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Performance evaluation of nine varieties of Miscanthus in Iowa

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Performance evaluation of nine varieties of *Miscanthus* in Iowa

by

Muhammad Aurangzaib

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Crop Production and Physiology

Program of Study Committee:

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Ames, Iowa

2012

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CHAPTER 1

GENERAL INTRODUCTION

Global Concern

The world's population is increasing rapidly; by 2050 it is expected to be 9.2 billion, 34 % higher than today (United Nations 2008). Along with this drastic increase in population, the demand for food and fiber is expected to increase 70 % by the first half of this century (FAO 2009). Human survival mainly depends on the resources present in the environment. Our natural resources (soil, water, gas and oil) are being deteriorated and depleted. It has been reported that from 2007 world's natural gas and oil reserves have started declining (Youngquist 2003; Campbell 2006; IEA 2007). Additionally, global climate change is stressing natural and managed ecosystems (Ansuategi and Escapa 2002; Smith, Fang et al. 2008; Gaffney and Marley 2009). Significant variations in world crude oil price with generally an increasing trend is also a major driving force to find alternate sources of energy such as biofuel. Also, due to rapid environmental changes we should also limit the use of conventional energy sources like oil, coal and gas and should focus towards biorenewable sources. Crops grown for biofuels could not only help us in this context, it will also be a step towards sustainability, biodiversity, environmental and energy security. In this regard in 2007 the Energy Independence Security Act (EISA) was passed to ensure a continued supply of clean and safe biofuels.

According EISA, it is expected that by 2022, 36 billion gallons of ethanol will be produced in the USA to meet energy demands and to reduce dependence on foreign oil. Out

of this 36 billion, 21 billion gallons should be produced by non-food sources like cellulosic and hemi-cellulosic material, and the rest will come from grain producing crops like maize, soybean etc (EISA 2007; Perlack, Wright et al. 2011). In the United States, starch based biofuel produced provides ~ 2 percent share of total energy needs (Ragauskas, Williams et al. 2006). Using food crops to produce fuel leads to a direct, and highly contentious, competition between food and fuel. Crops grown for energy purposes are also competing with food production due to limited fertile land resources (Tilman, Hill et al. 2006). Biofuels produced from lignocellulosic crops that can grow on land marginal to food crop production offer a solution to this problem (Cai X et al. 2011). Specifically, perennial crop varieties that can produce large amounts of lignocellulosic feedstock per unit land, nutrient and energy can not only meet fuel demands while sparing land for food production, but can also provide ecosystem services, such as soil and water conservation, wildlife habitat and greenhouse gas mitigation. Currently *Miscanthus × giganteus* (Greef et Deu ex. Hodkinson et Revoize) is one of the most promising such crops for the Midwest US (Heaton, Voigt et al. 2004; Heaton, Dohleman et al. 2008; Dohleman 2009; Heaton, Boersma et al. 2010).

***Miscanthus* Introduction**

Miscanthus × giganteus is a bioenergy crop grown primarily for heat and electricity production and can also be used to produce biofuel (Christian, Yates et al. 2009). It is already being grown commercially in the USA and several other countries, especially in Europe for biofuel production. *Miscanthus × giganteus* is warm season perennial grass originating from Asia, and was introduced to Europe in 1935 as an ornamental plant (Greef and Deuter 1993; Hodkinson, Chase et al. 2002). *Miscanthus × giganteus* is an allotriploid ($2n = 3x = 57$)

(Linde-Laursen 1993), and is a cross between *Miscanthus sinensis* ($2n = 2x = 38$) and *Miscanthus sacchariflorus* ($2n = 2x = 38$) (Hodkinson, Chase et al. 2002). *Miscanthus* × *giganteus* is also known as *M. giganteus*, *M. sinensis* Anderss, and *M. ogiformis* Honda (Hodkinson, Chase et al. 2002). The hybrid is sterile, minimizing invasive potential from seed spread, and thus the main method of propagation is rhizome or from micropropagules (Lewandowski, Clifton-Brown et al. 2000; Heaton, Boersma et al. 2010; Boersma and Heaton 2011). It is of agronomic interest due to its high yield potential and the diverse uses of its biomass. In productivity trials in Germany and USA, *Miscanthus* has proved itself a high yielding biomass producing crop with yields of up to 30-43 tons of dry matter (DM) ha⁻¹ (Lewandowski and Kicherer 1997; Heaton, Clifton-Brown et al. 2004).

Suitability of biomass used for the production of energy is affected by the concentration of minerals present in the feedstock at the time of harvest (Jenkins, Baxter et al. 1998; Boateng, Hicks et al. 2006). Presence of less mineral contents ensures efficient combustion and thermochemical conversion of harvested biomass (Nordin 1994; Jenkins, Baxter et al. 1998). *Miscanthus* is a good solid biofuel when harvested in spring, with its low contents of water, ash and minerals such as N, P, K, Cl, Ca, S, Mg and Si (Clifton-Brown 2000; Woli, David et al. 2011).

***Miscanthus* Winter Hardiness**

Generally C4 plant species are not found in cold environments, but *M. × giganteus* is an exception, and has been found to produce new leaves and shoots even at < 10 °C (Beale, Bint et al. 1996), and is considered to be one of the most productive crops grown at the cool temperatures and high latitudes of northern Europe (Long 1999). Despite this tolerance, cold

can still adversely affect *M. × giganteus* productivity. One of the important problems is the occurrence of chilling damage, mostly during the first winter after planting (Bullard 1994; Greef 1994; Clifton-Brown and Lewandowski 2000). Almost 100 % winter losses were observed in some trials of *Miscanthus* establishment in Europe (Clifton-Brown 2001).

Though *M. × giganteus* has shown promising yields throughout Europe, field trials in northern Europe particularly had poor overwinter survival in the first winter after planting, perhaps indicating less suitability of this genotype for northern climatic zones (Schwarz, Greef et al. 1995; Pude 1998). Christian and Haase (2001) also reported that winter survival is an obstacle for the establishment of the high yielding *M. × giganteus* genotype. In one of the few examinations of *Miscanthus* genetics in different environments, J.C. Clifton-Brown and Lewandowski (2000) established a field trial of 5 different genotypes of *Miscanthus* and suggested that *M. sinensis* genotypes have better winter survival than *M. × giganteus* and *M. sacchariflorus* genotypes in areas where there is a risk of soil temperatures falling below -3 °C. They expanded their scope to test 15 *Miscanthus* genotypes for three years at five different locations, measuring growth characteristics like plant height, tiller number, stem diameter, flowering and senescence rate in fall. In areas where soil temperatures in the first winter following planting fell below -4.5 °C, *M. × giganteus* and *M. sacchariflorus* had the highest mortality rates, and *M. sinensis* genotypes the least. In locations where winter soil temperature was > -2.8 °C, all genotypes were able to survive, suggesting this is a threshold for survival. Further, when there was no severe winter, *M. × giganteus* genotypes produced higher biomass yields than did the *M. sinensis* and *M. sacchariflorus* genotypes, but where winter soil temperatures were below - 4.5 °C, *M. sinensis* genotypes were the highest

yielding, indicating this species has the most potential for producing cold-tolerant crop varieties.

Many factors could be influencing the winter survival of newly planted *Miscanthus* varieties, including inherent genotypic cold tolerance and environmental conditions. Many cold tolerance studies have identified changes in chemical composition, e.g., carbohydrates, lipids and proteins, in plant cells during cold temperatures (Griffith, Marentes et al. 1993; Nishida and Murata 1996; Jones, Paroschy et al. 1999). Christian and Haase (2001) reported that winter survival of *Miscanthus* can also be related to dormancy during chilling temperatures; the only organs that were frost tolerant were those that developed dormancy during autumn and early winter. Pude (1997) and J. C. Clifton-Brown (2000) linked overwintering survival in *Miscanthus* × *giganteus* with early senescence. Understanding the processes of dormancy induction and senescence are difficult and poorly studied in perennial, warm-season grasses like *Miscanthus*, where more work has focused on drought tolerance and fire-avoidance, e.g., (Heckathorn and Delucia 1994), and new research is needed to understand how overwinter mortality can be mitigated in this promising crop for northern climates.

Given the wide genetic variability within the *Miscanthus* genus, and variability in morphology associated with senescence and winter survival as well as biomass yield, this project used a range of cold-tolerant *Miscanthus* varieties to address two general questions:

- 1) What metrics can be used to assess senescence in field-grown *Miscanthus* varieties?
- 2) How do morphologic metrics and biomass yield relate to winter survival?

Dissertation Organization

This dissertation is organized into four chapters, and focuses on two research papers that are intended for submission to *Agronomy Journal*. Chapter 1 is a general introduction. Chapter 2 is the first research paper, examining indicators of senescence in cold-tolerant experimental varieties of *Miscanthus*. Chapter 3 is the second research paper that evaluates the relationship between morphological characters, winter survival and biomass yield of those same *Miscanthus* varieties, followed by chapter 4, a general conclusion.

CHAPTER 2

GENOTYPIC VARIATION IN *MISCANTHUS* SENESCENCE

A paper to be submitted to Agronomy Journal

Muhammad Aurangzaib¹², Emily A. Heaton¹³⁴

Abstract

Miscanthus is a biomass crop that has the potential to produce high dry matter yields across a wide range of environmental conditions, but may be limited by cold damage in the winter following planting. Winter losses could be exacerbated by incomplete or late senescence. A field trial was established in spring 2010 as a split-plot randomized complete block design with four replicate blocks to evaluate plant senescence timing and rates in nine different varieties of *Miscanthus* selected in part for cold tolerance. Photosynthetic assimilation, stomatal conductance and total leaf nitrogen (N) concentration ([N]) were measured beginning in late summer and ending at the first hard freeze as indicators of senescence timing and rates. All nine varieties of *Miscanthus* significantly differed in photosynthetic assimilation, stomatal conductance and leaf [N] ($P = 0.0001$, $P = 0.0006$, $P = 0.0001$ respectively). Four out of five Amuri-type varieties responded earlier to environmental signals for autumn and started senescence sooner and at a more rapid pace than the other six varieties of *Miscanthus*. Earlier senescing varieties tended to have lower

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final leaf [N] than varieties with late senescence, suggesting selecting for senescence traits could also influence nutrient cycling and biofuel quality characteristics.

Introduction

Interest in the cultivation of biomass crops, including *Miscanthus* species, is increasing as global demand for biofuel grows. *Miscanthus × giganteus* is now widely promoted as a biofuel crop because of its relatively high dry matter yields, and its potential to perform well over a wide range of climatic and soil conditions. *Miscanthus* species are C₄, perennial grasses native to parts of Asia, and found on a wide range of environmental and soil conditions (Stewart, Toma et al. 2009), *Miscanthus × giganteus*, the one most commonly used for biomass production, is a naturally occurring sterile hybrid of *M. sacchariflorus × M. sinensis* (Greef and Deuter 1993; Hodkinson, Chase et al. 2002; Ma, Jensen et al. 2012).

Generally, C₄ species are not found in cold environments, but a few species occur in cool, temperate climate zones (Naidu and Long 2004). Found in highland areas of Asia (Stewart, Toma et al. 2009), *M. × giganteus* is exceptional among C₄ species for its high productivity in cold climates (Beale and Long 1995; Bullard, Heath et al. 1995; Beale, Bint et al. 1996; Heaton, Clifton-Brown et al. 2004). *Miscanthus × giganteus* has been found to produce photosynthetically active leaves in chilling temperatures (e.g., <10 °C) (Beale, Bint et al. 1996). It's also reported that *M. × giganteus* maintains relatively high photosynthetic rates when grown at either 14 °C or 25 °C (Naidu, Moose et al. 2003). Wang and Portis et al (2008) found that maintenance of photosynthetic capacity could be explained by the amount and activity of the C₄ enzyme PPDK; the amount of PPDK was reported to increase

significantly in *M. × giganteus* when grown at low temperatures and found to decrease in maize grown at the same temperatures (Naidu, Moose et al. 2003). *Miscanthus × giganteus* is closely related to sorghum (*Sorghum bicolor*), sugarcane (*Saccharum officinarum*), and maize (*Zea mays*) and uses the same NADP-malic enzyme C4 pathway (Naidu, Moose et al. 2003; Wang, Portis et al. 2008), thus there is hope that elucidation of the mechanism that allows *M. × giganteus* to maintain productivity in cool temperatures will enable adaptation of these crops to cold weather and climates.

So far *M. × giganteus* has shown promising yields throughout Europe, but some field trials of *M. × giganteus* in northern Europe have shown high mortality in their first winter after planting, indicating poor adaptation of this *Miscanthus* species to all climatic zones (Schwarz, Greef et al. 1995; Pude 1998; Christian and Haase 2001). Schwarz and Greef et al. (1995) reported overwintering mortality in *M. sacchariflorus* due to incomplete dormancy. It has been observed that *Miscanthus* in its first year does not senesce properly, thus translocation of nutrients to underground storage organs (i.e. rhizomes) is likely not optimum (Clifton-Brown and Lewandowski 2000). These underground storage organs are responsible for regrowth in spring. Rhizome development occurs during the active growing season and in autumn, and size might depend upon the quantity of metabolites translocated from above ground plant parts (Koike, Shoji et al. 1975; Jodl and Hotz et al. 1996). Incompletely developed rhizomes may not contain enough reserves to support spring regrowth.

It has been reported that *Miscanthus* in its first year does not follow a normal senescence process and the leaves just turned brown after first killing frost and high mortality losses were observed; however, older plants did follow a normal senescence process and

plant leaves started changing their color in late summer and early autumn and plant losses were least (Clifton Brown and Lewandowski, 2000). Clifton Brown et al (2001) reported almost 100 % mortality rates in overwintering *M. × giganteus* genotypes established in a trial in Europe. To understand the suitability of different *Miscanthus* genetics in different locations, Clifton Brown et al (2001) tested 15 *Miscanthus* genotypes, including *M. × giganteus* and its parent species, for three years across five locations in Europe. They measured growth characteristics like plant height, tiller number, stem diameter, flowering and senescence rate in autumn and reported that *M. × giganteus* was the highest biomass producing genotype among all the genotypes used, but only when there was no severe winter.

Literature findings indicate a strong relationship between plant senescence time and winter mortality in *Miscanthus* but in their pioneering study of the interaction between *Miscanthus* genetics and growth environment, Clifton Brown et al. (2001) used subjective metrics to assess “senescence”, making visual observations of greenness of the aerial parts (i.e shoots) of the plant in each autumn. If the length of the green part of a fully expanded leaf was > 60 %, it was counted as green, and if the green part was < 60 % it was counted as dead. Use of this protocol could be a quick and easy method, but in fact does not really illuminate senescence timing and rates, as whole-plant senescence is a complex syndrome comprised of many processes, including dismantling of the photosynthetic apparatus and translocation of plant metabolites to the storage organs before death of aerial tissues.

The present study was conducted to better quantify senescence in cold-tolerant genotypes of *Miscanthus*. Our approach was to assess the ecophysiological response of key indicators, i.e., photosynthetic gas exchange and leaf N concentration ([N]) to changing

environmental cues during the period from active growth in the late summer to the freezing of aerial tissues in the autumn. In addition to assessment in the critical establishment year, we followed plants in their second year after growth, to learn if genotypes differentially respond to environmental cues in the planting vs subsequent growing season, as has been suggested by the literature.

Materials and Methods

Experimental design and establishment

A two year field trial was established in June-01-2010 at the Iowa State University, Hinds Research Farm in Ames, Iowa. The experiment was arranged as a split-plot randomized complete block design with four replications. The split in this design was dedicated for destructive plant sampling to determine biomass yield for both years. Each plot was 4.57 m × 11.43 m. Nine different cultivars of putatively cold tolerant *Miscanthus* (Table 1) were planted using plugs received from Mendel Biotechnology, Inc. (Hayward, CA). Plugs were stored at 4 °C until the transplanting was complete. Plants were planted by hand at an inter- and intra-row distance of 0.76 m. In the first year, weeds were controlled manually as needed in addition to a June application of 2,4-D (2,4-dichlorophenoxyacetic acid) with pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine). In the second year, weed control was done once manually early in the growing season coupled with a single June application of 2,4-D (2,4-dichlorophenoxyacetic acid) with acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)).

Measurements

Senescence time and rate

The senescence time and rate was estimated by measuring photosynthetic gas exchange using LI-6400-XT portable photosynthesis system (LI-COR Biosciences, Lincoln, Nebraska). Measurements were taken once a week on clear sunny days within two hours of solar noon from early September to the first killing frost. The photosynthetically active radiation (PAR) in the chamber and block temperature were adjusted close to the ambient light and temperature while providing a constant supply of CO₂ (400 μmol CO₂ mol⁻¹air). To take the measurement, a recently fully expanded leaf was enclosed in the chamber. Once the system was stabilized, the leaf CO₂ assimilation and stomatal conductance rates were recorded. Two randomly selected plant leaves per plot were used to record gas exchange and then removed from the plant to be used for elemental analysis of C and N.

Leaf nitrogen and carbon

The sampled leaves were dried and ground to 1 mm using a Thomas Wiley mill (Thomas Scientific, Philadelphia, PA). Approximately 0.125 g of ground leaf sample was weighed and used to determine total percentage of leaf N and C using a LECO True SpecTM CN analyzer (LECO Corp. St. Joseph, MI).

Statistical analysis

A mixed model analysis of variance was done with SAS 9.2 (SAS Institute Cary, NC, USA), using PROC GLIMMIX. *Miscanthus* varieties, block, year, variety × year were considered as fixed effects and variety × block was considered as random effect. Significant

effects were estimated by F-Statistics at $\alpha = 0.05$. Differences between least-squared means were determined with Tukey's adjustment.

Results

In both years all varieties of *Miscanthus* used in this experiment performed well. No plant mortality was observed in either year of the experiment. Temperatures were fairly typical over the course of the project: the lowest mean monthly air temperature was $-10\text{ }^{\circ}\text{C}$ in January, 2010, and the highest mean monthly temperature was $24\text{ }^{\circ}\text{C}$ in July, 2011 (Figure 5). 2010 was wet compared to the 20 year average monthly precipitation; in June and August 2010 heavy rainfall caused widespread flooding in the field but all plants survived (Figure 6).

Photosynthetic C assimilation rates

Variety, date, year and their respective two-way interactions all significantly affected photosynthetic C assimilation rates (A) (Table 2, Fig. 1). Averaged over both years, the fertile Amuri clone AM-905 had the lowest A at $12.49 (\pm 0.87)\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ and $M\times g$ -IL had the highest at $17.46 (\pm 0.87)\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$.

Because there was a significant variety \times year interaction, each year was also analyzed separately. Overall, average A rates in 2010 ranged from $19.89 (\pm 0.57)\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ for AM-900 to $14.65 (\pm 0.57)\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ for AM-905. In 2011, average rates were lower, ranging from $17.31 (\pm 1.13)\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ for $M\times g$ -IL to $10.35 (\pm 1.13)\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$ for AM-905. Within each year, variety, date and their interaction were also significant ($P < 0.0001$, $P < 0.0001$, $P = 0.007$ respectively for 2010, Table 3, and $P = 0.008$, $P < 0.0001$, $P < 0.0001$ respectively for 2011, Table 4).

The interaction with date became apparent after the first chilling temperature in each year; in 2010 this occurred on Oct. 4, when the low temperature reached 2.7 °C, and in 2011 on Oct.1, when the temperature dropped to 1.1 °C (Fig. 7). Interestingly, in 2010, *A* rates seem to mirror daily temperatures, rising and falling similarly among all varieties (Fig. 1, Fig. 7). For example, all varieties experienced a significant drop in *A* on Oct. 4, 2010 compared to earlier dates ($P < 0.0001$), then rose again by Oct. 9, when the temperature climbed back to 19.4 °C, to rates that were not significantly different than they were at the beginning of measurements on Sept. 8 ($P = 0.507$). However, in 2011, some varieties recovered after the cold shock on Oct. 01, 2011, while other seemed to go into senescent decline. For example, *A* again decreased in all varieties on Oct. 1 ($P < 0.0001$), but only five varieties returned to their pre-chilling rates before the killing frost came on Oct. 17, 2011. The other four varieties, the fertile Amuri clones AM-900, AM-903, AM-905 and AM-985, all had significantly lower *A* rates by Oct. 13 ($P = 0.002$, $P = 0.02$, $P < 0.0001$ and $P = 0.0008$, respectively), dropping to a low of $2.78 \mu\text{mol m}^{-2} \text{s}^{-1}$ in AM-905.

Stomatal conductance to water vapor (g_s)

Variety, date, year, and their respective two- and three-way interactions all significantly affected g_s (Table 2, Fig. 2). Averaged over both years, AM-905 and AM-903 had the lowest g_s (0.10 and $0.11 (\pm 0.0059) \text{ mol m}^{-2} \text{ s}^{-1}$, respectively), and $M \times g\text{-IL}$ had the highest at $0.14 (\pm 0.006) \text{ mol m}^{-2} \text{ s}^{-1}$.

Because there was a significant variety \times year interaction, each year was also analyzed separately, where it was observed that the effect of variety, date and their interaction were also significant for each year (Table 3, Table 4). Average g_s rates in 2010 ranged from $0.13 (\pm 0.004) \text{ mol m}^{-2} \text{ s}^{-1}$ AM-905 to $0.16 (\pm 0.004) \text{ mol m}^{-2} \text{ s}^{-1}$ for $M \times g\text{-IL}$. In

2011 average g_s rates were lower than 2010, likely reflecting the lower average temperatures during the measurement period (Fig. 5, 7), ranging from $0.07 (\pm 0.006) \text{ mol m}^{-2} \text{ s}^{-1}$ AM-905 to $0.11 (\pm 0.006) \text{ mol m}^{-2} \text{ s}^{-1}$ for $M \times g$ -IL.

The same chilling temperatures on Oct. 04, 2010 and Oct. 01, 2011 that affected A also significantly affected g_s , but only in the Amuri clones AM-903 and AM-905 which had significantly lower g_s rates after Oct. 4 in 2010 ($P < 0.0001$ and $P = 0.04$, respectively; Fig. 2). AM-903 maintained significantly lower g_s rates to the end of the season ($P < 0.0001$) while AM-905 did not. In 2011, only AM-905 had significantly lower g_s rates at $0.06 (\pm 0.006) \text{ mol m}^{-2} \text{ s}^{-1}$ ($P = 0.02$) following chilling temperatures on Oct. 1, 2011, which it maintained to the end of the season, despite subsequent increases in air temperature ($P = 0.0003$; Fig. 2, 7).

Leaf [N]

Variety, date, year and their respective two-way interactions all significantly affected [N] rates (Table 2, Fig. 3). The fertile clone AM-905 had the lowest leaf [N] of $1.05 (\pm 0.05) \%$, which was significantly different from all other varieties except M-912 and AM-903, and $M \times g$ -IL had the highest leaf [N] at $1.35 (\pm 0.05) \%$.

Because there was a significant variety \times year interaction, each year was also analyzed separately. In 2010, variety and date were found to significantly affect leaf [N] ($P < 0.0001$, $P < 0.0001$ respectively, Table 2, Fig. 3), but unlike A and g_s , there was no significant variety \times date interaction ($P = 0.2$ Table 2). However, in 2011 the effects of variety, date and their interaction were all significant ($P = 0.009$, $P < 0.0001$, $P < 0.0001$ respectively, Table 2), suggesting that the leaf [N] among varieties changed differentially in the second year of growth compared to the first year.

Generally, the leaf [N] among varieties did not rank consistently within each year of growth, though AM-905 finished both years with the lowest leaf [N] (Fig. 3). In 2010, varieties leaf [N] ranged from 1.29 (± 0.03) % for AM-905 to 1.64 (± 0.03) % for *M* \times *g*-IL. In 2011 [N] rates were lower, ranging from 0.82 (± 0.05) % for AM-905 to 1.11 (± 0.05) for AM-900. In both years earlier dates were found significantly different from the last date of measurement. The chilling temperatures on Oct. 04, 2010 that caused a drop in *A* and *g_s* did not immediately decrease leaf [N] compared to earlier dates of measurement, but AM-903, AM-905, AM-985 *M* \times *g*-IL, *M* \times *g*-Nagara and M-912 had significantly lower leaf [N] by ($P = 0.0006$, $P = 0.001$, $P = 0.006$, $P = 0.02$, $P = 0.02$, $P = 0.02$, $P = 0.05$ respectively) the last day of measurement. In 2011 in their second year of growth, varieties did not follow the same pattern.

After chilling temperatures on Oct. 01, 2011 the leaf [N] in AM-905 and *M* \times *g*-IL dropped significantly ($P = 0.04$ and $P = 0.01$ respectively), but on the last day of measurement only AM-905 and AM-900 were found to have significantly different leaf [N] from the beginning of the measurement period.

Discussion

The above results provide quantitative metrics to assess senescence in *Miscanthus* cultivars using photosynthetic gas exchange, and to a lesser extent, leaf [N]. The methods described offer a more objective means to assess senescence than the greenness ratings used previously to differentiate *Miscanthus* cultivars (Robson, Mos et al. 2012) and allow for more physiological insight into the environmental cues for whole-plant senescence.

As the growing season progressed and temperature started decreasing, A and g_s rates also start decreasing; in both years earlier dates of measurement for A and g_s were significantly different from later dates. The same thing was observed for leaf [N]. In the active growing season the leaf [N] was high, but as the plants started flowering and the air temperature dropped, visual changes in leaf color became apparent in some varieties, and were accompanied by a concomitant and significant drop in leaf [N]. This change in leaf [N] has been shown before at a courser scale in *M. × giganteus* (Dohleman, Heaton et al. 2009; Heaton, Dohleman et al. 2009; Heaton, Dohleman et al. 2009) but this is the first time it has been used at this temporal resolution to distinguish differences between *Miscanthus* genotypes. Our findings support prior studies of senescence and N cycling in perennial grasses that at the end of active growing season, above-ground plant parts start changing their color and transfer their metabolites to the storage organs (Beale and Long 1997). While many species begin N translocation back to storage organs relatively early in the season, the phenomenon is frequently a sign of whole-plant, rather than tissue, senescence. Another major finding of this work was that photosynthetic gas exchange and leaf [N] vary significantly between the establishment and second year, and that this variation differed among varieties. Such differences have been anecdotally observed by researchers (E. Heaton, J.Clifton-Brown, personal observation) but this is the first time those observations have been statistically tested and supported. In the first growing season we observed incomplete senescence in almost all of the cultivars except AM-905, as indicated by A rates that were not significantly different by the end of the growing season than they were at the beginning of measurement in late summer (Fig. 1, Fig. 3). Four out of five Amuri clones (AM-905, AM-903, AM-900 and AM-985) also flowered early and showed earlier and stronger decreases in

leaf [N], reflecting its earlier maturity than other varieties. These results support Clifton Brown and Lewandowski (2000) who reported similar findings, and found that first year stands of five genotypes did not senesce before first killing frost, and winter mortality rates were higher in following spring, but older plants did senesce earlier and rare plant deaths were observed thereafter. We did not observe plant death in either year, but clear differences in patterns of senescence indicators were observed in the second growing season in four out of five Amuri clones i.e. AM-905, AM-985, AM-900 and AM-903. These cultivars had the lowest *A* rates and leaf [N] at the end of their second growing season, suggesting the photosynthetic apparatus was being shut down and dismantled in preparation for dormancy. The rest of the cultivars (i.e. *M*×*g*-IL, AM-904, MF-901, M-912 and *M*×*g*-Nagara) remained green with higher *A* rates and leaf [N]. All above-ground plant parts of these cultivars were just killed after the first killing frost.

In concert with leaf [N], the C-N ratio changed dramatically during the course of this experiment. This could simply be a result of leaf N depletion, but may also reflect a purposeful increase in leaf carbohydrate concentration to protect from cooler temperatures. Such an increase would support Cook et al. (2004), who found that cold acclimated *Arabidopsis* plants accumulated carbohydrates like fructose, glucose, galactinol, raffinose in their leaves 5-25 fold more than non-acclimated plants.

In summary, all of the *Miscanthus* varieties used in this experiment are capable of senescing prior to a killing frost in Iowa, but this process occurs at different rates and times among varieties. The fertile Amuri clones AM-905 and AM-903 responded early to changes in temperature and matured more early than other varieties, showing no increase in photosynthetic gas exchange after a cold shock in the second year of growth, and suggesting

they will be more adapted to short-season, northern locations. By contrast, *M*×*g*-IL, MF-901, *M*×*g*-Nagara and M-912 responded late to the temperature drop, and were slow to translocate N. These varieties stayed green and maintained higher photosynthetic rates until all above-ground plant parts were killed by the first killing frost. A longer growing season may be advantageous for biomass accumulation, but reduce the viability of such varieties in northern locations in the long-run. The Amuri types AM-985, AM-900 and AM-904 had an intermediate response that may be best matched to the growing season in central Iowa, but will require testing in more locations and years to determine.

Tables

Table 1. Varieties used

Code	Name	Ploidy	Sterility
AM-900	Amuri Clone	Diploid <i>M. sinensis</i> × Diploid <i>M. sacchariflorus</i>	Fertile
AM-903	Amuri Clone	Diploid <i>M. sinensis</i> × Diploid <i>M. sacchariflorus</i>	Fertile
AM-904	Amuri Clone	Diploid <i>M. sinensis</i> × Diploid <i>M. sacchariflorus</i>	Fertile
AM-905	Amuri Clone	Diploid <i>M. sinensis</i> × Diploid <i>M. sacchariflorus</i>	Fertile
AM-985	Amuri Clone	Diploid <i>M. sinensis</i> × Diploid <i>M. sacchariflorus</i>	Fertile
M-912	<i>M. sinensis</i>	Diploid <i>M. sinensis</i>	Fertile
<i>M</i> × <i>g</i> -Nagara	<i>M. × giganteus</i> -Nagara	Diploid <i>M. sinensis</i> × Tetraploid <i>M. sacchariflorus</i>	Sterile
MF-901	<i>M. × giganteus</i> clone “Freedom”	Diploid <i>M. sinensis</i> × Tetraploid <i>M. sacchariflorus</i>	Sterile
<i>M</i> × <i>g</i> -IL	<i>M. × giganteus</i> clone “IL”	Diploid <i>M. sinensis</i> × Tetraploid <i>M. sacchariflorus</i>	Sterile

Table 2. ANOVA for physiological indicators of plant senescence rate and times during 2010 and 2011

Analysis	Source	DF	F Value	P>F
Photosynthetic C assimilation rate (A)				
	Block	3	9.81	0.0002
	Variety	8	6.75	0.0001
	Year	1	190.41	<.0001
	Variety × Year	8	5.74	<0.0001
	Date	5	60.84	<0.0001
	Variety × Date	40	3.14	<0.0001
	Year × Date	5	4.01	0.0016
	Variety × Year × Date	40	1.41	0.06
Stomatal Conductance to water vapor (g_s)				
	Block	3	8.56	0.0005
	Variety	8	5.37	0.0006
	Year	1	617	<0.0001
	Variety × Year	8	2.1	0.036
	Date	5	45.7	<0.0001
	Variety × Date	40	3.65	<0.0001
	Year × Date	5	15.98	<0.0001
	Variety × Year × Date	40	1.92	0.0012
Total leaf nitrogen ([N])				
	Block	3	2.59	0.07
	Variety	8	6.85	0.0001
	Year	1	1479.35	<.0001
	Variety × Year	8	2.68	0.007
	Date	5	33.76	<0.0001
	Variety × Date	40	1.73	0.006
	Year × Date	5	7.37	<0.0001
	Variety × Year × Date	40	1.8	0.003

DF= Degrees of freedom

Table 3. ANOVA for physiological indicators of plant senescence rate and times during 2010

Analysis	Source	DF	F Value	P>F
Photosynthetic C assimilation rate (A)				
	Block	3	4.41	0.013
	Variety	8	8.02	< 0.0001
	Date	5	30.86	< 0.0001
	Variety × Date	40	5.74	0.007
Stomatal conductance to water vapor (g_s)				
	Block	3	7.85	0.0008
	Variety	8	4.24	0.002
	Date	5	48.6	< 0.0001
	Variety × Date	40	3.88	< 0.0001
Total leaf nitrogen ([N])				
	Block	3	4.89	0.008
	Variety	8	8.01	<0.0001
	Date	5	16.92	<0.0001
	Variety × Date	40	1.15	0.27

DF= Degrees of freedom

Table 4. ANOVA for physiological indicators of plant senescence rate and times during 2011

Analysis	Source	DF	F Value	P>F
Photosynthetic C assimilation rate (A)				
	Block	3	5.62	0.004
	Variety	8	3.47	0.008
	Date	1	48.6	<0.0001
	Variety × Date	8	3.88	<0.0001
Stomatal Conductance to water vapor(g _s)				
	Block	3	3.95	0.02
	Variety	8	3.19	0.013
	Date	1	33.18	<0.0001
	Variety × Date	8	2.54	<0.0001
Total leaf nitrogen ([N])				
	Block	3	0.7	0.56
	Variety	8	3.42	0.009
	Date	1	33.49	<0.0001
	Variety × Date	8	3.43	<0.0001

DF= Degrees of freedom

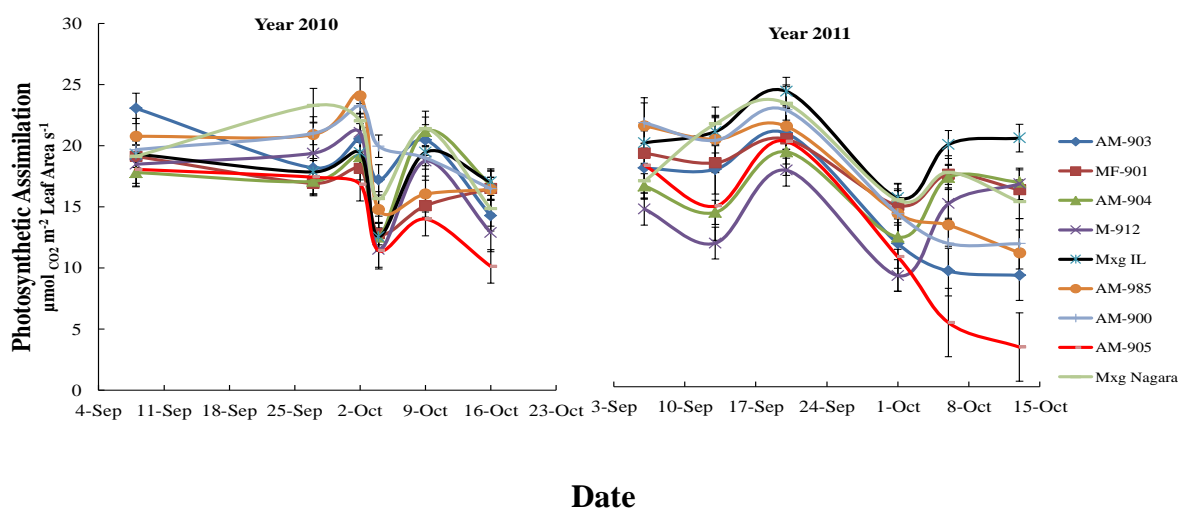
Table 5. Tukey's grouping for variety Least Squares Means (Alpha=0.05)

LS-means with the same letter are not significantly different

Varieties	<i>A</i>	<i>g_s</i>	Leaf [N]
	$\mu \text{ mol CO}_2 \text{ m}^{-2} \text{ Leaf Area S}^{-1}$	$\text{mol H}_2\text{O m}^{-2} \text{ Leaf Area S}^{-1}$	%
AM-900	17.26a	0.1270bac	1.3308a
AM-903	15.697a	0.1162bc	1.21bac
AM-904	15.59a	0.1290ba	1.253ba
AM-905	12.49b	0.106c	1.05c
AM-985	16.68a	0.130ba	1.245ba
<i>M</i> × <i>g</i> -IL	17.46a	0.141a	1.356a
<i>M</i> × <i>g</i> -Nagara	17.37a	0.130ba	1.302ba
MF-901	15.82a	0.1208bac	1.2701ba
M-912	14.58ba	0.1242bac	1.155bc

Figures

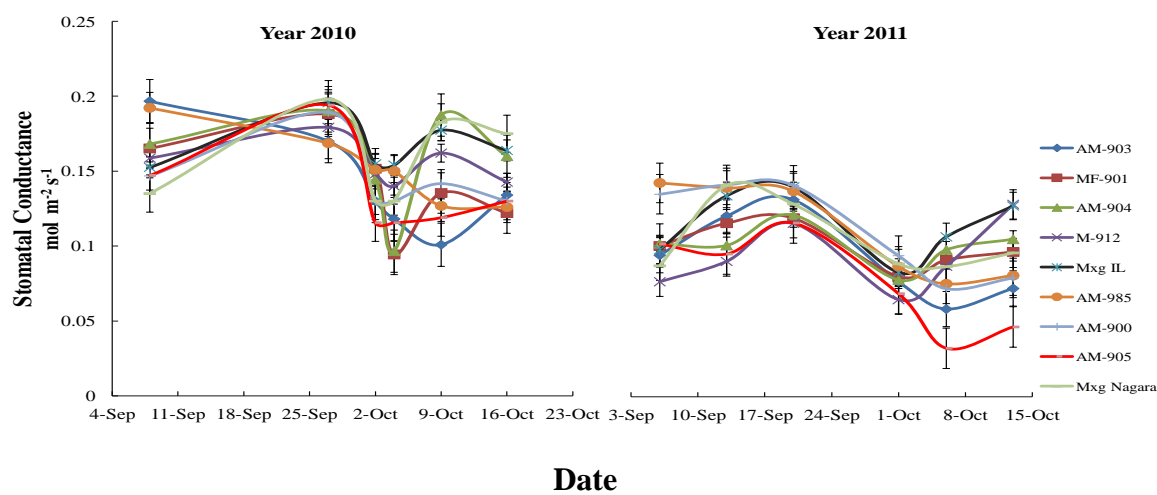
Figure 1. Photosynthetic C assimilation (A) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ Leaf Area s}^{-1}$)



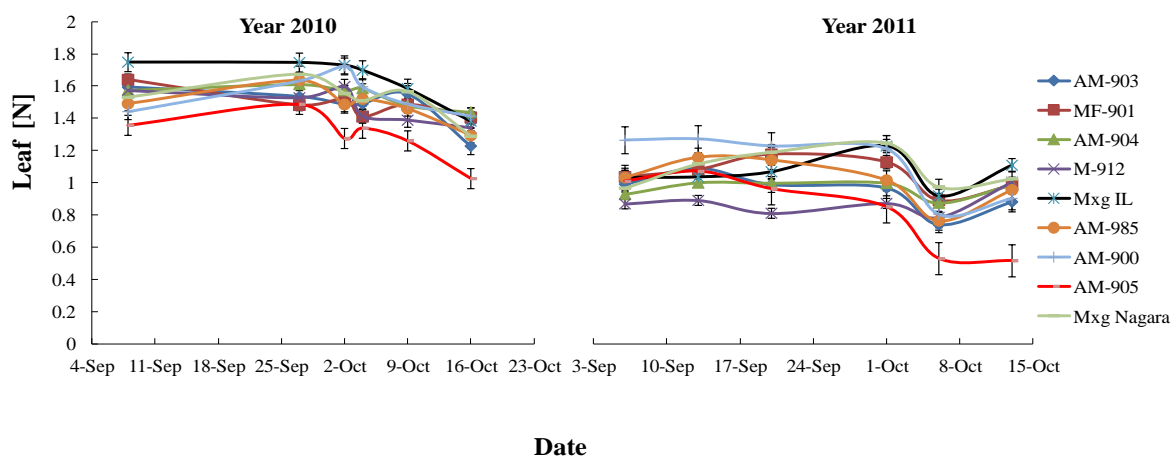
Photosynthetic assimilation rates for nine varieties of *Miscanthus* during year 2010-2011.

Least squared means for photosynthetic assimilation were calculated from $n=4$ blocks and error bars indicate ± 1 standard error of means.

Figure 2. Stomatal conductance to water vapor (g_s) ($\text{mol}_{\text{H}_2\text{O}} \text{m}^{-2} \text{Leaf Area S}^{-1}$)

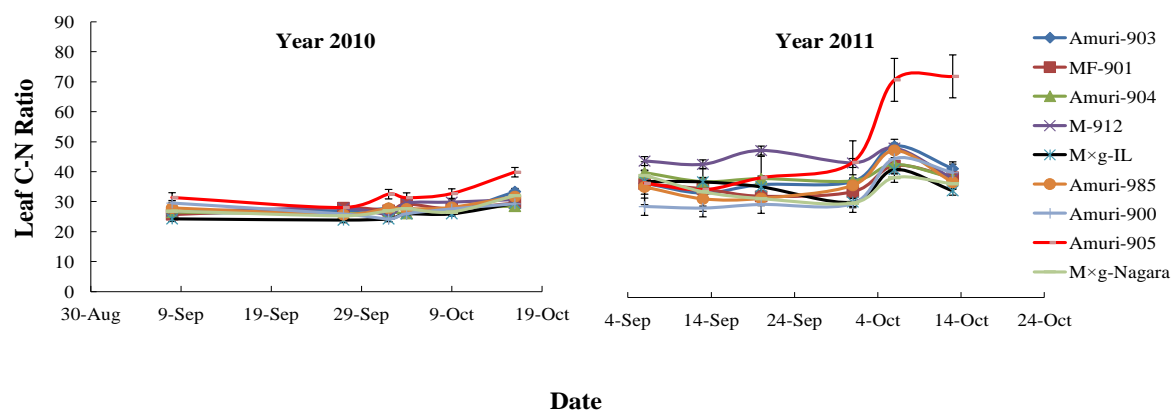


Stomatal conductance to water vapor (g_s) for nine varieties of *Miscanthus* during year 2010-2011. Least squared means for stomatal conductance were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

Figure 3. Leaf [N]

Leaf [N] for nine varieties of *Miscanthus* during year 2010-2011. Least squared means for leaf [N] were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

Figure 4. Leaf carbon-nitrogen ratio



Leaf C-N ration for nine varieties of *Miscanthus* during year 2010-2011. Least squared means for leaf C-N ratio were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

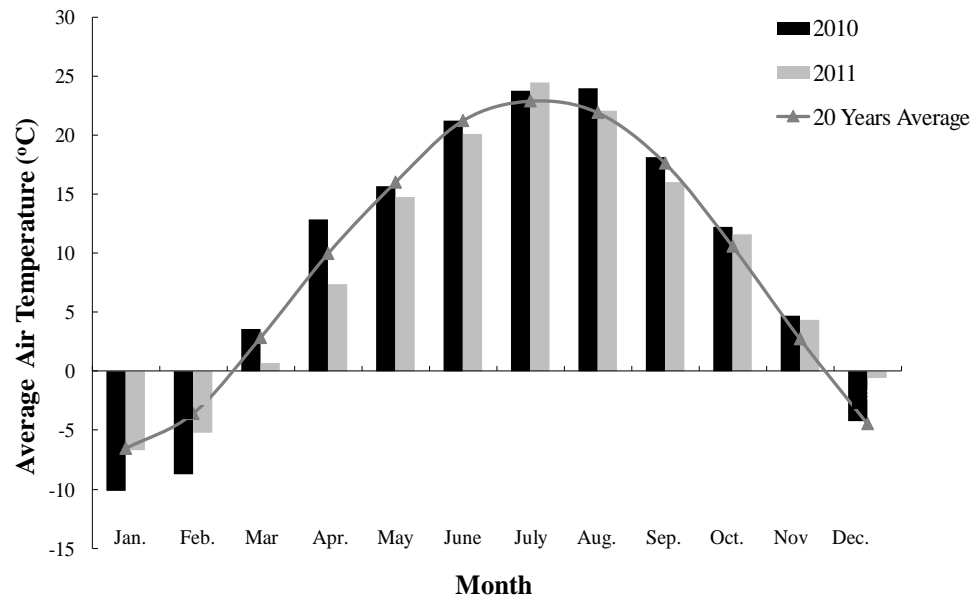


Figure 5. Mean monthly air temperature at Hinds farm in Ames, Iowa for year 2010 and 2011 with average over 20 years

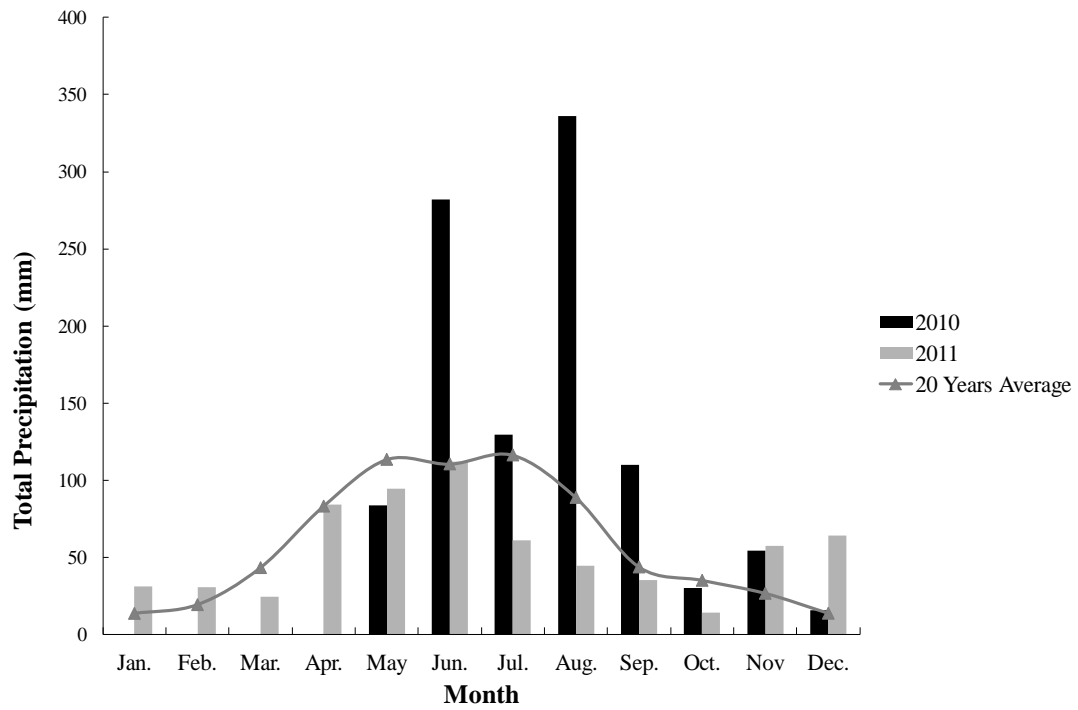


Figure 6. Mean monthly precipitation at Hinds farm in Ames, Iowa for year 2010 and 2011 with average over 20 years

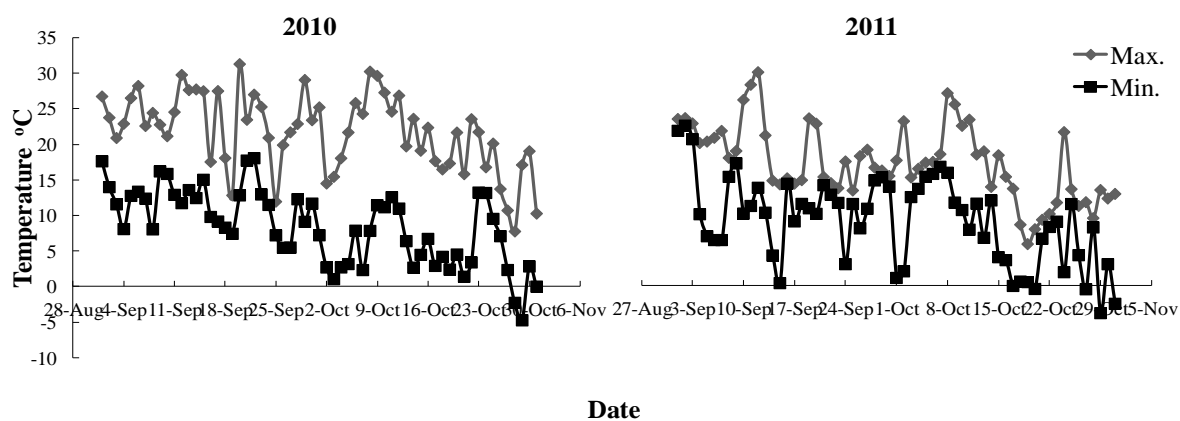


Figure 7. Daily maximum and minimum temperature during September and October at Hinds farm in Ames, Iowa for year 2010 and 2011

CHAPTER 3

VARIATION IN MORPHOLOGICAL CHARACTERISTICS AND BIOMASS YIELD OF NINE VARIETIES OF *MISCANTHUS*

A paper to be submitted to Agronomy Journal

Muhammad Aurangzaib¹², Emily A. Heaton¹³⁴

Abstract

Miscanthus has the potential to produce higher dry matter yields per unit area of land on a wide range of environmental and soil conditions than other perennial grasses used for biofuel production. Currently *M. × giganteus* is the most widely used genotype for biomass production. But cultivation of a single clone for a longer time and in the same areas could face many problems such as disease, pest attack, abiotic stresses etc, so there is a great need to identify and develop new varieties of *Miscanthus* which better withstand these stresses and allow the production of higher quality and quantity biomass. A field trial was established in spring 2010 as a split-plot randomized complete block design with four blocks to evaluate different varieties of *Miscanthus* for their morphological characters and yield potential in Iowa. All varieties performed well in both years (2010-2011), with no plant mortalities in any of the variety. Significant differences between varieties for their morphological characters such as plant height ($P < 0.0001$), tiller density ($P < 0.0001$), basal circumference ($P < 0.0001$) and leaf width ($P < 0.0001$) were observed. Varieties were also significantly different for their above-ground biomass yields ($P = 0.01$), but no significant differences were

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observed between varieties for their below-ground biomass yields. In all varieties year had a significant difference ($P < 0.0001$) for all morphological and yield characters except stem diameter.

Introduction

Due to increased demand of fuel, food and a cleaner environment, the world has started paying attention to clean and renewable fuel production. In the United States most of the biofuel is produced from 1st generation energy crops (e.g., maize, sugarcane), by fermentation of sugars and starch. But crops grown for the purpose of biofuel production also compete with food producing crops for limited resources like fertile land and irrigation water (Tilman, Hill et al. 2006), leading to a competition between food and fuel. According to a recent energy mandate, it is expected that the United States will be producing 36 billion gallons of renewable fuel to meet energy demands by 2020, and 21 of those 36 billion gallons should come from non-food crops, i.e., lignocellulosic feedstock (EISA 2007). Consequently, use of 2nd generation energy crops, like *Miscanthus*, is being promoted for the production of biofuel, because of their potential to produce high biomass yields per unit area (Clifton-Brown 2004; Heaton, Clifton-Brown et al. 2004; Heaton, Dohleman et al. 2008; Hastings, Clifton-Brown et al. 2009; Cai X et al. 2011).

Biofuel produced from lignocellulosic feedstock is reported to reduce greenhouse gas emissions by 85 % compared to gasoline (Wang, Wu et al. 2007). Among lignocellulosic feedstock producing crops, *Miscanthus* is getting much attention due to the fact that it not only produces high biomass per unit area, but also as a perennial with the C4 photosynthetic pathway, it maintains high rates of photosynthesis with efficient utilization of resources like

water, nutrients and radiation, so it is a relatively more environmentally friendly crop (Beale and Long 1995; Beale, Morison et al. 1999; Lewandowski and Clifton Brown 2000).

At present, *Miscanthus* × *giganteus* is the most widely used genotype of *Miscanthus*, and most of the research in Europe and United States has focused on this single genotype. *Miscanthus* × *giganteus* is a sterile hybrid produced by the cross of diploid *M. × sinensis* and tetraploid *M. × sacchariflorus* (Greef and Deuter 1993; Hodkinson, Chase et al. 2002). So far *M. × giganteus* has shown promising yields throughout Europe, but there are some limitations in the wide spread cultivation of this genotype, namely plant losses during winter and high establishment costs. Furthermore, use of a single genotype in an area for a longer time could increase the chances of yield losses due to insect, pest attack. Such problems could be overcome by the use of different genotypes of *Miscanthus*.

Because it is sterile, establishment of *M. × giganteus* is done vegetatively, typically by rhizomes, which is not only an expensive method of propagation, but also makes its cultivation more challenging on a large scale as compared to the cultivars which can be planted with seeds (Clifton-Brown and Lewandowski 2000; Lewandowski, Clifton-Brown et al. 2000; Munzer 2000; Pude 2000).

Plant losses during winter are also a major issue in cultivation of *M. × giganteus* in areas with harsh winters. Field trials of *M. × giganteus* in northern Europe have shown poor overwintering potential for their first winter after planting, indicating less adaptability to temperate climatic zones (Schwarz, Greef et al. 1995; Pude 1998; Christian and Haase 2001). Clifton Brown (2001) tested 15 *Miscanthus* genotypes for three years in five different locations, and studied *Miscanthus* growth characteristics like plant height, tiller number, stem

diameter, flowering and senescence rate in fall. He reported that in areas where soil temperatures in the first winter following planting fell below -4.5°C , *M. × giganteus* and *M. sacchariflorus* genotypes experienced the highest plant losses while *M. sinensis* had the lowest mortality rates, and produced higher biomass yields than both *M. × giganteus* and *M. sacchariflorus*. In locations with winter temperature $> -2.8^{\circ}\text{C}$, however, all genotypes were able to survive and *M. × giganteus* produced more biomass than *M. sinensis* or *M. sacchariflorus*. These findings indicate that not all *Miscanthus* species could be a best fit for all environmental and weather conditions.

The present study was conducted in order to investigate variation in the adaptation and performance of nine different varieties of *Miscanthus* in Iowa under the same management practices and environmental system. Developmental morphology of perennial grasses is very important for determining improved management and cultural practices. Parameters like plant height, stem diameter, basal circumference, tiller density and biomass yield were recorded to evaluate the genotypic differences among these varieties for their morphological and yield characteristics.

Material and Methods

Experimental design and establishment

A two year field trial was established in June-01-2010 at the Iowa State University, Hinds Research Farm in Ames, Iowa. The experiment was arranged as a split-plot randomized complete block design with four replications. The split in this design was dedicated for destructive plant sampling to determine biomass yield for both years. Each plot was $4.57\text{ m} \times 11.43\text{ m}$. Nine different cultivars of *Miscanthus* (Table 1) were planted using

plugs received from Mendel Biotechnology, Inc. (Hayward, CA). Plugs were stored at 4 °C until the transplanting was complete.

Plants were planted by hand at an inter- and intra-row distance of 0.76 m. In the first year, weeds were controlled manually as needed in addition to a June application of 2,4-D (2,4-dichlorophenoxyacetic acid) with pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine). In the second year, manual weed control was done once early in the growing season coupled with a single June application of 2,4-D (2,4-Dichlorophenoxyacetic acid) with acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl).

Measurements

All morphological measurements were taken on three randomly selected plants in each plot.

Plant height

Plant heights were taken by using a marked poll (cm). Average canopy height of three plants per plot was taken for the estimation of plant height.

Tiller density

All living tillers above 5 cm were counted to determine tiller density.

Basal circumference

A seamstress tape was used to determine the basal circumference of a plant by putting it around the plant 1 inch above the ground surface.

Stem diameter

A Vernier caliper (Fisher Scientific Inc. Waltham, Massachusetts) was used to determine stem diameter by clamping it on to a random plant tiller 1 inch above the ground surface.

Biomass yield

To estimate above- and below-ground biomass destructive samples of two randomly selected plants per plot were taken at the end of each growing season, avoiding border rows. Each plant was cut close to the ground surface with a knife, then above-ground biomass was weighed and dried to constant weight at 60 °C. These dry samples were then weighed to estimate the above-ground dry matter yield. For below-ground measurements, the samples were dug with shovels to extract the visible rhizome mass (approximately 25 cm deep) which typically comprises ~80-90% of *Miscanthus* below-ground biomass (Dohleman, Heaton et al. 2012). These samples were completely washed free of soil, dried and weighed using the same method as used for above-ground biomass.

Statistical analysis

A mixed model analysis of variance was performed with SAS 9.2 (SAS Institute Cary, NC, USA), using PROC GLIMMIX. *Miscanthus* varieties, block, year, variety \times year were considered as fixed effects and variety \times block was considered as random effect. Significant effects were estimated by F-Statistics at $\alpha = 0.05$. Differences between least-squared means were determined with Tukey's adjustment. PROC CORR was used to estimate correlation between biomass yield and morphologic characteristics.

Results

All varieties used in the experiment appeared to perform well in both years. During the summer the mean monthly air temperature ranged from 14 – 24 °C (Chapter 2, Figure 5) which is in the range conducive to *Miscanthus* growth (Clifton-Brown, Neilson et al. 2000; Heaton, Voigt et al. 2004). 2010 was wet compared to the 20 year average monthly precipitation (Fig. 6); in August 2010 the research field received a flood from a nearby river but all plants survived.

Plant height

Variety, year and their interaction all significantly affected plant height ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$ respectively, Table 1, Fig. 1). Averaged over both years, the Amuri clone AM-905 was tallest at 171.78 (± 5.63) cm (Table 2, Fig. 1). AM-904 was shortest at 125.78 (± 5.63) cm and was significantly different from rest of the Amuri clones. Since there was a significant variety by year interaction, each year was also analyzed separately. In the first year of growth in 2010, the fertile Amuri clones were taller than the sterile *M. × giganteus* varieties, with heights ranging from 133.45 (± 3.27) cm for AM-905 to 55.55 (± 3.27) cm for *M × g*-IL. In 2011, all plants were taller and more similar in height than 2010 and from the sterile *M. × giganteus* Freedom clone was tallest at 216.34 (± 6.97) cm while the *M. sinensis* variety was shortest at 185.95 (± 6.97) cm for M-912 (Table 2, Fig. 1).

Not unexpectedly, the effects of variety and date were also found significant within each year, as plants grew taller over the growing season, especially during flowering ($P < 0.0001$, $P < 0.0001$ respectively for 2010, Table 3, and $P = 0.05$, $P < 0.0001$ respectively for 2011, Table 4). Importantly, the interaction between variety \times date in 2010 was highly

significant ($P < 0.0001$, Table 3) but not in 2011 ($P = 0.08$, Table 4), suggesting that plant height is much more variable in the transplanting year.

Tiller density

Variety, year and their interaction all significantly affected tiller density ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, respectively Table 1, Fig. 2). Averaged over two years, AM-985 had the most tillers at $56.63 (\pm 1.29)$, and $M \times g$ -IL had the least at $24.54 (\pm 1.29)$ (Table 2, Fig. 2).

Because there was a significant variety \times year interaction, each year was analyzed separately. As with plant height, variety and date significantly affected tiller density in each year, but the interaction was significant only in 2010, the establishment year (Table 3, 4). In 2010, average tiller numbers ranged from $47.22 (\pm 3.15)$ for $M \times g$ -Nagara to $25.33 (\pm 3.15)$ for $M \times g$ -IL, and in 2011 average tiller numbers ranged from $70.43 (\pm 2.34)$ for AM-985 to $24.50 (\pm 2.34)$ for $M \times g$ -IL. According to the Tukey's grouping $M \times g$ -IL was significantly different from rest of the varieties and had the least number of tillers in both years (Table 2, Fig. 2).

Basal circumference

Variety, year and their interaction all significantly affected basal circumference ($P = 0.001$, $P = < 0.0001$, $P < 0.0001$ respectively, Table 1, Fig. 3). Averaged over both years, AM-900 had the largest basal circumference at $108.39 (\pm 1.39)$ cm, and M-912 had the smallest at $80.51 (\pm 1.39)$ cm (Table 2).

Because there was a significant variety \times year interaction, each year was analyzed separately. As with plant height and tiller density, variety and date significantly affected basal circumference in each year, but the interaction was significant only in 2010, the establishment year (Table 3, 4). In 2010 the average basal circumference ranged from 91.13

(± 4.31) cm for AM-900 to 62.36 (± 4.31) cm for AM-905, and in 2011 average basal circumference ranged from 125.38 (± 3.45) cm for AM-900 to 97.68 (± 3.45) cm for AM-904 (Fig. 3).

Stem diameter

In analysis of variance for stem diameter variety and year and variety \times year were found significant ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$ respectively, Table 1, Fig. 4). Averaged over both years, *M* \times *g*-IL had the highest stem diameter at 7.42 (± 0.15) mm, and AM-985 with the least at 5.82 (± 0.15) mm. According to the Tukey's grouping, *M* \times *g*-IL was found significantly different from all other varieties except M-912, *M* \times *g*-Nagara and MF-901.

In individual year analysis for 2010, variety and date were significant ($P = 0.03$, $P = 0.04$ respectively, Table 3), variety \times date interaction was not significant ($P = 0.32$, Table 3), 2011 had highly significant effect for variety ($P < 0.0001$, Table 4), but was not significant for date and variety \times date interaction ($P = 0.06$, $P = 0.59$ respectively, Table 4), suggesting that differences between varieties became stronger after establishment, and that the stems had reached their maximum diameter by the time measurements commenced in 2011. In 2010, average stem diameter ranged from 7.37 (± 0.33) mm for MF-901 to 5.91 (± 0.33) mm for AM-985, and in 2011 average stem diameter ranged from 8.09 (± 0.26) mm for *M* \times *g*-IL to 5.77 (± 0.26) mm for AM-985.

Leaf width

Variety, year and their interaction all significantly affected leaf ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$ respectively, Table 1, Fig. 5). Averaged over both years, the Amuri clone AM-900 had the widest leaves 16.80 (± 0.23) mm while MF-901 and AM-904 had the narrowest leaves at 13.71 and 12.13 (± 0.23) mm respectively (Table 2).

Because there was a significant interaction of variety and year, each year was analyzed separately. Within each year, variety and date effects were found significant ($P < 0.0001$, $P < 0.0001$ respectively for 2010, Table 3, and $P < 0.0001$, $P < 0.0001$ respectively for 2011, Table 4) but their interaction was not significant in either year (Table 3, 4), suggesting leaves were at their maximum width at the commencement of measurements. In 2010 the average leaf width ranged from 15.88 (± 0.46) mm for AM-900 to 9.51 (± 0.46) mm for AM-904, and in 2011 average leaf width ranged from 18.83 (± 0.47) mm for $M \times g$ -IL to 15.10 (± 0.47) mm for AM-904.

Above-ground biomass yield

Variety, year and their interaction all significantly affected above-ground biomass yield (Table 5, Fig. 6). Averaged over both years $M \times g$ -Nagara had the highest yield of 12.26 (± 0.80) Mg ha⁻¹, but was not significantly different from all other varieties except $M \times g$ -IL and AM-900 which had the lowest least square means of 8.14 and 8.09 (± 0.80) Mg ha⁻¹ respectively. $M \times g$ -IL and AM-900 were not significantly different from all other varieties.

Because there was a significant interaction of variety and year, each year was analyzed separately. In 2010, varieties were found significantly different for their above-ground biomass yield ($P = 0.001$, Table 7). The Amuri clone AM-905 had the highest above-ground biomass yield at 8.55 (± 0.54) Mg ha⁻¹, but it was only significantly different from $M \times g$ -IL which had the lowest above-ground biomass yield at 3.06 (± 0.54) Mg ha⁻¹. In 2011 the effect of variety was only marginally significant ($P = 0.06$, Table 8). In 2011, average above-ground biomass yield ranged from 16.57 (± 1.12) Mg ha⁻¹ for $M \times g$ -Nagara to 11.17 (± 1.12) Mg ha⁻¹ for AM-900.

Below-ground biomass yield

No significant differences were observed among varieties for their below-ground biomass yield ($P = 0.42$ Table 2, Fig. 6). However, the effect of year was highly significant ($P < 0.0001$), with no interaction between variety \times year ($P = 0.3793$).

Within each year varieties were not significantly different for their below-ground biomass yield ($P = 0.30$ for 2010 and $P = 0.36$ for 2011, Table 7 and Table 8, respectively); however, below-ground biomass yields for all varieties in 2011 were significantly higher than 2010. In 2010, average below-ground biomass yield ranged from $3.96 (\pm 0.54)$ Mg ha⁻¹ for MF-901 to $2.01 (\pm 0.54)$ Mg ha⁻¹ for *M* \times *g*-IL, and in 2011 below-ground biomass yields ranged from $10.5 (\pm 1.22)$ Mg ha⁻¹ for AM-905 to $7.14 (\pm 1.22)$ Mg ha⁻¹ for *M* \times *g*-Nagara.

Correlation between morphologic characteristics and biomass yield

In the analysis of correlation between morphological characteristics of *Miscanthus* varieties and above-ground biomass yield averaged over both years, significant correlations were found for plant height, stem diameter, basal circumference and leaf width ($P < 0.0001$, $P = 0.02$, $P < 0.0001$, $P = 0.04$ respectively, Table 9, Fig. 7). Tiller density did not significantly correlate with above-ground biomass ($P = 0.73$, Table 9). Plant height and basal circumference were found to have the highest r values at 0.77 and 0.56 respectively (Table 9, Fig. 7).

Discussion

Results of two years of growth and developmental study of *Miscanthus* revealed significant variability among all varieties for their biomass yields and morphological characteristics, suggesting there is considerable scope for targeting improved *Miscanthus* genotypes in the Midwest.

This study showed that plant height, tiller density, basal circumference and leaf width was significantly different for all nine varieties. Jezowski (2008) studied different genotypes of *M. × giganteus* and reported correlation between *Miscanthus* biomass yield and tiller density, and between *Miscanthus* biomass yield and tuft diameter in first year old plants, but for second and third year stands he reported a stronger correlation. He also reported that plant heights significantly affect yield in second year old plants. Angelini et al. (2009) also reported a correlation of 0.75 between tiller density and above-ground biomass yield in a 10 year study of *M. × giganteus*. We did not find a significant correlation between tiller density and biomass yield, but this study lasted only 2 years, one of which was the establishment year. Data from subsequent years would be necessary to confirm or deny a relationship between tiller density and yield in these varieties in Iowa.

Bullard et al. (1997) also reported stem number and height as important factors contributing towards *Miscanthus* biomass yield. Results of this study also revealed that all high yielding varieties were also taller and had more tillers in both years. Looking at the mean yields of nine varieties over the two year period, it was observed that varieties AM-905, AM-903 and AM-985, which produced highest above-ground biomass yields at the end of first growing season, had the greatest heights among all varieties. *M×g*-Nagara was also one of the high above-ground biomass producing varieties at the end of first growing season,

but it was not among the tallest varieties. Instead, it produced the most tillers. Almost similar results were observed in second year, when the varieties with greater heights and tiller densities like AM-905, AM-903, *M*×*g*-Nagara, AM-901, AM-900, had the greater above-ground biomass yields at the end of second growing season. Similar results were observed in correlation analysis of above-ground biomass yield and morphologic characteristics, where plant heights significantly correlated with above-ground biomass yield ($r = 0.77$, Table 9; Fig. 7). Similarly, tiller number and basal circumference also had a respective correlation of 0.41 and 0.56 with above-ground biomass yield. Leaf width and stem diameter for varieties had a significant affect on above-ground biomass yield ($P = 0.02$, $P = 0.04$ respectively, Table 9, Fig. 7), but only had a weak correlation for above-ground biomass yield ($r = 0.27$, $r = 0.24$ respectively).

According to the analysis of variance no significant differences were found among varieties for their below-ground biomass yields, but year had a significant effect over varieties for their below-ground biomass yield. Looking at the mean yield data for below-ground biomass yields, it was observed that AM-905 and AM-903 had the highest below-ground yields at the end of second growing season. Interestingly, *M*×*g*-Nagara, which produced highest above-ground biomass, had the lowest below-ground biomass yield at the end of second growing season. *M*×*g*-Nagara is a close relative of *M. × giganteus* IL, but performed better than the IL clone in most aspects of this trial, suggesting it is better adapted to central Iowa, and possibly other areas of the Midwest.

Selection of a *Miscanthus* genotype based on two years of research is not optimal; atleast three to four years of research should be conducted before making any recommendations for perennial crops. These results, however, provide an early look at

possible new varieties that are adapted to Midwestern growing conditions, and suggest that *M*×*g*-Nagara and all of the Amuri varieties except AM-900 could be a good fit for Iowa.

Tables

Table 1. ANOVA tables for morphological characteristics

Analysis	Source	DF	F Value	P>F
Plant height (cm)				
	Block	3	1.60	0.22
	Variety	8	8.00	<.0001
	Year	1	758.27	<.0001
	Variety × Year	8	9.70	<.0001
Tiller density				
	Block	3	0.66	0.5783
	Variety	8	54.08	<.0001
	Year	1	258.54	<.0001
	Variety × Year	8	29.22	<.0001
Basal circumference (cm)				
	Block	3	11.84	<.0001
	Variety	8	41.27	<.0001
	Year	1	1197.61	<.0001
	Variety × Year	8	8.53	<.0001
Stem diameter (mm)				
	Block	3	0.97	0.4057
	Variety	8	1.16	0.3209
	Year	1	3.28	0.0709
	Variety × Year	8	1.53	0.1451
Leaf width (mm)				
	Block	3	3.32	0.02
	Variety	8	26.97	<.0001
	Year	1	316.61	<.0001
	Variety × Year	8	7.46	<.0001

DF= Degrees of freedom

Table 2. Tukey's grouping for variety Least Squares Means (Alpha=0.05)
LS-means with the same letter are not significantly different

Varieties	Plant height	Tiller density	Stem diameter	Basal circumference	Leaf width
	cm	number	mm	cm	mm
AM-900	156.24ab	43.17cde	6.28cd	108.39a	16.80a
AM-903	160.61ab	55.26a	5.90d	92.04c	16.37ab
AM-904	125.78c	41.12de	6.43abcd	80.51f	12.13e
AM-905	171.78a	49.01b	6.09d	83.31ef	14.62cd
AM-985	159.70ab	56.63a	5.88d	98.54b	16.75ab
<i>M</i> × <i>g</i> -IL	127.54c	24.98f	7.42a	84.99def	15.15bc
<i>M</i> × <i>g</i> -Nagara	148.31abc	47.78bc	6.76abc	87.88cde	16.23ab
MF-901	136.83bc	39.09e	7.01ab	90.70cd	13.72d
M-912	139.62bc	46.66bcd	6.78abc	82.28f	14.50cd

Table 3. ANOVA tables for morphological characteristics for 2010

Analysis	Source	DF	F Value	P>F
Plant height (cm)				
	Block	3	16.98	<0.0001
	Variety	8	96.34	<0.0001
	Date	4	142.18	<0.0001
	Variety*Year	32	4.36	<0.0001
Tiller density				
	Block	3	3.35	0.035
	Variety	8	5.07	0.0009
	Date	4	38.82	<0.0001
	Variety*Year	32	1.47	0.07
Stem diameter (mm)				
	Block	3	1.38	0.27
	Variety	8	2.6	0.03
	Date	4	2.54	0.04
	Variety*Year	32	1.12	0.33
Basal circumference (cm)				
	Block	3	1.22	0.32
	Variety	8	4.78	0.001
	Date	4	22.37	<0.0001
	Variety*Year	32	2.11	0.0023
Leaf width (mm)				
	Block	3	3.94	0.02
	Variety	8	18.29	<0.0001
	Date	4	19.21	<0.0001
	Variety*Year	32	1.46	0.07

Table 4. ANOVA tables for morphological characteristics for 2011

Analysis	Source	DF	F Value	P>F
Plant height (cm)	Block	3	0.22	0.88
	Variety	8	2.33	0.05
	Date	8	201.93	<0.0001
	Variety*Year	64	1.3	0.08
Tiller density	Block	3	2.87	0.06
	Variety	8	37.08	<0.0001
	Date	8	13.89	<0.0001
	Variety*Year	64	1.29	0.1
Stem diameter (mm)	Block	3	0.68	0.57
	Variety	8	9.89	<0.0001
	Date	8	1.89	0.06
	Variety*Year	64	0.94	0.59
Basal circumference (cm)	Block	3	4.49	0.01
	Variety	8	8.76	<0.0001
	Date	8	6.59	<0.0001
	Variety*Year	64	0.93	0.62
Leaf width (mm)	Block	3	0.69	0.57
	Variety	8	8.38	<0.0001
	Date	8	8.56	<0.0001
	Variety*Year	64	1.25	0.12

Table 5. ANOVA tables for biomass yield

Analysis	Source	DF	F Value	P>F
Above-ground yield (Mg ha ⁻¹)				
	Block	3	0.61	0.6166
	Variety	8	3.20	0.01
	Year	1	272.26	< .0001
	Variety × Year	8	2.9	0.01
Below-ground yield (Mg ha ⁻¹)				
	Block	3	2.73	0.0536
	Variety	8	1.04	0.4217
	Year	1	137.89	< .0001
	Variety × Year	8	1.1	0.3793

DF= Degrees of freedom

Table 6. Tukey's grouping for variety Least Squares Means (Alpha=0.05)

LS-means with the same letter are not significantly different

Varieties	Above ground yield	Below ground yield
	Mg ha ⁻¹	Mg ha ⁻¹
AM-900	8.09b	6.04a
AM-903	11.34ab	7.03a
AM-904	9.93ab	5.48a
AM-905	11.23ab	6.41a
AM-985	10.62ab	5.35a
<i>M</i> × <i>g</i> -IL	8.14b	4.74a
<i>M</i> × <i>g</i> -Nagara	12.26 a	5.14a
MF-901	10.65ab	6.05a
M-912	9.28ab	5.29a

Table 7. ANOVA table for biomass yield 2010

Analysis	Source	DF	F Value	P>F
Above-ground yield (Mg ha ⁻¹)				
	Block	3	1.4	0.26
	Variety	8	4.64	0.001
Below-ground yield (Mg ha ⁻¹)				
	Block	3	1.56	0.22
	Variety	8	1.28	0.3

Table 8. ANOVA table for biomass yield 2011

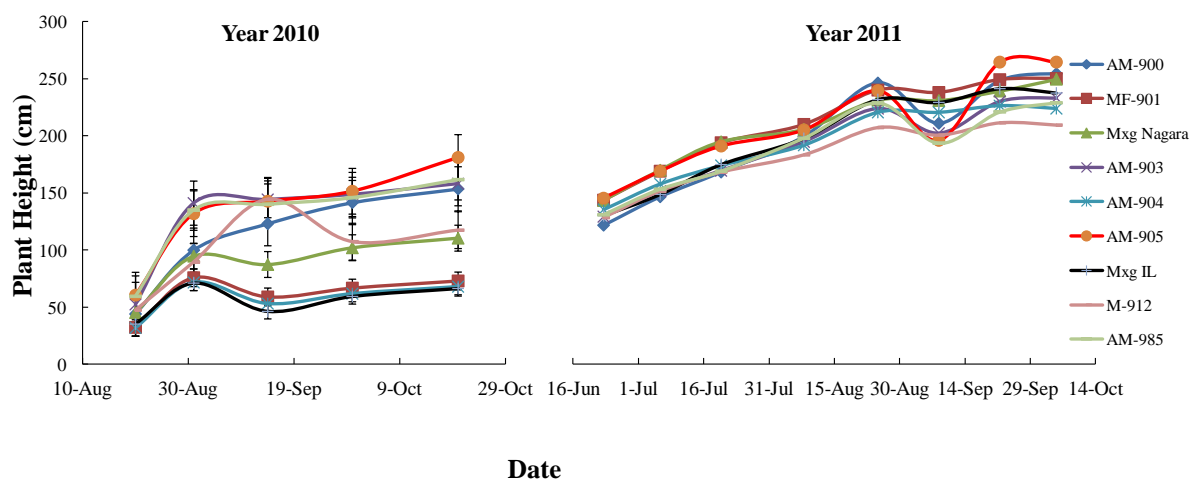
Analysis	Source	DF	F Value	P>F
Above-ground yield (Mg ha ⁻¹)				
	Block	3	0.85	0.48
	Variety	8	2.25	0.06
Below-ground yield (Mg ha ⁻¹)				
	Block	3	3.16	0.04
	Variety	8	1.16	0.36

Table 9. Correlation between morphologic characteristics and above-ground biomass yield

Pearson Correlation coefficients					
N = 72, Prob > r under Ho: rho = 0					
Yield	Plant height	Tillers density	Stem diameter	Basal circumference	Leaf width
1	0.77	0.41	0.27	0.56	0.24
	<0.0001	0.73	0.02	<0.0001	0.04

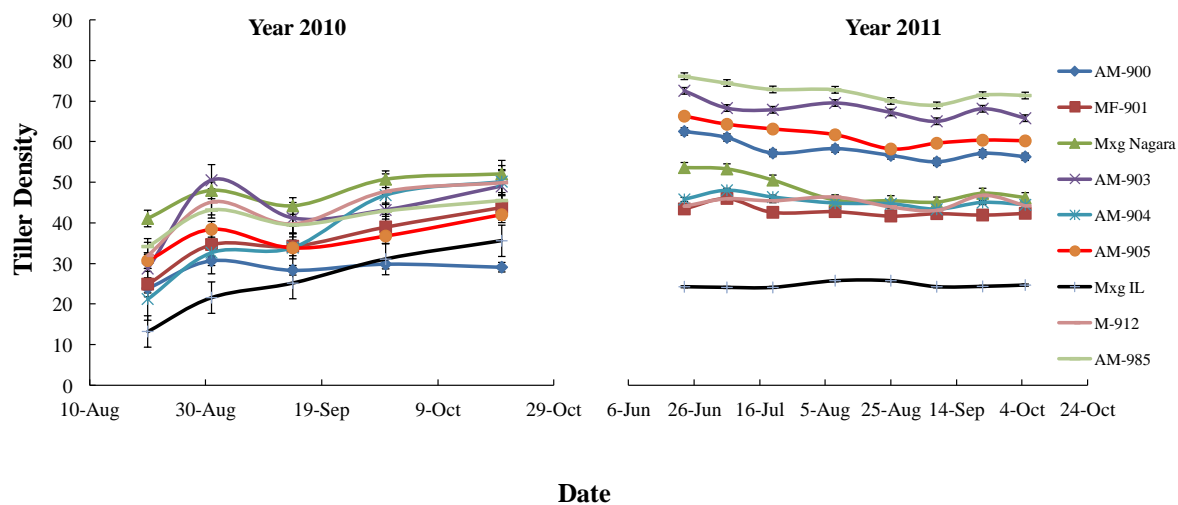
Figures

Figure 1. Plant height (cm)



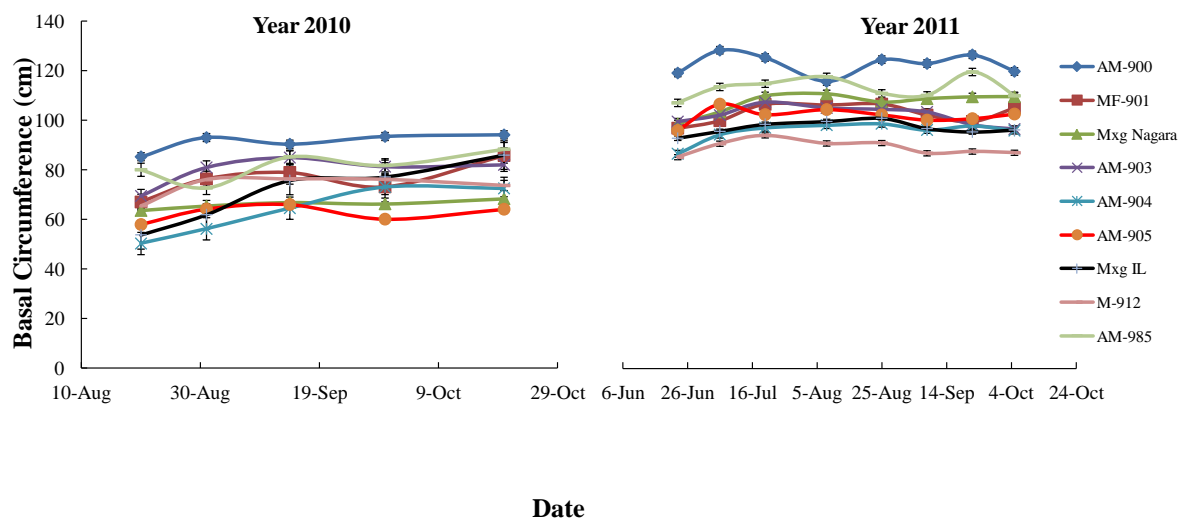
Plant heights for nine varieties of *Miscanthus* during year 2010-2011. Least squared means for plant height were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

Figure 2. Tiller density



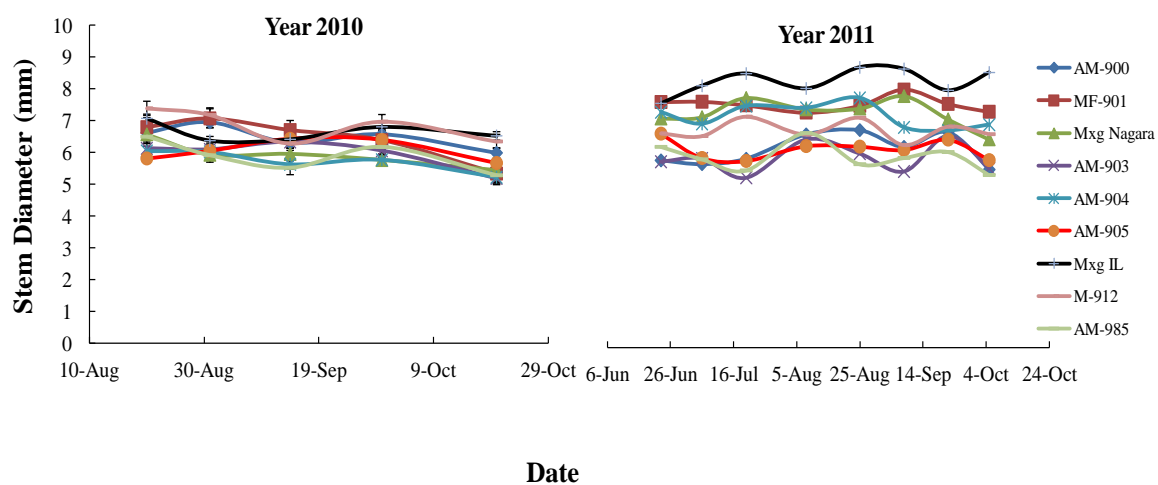
Tiller numbers for nine varieties of *Miscanthus* during year 2010-2011. Least squared means for tiller numbers were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

Figure 3. Basal circumference (cm)



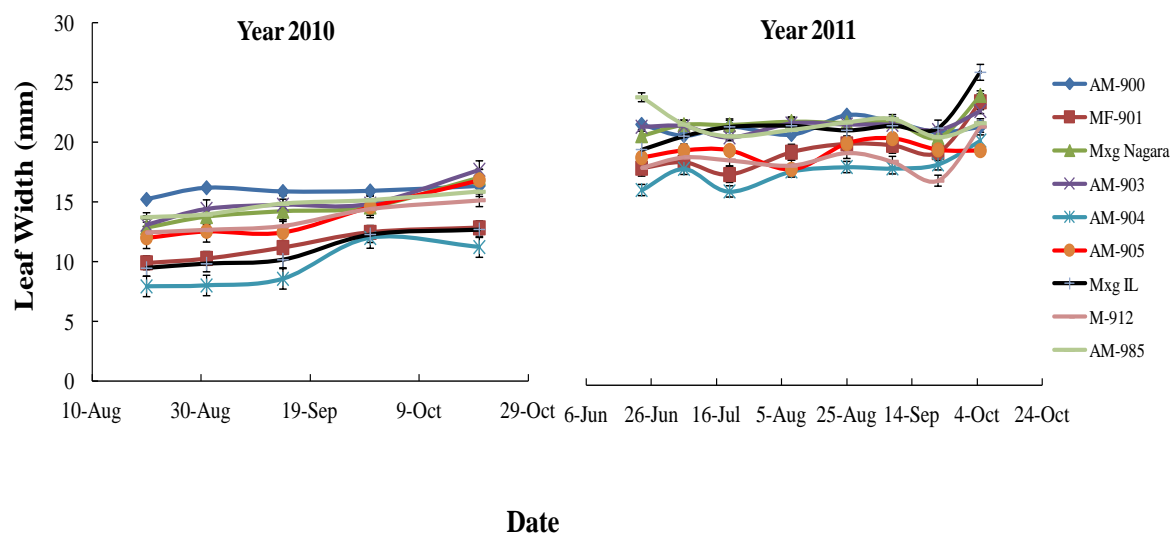
Basal circumference for nine varieties of *Miscanthus* during year 2010-2011. Least squared means for basal circumference were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

Figure 4. Stem diameter (mm)



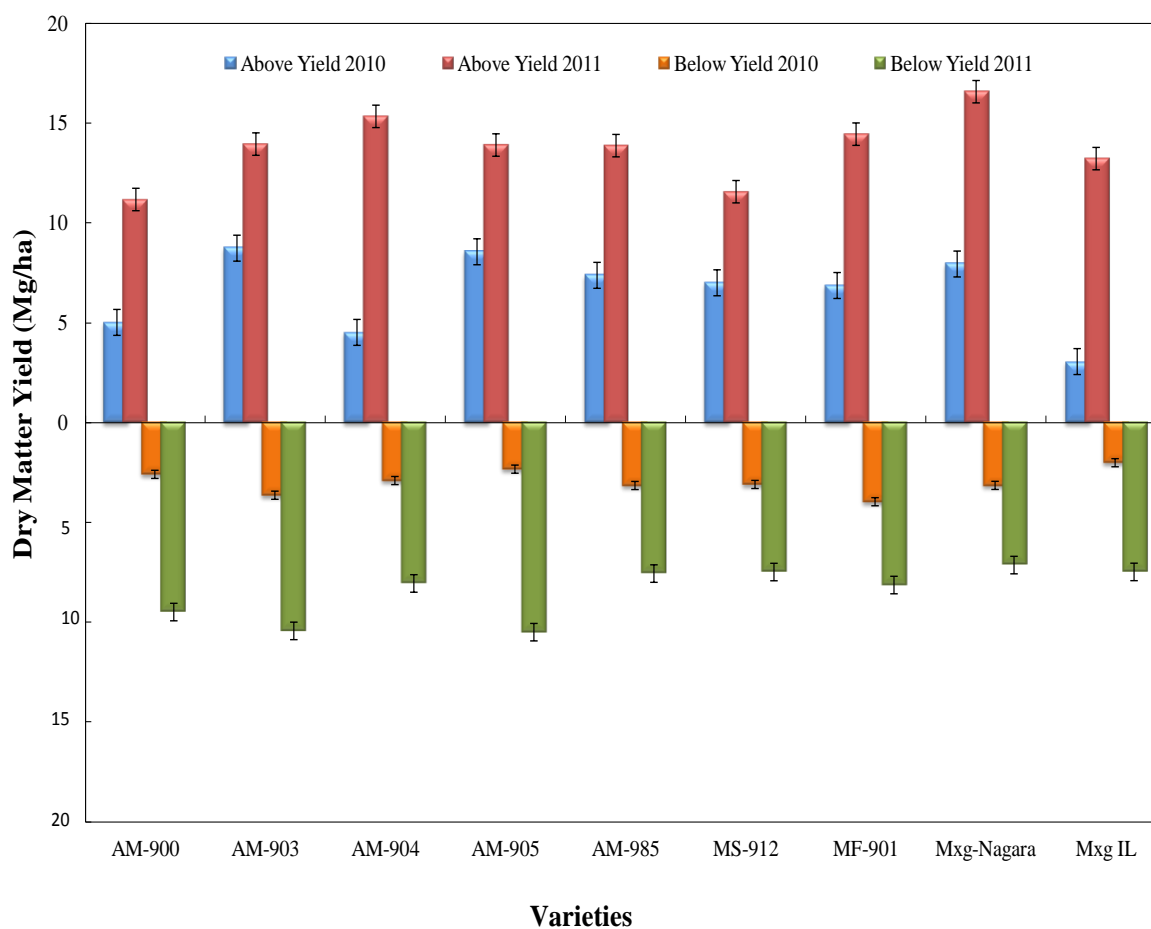
Stem diameter for nine varieties of *Miscanthus* during year 2010-2011. Least squared means for stem diameter were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

Figure 5. Leaf width (mm)

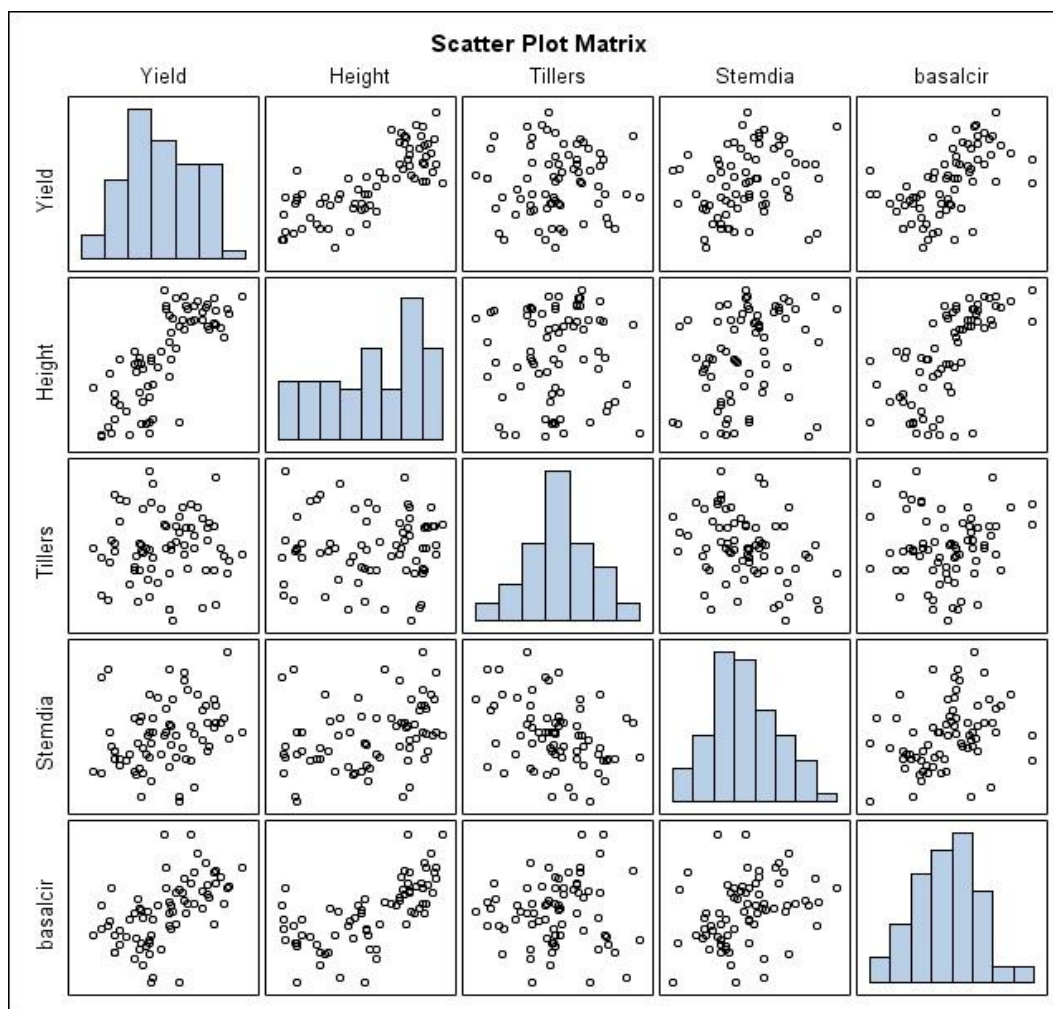


Leaf width for nine varieties of *Miscanthus* during year 2010-2011. Least squared means for leaf width were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

Figure 6. Biomass yield (above & below-ground) (Mg ha^{-1})



Above and below-ground dry matter biomass yields for nine varieties of *Miscanthus* for year 2010-2011. Least squared means for above and below ground biomass yields were calculated from $n = 4$ blocks and error bars indicate ± 1 standard error of means.

Figure 7. Correlation between morphologic characters and above-ground biomass yield

CHAPTER 4

GENERAL CONCLUSION

In this study we investigated physiological and morphological responses of different varieties of *Miscanthus* planted in Iowa. *Miscanthus* has proved itself to produce high yields in diverse climatic conditions including cold weather, but still one of the major obstacles in its production is overwintering in the establishment year. The purpose of this study was to investigate the process of senescence, which helps the overwintering *Miscanthus* plants to survive in freezing temperatures, and to see if there is any difference in these varieties for their morphological and biomass yield characteristics.

Results of two years of study indicate that all varieties of *Miscanthus* performed well, and no plant mortalities were observed over two years of *Miscanthus* growth and development. Varieties in this experiment significantly differed from each other for their senescence time and rate. In first autumn of growth and development clear senescence was not observed in *Miscanthus* varieties except AM-905 and AM-903, but during the second year of growth all Amuri varieties, except AM-904, did show clear senescence as indicated by declines in A and g_s . Our study results revealed that all varieties responded to the rise or fall in air temperature, but the level of this response was different for each variety. For example, AM-905, AM-903, AM-900, AM-985 seemed to respond rapidly to a drop in air temperature and in both years these varieties started senescence early in autumn and had the lowest A , g_s and leaf [N] before the first killing frost. On the other hand $M \times g$ -IL, $M \times g$ -Nagara, MF-901, AM-904 and M-912 maintained higher rates of A and g_s and leaf [N], indicating a slower senescence rate, until the first killing frost, when their above-ground plant

parts died. This phenomenon possibly halted the translocation of nutrients from above-ground plant parts to the rhizomes and reducing their capacity for regrowth in spring.

Although there were no significant differences among varieties for their below-ground biomass yields, the mean data for below-ground biomass yields (Chapter 3, Fig. 6) suggests that the Amuri varieties AM-905 and AM-903, which have a trend of early senescence, might yield more below-ground biomass in the second growing season than other varieties. Though these two varieties did not produce the most above-ground biomass at the end of the second growing season, they did have the highest above-ground biomass at the end of first growing season.

The varieties used in this project also significantly differed from each other for their morphological characters. Being a biomass crop, the important yield components in *Miscanthus* are tiller density, plant height, stem diameter, leaf width and the number of leaves. One or more of these factors could contribute greatly for enhancing *Miscanthus* biomass yield.

More studies should be done on growth and morphology of *Miscanthus* varieties to understand long term results, and the same work should also be done in other areas with cold winters to see how these varieties perform there.

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