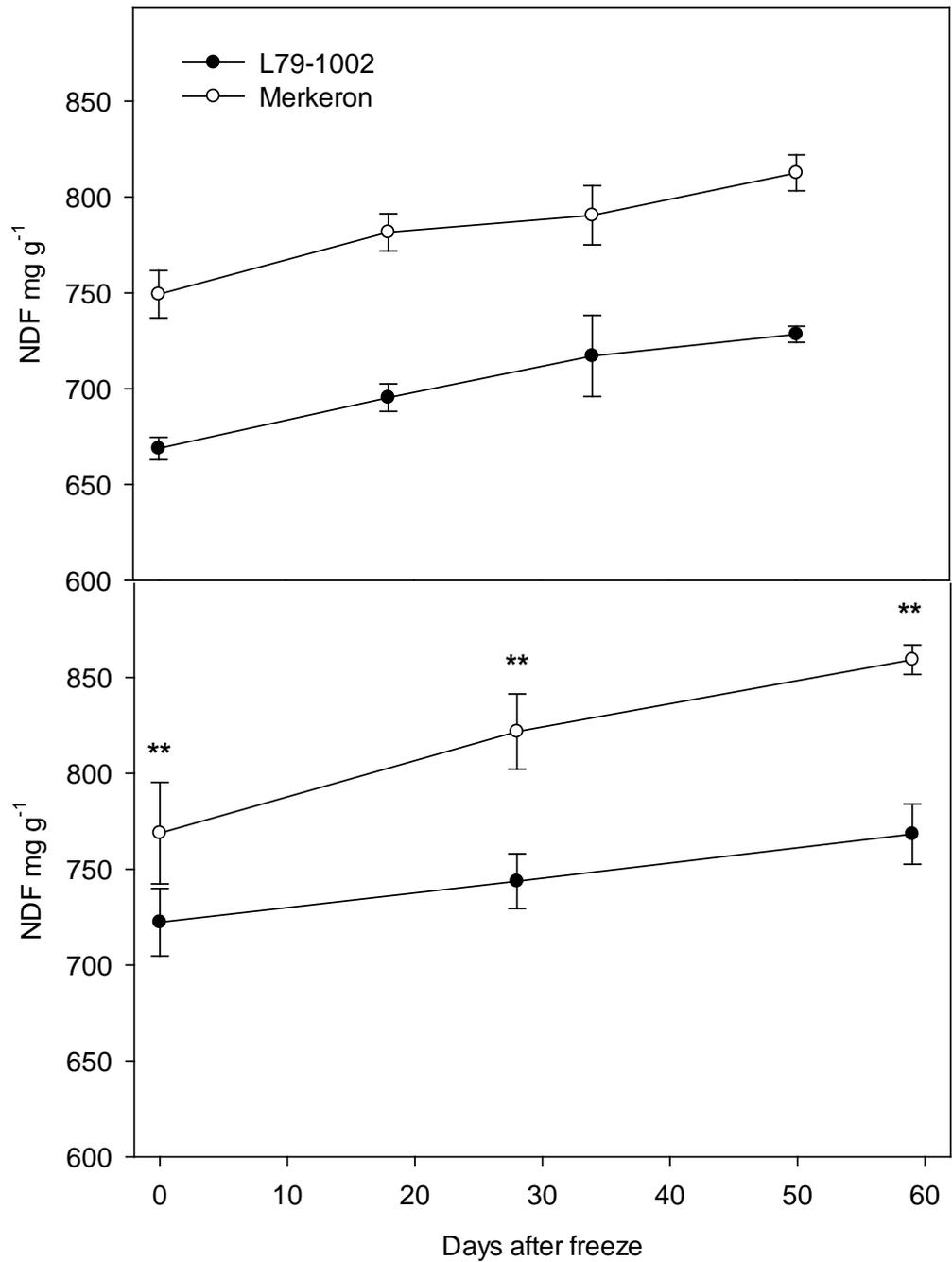


Figure 7-6. Effect of days after a freeze event on neutral detergent fiber (NDF) concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). Entry, sampling date, and their interaction  $P$  values were  $< 0.001$ ,  $< 0.001$ , and  $0.714$ , respectively, in 2010-2011; and  $< 0.001$ ,  $< 0.001$ , and  $0.026$ , respectively, in 2011-2012. Bars show mean  $\pm$  one standard error. Entries within a date, \*\*  $P \leq 0.01$ .

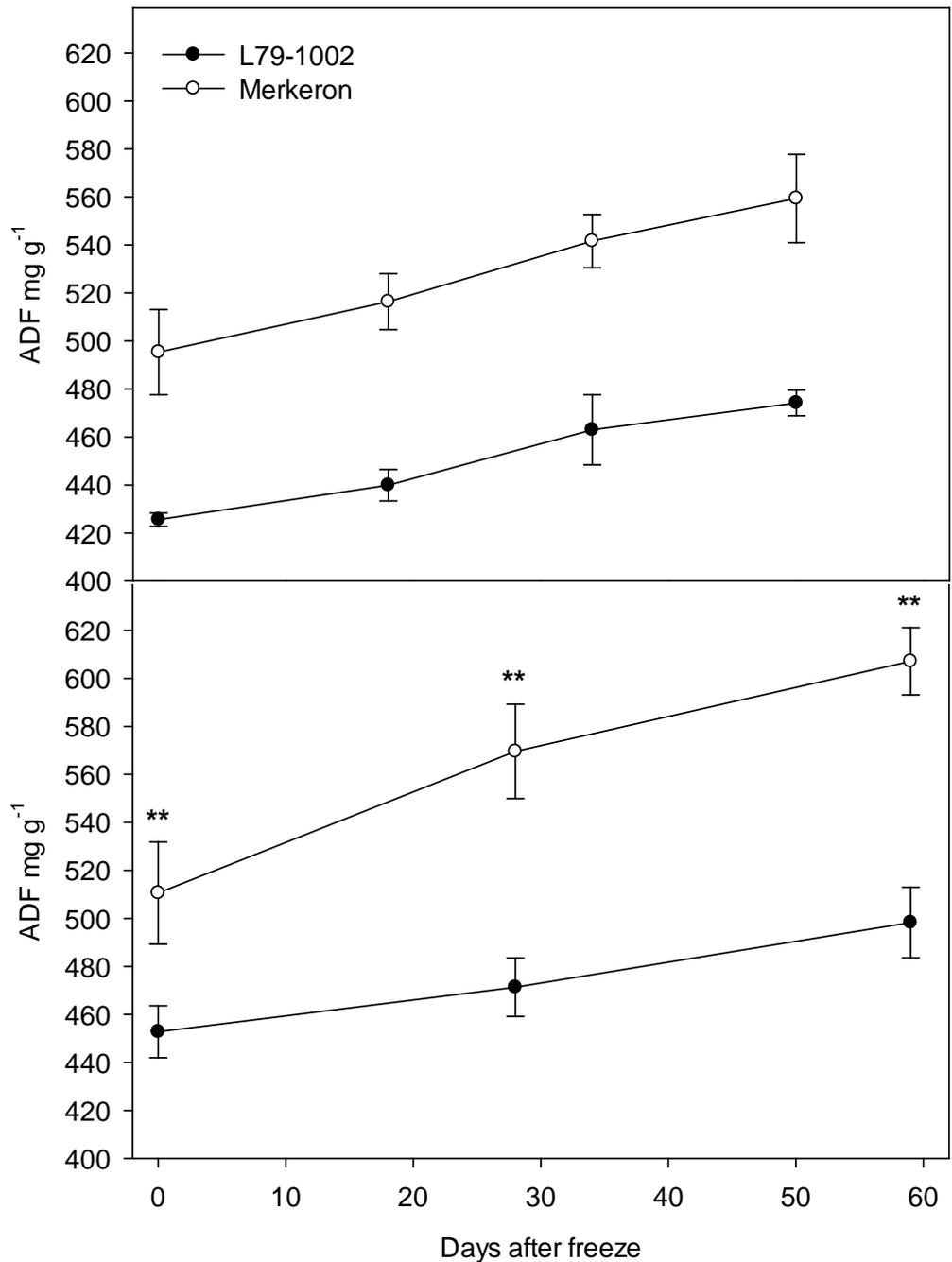


Figure 7-7. Effect of days after a freeze event on acid detergent fiber (ADF) concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). Entry, sampling date, and their interaction  $P$  values were  $< 0.001$ ,  $< 0.001$ , and  $0.627$ , respectively, in 2010-2011; and  $< 0.001$ ,  $< 0.001$ , and  $0.001$ , respectively, in 2011-2012. Bars show mean  $\pm$  one standard error. Entries within a date, \*\*  $P \leq 0.01$ .

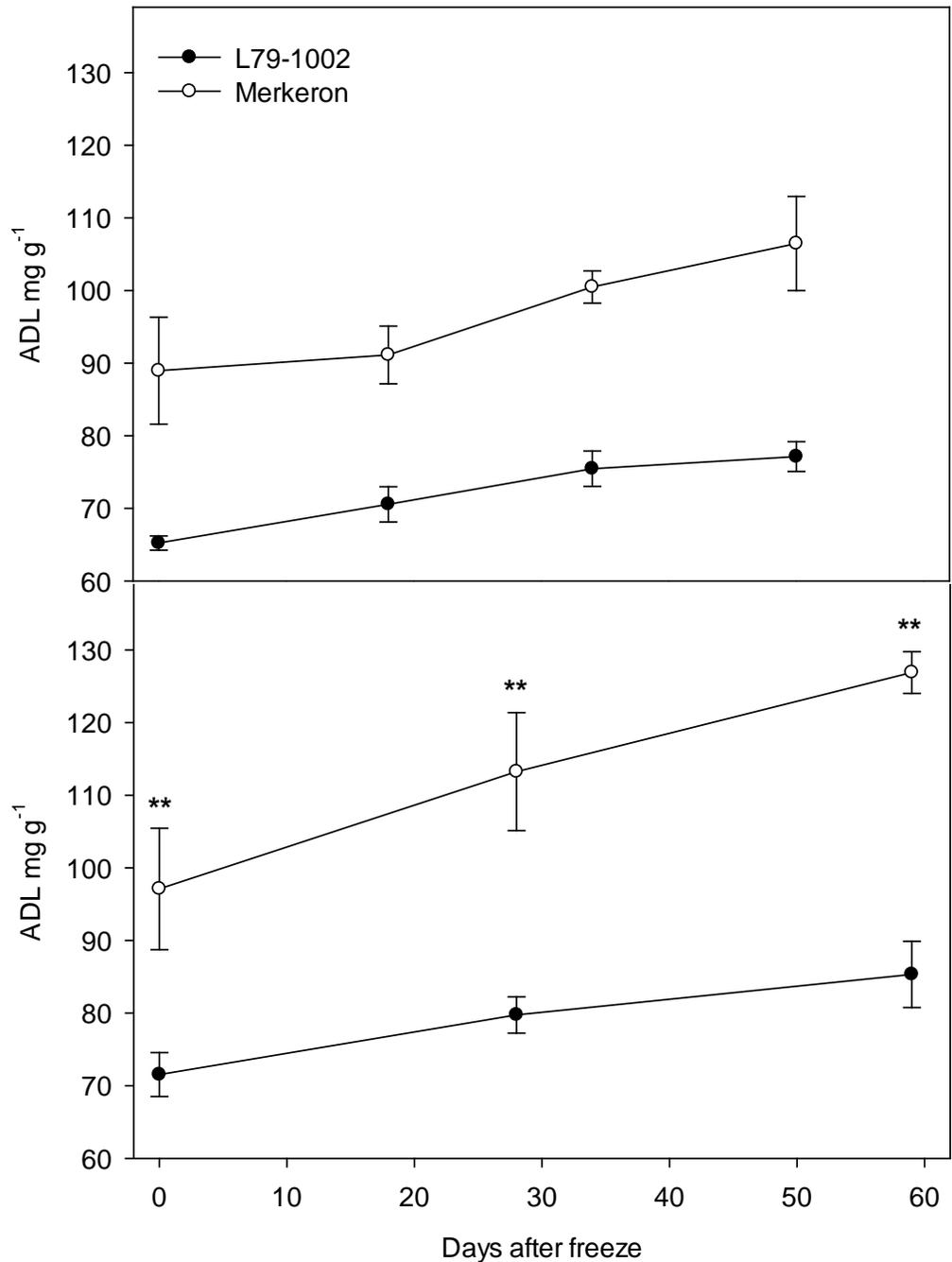


Figure 7-8. Effect of days after a freeze event on acid detergent lignin (ADL) concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). Entry, sampling date, and their interaction *P* values were < 0.001, < 0.001, and 0.174, respectively, in 2010-2011; and < 0.001, < 0.001, and 0.019, respectively, in 2011-2012. Bars show mean  $\pm$  one standard error. Entries within a date, \*\* *P*  $\leq$  0.01.

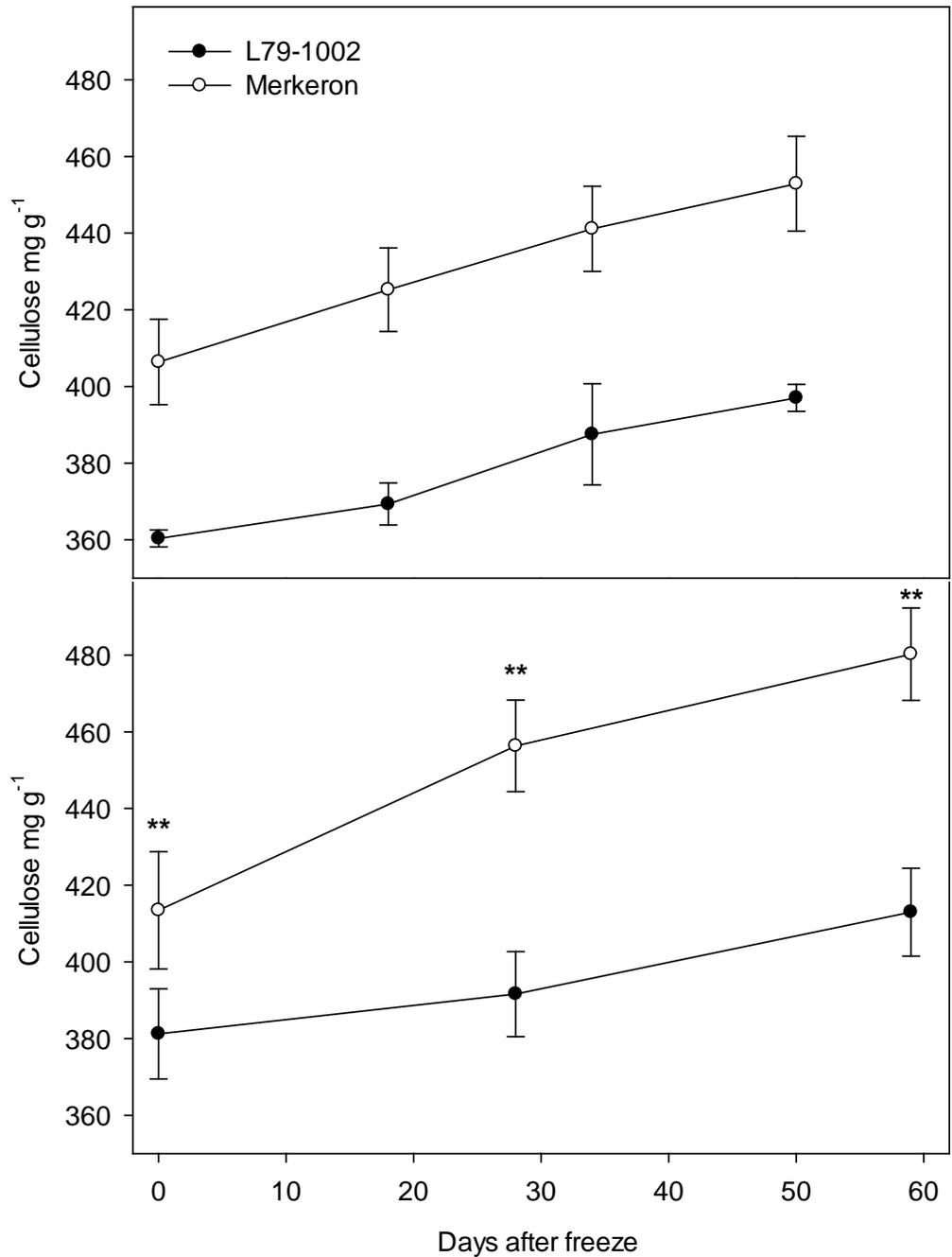


Figure 7-9. Effect of days after a freeze event on cellulose concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). Entry, sampling date, and their interaction P values were < 0.001, < 0.001, and 0.678, respectively, in 2010-2011; and < 0.001, < 0.001, and 0.001, respectively, in 2011-2012. Bars show mean  $\pm$  one standard error. Entries within a date, \*\* P  $\leq$  0.01.

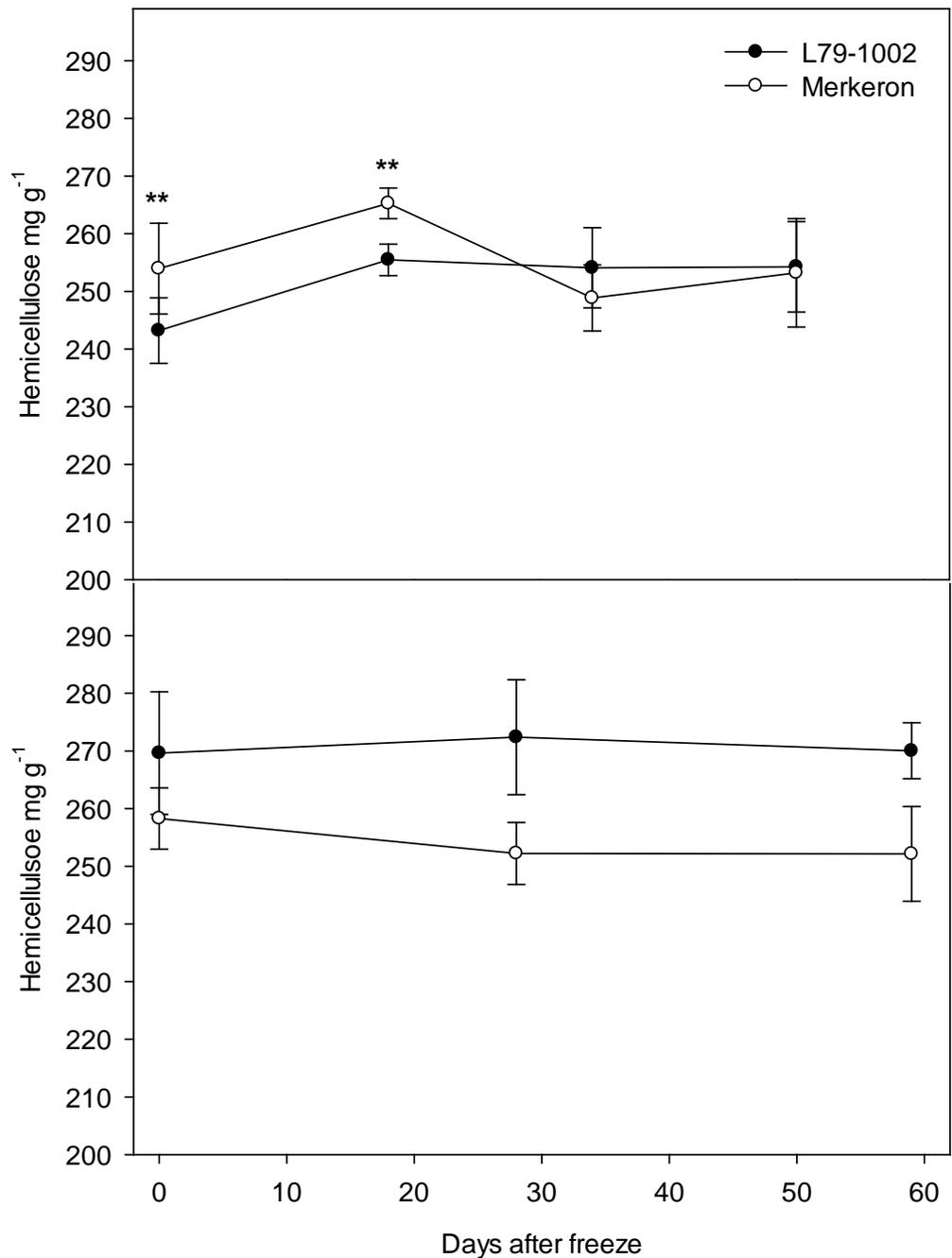


Figure 7-10. Effect of days after a freeze event on hemicellulose concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). Entry, sampling date, and their interaction *P* values were 0.240, 0.006, and 0.040, respectively, in 2010-2011; and 0.009, 0.638, and 0.272, respectively, in 2011-2012. Bars show mean  $\pm$  one standard error. Entries within a date, \*\* *P*  $\leq$  0.01.

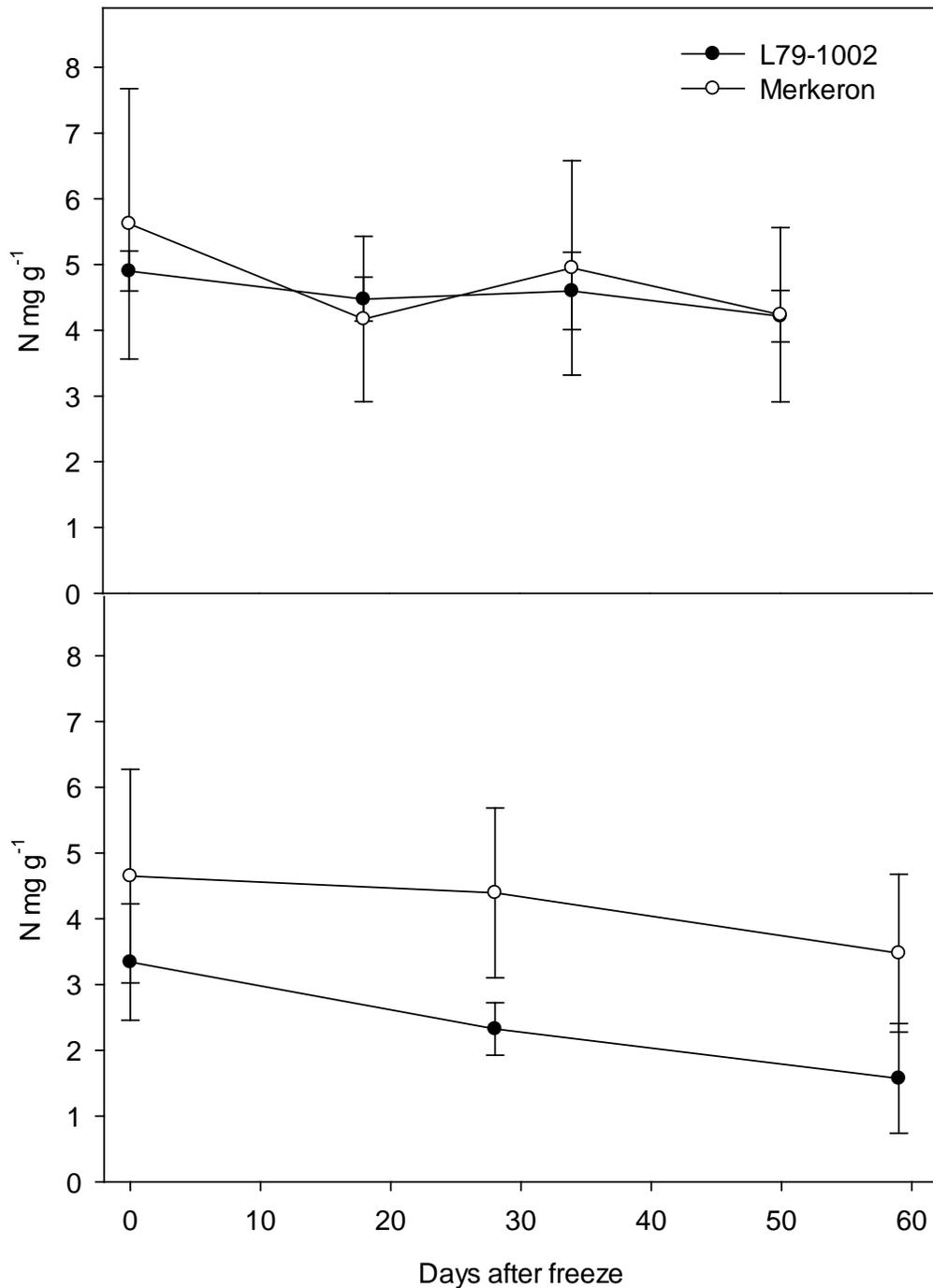


Figure 7-11. Effect of days after a freeze event on N concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). Entry, sampling date, and their interaction *P* values were 0.666, 0.285, and 0.832, respectively, in 2010-2011; and 0.032, 0.011, and 0.622, respectively, in 2011-2012. Bars show mean  $\pm$  one standard error.

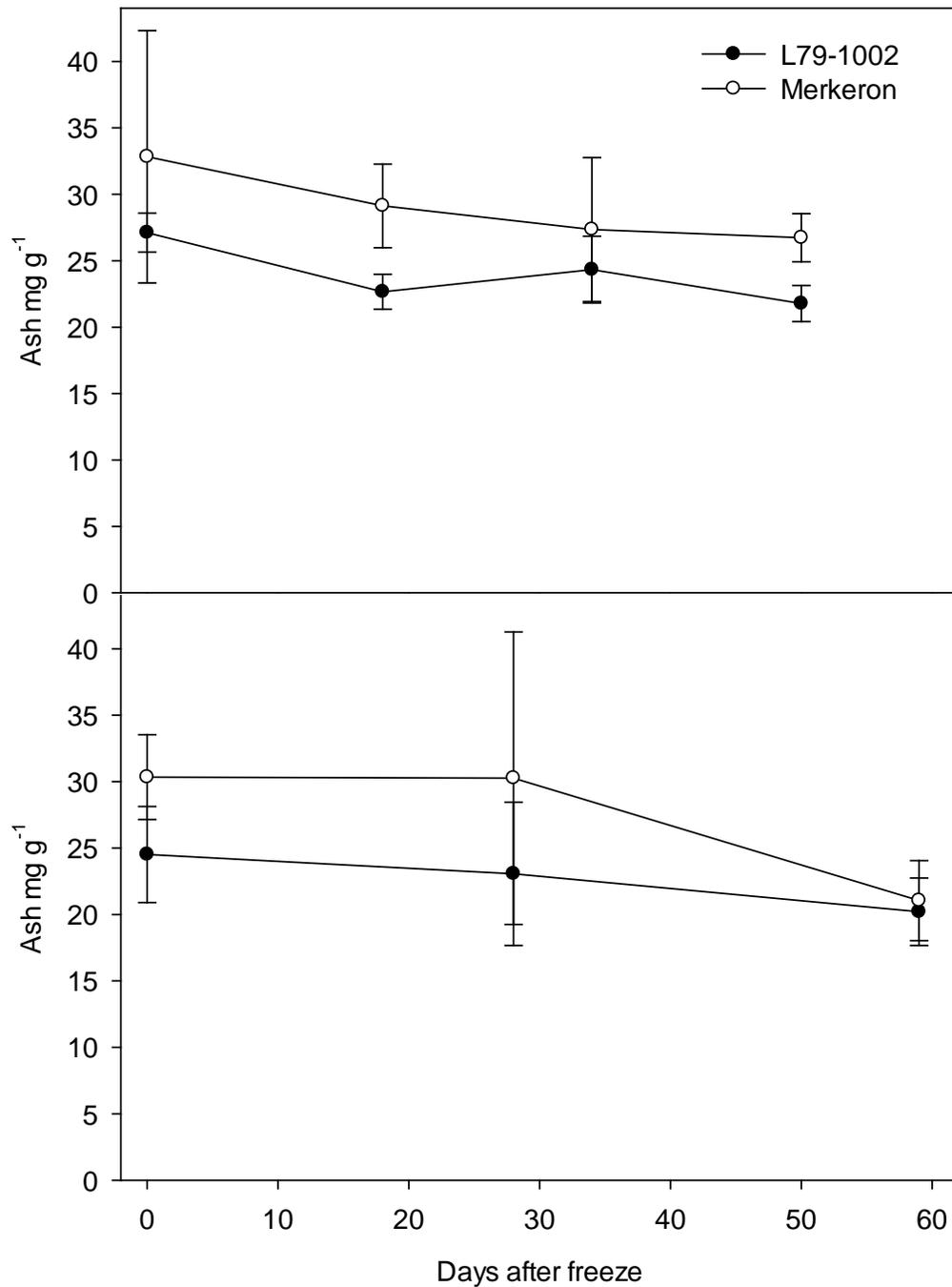


Figure 7-12. Effect of days after a freeze event on ash concentration of two perennial grass entries in 2010-2011 (upper) and 2011-2012 (lower). Entry, sampling date, and their interaction *P* values were 0.042, 0.040, and 0.815, respectively, in 2010-2011; and 0.182, 0.017, and 0.342, respectively, in 2011-2012. Bars show mean  $\pm$  one standard error.

## CHAPTER 8 CONCLUSIONS

Outstanding biomass yield potential of warm-season perennial grasses is attractive for cellulosic biofuel production systems. In the southeastern USA, elephantgrass (*Pennisetum purpureum* Schum.) and energycane (*Saccharum* spp. hybrid) are widely recognized for their biomass production and therefore are candidate species for second generation biofuel production in this region. Although regional biomass production is high, a logistical challenge facing processing plants that convert feedstock to fuel is seasonality of biomass production. This results in uneven supply of feedstock to the conversion facility and limits efficiency of operation. Different grass species may be better adapted to flexible harvest management, but there is relatively little information available describing such differences for elephantgrass and energycane.

The research reported in this dissertation was conducted to address the following objectives: 1) determine the effect of harvest management of elephantgrass and energycane on biomass yield and composition when plants are managed as bioenergy feedstocks (Chapters 3 and 4); 2) assess morphological and chemical changes of elephantgrass and energycane throughout the growing season (Chapters 5 and 6); and 3) investigate effects of delayed harvest after a killing freeze on yield and chemical composition of elephantgrass and energycane (Chapter 7).

### **Effect of Harvest Management on Perennial Grasses – Chapters 3 and 4**

The experiment was conducted during 2010, 2011, and 2012 at the Plant Science Research and Education Unit (PSREU) at Citra, FL (29.41° N, 82.17° W). The three grass entries included two elephantgrasses, 'Merkeron' and a breeding line UF-1,

and 'L79-1002' energycane. Three harvest management treatments were imposed that included different frequencies and timing of harvest. These were i) two harvests per year (2X; summer and fall ratoon), ii) one harvest per year in fall (1X-Nov), and iii) one harvest per year in winter (1X-Dec). Biomass harvested and related responses (Chapter 3) and chemical composition characteristics (Chapter 4) were measured.

Delaying a single harvest until after a freeze event or harvesting biomass twice per year increased the effective harvest period of biomass and would therefore likely improve seasonal distribution of biomass to the biorefinery. However, increasing harvest frequency to twice per year may compromise long-term biomass production (42% biomass reduction for this treatment in the third year). Energycane biomass decreased 41% from Year 2 to Year 3 due to damage from sugarcane smut (*Sporisorium scitamineum*) disease. Delaying harvest until after a freeze event reduced the leaf percentage in harvested biomass and increased the biomass dry matter concentration. Persistence evaluation suggested that UF-1 likely has capability to adapt to loss of tillers in the stand and achieve the same or even greater biomass yield than Merkeron.

Harvest frequency affects compositional quality of perennial grasses. Delaying a single harvest until fall maximized the concentration of cellulose in total biomass, but there was little change thereafter in elephantgrass. In contrast, increasing soluble sugar concentration in energycane caused a decrease in structural carbohydrate concentration in plants not harvested until first freeze. A major factor affecting concentration differences due to harvest management was differences in leaf proportion. Later harvests were associated with lesser leaf percentage in total biomass and this caused N, P, and ash to decrease in 1X-Nov and 1X-Dec relative to 2X.

Decrease in N and ash was associated with amount of leaf abscission. For single harvest treatments, because of decreased leaf proportion, compositional characteristics of total biomass usually were very similar to those of stem. 1X-Nov and 1X-Dec treatments of all entries had lesser concentrations of the components N and ash, but they had greater lignin concentration than for 2X.

### **Morphological and Chemical Changes of Perennial Grasses During the Growing Season – Chapters 5 and 6**

Plots used were a subset of those from the experiment described in Chapter 3. Full-season growth sampling occurred on plots that were harvested once per year and first growth/ratoon growth sampling occurred on the plots that were harvested twice per year with first harvest on 30 July 2010 and 21 July 2011. Morphological characteristics (Chapter 5) and chemical constituents (Chapter 6) were measured throughout the growing season.

Merkeron and UF-1 elephantgrasses generally demonstrated similar seasonal patterns in morphological characteristics. Differences in seasonal morphological characteristics between elephantgrass and energycane were detected. Relative to energycane, the elephantgrasses generally had 1) a more consistent number of tillers throughout the season, 2) greater tiller mass and earlier development of LAI than energycane, and 3) greater stem proportion and biomass dry matter concentration than energycane. Elephantgrass UF-1 showed desirable characteristics for biomass production including increasing tiller mass and canopy height until late in the growing season and a greater proportion of stem. During ratoon growth, grasses rapidly achieved the same LAI as full-season growth which explains why there were no yield differences between one and two harvests per year treatments in the first 2 yr of the

study reported in Chapter 3. These data provide evidence of the value of detailed characterization of morphological responses in understanding plant biomass accumulation and response to defoliation.

Seasonal changes in chemical composition were quantified by assessing cell wall constituents using the Van Soest fiber analyses and through mineral analyses. Different patterns of response were observed between elephantgrass and energycane. Cell wall constituents of the two elephantgrasses responded similarly. Cell wall constituents except hemicellulose increased until late summer and either remained relatively constant (UF-1) or slightly increased (Merkeron) during the remainder of the growing season. In contrast, once energycane cell wall constituents peaked in late summer, they decreased thereafter because of increasing concentrations of non-structural components.

Nitrogen and ash are considered to be anti-quality factors in some biomass conversion processes. Because leaf N and ash concentrations are much greater than those in stem, N and ash can be returned to the field efficiently by leaf abscission as leaves mature. There may be value in returning remaining leaf biomass to the production field at time of harvest. Data for both N and ash indicate that returning leaf to the production field is a more attractive option for elephantgrass than for energycane. In elephantgrass, leaf biomass as a percentage of total biomass is considerably smaller than leaf N and ash content as a percentage of total N and ash. Thus, there is an option to reduce N and ash in harvested biomass to a greater degree than biomass yield if leaf were to be returned at harvest. Currently, it is not certain the degree to which the loss of

biomass is compensated for by greater biomass quality or by return of nutrients for use in subsequent growing seasons.

### **Effects of Delaying Harvest After a Freeze Event on Biomass Harvested and Chemical Composition – Chapter 7**

The experiment was conducted during the winter seasons of 2010-2011, 2011-2012, and 2012-2013 at the Plant Science Research and Education Unit (PSREU) adjacent to the site where the experiments were conducted that are reported in Chapters 3 through 6. Harvest of two grass entries ('Merkeron' elephantgrass and 'L79-1002' energycane) was delayed incrementally for up to 2 mo after the first killing freeze event and change in biomass harvested and biomass composition were quantified.

Delayed harvest after a freezing event can increase the duration of the biomass harvest period. Elephantgrass biomass yield and composition were more sensitive to weather conditions after the freeze event than energycane. Energycane biomass yield was unaffected by delayed harvest, but elephantgrass biomass decreased with increasing number of days in the delayed harvest period. Leaf proportion in biomass decreased with increasing time between freeze and harvest, especially for elephantgrass, and this caused N and ash concentration to decrease during the delayed harvest period. Fiber components of elephantgrass generally increased after the freeze because stem of this species appeared to lose a greater amount of non-structural constituents. These data suggest that energycane may be better suited for delayed harvest following a freeze because its biomass loss is minimal. Elephantgrass appears to be more susceptible to loss of biomass and non-structural constituents following a freeze event, particularly when warm weather follows the occurrence of freezing temperatures. Thus, post-freeze weather conditions will likely determine the viability of

delaying harvest, especially with elephantgrass, with colder temperatures following a freeze event likely having less negative impact on biomass harvested and composition.

### **Implications of the Research**

It is possible to increase the period when biomass can be provided to the biorefinery by delaying harvest until first freeze without compromising quality and biomass yield of elephantgrass and energycane. In contrast, multiple harvests during the growing season, at least in the colder subtropics like northern Florida, may sacrifice long-term biomass yield and persistence as well as increase concentrations of minerals and ash in the biomass that can interfere with some conversion processes. Leaf fall from full-season growth, especially when harvest is delayed until after a freeze event will likely increase nutrient recycling for the next growing season, transportation efficiency, and feedstock quality for the biorefinery. The final decision regarding harvest frequency and timing must take into account the species being used and the regional circumstances related to availability of feedstock to the conversion plant. It may well be most efficient if large production areas are divided into management units such that a given unit is harvested twice annually only one year out of three or four. In other years it can be harvested once per year some time during the fall through winter period. Because energycane loses less biomass than elephantgrass during the winter, it may be an excellent candidate to supply biomass later in the winter season.

Season-long evaluation of morphological characteristics provides information for determining optimal harvest dates. A large decrease in mineral nutrient and ash concentrations over the season, associated with leaf abscission, indicated that nutrient recycling and feedstock quality may be improved if harvest occurs when grasses are mature. Elephantgrass UF-1 showed excellent feedstock characteristics including

extended growth, high biomass yield, and late flowering, and it likely merits release as a biomass biofuel crop. If energycane cultivars are to have a future for use as sources of feedstock for bioenergy in this region, smut resistance will be a necessary plant characteristic. In the absence of such resistance, long-term yield is compromised and persistence for more than three growing seasons appears unlikely. Changes in biomass yield and composition of perennial grasses are dependent on species and weather following the initial freeze. Elephantgrass is more sensitive to weather conditions than energycane, and if warm weather follows the first freeze elephantgrass should be harvested in a relatively narrow time window to avoid loss of biomass.

### **Future Research Needs**

Current experiments have answered important questions, but a number of newly emerging challenges and questions will need to be addressed by future research. Although efficiency of nutrient recycling is an important consideration for biomass production, the amount of nutrient that remains on the field and the degree to which it contributes to production the following year have not been established. Measurement of N allocation and translocation for the next season will provide further information regarding nutrient use efficiency of perennial grasses. It was not possible to fully assess the performance of energycane because the impact of sugarcane smut was so devastating. Energycane cultivars selected for smut resistance should be evaluated and compared with smut-susceptible types and elephantgrass. To more fully assess the benefits of cultivation of perennial grasses, it is important to evaluate impacts on ecosystem services. For instance, the degree of C sequestration associated with long-term imposition of various harvest management practices has not yet been investigated. Field observations suggest that bioenergy grass plots provide habitat for many wildlife

species. Assessment of species richness and population sizes of various wildlife species are needed to more clearly establish the value of production fields as wildlife habitat. Especially for elephantgrass, there were large border effects such that over time plants in border rows became more robust than in inner rows and likely competed aggressively with plants in inner rows for light and nutrients. Greater row spacing may be needed to address this constraint in future research.

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## BIOGRAPHICAL SKETCH

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