

## Miscanthus: A Promising Biomass Crop

EMILY A. HEATON,<sup>\*,1</sup> FRANK G. DOHLEMAN,<sup>†,2</sup>  
A. FERNANDO MIGUEZ,<sup>\*</sup> JOHN A. JUVIK,<sup>‡</sup> VERA LOZOVAYA,<sup>‡</sup>  
JACK WIDHOLM,<sup>‡</sup> OLGA A. ZABOTINA,<sup>§</sup> GREGORY F. MCISAAC,<sup>||</sup>  
MARK B. DAVID,<sup>||</sup> THOMAS B. VOIGT,<sup>‡</sup> NICHOLAS N. BOERSMA<sup>\*</sup>  
AND STEPHEN P. LONG<sup>†</sup>

<sup>\*</sup>*Department of Agronomy, Iowa State University, Ames, IA, USA*

<sup>†</sup>*Energy Biosciences Institute, University of Illinois at  
Urbana-Champaign, Urbana, IL, USA*

<sup>‡</sup>*Department of Crop Sciences, University of Illinois at  
Urbana-Champaign, Urbana, IL, USA*

<sup>§</sup>*Department of Biochemistry, Biophysics and Molecular Biology, Iowa  
State University, Ames, IA, USA*

<sup>||</sup>*Department of Natural Resources and Environmental Sciences,  
University of Illinois at Urbana-Champaign, Urbana, IL, USA*

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<sup>1</sup>Corresponding author. E-mail: [heaton@iastate.edu](mailto:heaton@iastate.edu)

<sup>2</sup>Current address: Monsanto Company, St. Louis, MO, USA.

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## ABSTRACT

The C<sub>4</sub> grass *Miscanthus × giganteus* is of increasing interest as a biomass feedstock for renewable fuel production. This review describes what is known to date on *M. × giganteus* from extensive research in Europe and more recently in the US. Research trials have shown that *M. × giganteus* productivity is among the highest recorded within temperate climates. The crop's high productivity results from greater levels of seasonal carbon fixation than other C<sub>4</sub> crops during the growing season. Genetic sequencing of *M. × giganteus* has identified close homology with related crop species such as sorghum (*Sorghum bicolor* (L.) Moench) and sugarcane (*Saccharum officinarum* L.), and breeding of new varieties is underway. *Miscanthus × giganteus* has high water use efficiency; however, its exceptional productivity causes higher water use than other arable crops, potentially causing changes in hydrology in agricultural areas. Nitrogen use patterns are inconsistent and may indicate association with N fixing microorganisms. *Miscanthus × giganteus* has great promise as an economically and ecologically viable biomass crop; however, there are still challenges to widespread commercial development.

## I. INTRODUCTION

### A. A ROLE FOR BIOMASS CROPS

Increasing the share of world energy that comes from renewable sources is critical to stabilizing the global climate (IPCC, 2007). Among renewable energy sources, only biomass can provide fuel and electricity in a form and scale that is compatible with existing transportation and power generation infrastructure (DOE, 2006). Unlike wind and solar energy, biomass can be converted directly

into liquid fuel by a variety of conversion routes, as is current practice with petroleum, or it can be stored to generate electricity on-demand, as is the current practice with coal. It also provides raw material for renewable alternatives to fossil-based products. Biomass is also the only available source of renewable carbon for products currently made from fossil carbon sources.

How much biomass is needed? Of the 105 exajoules (EJ,  $10^{18}$  J) of energy consumed in the US in 2008, only 4% or 4.1 EJ came from biomass sources, mainly from combustion of wood residues for heat and power by paper manufacturers (DOE, 2009). Energy consumption is expected to increase by 14% by 2034, to 120.8 EJ (DOE, 2010). Multiple acts of legislation currently under consideration in the US could further increase renewable energy demand 10–40%, leading it to comprise 14% of the total US energy demand, or 17 EJ  $y^{-1}$ , by 2035 (DOE, 2010).

Over 900 million Mg of biomass per year is needed to produce 17 EJ  $y^{-1}$ , assuming biomass to contain 18 MJ  $kg^{-1}$  (Jenkins *et al.*, 1998) and energy conversion to be 100% efficient. Of course, conversion of biomass energy into useful forms like liquid fuels or electricity is not 100% efficient, and typical efficiencies range between 30% and 70%, depending on methods and accounting (Brown, 2003; Jenkins *et al.*, 1998; Mohan *et al.*, 2006). Assuming an average conversion efficiency of 50%, the US will require more than 1.8 billion Mg of biomass per year to meet renewable energy demands through bioenergy, or a little more than 50% of the entire US maize crop in 2009 (NASS, 2010).

Even if only a portion of US renewable energy comes from biomass, it will still have a major impact on cultivated and natural lands. The feasibility and impact of large-scale biomass production have been intensely debated and investigated in recent years (Dohleman *et al.*, 2010; Dornburg *et al.*, 2010; Fargione *et al.*, 2008; Hertel *et al.*, 2010; Hill *et al.*, 2009; Kim *et al.*, 2009; Levasseur *et al.*, 2010; Reijnders, 2010; Smeets and Faaij, 2010; Solomon, 2010; Taheripour *et al.*, 2010). Despite a wide range of conclusions, it is generally agreed that (1) resources are limited and (2) future agricultural systems must be sustainable.

## B. FOOD VERSUS FUEL AND THE CASE FOR HIGH-YIELDING CROPS

It is reasonable to propose that crops that produce high biomass yields per unit land area be used to meet bioenergy demand, since they will require less land than low-yielding crops, and this is a key principle of biomass crop development (Heaton *et al.*, 2008b). For example, the high-yielding perennial *Miscanthus × giganteus* could require 87% less land to produce the same amount of biomass as a low-input, high-diversity mixture of prairie species,

because the yield of the *M. × giganteus* monoculture is nearly eightfold greater (Heaton *et al.*, 2008a). However, while yield might be a driving selection criterion, it is not the only one, and future crop systems must be evaluated on their environmental and social functions, in addition to traditionally valued economic functions (Boody *et al.*, 2005; Schulte *et al.*, 2006).

Diverse cropping systems that fill all available environmental niches can provide more ecosystem services such as nutrient cycling, water retention and filtration and biodiversity than annual monocultures, but they are inherently more difficult to manage for biomass production because each species prefers different conditions in a given year (Russelle *et al.*, 2007; Tilman *et al.*, 2006). High-yielding perennials that are on the field for most of the year can offer a compromise by simplifying crop management over diverse mixtures while still providing ecosystem services (Heaton *et al.*, 2004b; Schmer *et al.*, 2008).

### 1. Sustainability

‘Sustainable’ has many definitions, most of them contentious with reference to agriculture. A useful metaphor to discuss sustainability is the ‘sustainability stool’. The legs of the stool are environmental, economic and social sustainability; if an agricultural system has inadequate performance in any of the three areas, the system will eventually collapse (Douglass, 1984). Perennial energy crops potentially can provide a solid foundation for sustainability with performance that is equal to or improved over that of annual arable crops.

*a. Economic sustainability.* Of the three legs of the sustainability stool, economic sustainability of agriculture receives the most attention. Globally, there has been a trend away from diverse crop rotation to simplified annual crop systems that has been accompanied by increases in yield and farm labour productivity, made possible through increased reliance on synthetic fertilizer, pesticides and subsidy payments for crops in surplus (Bullock, 1992; Malezieux *et al.*, 2009; Schulte *et al.*, 2006; Tegtmeier and Duffy, 2004). Beginning with the Soil Conservation Act of 1935, the US government has, like many developed countries, paid farmers to set aside land from arable cropping, and instead plant it to perennials as a soil conservation tool. As demand grows for highly productive land to produce food, feed, fibre and now fuel, however, the value of these government programmes fades in comparison to what a farmer can earn by producing a subsidy-protected grain crop.

Traditionally, it has been difficult and nebulous to value the ecosystem services provided by perennial agriculture (Farber *et al.*, 2002; Liu *et al.*, 2010; Porter *et al.*, 2009), and without a harvested product for sale,

perennials usually lose against annual crops in the marketplace. With the advent of a clear demand for energy from perennial biomass, farmers and conservationists may have their cake and eat it too, as the crops grown can be harvested and sold for a profit while still providing ecosystem services similar to those from set-aside land.

How does the economic return of biomass crops compare to that of traditional arable crops in the US? James *et al.* (2010) calculated the break-even price for a farmer in the Midwestern US to switch to a range of perennial energy crops and found that currently, none was economically viable against continuous maize production on highly fertile land. However, they evaluated *M. × giganteus* using current prices for rhizomes (\$1.80 ea) and a future price anticipating improved production practices (\$0.05 ea) and found that of all the crops evaluated, future *M. × giganteus* is more profitable than continuous maize, with a break-even price of only \$45 Mg<sup>-1</sup> (James *et al.*, 2010). In on-farm trials with co-operators in Nebraska, South Dakota and North Dakota, Perrin *et al.* (2008) found that switchgrass could be grown at a commercial scale for about \$50 Mg<sup>-1</sup>. By comparison, the costs for continuous maize production on prime farmland in Iowa are about \$150 Mg<sup>-1</sup> in 2010 (Duffy, 2010), suggesting that perennial crops are profitable and will be economically sustainable even on prime farmland in the US.

*b. Environmental sustainability.* Perennial plants have long been associated with good environmental performance and improved ecosystem health. Without the disturbance of annual soil tillage above- and below-ground biomass accumulates, perennials protect and hold the soil against wind and water erosion while increasing soil quality and organic matter (Blanco-Canqui, 2010; Luo *et al.*, 2010). An increased proportion of perennials in the landscape are also associated with an increase in biodiversity, as perennials provide habitat for animals and insects (Malezieux *et al.*, 2009; Schulte *et al.*, 2006). Additionally, perennial crops can increase the quantity and diversity of mineral nutrients available in the rhizosphere by establishing complex and often long-term relationships with the microbial community (Davis *et al.*, 2010; Nehls *et al.*, 2010).

The larger and active root system of perennial grasses is particularly effective at scavenging available nutrients and preventing them from leaching with draining water where they may act as pollutants (Allan, 2004; Randall *et al.*, 1997). In the US Environmental Protection Agency's recent Science Advisory Board report on hypoxia in the Gulf of Mexico, the high losses of nitrate from current corn-soybean production systems on tile-drained landscapes in the Mississippi River Basin were clearly identified as a major source of the nutrients causing hypoxia (EPA, 2008). These losses occurred even

when best management practices were applied. In that report, it was suggested that perennials were the best option to substantially reduce nitrate losses, but such a shift was unlikely, given current agricultural policies.

In a more specific example of how nitrate losses from current production systems could be reduced using perennials, [Hatfield \*et al.\* \(2009\)](#) evaluated a watershed in central Iowa. They observed that mean annual  $\text{NO}_3\text{-N}$  concentrations in water have been increasing since 1970 in spite of no significant change in N fertilizer use for the past 15 years, and a decrease in cattle and hog production in the watershed. Upon evaluation of regional crop yields, land-use change and precipitation, they found that an increase in land planted to maize and soybean, at the expense of perennial pasture, were highly correlated with the increase in  $\text{NO}_3\text{-N}$  concentrations. The authors concluded that the narrow window of nutrient uptake in maize–soy systems allowed more nutrients to leave the system, even though the amount of fertilizer applied was steady and crop yields were increasing. One suggested solution to reduce nutrient loading in the watershed was to plant more perennials with water use patterns that complement those of maize–soy ([Hatfield \*et al.\*, 2009](#)).

*c. Social sustainability.* Biomass energy may help revitalize languishing rural economies ([Solomon, 2010](#)). Even as industrial agriculture has delivered record crop yields and gross revenue in the past 50 years, farmer employment and profit have deteriorated ([Fig. 1](#)). The US Department of Agriculture (USDA) reports that a rural society that used to be characterized by small farms supported by farm sales has changed to large, concentrated farms, and over 40% of documented farms are in the ‘residential/lifestyle’ category. While the majority of US farms are still small farms, over 50% of their operators are retired or rely on another job as their principal occupation ([NASS, 2007](#)). Conversely, large farms, that is, those with revenue over \$100,000 per year, comprise only 15% of all US farms, yet account for 88% of sales. In short, only a fraction of farmers can still make a living from farming ([Duffy, 2008](#)), and this is reflected in the steady decline of rural populations ([US Census Bureau, 1990](#)).

Job creation in the renewable energy economy supports the social sustainability of biomass cropping systems. A review of clean energy finance by the Pew Charitable Trust found global investment up by 230% since 2005, despite the largest economic downturn in at least 50 years, and clean energy investments are expected to grow to \$200 billion by 2010 ([The Pew Charitable Trusts, 2010](#)). ‘Green jobs’ have been touted as the solution to the economic and environmental woes of many countries, and have received priority in economic recovery spending. Despite inconsistent government

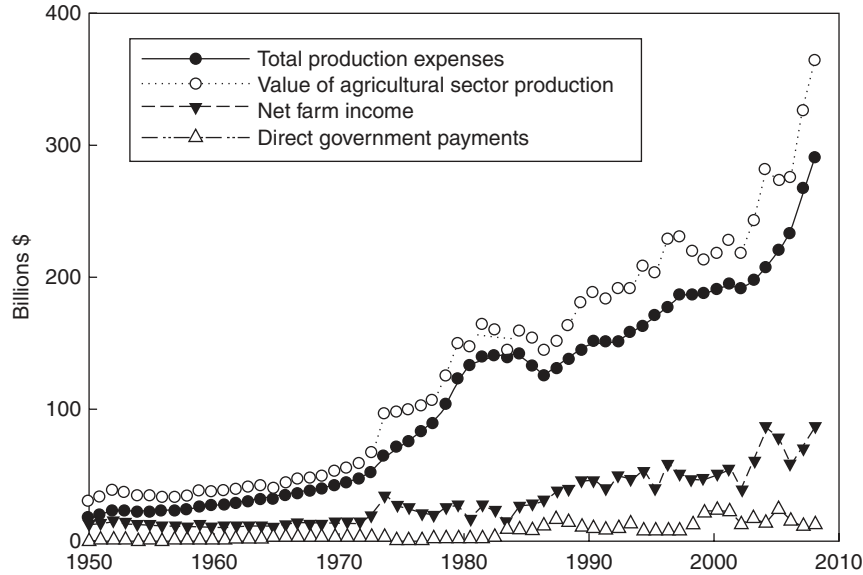


Fig. 1. The value of U.S. agricultural production, total production expenses, net farm income and direct payments from the government, that is, subsidies, from 1949 through 2008 (USDA/ERS, 2010).

support, there are already more green jobs than biotechnology-related jobs, though biotech has seen steady government support (Fig. 2) (The Pew Charitable Trusts, 2009).

The low bulk density of biomass makes it inherently inefficient to transport (Fales *et al.*, 2007; Rentizelas *et al.*, 2009; Shinnars and Binversie, 2007), necessitating local processing and handling, thus ensuring distributed jobs within regions irrespective of the fuel produced. In an analysis of case studies in Brazil and the Ukraine, Smeets and Faaij (2010) found that instilling a ‘strict’ set of sustainability criteria, for example, restriction of child labour, education of the workforce and mandatory healthcare, had positive community impacts with only a limited effect on the cost of bioenergy production from perennials. This was largely attributed to the reduced costs of perennial agriculture compared to annual row cropping systems.

### C. A ROLE FOR *MISCANTHUS* × *GIGANTEUS*

#### 1. Origins and uses

*Miscanthus* is a genus comprising 14–20 species of perennial, C<sub>4</sub> grasses native to eastern Asia, N. India and Africa (Clayton *et al.*, 2008; Hodkinson *et al.*, 2002a; Scally *et al.*, 2001). As described in a review by

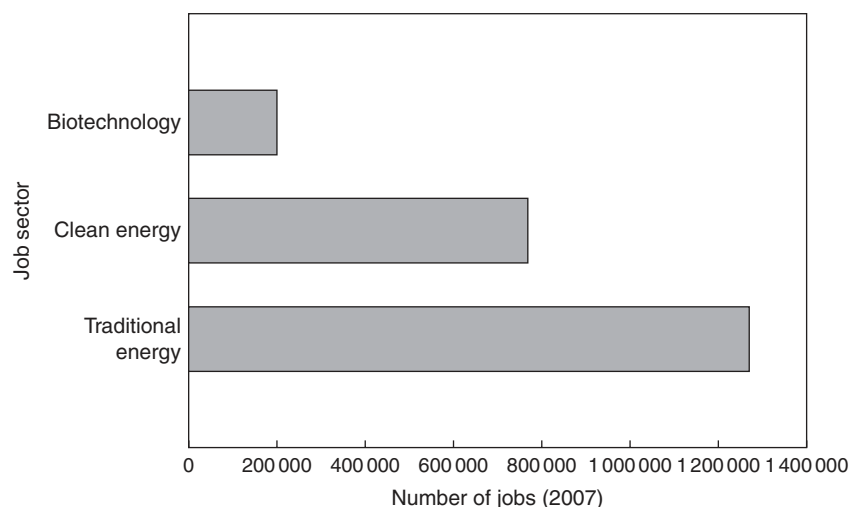


Fig. 2. Number of U.S. jobs in biotechnology, clean energy and traditional energy industries in 2007 (Pew Charitable Trust, 2009).

Stewart *et al.* (2009), *Miscanthus* species have long been used for grazing and structural materials in China and Japan and have only recently become of interest for energy. Long recognized for their ornamental value, and as a germplasm source of stress tolerance in sugarcane breeding, *Miscanthus* species are now found and commonly naturalized in North and South America as well as in Europe, Africa, Asia and Europe (Clayton *et al.*, 2008; Scally *et al.*, 2001).

In 1935, Aksel Olsen brought a sterile *Miscanthus* hybrid that was of horticultural interest back from Yokohama, Japan to Denmark, where it was cultivated by Karl Foerster and observed to have vigorous growth (Lewandowski *et al.*, 2000; Linde-Laursen, 1993; Scally *et al.*, 2001). Originally named *Miscanthus sinensis* 'Giganteus' hort. (Greef and Deuter, 1993), it has gone by many names, including *M. giganteus*, *M. sinensis* Anderss. 'Giganteus' and *M. ogiformis* Honda (Hodkinson *et al.*, 2002c). By using DNA sequencing, AFLP and fluorescent *in situ* DNA hybridization, Hodkinson *et al.* (2002c) confirmed suspicions that it was an allotriploid ( $2n = 3x = 57$ ) hybrid of *M. sinensis* and *Miscanthus sacchariflorus* and subsequently formally classified it with the Royal Botanic Gardens, Kew in the UK as *M. × giganteus* (Greef & Deuter ex Hodkinson & Renvoize) (Hodkinson *et al.*, 2002b).

Following concern over fossil fuel dependence beginning in the 1970s, *M. × giganteus* was evaluated along with several other species for potential as a bioenergy crop. The sterile clone from trials in Hornum, Denmark was



spread across Europe, and included in both public and private trials (Jorgensen and Schwarz, 2000; Lewandowski *et al.*, 2000).

## 2. Overview of *Miscanthus* research history

*Miscanthus* × *giganteus* has been studied across Europe since 1983 under a multitude of national and EU programmes (Jones and Walsh, 2001a; Lewandowski *et al.*, 2000). Two EU-wide projects, the *Miscanthus* Productivity Network (MPN) and the European *Miscanthus* Improvement (EMI), have been particularly influential on the availability of *Miscanthus* data today (Fig. 3).

In 1992, the 3-year MPN began as part of the European Agro-Industry Research programme (contract no. AIR1-CT92-0294). With 17 partners in 10 countries, the MPN aimed to ‘...generate information on the potential of *Miscanthus* as a non-food crop in Europe’, (Jones and Walsh, 2001b). Most trials used similar methods to assess potential productivity associated with water, nitrogen and low temperature limitation across different environments. Harvest, storage and utilization of biomass were also studied, along with genotype screening of other *Miscanthus* species. Generally, the MPN found *M.* × *giganteus* to be broadly adapted to a wide range of growing

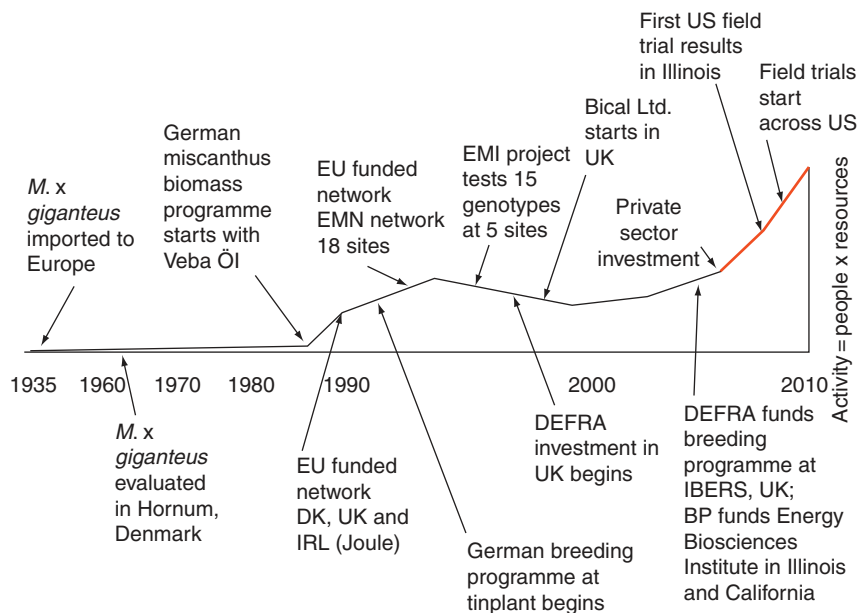


Fig. 3. Timeline of key activities in the investigation of *Miscanthus* as a biomass crop, adapted courtesy of J. Clifton-Brown.

conditions, but was not the optimal choice in all locations tested (McCarthy, 1992). For a complete description of MPN results, see Jones and Walsh (2001a).

Following on from the MPN, the EMI project began in 1997 to address the limitations imposed by a narrow genetic base within *M. × giganteus* clones and better match genotypes with environments (Lewandowski and Clifton-Brown, 1997). Similar in structure to the MPN, EMI focused on crop improvement by developing breeding methods and assessing the genotype × environment interaction of 15 selected *Miscanthus* genotypes in five countries (Clifton-Brown *et al.*, 2001a). The EMI project successfully identified genotypic variation in environmental performance among *Miscanthus* genotypes and has paved the way for current private and public breeding programmes in the US and Europe (Clifton-Brown *et al.*, 2008).

In contrast to Europe, *Miscanthus* species were not included in initial screening of potential biomass crops in the US. There, research, supported primarily by the US Department of Energy (DOE), focused on switchgrass (*Panicum virgatum* L.) as a model herbaceous species beginning in the 1980s (McLaughlin, 1992; Parrish and Fike, 2005; Sanderson *et al.*, 1996). In fact, it was not until 2004 that Heaton *et al.* (2004b) used the model MISCANMOD, developed by Clifton-Brown *et al.* (2000) in Ireland, to project potential *M. × giganteus* productivity in the US. Following promising modelled productivity, Heaton *et al.* (2004a) Heaton *et al.* went on to show that *M. × giganteus* was likely to produce more biomass per unit input of water, nitrogen or heat, than would switchgrass under the same conditions, and thus field research in the US was warranted. Superior yield of *M. × giganteus* over switchgrass was later confirmed in the first replicated trials of *M. × giganteus* in the US, at three sites in Illinois where measured yields of *M. × giganteus* were two- to fourfold higher than those of switchgrass, var. Cave-In-Rock (Heaton *et al.*, 2008a).

#### D. STRUCTURE OF THIS REVIEW

Following promising initial results, a Strategic Research Initiative (SRI) was initiated at the University of Illinois at Urbana-Champaign to further investigate *M. × giganteus* in Illinois. Initial work by 14 investigators focused on a clone of *M. × giganteus* collected by the Chicago Botanic Garden and brought to the Urbana, Illinois campus in 1988 where it had thrived in a demonstration planting (Heaton *et al.*, 2008a). This review will highlight research areas addressed by the SRI through support from the Illinois Council on Food and Agriculture Research from 2004 to 2009 (award 04-SRI-036) (Long, 2005). Research in Illinois has expanded exponentially

in recent years, and has been the provenance of work on *Miscanthus* in the US, which has grown from non-existence 10 years ago to being underway in nearly every state today.

Focusing on *M. × giganteus*, this review will address modelled and observed productivity (Section II), the physiological basis for that productivity (Section III), breeding and genetic engineering efforts (Section IV), the environmental impacts of production (Section V) and the technical challenges to commercial production (Section VI).

## II. PRODUCTIVITY

### A. EUROPEAN AND US TRIALS

Here, we review the biomass production of *M. × giganteus* reported from trials over a wide geographic range, with emphasis on how yield varies with precipitation, temperature and soil conditions. While other reviews of *Miscanthus* productivity and suitability can be a good source of data that might be otherwise difficult to find (Jones and Walsh, 2001a; Lewandowski *et al.*, 2000; Miguez *et al.*, 2008; Smeets *et al.*, 2009; Zub and Brancourt-Hulmel, 2010), our goal here is to provide an overview of *M. × giganteus* productivity, key factors that influence it and how it may be modelled and predicted.

Productivity of *M. × giganteus* has been tested in field trials across Europe since 1983 under a multitude of national and EU programmes (Lewandowski *et al.*, 2003b). Only a portion of the numerous academic and industrial field trials that have been conducted is reported in English and published in easily accessed, peer-reviewed publications. Most studies cover a 2–5-year growth period, even though the lifetime of a *M. × giganteus* stand can range from 15 to 30 years (Hastings *et al.*, 2009a; Heaton *et al.*, 2004b), and only a few studies have followed *M. × giganteus* growth over a longer term, for example, 10 or more years (Christian *et al.*, 2008; Clifton-Brown *et al.*, 2007).

*Miscanthus × giganteus* is not typically harvested in the year, it is planted because of low yields and possible negative impacts on survival during the crop's critical first winter. Generally, winter kill is only a problem in the first season; if a plant makes it through the first winter, it will nearly always survive subsequent winters, even if they are much harsher (Clifton-Brown *et al.*, 2001b; Heaton *et al.*, 2008a; Lewandowski *et al.*, 2000). A typical growing season for a mature stand of *M. × giganteus* is shown in Fig. 4. Shoots emerge from the rhizomes in early spring when soil temperatures are between 6 and 10 °C. Though leaves can extend at lower temperatures, 10 °C is considered the standard for consistent leaf extension (Clifton-Brown and

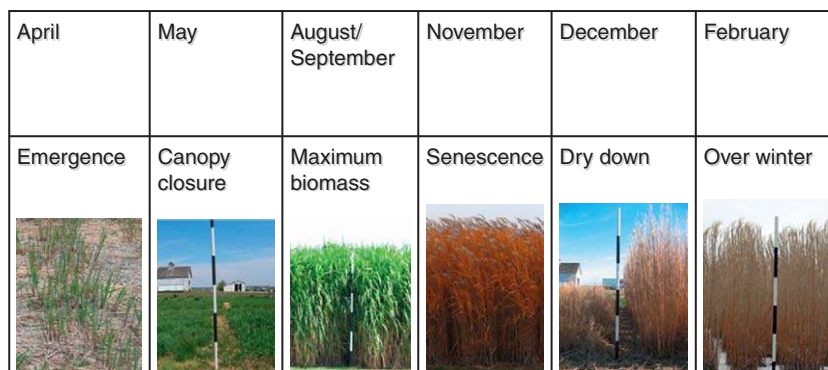


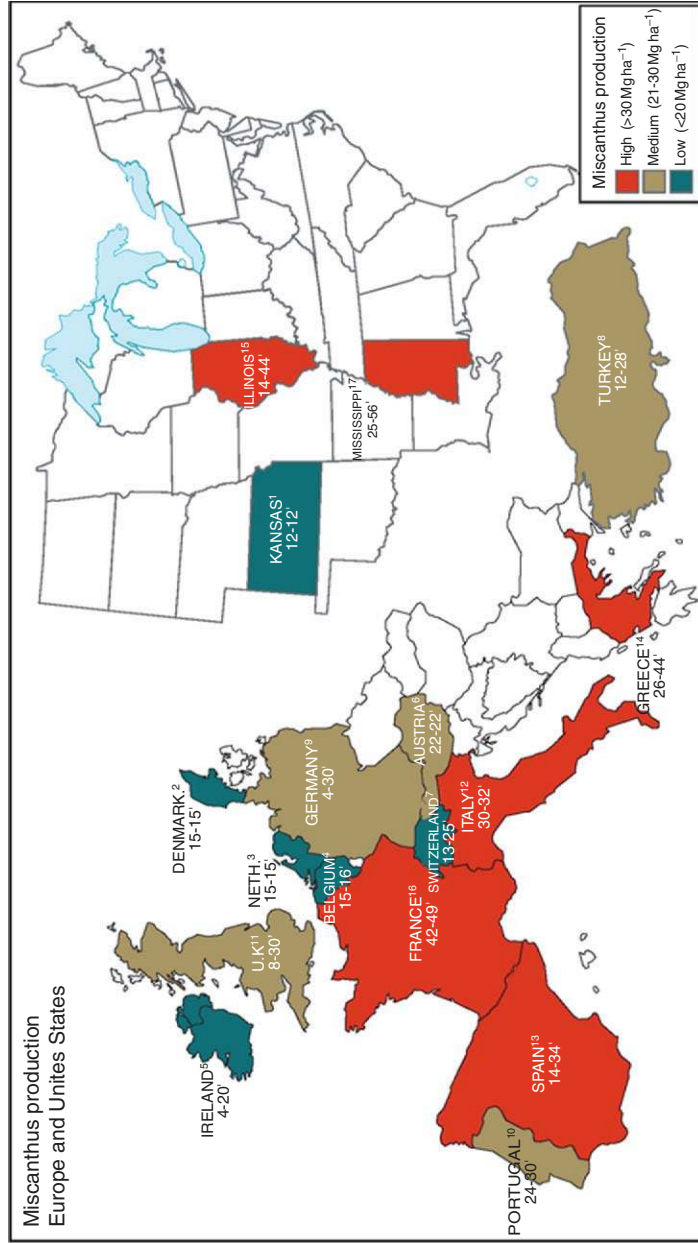
Fig. 4. Annual growth cycle of a mature *M. × giganteus* stand. Typically 30% of peak biomass can be lost during senescence and overwintering as leaves drop and nutrients are remobilized to rhizomes.

Jones, 1997; Hastings *et al.*, 2009a; Zub and Brancourt-Hulmel, 2010). Biomass rapidly accumulates through summer, peaking around September (Beale and Long, 1997; Heaton *et al.*, 2008a). Though biomass yields are highest in late summer, so are moisture contents and nutrient take-off rates. The crop is typically harvested after senescence and associated nutrient remobilization and crop drying have occurred, that is, in the period after a killing frost but prior to spring growth. For example, the harvest window in Illinois is between November and March, depending on the demand for feedstock and ability to access the field under snowy winter conditions.

During senescence, 30–50% of harvestable dry matter can be lost as leaves drop (Clifton-Brown *et al.*, 2007; Heaton *et al.*, 2008a) and nutrient reserves are translocated to the perennating rhizomes (Section III) (Beale and Long, 1997; Dohleman, 2009). Efficient translocation not only helps to ensure adequate nutrient supply for growth in the following season, thus reducing the need for additional fertiliser (Himken *et al.*, 1997; Lewandowski and Kicherer, 1997), it also minimises the inorganic compounds in the harvested feedstock that could become pollutants in fuel (Jenkins *et al.*, 1998).

### 1. Productivity overview

Overall, studies show that the range of harvestable *M. × giganteus* yields to be between 5 and 55 Mg ha<sup>-1</sup> (Fig. 5), making it one of, if not the most, productive land plants in temperate climates. The underlying physiologic basis of this exceptional productivity is discussed in the next section (Section III); here, we outline the results of geographically diverse yield trials.



<sup>1</sup> Propher *et al.* (2010); Scott Staggenborg, unpublished data; Lewandowski *et al.* (2000)  
<sup>2</sup> Lewandowski *et al.* (2000)  
<sup>3</sup> Clifton-Brown *et al.* (2000); Clifton-Brown *et al.* (2004)  
<sup>4</sup> Clifton-Brown *et al.* (2000)  
<sup>5</sup> Clifton-Brown *et al.* (2007)  
<sup>6</sup> Lewandowski *et al.* (2000)  
<sup>8</sup> Lewandowski *et al.* (2000); Acaroglu and Aksoy (2005)  
<sup>9</sup> Lewandowski *et al.* (2000)  
<sup>10</sup> Clifton-Brown *et al.* (2000); Clifton-Brown *et al.* (2004)  
<sup>11</sup> Beale and Long (1997)  
<sup>12</sup> Lewandowski *et al.* (2000); Mantinea *et al.* (2009)  
<sup>13</sup> Lewandowski *et al.* (2000)  
<sup>14</sup> Lewandowski *et al.* (2000)  
<sup>15</sup> Heaton *et al.* (2008)  
<sup>16</sup> Tayot *et al.* (1995); Clifton-Brown *et al.* (2004)  
<sup>17</sup> Batchelor and Steele (2009)

Fig. 5. Map of *M. × giganteus* trials and the range of biomass yields reported in the peer-reviewed scientific literature.

Numerous studies have evaluated *M. × giganteus* in England and Ireland. [Beale and Long \(1997\)](#) measured above- and below-ground productivity of 3–4-year-old stands of *M. × giganteus* and another C4 perennial grass, *Spartina cynosuroides* in England at 51° N latitude. The above-ground biomass of *M. × giganteus* peaked at 30 Mg ha<sup>-1</sup> in mid-September, then declined to about 20 Mg ha<sup>-1</sup> by February, while *S. cynosuroides* reached peak production in August (~20 Mg ha<sup>-1</sup>) but maintained that level throughout the fall and early winter as leaves were retained in the aerial biomass instead of dropping as in *M. × giganteus*. Below the ground, the biomass of rhizomes and roots extracted from a 20-cm depth was found to vary over the course of the year in a pattern suggestive of nutrient mobilization, with depletion of biomass in the late spring and early summer, followed by increases that peaked around the end of the growing season in early November ([Beale and Long, 1997](#)). This pattern has also been observed in *M. × giganteus* in Germany ([Himken et al., 1997](#)) and the US ([Dohleman, 2009](#)).

Two of the rare longer-term trials of *M. × giganteus* productivity were conducted in England at the Rothamsted Research Farm (51°48'N) and in Cashel Co., Ireland (52°39'N). In the cool climates at both locations, harvestable biomass continued to increase during the first 5 years of growth ([Christian et al., 2008](#); [Clifton-Brown et al., 2007](#)). This is in contrast to warmer locations where ceiling yields were achieved in 2–3 years ([Clifton-Brown et al., 2001a](#); [Heaton et al., 2008a](#)). Changes in harvestable biomass following achievement of plateau yields were attributed to annual weather conditions, namely moisture availability, and possibly to K deficiencies, as K take-off rates were high in both fertilized and unfertilized treatments. In Ireland, soil and tissue analysis indicated K to contribute more to observed yield increases than N or P fertilizer ([Clifton-Brown et al., 2007](#)), which showed no significant impact at either location.

Germany was the home to some of the largest plantations of *M. × giganteus* in the 1990s, though losses of micropropagated plants during the first winter ([Jorgensen and Schwarz, 2000](#)) tempered enthusiasm and slowed *M. × giganteus* adoption across the country. Multiple trials in Germany have shown good potential for *M. × giganteus* as well as *M. sinensis* varieties for biomass production ([Clifton-Brown and Lewandowski, 2002](#); [Clifton-Brown et al., 1998](#); [Clifton-Brown et al., 2001a](#)) with efficiencies of nutrient and energy use that are comparable to or better than willow (*Salix* spp.), switchgrass, reed canary grass (*Phalaris arundinacea*) and maize ([Boehmel et al., 2008](#); [Lewandowski and Schmidt, 2006](#)).

In the warmer, drier climates of Italy, Greece, Portugal and Turkey, *M. × giganteus* has produced >35 Mg ha<sup>-1</sup> under irrigation and N fertilization. Such locations present the best documentation of *M. × giganteus* yield

response to N fertilizer (Acaroglu and Aksoy, 2005; Ercoli *et al.*, 1999), though the response is still not consistent (Danalatos *et al.*, 2007; Mantineo *et al.*, 2009), as reviewed by Miguez *et al.* (2008). Without irrigation, *M. × giganteus* is not likely to be viable in dry Mediterranean climates, though some types of *M. sinensis* are promising (Clifton-Brown *et al.*, 2001a; Danalatos *et al.*, 2007).

Yield trials of *M. × giganteus* started much later in the US, where the focus was on switchgrass instead of *M. × giganteus* as a model herbaceous energy crop (Heaton *et al.*, 2004b). The first replicated trials were conducted in the Midwestern US where trials at three locations in Illinois (37°45'N–41°85'N) demonstrated some of the highest productivity on record, with average harvestable yields of 30 Mg ha<sup>-1</sup> without irrigation and only 25 kg ha<sup>-1</sup> of N fertilizer applied in one season (Heaton *et al.*, 2008a). Such high yields, 2–4 times those of the regionally adapted Cave-In-Rock switchgrass, even under a low-input management scheme, stimulated considerable interest in *M. × giganteus* in the US. Furthermore, the sterile nature of this clone is considered particularly advantageous in of light invasion potential from new biomass crops (Barney and Ditomaso, 2008; Jakob *et al.*, 2009).

Dohleman and Long (2009) demonstrated that *M. × giganteus* is 60% more productive than maize, even in the heart of the US 'Corn Belt'. How is this possible? Even though maize had higher light-saturated photosynthetic rates as well as higher rates of primary carboxylation and substrate regeneration, *M. × giganteus* had more leaf area and a longer canopy duration, allowing it to assimilate more carbon into biomass over the entire growing season (Dohleman and Long, 2009). A similar result has been observed in Kansas, where *M. × giganteus* has yielded more than maize (12.8 Mg ha<sup>-1</sup> vs. 10.1 Mg ha<sup>-1</sup>), though yields of both crops were half of those realized by the photoperiod-sensitive *S. bicolor* (Propheter *et al.*, 2010).

Why has such large variation been observed in such genetically similar material? In the following sections, we explore the relationship of biomass yield to environmental factors that might limit it.

## 2. Water

As a C<sub>4</sub> crop, *M. × giganteus* has a high efficiency of water use, typically requiring between 100 and 300 l of water to produce 1 kg of biomass (Beale *et al.*, 1999; Lewandowski *et al.*, 2000; Mantineo *et al.*, 2009). For comparison, typical values for an annual maize or sorghum crop are near the upper end of this range, around 300 l kg<sup>-1</sup> (Hanson and Hitz, 1983; Howell *et al.*, 1998). At high yields, efficient use of water does not necessarily confer low water use overall, and there is some concern that water availability will limit the use of *M. × giganteus* (Richter *et al.*, 2008). For instance, an



*M. × giganteus* crop of 25 Mg ha<sup>-1</sup> at an average water use efficiency would require 200 l kg<sup>-1</sup> × 25,000 kg = 5,000,000 l or the equivalent of 500 mm of rainfall during the growing season. More information on water use by *M. × giganteus* can be found in [Section V](#).

While *M. × giganteus* has been shown to have a root–shoot ratio of approximately 1 to 1 ([Dohleman, 2009](#)) and roots that extend down at least 2 m ([Neukirchen \*et al.\*, 1999](#)), it does not appear that *M. × giganteus* draws water from this entire depth. Studies of soil water depletion in England and Italy show that *M. × giganteus* obtains most of its water from the top 1.5 m of the soil profile, leading [Finch and Riche \(2008\)](#) to suggest that 1.7 m should be considered the ‘effective maximum rooting depth’ concerning soil moisture ([Beale \*et al.\*, 1999](#); [Finch and Riche, 2008](#); [Monti and Zatta, 2009](#)). [Beale \*et al.\* \(1999\)](#) found that *M. × giganteus* grown in southern England extracted most of its water from the first 0.8 m of the soil profile and 90% of the root biomass was found in the first 0.5 m of the soil profile. In this study, the water use efficiency of *M. × giganteus* was higher than another C<sub>4</sub> grass, *S. cynosuroides*, but the higher productivity of *M. × giganteus* was mainly due to its extended growing period, since the flowering of *S. cynosuroides* commenced in early July and *M. × giganteus* remained vegetative until September, reaching 29 Mg ha<sup>-1</sup> of dry biomass in an irrigated field. In a container experiment, [Clifton-Brown and Lewandowski \(2000b\)](#) found *M. sacchariflorus* to have the highest water use efficiency (4.1 g DM kg<sup>-1</sup> H<sub>2</sub>O) when compared to *M. sinensis* and *M. × giganteus*. They concluded that *M. sinensis* would be more suitable to drier environments than the other two genotypes since it responded early to drought stress by reducing stomatal conductance and leaf growth ([Clifton-Brown and Lewandowski, 2000b](#)).

Quantification of the impact of soil moisture deficit on biomass yield has been attempted within single experiments, but is perhaps best summarized by the inputs into MISCANFOR, a crop growth model developed for *M. × giganteus* and a stress-tolerant *M. sinensis* variety ([Hastings \*et al.\*, 2009a,b](#)).

MISCANFOR calculates the actual evapotranspiration (ET) a simulated crop would experience using a three-step process and available meteorological data. First, it considers evaporation of rainfall intercepted by the canopy, then leaf transpiration, which is in turn related to the leaf area index of the canopy and the limitation of available soil moisture, and finally, evaporation from the soil through diffusion from soil pores ([Hastings \*et al.\*, 2009a](#)). This process is more holistic than other approaches in that it considers the soil moisture holding capacity of soils in addition to precipitation/irrigation and ET, and has been used to estimate the likely growing range and productivity of *M. × giganteus* in Europe ([Hastings \*et al.\*, 2009b](#)).



### 3. Temperature

*a. Seasonal growth.* Typically, *M. × giganteus* begins to grow from dormant rhizomes when soil temperatures reach 10–12 °C, while leaves begin expanding after air temperatures average 5–10 °C (Clifton-Brown and Jones, 1997; Farrell *et al.*, 2006; Lewandowski *et al.*, 2000). Though chilling temperatures (below 12 °C) frequently limit productivity of C<sub>4</sub> crops (Long, 1999), *M. × giganteus* has proved an exception to this trend by remaining productive and with high quantum efficiencies of CO<sub>2</sub> assimilation, even at cool temperatures in the field (Beale and Long, 1995; Beale *et al.*, 1996; Dohleman *et al.*, 2009). When evaluating growth rates of different genotypes under low temperature in a controlled environment, Clifton-Brown and Jones (1997) found that *M. × giganteus* was also able to expand leaves more rapidly between 10 and 20 °C, allowing it to close canopy faster and yield more biomass, while Farrell *et al.* found genetic variation in temperature thresholds for emergence that should allow some genotypes to begin growing earlier in the season.

*b. Overwinter survival.* One of the major limitations to the production of *M. × giganteus* in temperate climates is consistent overwinter survival, particularly in the establishment year (Clifton-Brown and Lewandowski, 2000a; Farrell *et al.*, 2006; Heaton *et al.*, 2004b; Lewandowski *et al.*, 2000, 2003b). Currently, it is generally accepted that temperatures less than –3.4 °C are lethal to *M. × giganteus*, thus, this is the lower limit used to determine its potential distribution and productivity (Hastings *et al.*, 2009a,b).

Although Clifton-Brown and Lewandowski (2000a) identified –3.4 °C as sufficient to kill rhizomes removed from the field in an artificial freezing test, this temperature is not consistent with the observations in the US, where established *M. × giganteus* has regularly survived soil temperatures below –6 °C at a 10-cm depth (E. Heaton, unpublished data). Little work has yet to be done on the importance of cold acclimation to overwinter survival in *Miscanthus* species, but it is likely to be as important as it is in other cool-season perennial grasses, where a distinction is made between cold acclimation and freezing tolerance (e.g. Hulke *et al.*, 2008; Stier *et al.*, 2003; Zhou and Zhao, 2004).

### 4. Soil conditions

Generally, *M. × giganteus* performs well over a range of soil conditions when water is not limiting. Under water-limited conditions, it has performed best when planted in clay soils and worse when planted in sandy soils, likely to do with the higher water holding capacity of clay soils. Christian and Haase

(2001) report on Austrian trials aimed at testing the influence of soil type on the yield and stem number of *M. × giganteus*, where it was found that a good soil aggregate structure, as indicated by pore volume and size distribution, was more important than the soil type or pH. The authors concluded that the most suitable soils for *M. × giganteus* have an intermediate texture that allows good air movement, a high water holding capacity and high organic matter content (Christian and Haase, 2001). While they also assert that shallow soils reduce potential productivity, Clifton-Brown *et al.* (2007) found that *M. × giganteus* still produced 15–20 Mg ha<sup>-1</sup> y<sup>-1</sup> even when grown on marginal soil that had an effective rooting depth of 40 cm. Over a period of 15 years, productivity declined at this site, but was attributed to potassium deficiency and thus considered manageable. Heavy, waterlogged soils have also been shown to reduce plant height and delay achievement of plateau yields from 2 to 5 years in *M. × giganteus* (Christian and Haase, 2001).

In Germany, *M. × giganteus* was grown on two sandy soils, a silt-dominated soil and a clay-dominated soil. Above- and below-ground biomass was highest at the site with the silt soil, and *M. × giganteus* cropping was found to influence the soil organic matter (SOM) composition at all locations (Kahle *et al.*, 2001). Pre-harvest losses and harvest residues supplied 2.2–5 Mg C per year to the soil and lead to an increase of 0.5–1.2 g kg<sup>-1</sup> SOM per year, with higher contributions on the sandier soils. Further, it was found that *M. × giganteus* disproportionately enriched lipids, sterols and fatty acids in SOM that are less available for decomposition by soil microorganisms, thus increasing the hydrophobic components of SOM that are important for soil aggregation and stability and improving soil quality (Kahle *et al.*, 2001).

## B. CROP MODELLING

### 1. Productivity modelling

The potential of *M. × giganteus* as a dedicated bioenergy crop, as evidenced from the extensive network of European field trials (e.g. MPN), has been extended throughout the rest of Europe using semi-mechanistic crop models (Clifton-Brown *et al.*, 2000, 2004; Hastings *et al.*, 2009a; Miguez *et al.*, 2009; Price, 2004).

Initially, Clifton-Brown *et al.* (2000), using a simple model based on radiation use efficiency (RUE), simulated potential productivity of *M. × giganteus* for Ireland with yields ranging from 16 Mg ha<sup>-1</sup> in northern Ireland to 26 Mg ha<sup>-1</sup> in southern Ireland, where the total annual solar radiation and the length of the growing season are longer (Clifton-Brown *et al.*, 2000, 2001b). These predictions, however, were only based on radiation and temperature and ignored limitations due to water and nutrient

stress. Price (2004), using a similar approach that included effects due to water stress, estimated yields in the range 7–24 Mg ha<sup>-1</sup> for England and Wales. An important consideration that can be explored using crop models is the year-to-year variability in yields, which Price (2004) estimated to be 10–25%. This variability, which is typically poorly estimated from short-duration field trials, is a crucial component in planning for feedstock availability for a biorefinery.

Incorporating site-specific information about soil water availability and improving upon the previous version of the model, Clifton-Brown *et al.* (2004) showed that the water-limited potential for *M. × giganteus* biomass production in Europe ranged from 17 Mg ha<sup>-1</sup> in Sweden to 41 Mg ha<sup>-1</sup> in Portugal. Under non-limiting conditions, the highest estimated peak yield was of 60 Mg ha<sup>-1</sup>, which reflects the maximum potential of *M. × giganteus*, and it is close to the highest values measured in Italy and Greece (50 and 54 Mg ha<sup>-1</sup>, respectively). However, another important consideration in *M. × giganteus* productivity is the inevitable reduction in harvestable biomass between the peak biomass in the fall to that of late winter. Clifton-Brown *et al.* (2004) estimated this to be 0.36% loss per day and an average total of 33% by the late winter harvest.

Simple models, such as MISCANMOD, are valuable for assessing the potential of *M. × giganteus* productivity outside the range where it has been cultivated. However, there are limitations in its ability to extrapolate to other regions since the model strongly depends on a parameter that describes the efficiency of the crop in converting radiation to biomass (RUE, g MJ<sup>-1</sup>). Although in this model, RUE has been treated as a constant, Clifton-Brown *et al.* (2000, 2004) reported that the value of  $e_c$  for *M. × giganteus* ranged from 2.4 to 4.2 g MJ<sup>-1</sup> PAR in different environments. These authors recognized that the model depends strongly on RUE and that a more mechanistic model would be more appropriate (Clifton-Brown *et al.*, 2001b). Empirical models are appealing due to simplicity, but by their design, they cannot provide insights into the physiological basis of RUE variation, or growth and the physiology of water use.

The model MISCANMOD has been further refined and renamed MISCANFOR (Hastings *et al.*, 2009a), with improved descriptions of the relationship between potential and actual ET, which impacts calculation of water stress; variable RUE which depends on temperature, nutrient and water stress; and additional modifications that reflect recent findings in *M. × giganteus* physiology such as photoperiod sensitivity. Their results suggest that although *M. × giganteus* can be highly productive in southern Europe, a 20% variability in biomass productivity should be expected due to year-to-year fluctuations in weather patterns (Hastings *et al.*, 2009a).

To make more detailed predictions of *M. × giganteus* physiology and growth, a different type of model with a higher degree of mechanism is needed. WIMOVAC (Windows Intuitive Model of Vegetation response to Atmospheric and Climate Change) is a more suitable model as a guide to future experiments and breeding (Humphries and Long, 1995). It was shown that theoretically, *M. × giganteus* can increase its productivity by 4 Mg ha<sup>-1</sup> if the threshold temperature for growth could be lowered by 2 °C and degree-day requirements were increased so that flowering occurred uniformly (Clifton-Brown *et al.*, 2001b). Miguez *et al.* (2009) also showed that in addition to peak productivity, WIMOVAC was able to accurately simulate plant CO<sub>2</sub> uptake, leaf area index and biomass partitioning among leaf, stem, root and rhizome; this last part being limited by available data.

Although results from models are useful for evaluating the biomass potential of *M. × giganteus* in different regions, it is also important for crop models to integrate new information on plant growth and physiology generated in recent laboratory and field experiments. In addition, crop models can be used as an aid in breeding programmes if appropriate connections can be made between relevant traits that can be quickly phenotyped, included in crop models and evaluated for productivity (Boote *et al.*, 1996). Crop models are also the only tool available to produce estimates of *M. × giganteus* performance under future projected climate change scenarios or to evaluate the impact of increasing the land use devoted to bioenergy crops on carbon sequestration and reduction in greenhouse gas emissions (Clifton-Brown *et al.*, 2007; Davis *et al.*, 2010; Tuck *et al.*, 2006).

## 2. Modelling for crop improvement

Crop models can also be used as a guide for breeding programmes or as a means to envision a crop ideotype (Boote *et al.*, 1996). While simulation models can be used to predict appropriate trait phenotypes and selection protocols in breeding programmes to achieve ideotypes (Boote *et al.*, 1996), for a true integration of crop models and breeding, the inheritance of model parameters is required (Yin *et al.*, 2003). One objective that can be pursued in a breeding programme is to optimize plant carbon allocation among plant components (i.e. leaf, stem, rhizome and root), which requires at least (1) phenotypic and genotypic data, and (2) a crop model that can capture the impact of different carbon allocation schemes on growth and biomass production.

This approach can be used to study the effects of genotypes with different biomass partitioning schemes. However, there is clearly a balance between the support and nutrient acquisition provided by rhizomes and roots and the benefit of partitioning more biomass to above-ground organs that can be

harvested. One factor that is likely to have a major impact on carbon allocation is the manipulation of flowering time (Sticklen, 2007). By reducing the energy invested in reproductive structures, the proportion of biomass available for harvest can be increased (Ragauskas *et al.*, 2006) and optimized to develop cultivars adapted to particular regions. For example, an improved carbon allocation scheme can result in reduced leaf area by increasing the number of stems and/or their thickness. In addition, maintaining leaf area index at optimum values (Hay and Porter, 2006) also has the potential of reducing crop transpiration and thus improve water use efficiency which can be especially important for biomass production in dry environments (Richards *et al.*, 2002). This reduction in leaf area index will be most beneficial if it does not impact on the timing of canopy closure and maximum light interception.

It should also be considered that flowering is an important component in triggering senescence processes which, in perennial crops, initiate translocation of nutrients and carbohydrates to below-ground storage (Heaton *et al.*, 2009). If delayed flowering prevents this from happening, the nutrient use efficiency will decrease, impacting the sustainability of the cropping system, since synthetic fertilizers need to be added and the excess N in the exported biomass needs to be removed or treated (Beale and Long, 1997).

### III. PHYSIOLOGY

As reviewed in the previous section, *M. × giganteus* has proved to be one of the most, if not the most, productive terrestrial plants in mid-latitude northern climates (35–60° N). The first replicated trials of this crop in the US showed yields of 30–40 Mg ha<sup>-1</sup> y<sup>-1</sup> across three sites in Illinois (Heaton *et al.*, 2008a). In central Illinois, where some of the highest yields of maize in the world are recorded, *M. × giganteus* yielded 60% more shoot biomass, even though the maize crop was heavily fertilized and no fertilizer added to *M. × giganteus* during the comparison (Dohleman and Long, 2009). In E. England at 52° N, dry matter yield in similar replicated trials was 20 Mg ha<sup>-1</sup> with a peak biomass of 30 Mg ha<sup>-1</sup>. These are the highest annual dry matter yields for any crop in the UK (Beale and Long, 1995, 1997). Taking account of the large amount of root and rhizome simultaneously produced, the efficiency of conversion of visible sunlight energy intercepted by the leaves into total biomass energy was 7.8%, again one of the highest conversion efficiencies recorded and equal to that obtained by the Amazonian grass *Echinochola polystachya* which holds the record annual dry matter yield for any terrestrial vegetation (Beale and Long, 1995; Piedade *et al.*, 1991). This

represents 65% of the theoretical maximum efficiency of C<sub>4</sub> photosynthesis, that is, 12% of visible sunlight (Zhu *et al.*, 2008). The potentially higher water and nitrogen use efficiencies associated with C<sub>4</sub> photosynthesis are also realized (Beale and Long, 1997; Beale *et al.*, 1999). This section analyses the possible physiological basis of this exceptional productivity and resource use efficiency.

#### A. PHYSIOLOGICAL BASIS FOR *M. × GIGANTEUS* PRODUCTIVITY

Why is *M. × giganteus* so high yielding? Crop yield is determined by the product of total incident solar radiation ( $Q_{\text{tot}}$ ), the efficiency of radiation interception ( $\epsilon_i$ ), the efficiency of conversion of intercepted radiation to above-ground biomass ( $\epsilon_{\text{ca}}$ ) and the efficiency of partitioning biomass to harvested material ( $\eta$ ; e.g. the grain in most crop plants). In biomass crops, all above-ground biomass is harvested, making  $\eta$  close to unity, therefore  $\epsilon_i$  and  $\epsilon_{\text{ca}}$  are crucial to the final yield.

*Miscanthus × giganteus* is an inter-specific hybrid, so one hypothesis would be that its exceptional yield is a result of hybrid vigour. However, side-by-side trials in Europe have shown that cultivars of one of the parent species, *M. sinensis*, achieve similarly high yields (Clifton-Brown *et al.*, 2001a, 2004). A second factor is that its parent species, in their native habitat, are primary colonizers (Stewart *et al.*, 2009). Many primary colonizers have proved to be highly productive; this may be a feature selected in evolution, since to colonize sites where other plants have not previously grown, high productivity may be crucial to gaining a foothold, as shown by the highly productive species *E. polystachya* (100 Mg ha<sup>-1</sup> y<sup>-1</sup>; Piedade *et al.*, 1991) and *Spartina alterniflora* (64 Mg ha<sup>-1</sup> y<sup>-1</sup>; Long and Mason, 1983). Thirdly, *M. × giganteus* uses C<sub>4</sub> photosynthesis, as apparently do all genera of the grass tribe Andropogoneae which includes *Sorghum*, *Zea*, *Saccharum* and *Andropogon* (Kellogg, 1998).

C<sub>4</sub> photosynthesis has an inherently higher efficiency of conversion of sunlight energy into carbohydrate because it avoids photorespiration. Photorespiration occurs in other plants (C<sub>3</sub>) because the primary carboxylase (Rubisco) catalyses both a carboxylation and an oxygenation reaction. The oxygenation reaction catabolizes recently formed carbohydrate back to CO<sub>2</sub>, and so imposes an average 30% yield penalty on C<sub>3</sub> crops, a penalty that increases with temperature (Long, 1991). C<sub>4</sub> photosynthesis has evolved independently from the more ancient and ubiquitous C<sub>3</sub> photosynthesis at least 45 times (Sage *et al.*, 1999). The elimination of photorespiration in C<sub>4</sub> species does come at a cost. More energy is required for each CO<sub>2</sub> assimilated, although it is less than the energy lost to photorespiration when

temperature exceeds 25 °C. In high light environments where photosynthesis is light-saturated, additional energy requirements are, by definition, irrelevant. Because of the higher efficiency of carboxylation in C<sub>4</sub> plants, intercellular CO<sub>2</sub> concentration is lower and typically only about 60% of that in C<sub>3</sub> leaves. As a result, a C<sub>4</sub> leaf in the same environment as a C<sub>3</sub> leaf will lose only 60% of the water lost by a C<sub>3</sub> leaf in assimilating a given amount of CO<sub>2</sub>. Because of the advantages that C<sub>4</sub> photosynthesis has under high temperature, high light and low moisture conditions, it evolved mostly in low-latitude and relatively arid regions (Sage *et al.*, 1999). It has been hypothesized that the process of C<sub>4</sub> photosynthesis is intrinsically limited to warm climates, and that its efficiency of light use will be intrinsically less efficient at low temperatures (Sage and Kubien, 2007).

*Miscanthus × giganteus* is proof that this is not the case. It is able to form and maintain leaves with high photosynthetic rates at temperatures about 6 °C cooler than maize cultivars bred for cool temperate climates. Even compared to other C<sub>4</sub> plants native to cool climates, *M. × giganteus* appears exceptional (Long, 1999). How does *M. × giganteus* differ? Gene sequences of Rubisco and the enzymes of the C<sub>4</sub> dicarboxylate cycle show 99% homology with its very close relative sugarcane (*S. officinarum*), and the few single nucleotide polymorphisms (SNPs) give no clue of any changes that would make these enzymes more cold-tolerant. This is supported by the observation that there appear to be no differences in the temperature dependence of the kinetics of the recombinant enzymes from these two species *in vitro* (Wang *et al.*, 2008a,b).

Metabolic control analysis suggests that two enzymes limit the rate of photosynthesis in C<sub>4</sub> plants: Rubisco and PPDK. When maize and *M. × giganteus* are transferred from a growth temperature of 25 to 14 °C, photosynthetic rates in both species decline over the first 2 days, but in *M. × giganteus*, it then recovers while continuing to decline without recovery in maize. What underlies this difference? In maize, amounts of Rubisco and PPDK decline continuously, while in *M. × giganteus*, the amount of Rubisco is unchanged and the amount of PPDK more than doubles. This corresponds to a large increase in the amount of mRNA coding for PPDK, suggesting an up-regulation of gene expression. *In vitro*, PPDK is cold-labile, dissociating into its monomers at 10–12 °C. Concentration of the enzyme *in vitro*, however, suppresses this dissociation, which might explain the ability of C<sub>4</sub> photosynthesis to continue functioning at much lower temperatures in *M. × giganteus* (Wang *et al.*, 2008b).

C<sub>4</sub> plants are also strongly affected by photoinhibition at low temperatures. Decreased ability to use absorbed light energy in carbon metabolism leads to oxidative inhibition and damage to the photosynthetic apparatus.



Even compared to the UK native C<sub>4</sub> plant, *Cyperus longus*, *M. × giganteus* has a significant advantage here. While the efficiency of use of electrons utilized in CO<sub>2</sub> assimilation declines significantly with decrease in temperature from 25 to 17 °C in *C. longus*, this efficiency did not decline in *M. × giganteus* until 10 °C (Farage *et al.*, 2006). How is this achieved? First, *M. × giganteus* maintains high rates of photosynthetic carbon metabolism down to 10 °C, allowing it to utilize more of the absorbed light energy. Secondly, the xanthophylls Zeaxanthin, which facilitates heat dissipation of excess absorbed light energy in the photosynthetic apparatus, increased some 20-fold from 0.8 μmol m<sup>-2</sup> in *M. × giganteus* grown at 25 °C to 16.8 μmol m<sup>-2</sup> when grown at 10 °C (Farage *et al.*, 2006).

How do these biochemical advantages lead to increases in yield in the field? In trials in the Corn Belt of the Midwestern US, *M. × giganteus* has an annual  $\epsilon_i$  that is 60% higher than maize, the major factor accounting for its higher productivity. This is due to the perenniality of *M. × giganteus* and also its ability to produce photosynthetically viable leaves during the cooler periods at the ends of the growing season within the temperate environment (Dohleman and Long, 2009). Estimates of canopy photosynthesis show that *M. × giganteus* is able to produce active leaves early in the spring, allowing for a great deal of net canopy photosynthesis near the summer solstice, when the maximum amount of solar radiation is available. Furthermore, a great deal of CO<sub>2</sub> assimilation occurs in the autumn, after the maize crop has completely senesced (Fig. 6).

*Miscanthus × giganteus* is exceptionally productive when compared to other perennials as well, with the advantage compared to those species driven by a greater  $\epsilon_{ca}$ . *Miscanthus × giganteus* was able to produce a closed canopy within one month and maintain it for 5 months even at the high latitude of 52° N, and also have a 60% higher  $\epsilon_{ca}$  than *S. cynosuroides* (Beale and Long, 1995). *Miscanthus × giganteus* has been shown to have a substantially higher  $\epsilon_{ca}$  than the regionally adapted perennial switchgrass. When integrated over two full growing seasons, the leaf-level photosynthesis of *M. × giganteus* was 33% higher than that of switchgrass (Dohleman *et al.*, 2009). This increased carbon assimilation came at a price, however, as stomatal conductance was also 25% higher in *M. × giganteus* and could explain why switchgrass tends to remain more productive under dry conditions (Heaton *et al.*, 2004a).

High productivity in cool environments is not simply a function of capacity to maintain high leaf photosynthetic efficiency at low temperature, but also the ability of the perennating organ, the rhizome, to survive sub-zero winter temperatures. Established stands of *M. × giganteus* and the parent species have survived for decades in botanical gardens where winter temperatures can drop below -25 °C. For example, the clone planted in



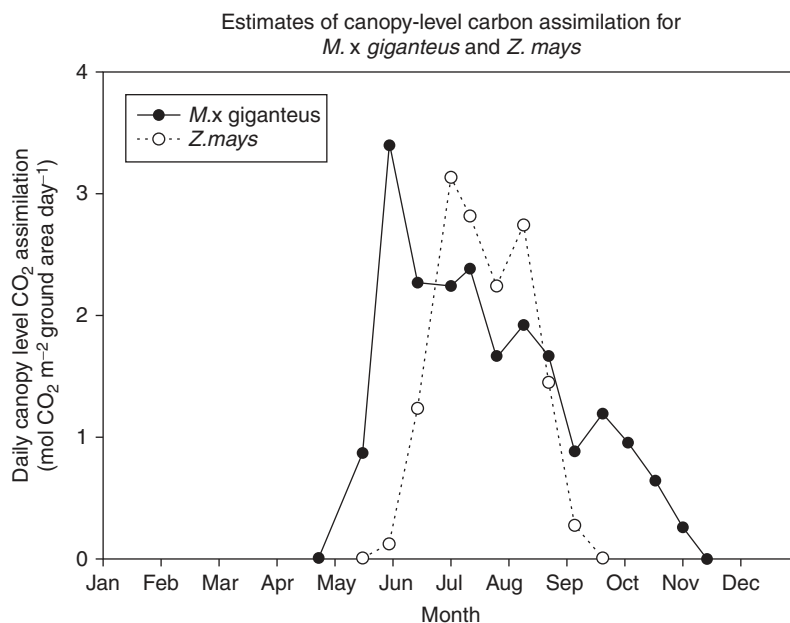


Fig. 6. Seasonal carbon accumulation for the perennial crop *M. × giganteus* and annual row crop *Z. mays*. While *Z. mays* is able to assimilate more carbon in the middle portion of the growing season, the extended growing season allows for 60% greater biomass accumulation in *M. × giganteus* (modified from [Dohleman and Long, 2009](#)).

the Illinois trials has survived without any evidence of winter loss in Urbana since 1988 and Chicago Botanical Gardens since 1970. This includes survival through the coldest winter temperature ever recorded in Chicago,  $-33\text{ }^{\circ}\text{C}$  in January 1985 ([Heaton \*et al.\*, 2008a](#)). However, *Miscanthus* appears more vulnerable to low temperature during the first year after planting.

Artificial freezing tests with rhizomes removed from the field showed that the lethal temperature at which 50% were killed (LT50) for *M. × giganteus* and *M. sacchariflorus* genotypes was only  $-3.4\text{ }^{\circ}\text{C}$  ([Clifton-Brown and Lewandowski, 2000a](#)). This represents a high risk for losses after planting of rhizomes or in the establishment of stands from seed. However, LT50 in one of the *M. sinensis* genotypes tested was  $-6.5\text{ }^{\circ}\text{C}$ , showing significant potential for selection of improved tolerance. Interestingly, among the genotypes, increased tolerance to freezing temperatures was not related to earliness in autumn shoot senescence or associated with size. This last point may be critical, since while *M. × giganteus* partitioned 35–40% of its biomass in rhizomes, the most cold-tolerant genotype partitioned only about 20% of its biomass into rhizomes ([Clifton-Brown and Lewandowski, 2000a](#)). This is

important, since if there is no over-wintering penalty for investing less in rhizomes, then it indicates the potential to select for lines which partition a higher proportion of photosynthate into stems. This will have the double benefit of accelerating shoot growth and harvestable biomass.

The clone(s) of *M. × giganteus* that have so far been examined in Europe and the US are thought to have been collected from Honshu island of Japan (Stewart *et al.*, 2009). The parent species, *M. sinensis* and *M. sacchariflorus*, range from the tropics to southern Siberia, so it is unlikely that results for this clone represent the limits to cold temperature tolerance, and there should be breeding resources available to improve the cold-tolerance of *Miscanthus* rhizomes.

#### B. NUTRIENT CYCLING IN *M. × GIGANTEUS*

One of the touted advantages of perennial grasses in general and *M. × giganteus* in particular is the ability to internally cycle or remobilize nutrients between above- and below-ground tissues. Himken *et al.* (1997) in Germany and Beale and Long (1997) in England independently documented a seasonal pattern in biomass and nutrient accumulation in shoots and rhizomes of *M. × giganteus*, with rhizome biomass peaking after 80% of above-ground dry matter had accumulated in the late summer/early autumn, then staying constant or decreasing slightly over the winter. Rhizome biomass then declined dramatically during shoot emergence in the spring, presumably as mobile carbohydrates and nutrients were translocated to the actively growing shoot tissue.

The concentrations of N and P in plant tissues generally mirrored the seasonal trends in biomass allocation, but K showed less fluctuation in below-ground tissues, possibly because more of the monovalent anion leached from senescing shoots before it could be remobilized to the rhizome (Beale and Long, 1997; Clifton-Brown *et al.*, 2007). Further, crop removal rates of K were higher and tissue and soil concentrations lower in long-term trials of *M. × giganteus* in England and Ireland (Christian *et al.*, 2008; Clifton-Brown *et al.*, 2007), suggesting that K is not translocated as efficiently as N and P and thus K may limit growth before other macronutrients do. It is important to be aware that the addition of K fertilizer is frequently achieved through application of KCl fertilizer, leading to complementary uptake of Cl (Lewandowski and Kicherer, 1997; Lewandowski *et al.*, 2003a) which leads to production of HCl during combustion of the feedstock. HCl can have negative environmental and economic consequences for energy producers, leading to emission of the poison dioxin, as well as corroding steam boilers (Lewandowski and Heinz, 2003). Mineral nutrients are

undesirable in biomass feedstock because they can ultimately become atmospheric pollutants that must be mitigated irrespective of the type of fuel that is produced.

Crop senescence and harvest time can dramatically impact the amount of nutrients removed from the field with short-term impacts on fuel quality and long-term impacts on environmental and economic sustainability. Green *M. × giganteus* shoot tissue typically has N, P and K concentrations of about 20, 2 and 20 mg g<sup>-1</sup>, respectively (Beale and Long, 1997; Heaton *et al.*, 2009; Himken *et al.*, 1997). Concentrations are highest during shoot emergence and become diluted as biomass rapidly accumulates in the first few months of growth. By winter, the concentrations of N, P and K have dropped by an order of magnitude, to 1–5 mg g<sup>-1</sup>. Nutrient budgets have shown that the shoots obtain some of their nutrients from the soil, and not all of the nutrients are taken off by the harvested crop or put back in the rhizome, and therefore, some are lost back to the soil every year (Beale and Long, 1997), though the impact of nutrient movement among plant, soil and detritus pools has not been thoroughly evaluated.

The N demands of a high-yielding *M. × giganteus* crop seem impossible to satisfy without external fertilizer or serious depletion of soil reserves: Heaton *et al.* (2009) found that *M. × giganteus* was capable of removing nearly 300 kg ha<sup>-1</sup> of N, despite only a single application of 25 kg ha<sup>-1</sup> N during the preceding 3 years. What is the source of this N? Even in fertile soils with high mineralization rates, balancing the N budget in Illinois was only possible when N fixation was included in the analysis (Davis *et al.*, 2010). N fixation may be a plausible explanation given that nitrogenase activity was found via acetylene reduction in rhizomes and in bacteria isolated from the rhizosphere.

Agricultural producers are faced with a difficult decision when it comes to choosing the best time to harvest *M. × giganteus*: harvest in the late summer when yields are highest, or wait until the first frost and lose 30–50% of biomass to leaf drop and weather? Multiple studies have examined this question (Heaton *et al.*, 2009; Huisman *et al.*, 1997; Lewandowski and Heinz, 2003; Lewandowski *et al.*, 2003a) and generally conclude that it is better to wait and harvest after nutrient concentrations have decreased than to harvest feedstock of reduced quality and be forced to apply costly and greenhouse gas-intensive fertilizer. Further, the biomass 'lost' due to leaf drop actually contributes to the SOM pool and could be valued in a carbon credit market. Because N is remobilized to the rhizome, the C:N ratio of standing biomass increases dramatically (Heaton *et al.*, 2009), making the remaining litter recalcitrant to microbial decomposition (Kahle *et al.*, 2001) and contributing to soil organic carbon (Kahle *et al.*, 2002).

## IV. BREEDING, GENOMICS AND GENETICS

### A. TAXONOMY AND ORIGINS

The tribe Andropogoneae within the family Poaceae includes several species of natural and agricultural value, including the C<sub>4</sub> grasses sorghum (*S. bicolor* L. Moench), maize (*Zea mays* L.) and sugarcane (*S. officinarum* L.). The subtribe Saccharinae includes the genera *Saccharum* L. and *Miscanthus* Anderss., species of which are currently under consideration as potential biomass crops for renewable energy production (Hodkinson *et al.*, 2002a). These two genera are closely related with evidence suggesting occasional inter-generic hybridization (Sobral *et al.*, 1994). Morphologically, *Miscanthus* species differ from *Saccharum* by their tough inflorescence rachis, with both spikelets of a pair being pedicellate (Hodkinson *et al.*, 2002a). The taxonomic status of the genus *Miscanthus* is in a state of flux, with relatively little information available about identity and inter-relationships of its species. According to Clayton and Renvoize (1986), the genus consists of approximately 20 species, most of which are endemic to eastern or southeastern Asia (China, Taiwan, Japan, Korea and south), with two species found in the Himalayas and four in sub-Saharan Africa.

Of particular relevance to this review are the species of *Miscanthus* endemic to southeastern Asia touted as potential dedicated bioenergy crops including *Miscanthus floridulus*, *Miscanthus lutarioriparium*, *M. sacchariflorus*, *M. sinensis* and the triploid inter-specific hybrid *M. × giganteus*. The basic chromosome number of these species is 19 (Adati and Shiotani, 1962), with most accessions being diploids, although some of the strongly rhizomatous species (*M. sacchariflorus* and *M. lutarioriparium*) include accessions that are triploid or tetraploid (Hirayoshi *et al.*, 1955; Hodkinson *et al.*, 2001). *Miscanthus sinensis* is endemic to East Asia ranging from New Guinea through Indonesia, north through Southeast Asia into China, Taiwan, Japan, Korea and Russia. The native distribution of *M. sacchariflorus* is limited to Northern China, Korea, Russia and Japan (Hodkinson *et al.*, 2002c). These species, particularly *M. sinensis*, have populations that have evolved to adapt to a broad range of environments and show substantial genetic diversity (Hodkinson *et al.*, 2002a). All of these species are perennial rhizomatous grasses with obligate out-crossing due to self-incompatibility and with the possible exception that *M. floridulus* can survive winters in temperate climates.

DNA evidence suggests that *M. × giganteus* ( $3n = 57$ ) is an allotriploid hybrid generated from a rare natural cross between diploid *M. sinensis* ( $2n = 38$ ) and a tetraploid *M. sacchariflorus* ( $4n = 76$ ) (Hirayoshi *et al.*, 1960; Lafferty and Lelley, 1994; Rayburn *et al.*, 2009) that occurred in

Japan and via clonal propagation transported in 1935 to Europe and later to North America by commercial nurserymen (Linde-Laursen, 1993). The University of Illinois clone was originally procured from the Chicago Botanic Garden, which in turn acquired their specimen from Europe. This clone shares genetic identity with the widely propagated *M. × giganteus* genotype grown throughout Great Britain (J. Clifton-Brown, personal communication). *Miscanthus × giganteus* is a sterile allotriploid and so does not produce viable seed, reducing its potential as an invasive species (Hodkinson *et al.*, 2002a). Much of the evidence as to the putative parents of *M. × giganteus* is based on morphological observations. Data obtained by Hodkinson *et al.* (2002b) using variation in the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA) supported the hypothesis that *M. sinensis* and *M. sacchariflorus* were the parents of *M. × giganteus* and that one species contributed two genomes while the other species contributed only one. The study was unable to elucidate which species contributed two genomes. Calculation of the nuclear genome size using flow cytometry suggests that the *M. × giganteus* accession at the University of Illinois comprises two genomes of *M. sinensis* and one of *M. sacchariflorus* (Rayburn *et al.*, 2009).

## B. MISCANTHUS SPECIES GENETIC IMPROVEMENT

### 1. Rationale

All *Miscanthus* species are presently genetically unimproved so one would expect that improvement in a number of key traits could be made using breeding and genetic engineering tools. The use of transformation technology is especially important in the case of *M. × giganteus*, which is sterile, thus seed is not produced and crosses cannot be made to generate variability. There also appear to be very few independently derived lines, again indicating little variation. Initial reports indicated that *M. × giganteus* had few pests and diseases, but more recent work has shown that indeed insects, nematodes and pathogens do attack the plants (see Section VI). Thus, it is likely that large plantings of genetically uniform unimproved clones will be subjected to the usual pests and diseases that can affect most crops.

### 2. Traits to improve

Overall yield is one of the most important traits for biomass crops and this is controlled by many factors, including growth rate and duration, and environmental limitations such as water, heat, cold, nutrient, pests and disease. One would expect photosynthetic efficiency to be very important for yield, but *Miscanthus* species already have the C<sub>4</sub> pathway and very efficient

photosynthesis (Section III). Populations of *M. sacchariflorus* endemic to Eastern Asia are found as far north as the Amur River Valley in Western Russia. Accessions from these species in Russia and Northern China have adapted to severe winter conditions and represent sources of cold-tolerant germplasm. If flowering is prevented, the active growth period can be increased, so delaying or preventing flowering by decreasing expression of the indeterminate gametophyte gene might be useful (Colasanti *et al.*, 1998). The growth rate might also be increased by increasing gibberillin levels (Eriksson *et al.*, 2000). There are also known genes that have shown promise for alleviating many biotic and abiotic stresses that affect plant performance (Allen, 2010; Datta, 2002).

Another trait that is most important to alter is composition of the cell walls. The bulk of mature plant biomass represents secondary cell walls consisting mainly of a complex polysaccharide framework, several types of highly glycosylated proteins and complex polymers of phenylpropanoid units, that is, lignin, the hydrophobic filler that provides physical strength to the cell wall. Various structural and chemical characteristics of plant cell walls that act as the first barrier between plant and environment have evolved in order to resist external stresses from pathogen attack, wounding or mechanical stimuli. These cell wall properties make it difficult to disassemble biomass when it is used for liquid biofuel production; however, high lignin can be advantageous for burning since it has a higher energy content than carbohydrate.

*Miscanthus* species, as all gramineous plants, have type II cell walls with a high content of arabinoxylans and 1,3:1,4- $\beta$ -glucans (b-glucans) and a low content of pectic polymers and xyloglucans, which predominate in the matrix of type I cell walls found in dicotyledons and other monocotyledons (Carpita, 1996). Another feature of gramineous cell walls is a high content of hydroxycinnamic acids, such as ferulic acid and *p*-coumaric acid, which are ester-linked to structural polysaccharides such as different arabinoxylans (Smith and Hartley, 1983). Glucuronoarabinoxylan, arabinoxylans and other xylan-rich hemicelluloses are the dominant hemicelluloses in the cell walls of different tissues of grasses, including their lignified supporting tissues. The amounts of lignin and cell wall-bound ferulic and diferulic acids as well as the composition of wall polysaccharides determine the gramineous plant cell wall rigidity, extensibility and digestibility (Grabber *et al.*, 2004).

Information on *Miscanthus* biomass composition is very limited at present, and the contents of main biomass constituents greatly vary in different publications. Thus, it was reported that dried biomass contains 18.30–

20.99% lignin and 69.78–78.63% holocellulose in *M. sacchariflorus* (Visser and Pignatelli, 2001), 38% cellulose, 24% hemicellulose and 25% Klason lignin in *M. × giganteus* (de Vrije *et al.*, 2002) and 41.9% cellulose, 26.6% hemicellulose and only 13.3% lignin determined as acid lignin fibre in *M. ogiformis* (analogous species to *M. × giganteus*) (Magid *et al.*, 2004). The reported different estimates of lignin concentrations could result from the application of various analytical procedures which do not give consistent results. We tested the biomass characteristics of several *Miscanthus* accessions from the germplasm collection grown on the University of Illinois experimental farm that were harvested at the end of the growing season applying the acetyl bromide method which was recommended for the prediction of biomass digestibility based on lignin levels (Fukushima and Hatfield, 2004). The results showed large variations across genotypes selected in major cell wall constituents which can have an influence on biomass biodegradability, with about 26% lignin, 40% cellulose and 20% xylan being typical (Fig. 7). There was a negative correlation between the lignin and ether-bound phenolic contents and sugar released by both enzymatic hydrolysis alone and that after acid pre-treatment (Fig. 8; A.V. Lygin, unpublished data) when saccharification of selected plant biomass and composition was carried out, as described by Chen and Dixon (2007).

For biochemical conversion to fuel, cost-effective pre-treatment (mechanical, physical, chemical or most promising enzymatic) of biomass is usually required. Pre-treatment can modify or remove unwanted by-products, such as lignin, to reduce cellulose crystallinity, and increase the porosity, thus improving hydrolysis (McMillan, 1994). As a consequence of these pre-treatments, cellulose is made accessible for hydrolysis to glucose and fermentation to alcohols. Using genetic engineering for the expression of glycosyl hydrolases that cleave only side chains in branched polysaccharides will give the possibility for fine modification of these polysaccharides, without complete breakdown, that can increase polysaccharide accessibility to enzymatic treatment during biomass conversion. Transgenic expression of glycosyl hydrolases with a well-characterized specificity provides a direct approach for post-synthetic modification of specific polymeric constituents in plant cell walls (Sticklen, 2007). On account of rapid advancements in the characterization of microbial hydrolases, the currently available number of these enzymes is sufficient to deconstruct cell wall polysaccharides completely.

Thus, generating transgenic plants with decreased cross-linking levels in cell walls and less lignin should result in higher efficiency of biochemical biomass conversion to fuel, while genotypes with high lignin would be ideal for burning.

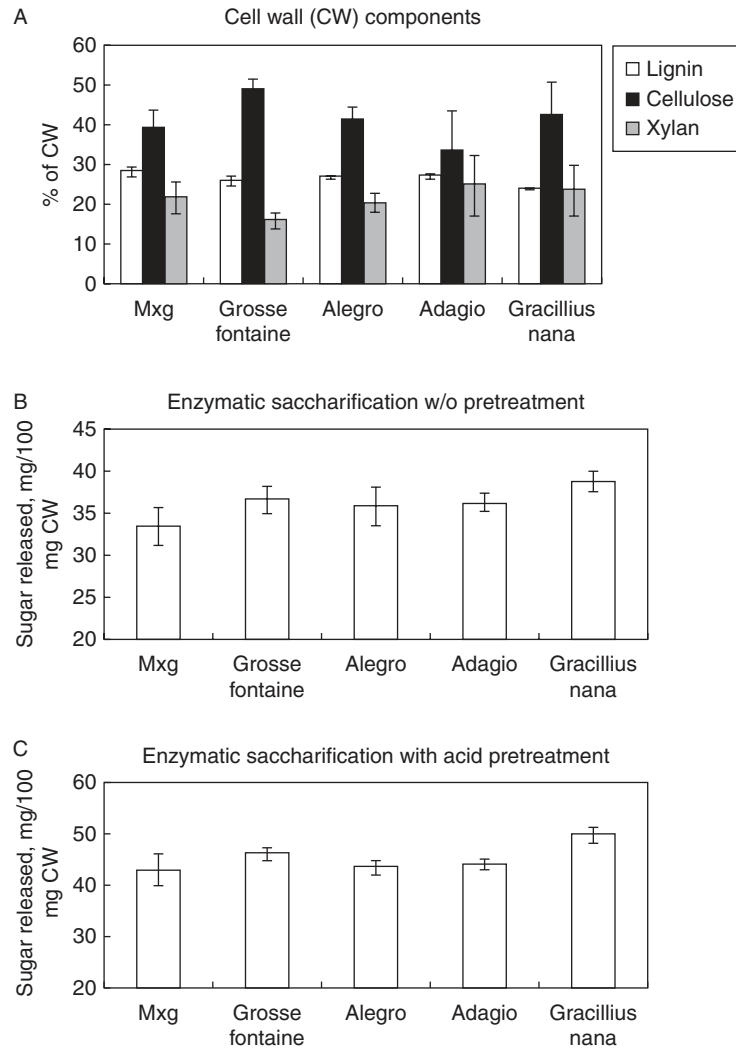


Fig. 7. Biomass characteristics of several *Miscanthus* genotypes (*Miscanthus giganteus*—*M*×*g* and four *M. sinensis* accessions: “Grosse Fontaine”, “Adagio”, “Gracillius Nana” and “Alegro”): (A) acetyl bromide lignin, cellulose and xylan concentrations in cell walls of dried mature tillers harvested in February 2010. Cellulose was estimated by treatment of CW with acetic-nitric reagent followed by phenol-sulfuric assay with glucose as a standard. Xylan was calculated using data from monosaccharide composition of hemicelluloses; (B) sugars (as glucose equivalent) released from grass cell walls by enzymatic hydrolysis (with cellulase and cellobiase for 72 h) without pretreatment and (C) with acid pretreatment. (Vertical bars represent the SD).



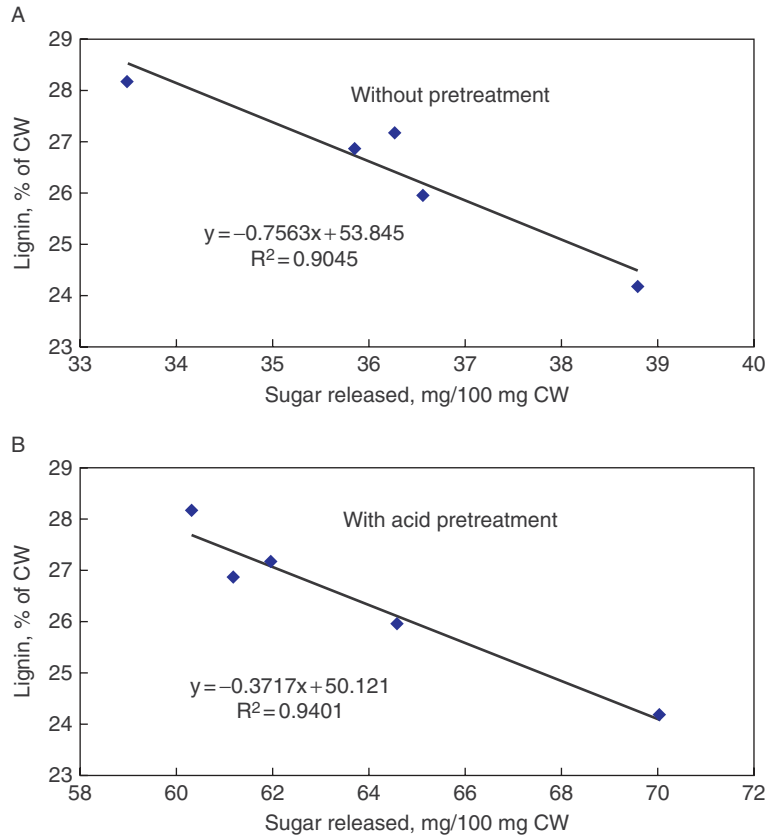


Fig. 8. Relationships between the lignin contents and saccharification of *Miscanthus* biomass. Each point represents an individual accession. Stem material was treated with cellulase and cellobiase for 72 h. Total sugar released is presented as a function of lignin content of untreated stems (A) or lignin content of acid pretreated stems (B).

### C. BREEDING

Genetic improvement of *Miscanthus* species as dedicated bioenergy crops is in its infancy. Introductions of *Miscanthus* accessions into Europe and North America from Southeast Asia were made in the late nineteenth and throughout the twentieth century. Until recently Kew Gardens in England had one of the most extensive collections. These collections represent much of the currently available germplasm for *Miscanthus* crop improvement. Increased interest and use of ornamental grasses in urban landscapes in the 1970s and 1980s lead to the propagation and commercial sale of *Miscanthus* accessions (primarily *M. sinensis*) by nurseries in Europe and North America. These

horticultural varieties were sold as vegetatively propagated clones and tended to have reduced stature, early flowering and showy inflorescences.

The oil embargoes of the 1970s and increasing crude oil costs initiated efforts in Europe and North America to investigate alternative and renewable sources of energy. This leads to the creation of the EU's MPN and the EMI programmes described earlier and ultimately to the formation of Tinplant Biotechnik und Pflanzenvermehrung GmbH in 1992, a commercial company partially dedicated to *Miscanthus* breeding in Germany. For 15 years, Tinplant conducted hybridizations and selection for improved accessions of *M. sinensis*, *M. sacchariflorus* and *M. × giganteus* for sale to the ornamental nursery industry and for enhanced biomass. Tinplant was acquired in 2007 by Mendel Biotechnology, Inc. of California, who has expanded the *Miscanthus* breeding programme. In addition, in 2004, the United Kingdom Department for Environment, Food and Rural Affairs initiated support for a *Miscanthus* breeding programme in Aberystwyth, Wales where they have acquired the Kew Garden collections and some materials from Tinplant and Southeast Asia (Clifton-Brown *et al.*, 2008). *Miscanthus* breeding efforts have recently been initiated at several other institutions, including the University of Illinois' Energy Biosciences Institute.

Current efforts by both private and public programmes are focused on the collection of *Miscanthus* genetic resources primarily from countries in Southeast Asia. Collection of germplasm from foreign countries requires compliance with the Convention on Biological Diversity (United Nations Environment Programme, 1993), which gives sovereignty to each country over its genetic resources and requires arrangement of formal partnerships before collection and export of germplasm to another country. Another factor that influences collection involves compliance with issues of plant quarantine where *Miscanthus* germplasm (seeds or propagules) must be tested and inspected by a government-approved plant pathologist before release.

The collection and use of diverse germplasm is a crucial factor in *Miscanthus* crop improvement programmes. While significant genetic variability has been found among the parental species (*M. sinensis* and *M. sacchariflorus*) (Jorgensen and Muhs, 2001), the few (3 or 4) existing triploid *M. × giganteus* accessions generated from inter-specific hybridization display very low levels (Greef *et al.*, 1997; Hodkinson *et al.*, 2002a). *Miscanthus × giganteus* displays remarkable heterosis for vegetative growth when compared to its putative parental species, although in competitive European trials, some accessions of *M. sinensis* produced up to 70% of the biomass of *M. × giganteus* (Clifton-Brown *et al.*, 2001a). Breeding efforts with *M. × giganteus* are hindered by difficulties in re-synthesizing new

accessions of triploid *M. × giganteus* due to incompatibility between the parents and the sterility associated with the triploid genome.

The requirement of labour-intensive and costly vegetative propagation for potential commercial production of triploid *M. × giganteus* germplasm favours the development of improved vegetative propagation techniques that result in higher multiplication rates and hardy propagules. The inherently high cost of vegetative propagation, however, also favours the development of *Miscanthus* genotypes that are fertile and bear seeds to facilitate planting and production. The recent creation of hexaploid *M. × giganteus* plants (Yu *et al.*, 2009) presents potential opportunities for the development of fertile germplasm and new breeding opportunities by conducting hybridizations between hexaploids or between hexaploids and diploid *M. sinensis* and *M. sacchariflorus* accessions that could generate viable seed. Early emphasis in public and private breeding programmes is on creation and selection within diploid *M. sinensis*, *M. sacchariflorus* and hybrid *M. sinensis × M. sacchariflorus* populations. These populations will be used to generate linkage maps for diploid *M. sinensis* and *M. sacchariflorus* and for associating the genome with beneficial phenotypic traits. DNA marker-saturated linkage maps of the *Miscanthus* genome will allow for association mapping and marker-assisted breeding. It must be cautioned that though seeded *Miscanthus* is highly favourable from an economic perspective, a thorough investigation about the invasive potential of fertile *Miscanthus* is critical prior to mass plantation of these species.

#### D. GENOMICS

One aim of the current Feedstocks Genomics Programme within the Energy Biosciences Institute at the University of Illinois is to generate resources that will enable genomics-directed improvement of *Miscanthus* germplasm. The genome of *M. × giganteus* is very large, estimated to be  $\sim 7.0$  Gbp by flow cytometry (Rayburn *et al.*, 2009). Using new generation genomic tools, 1 $\times$  skim sequencing of *M. × giganteus* DNA revealed that much of the genome consists of major repeated sequences with only 2% or about 165 Mbp as ‘genespace’, and the recently sequenced *S. bicolor* is a useful reference genome (Swaminathan *et al.*, 2010). Deep sequencing of the *M. sinensis*, *M. sacchariflorus* and *M. × giganteus* transcriptome found that contigs matched  $\sim 29,000$  of the estimated 36,000 *Sorghum* genes.

This sequence information will be used to generate SNPs, single sequence repeats (SSRs) and PCR-based markers that will be made available to the public for linkage studies, association mapping and marker-assisted breeding. We are in the process of identifying the most informative SNPs across

the transcriptome of these species to generate a Goldengate SNP array to apply to *M. sinensis* and *M. sinensis* × *M. sacchariflorus* hybrid segregating populations. This will provide genetic linkage maps for these species and be used to identify QTL and genes associated with desired phenotypes. These SNP arrays will be made available to the public, while the transcript and genomic sequences the programme generates will be available to online browsers by 2011.

## E. MICROPROPAGATION, GENETIC ENGINEERING AND CHROMOSOME DOUBLING

### 1. *Micropropagation*

Methods have been developed using several tissues of the *M. × giganteus* plant, most efficiently immature inflorescences, to initiate cultures that then can be multiplied and regenerated into whole plants (Holmes and Petersen, 1996; Kim *et al.*, 2010). These methods can be used for micropropagation for large-scale planting and also would be important for genetic transformation, since the genes are, in most cases, inserted into cultured cells.

### 2. *Genetic engineering*

While breeding may be able to manipulate a number of traits for *Miscanthus* species, there is very little presently known about what traits are available in the germplasm, and breeding systems are just being developed. Because *M. × giganteus* is sterile and breeding cannot be readily accomplished, being able to directly insert genes appears to have some real importance.

Most plant transformation utilizes tissue culture, and methods have been published for culture initiation, maintenance and plant regeneration as stated earlier (Holmes and Petersen, 1996; Kim *et al.*, 2010). Usually, the gene of interest and selectable marker gene are inserted into cells by particle bombardment or *Agrobacterium tumefaciens* co-cultivation. The transformed cells are selected using a selective agent that kills untransformed cells, but not those expressing the selectable marker gene, such as antibiotic resistance. Plants are then regenerated.

To date, the only published report of *Miscanthus* transformation is with *M. sinensis* using tissue cultures initiated from immature spiklets or germinating seeds and *A. tumefaciens* (Engler and Chen, 2009). Selection was carried out using the antibiotic G-418 with the *nptII* selectable marker gene and plants were generated from the selected callus.

### 3. *Chromosome doubling*

It is possible that doubling the chromosome number of the sterile triploid *M. × giganteus* could generate fertile hexaploids that could enable seed production and breeding. Since the cell size would also increase, it is possible that biomass production could increase and the cell wall composition change. We have applied methods that were used to double the chromosome number in maize callus (Wan *et al.*, 1991) to regenerable *M. × giganteus* callus and did produce chromosome-doubled plants (Yu *et al.*, 2009). Preliminary results indicate that the pollen is more viable, as shown by triphenyltetrazolium chloride staining, than that produced by the triploid (W.B. Chae, unpublished data). No seed was produced, but there may be a problem of self-incompatibility as seen with *Miscanthus* species.

## V. ENVIRONMENTAL IMPACTS

Compared to annually cultivated crops, perennial grasses are often considered environmentally favourable because the more dense and continuous vegetative cover provides protection to the soil against erosion, may reduce runoff and nutrient loss and sequester carbon in the soil (Blanco-Canqui, 2010). Because perennials begin growth earlier in the year than annuals, perennial grasses are thought to be more synchronous with soil nutrient availability (mineralization) and plant uptake throughout the growing season, which may limit nutrient losses. The degree to which these benefits are realized in practice depends on the specific management practices employed, in addition to past management and the environmental context. Reduced runoff may be beneficial in settings where erosion or downstream flooding is problematic. However, in some settings, runoff and drainage from agricultural cropland are important sources of water for human communities and aquatic ecosystems. In these situations, reduced runoff or drainage may be considered detrimental, especially during droughts. Thus, understanding and assessing the environmental impacts of *M. × giganteus* require some attention to specific management practices and the likely impacts in the various places it will be grown. Unfortunately, there has been relatively little research on the environmental impacts of *M. × giganteus* to date across the range of environmental conditions where it might be grown. Several biofuel crops have been reviewed for possible impacts on water use and nutrient loss (Powlson *et al.*, 2005), but more recent studies are now available on *M. × giganteus*.

## A. WATER

Although *M. × giganteus* is considered an efficient crop in terms of water use per unit of biomass produced (Beale *et al.*, 1999), its high productivity may lead to high water use compared to other crops. Empirical evidence from the US and Germany (Boelcke *et al.*, 1998) is consistent with this view, but a simulation study of the southern UK (Finch *et al.*, 2004) suggested less water use from *M. × giganteus* than from the existing land cover, which was largely a mixture of annual crops and perennial grasses.

At two locations in Germany, Boelcke *et al.* (1998) measured soil moisture in mature (4–6 years old) stands of *M. × giganteus* for a 4-year period. They concluded that soil moisture supply was limiting the biomass yield. They used soil moisture data to calibrate the model LEACHW, and used the model to estimate ET and drainage. The model simulations indicated that *M. × giganteus* provided significantly less groundwater recharge compared to a rotation of winter rye-phacelia-potato.

Finch *et al.* (2004) used a mechanistic model to predict long-term changes in ET resulting from increased plantings of *M. × giganteus*, switchgrass and short rotation willow coppice in the UK. Their modelling predicted that *M. × giganteus* and switchgrass would reduce ET by approximately 50 mm y<sup>-1</sup> compared to the existing mixture of cereal crops (e.g. wheat) and C<sub>3</sub> grasses, largely because the existing vegetation had a lower temperature threshold for photosynthesis which results in a longer growing season.

Model parameterization was partly based on measured characteristics of the plants. The cultivar of switchgrass and the age of the stands were not mentioned, but they measured greater leaf area index in switchgrass than *M. × giganteus*, which is opposite of the results reported by Heaton *et al.* (2008a) who compared *M. × giganteus* to Cave-in-Rock switchgrass. Finch *et al.* (2004) also reported that soil moisture under *M. × giganteus* tended to be lower than soil moisture under switchgrass during the later stages of the growing season. The soil moisture comparison was made at two sites over 2 years using replicate plots, but no statistical analysis of the differences was presented. The soil moisture measurements were used to calibrate the model they used to predict the hydrologic impact of the grasses. Finch *et al.* (2004) acknowledged a need for more data collection on energy grasses to confirm their results. Richter *et al.* (2008) reaffirmed this need for more data collection to verify the results of Finch *et al.* (2004).

Richter *et al.* (2008) presented an analysis of existing biomass yields of *M. × giganteus* in the UK, and concluded that soil water availability appeared to be the single most important factor in limiting biomass yield. Finch and Riche (2008) reported that the depth of soil water depletion under

*M. × giganteus* (1.7 m) was greater than under most crops grown in the UK, even though they estimated that stomatal conductance was lower than most crops. Finch and Riche (2010) also reported that approximately 20% of the precipitation falling on *M. × giganteus* from September till harvest was evaporated from the leaves and stem. This quantity of interception in the fall during winter is more typical of a forest than an agricultural crop, and may partly explain the greater depletion of soil moisture.

In the US, McIsaac *et al.* (in press) measured soil moisture to a depth of 90 cm under *M. × giganteus*, switchgrass (Cave-In-Rock cultivar) and a maize–soybean rotation (the predominant land use in the region) over four growing seasons (2005–2008) in central Illinois. At the end of the growing season, soil moisture under *M. × giganteus* was statistically less than under either switchgrass or maize–soybean. Based on simple water budget calculations, they estimated that the ET of *M. × giganteus* was on average 104 mm y<sup>-1</sup> greater than maize–soybean and 140 mm y<sup>-1</sup> greater than switchgrass (McIsaac *et al.*, in press). An increase of 104 mm y<sup>-1</sup> in ET could reduce surface water flows by 32% in the central Illinois region.

Both switchgrass and the *M. × giganteus* were harvested in winter and neither received N fertilizer in this experiment. Cave-In-Rock is not the most productive variety of switchgrass. Higher yielding varieties of switchgrass, treated with appropriate quantities of N fertilizer, would likely be more productive and consequently may use more water than reported in this study (Kiniry *et al.*, 2008; Vogel, 2004).

Hickman *et al.* (2010) used a micrometeorological residual energy budget method to estimate ET from *M. × giganteus*, switchgrass and maize in one growing season (2007). According to their estimates, ET from switchgrass and maize was similar (764 mm) over the 166-day growing season, while water use by *M. × giganteus* was about 190 mm greater. This study was conducted in a subset of the plots used by McIsaac *et al.* (in press), who estimated *M. × giganteus* used 109 mm more water than maize and 69 mm more than switchgrass during this growing season. Although both studies indicate substantially greater ET from *M. × giganteus* compared to the other two crops, differences in magnitude may reflect limitations of the measurement approaches used, as well as somewhat different time periods of observation during the growing season. If the results of Hickman *et al.* (2010) are more accurate and representative, the impact of *M. × giganteus* could reduce surface water flows in the region by 58%.

In a modelling study of the Raccoon River watershed in Iowa, US, Schilling *et al.* (2008) estimated that converting corn–soybeans to perennial grasses would increase ET by 47 or 58 mm y<sup>-1</sup>, depending on whether warm-season or cool-season grasses were planted, and this would reduce annual

water yields (annual stream flow per ha) by 46 and 54 mm  $y^{-1}$ , respectively. Compared to a baseline water yield of 193 mm  $y^{-1}$ , these values represent reductions in water yield of 24% and 28%, respectively. Modelling of warm-season grasses was based on characteristics of switchgrass, while cool-season grasses were modelled on the basis of fescue. *M. × giganteus* was not modelled because of lack of information about its physiological characteristics (Schilling *et al.*, 2008). Given the higher productivity of *M. × giganteus* and the greater soil moisture depletion, as demonstrated by McIsaac *et al.* (in press), it seems reasonable to expect that conversion to *M. × giganteus* could lead to greater reductions in water yield in Iowa than modelled by Schilling *et al.* (2008).

#### B. NITRATE LEACHING

Christian (1994) and Christian and Riche (1998) reported that nitrate leaching losses from plots of *M. × giganteus* grown in silty clay loam soil at Rothamstead Farm in the UK. Fertilizer treatments were 0, 60 and 120 kg N  $ha^{-1} y^{-1}$  applied in the spring. They measured soil water nitrate concentrations in water extracted using porous ceramic cups and estimated leaching from the drainage measured from separate soil monoliths during the dormant season. During the establishment year, they reported 154, 187 and 228 kg N  $ha^{-1}$  leached, respectively, from the 0, 60 and 120 kg N  $ha^{-1}$  fertilizer treatments (Christian, 1994; Christian and Riche, 1998). These high values were partly attributed to the prior cropping system, and to precipitation being 200 mm above average. In the second and third years of the experiment, precipitation was 37 mm above and 37 mm below normal, respectively, and the average nitrate leached in those 2 years was 5.5, 17.5 and 58.5 kg N  $ha^{-1} y^{-1}$ . Christian and Riche (1998) reported that there were no biomass yield differences among the N fertilizer treatments. Thus, under optimal N management in that setting (0 N fertilizer), nitrate leaching appears to be relatively low. Christian and Riche (1998) used porous cup lysimeters to obtain soil solution nitrate concentrations and then estimated leaching flux by combining these concentration data with a separate measurement of drainage flux. Tension lysimeter data may not always reflect drainage water concentration, which adds uncertainty to the overall flux estimate; see Fares *et al.* (2009) for review.

Curley *et al.* (2009) also used suction lysimeters to monitor nitrate concentrations in the soil under second and third year *M. × giganteus* in Ireland that had been treated with cattle manure slurry with N rates of 0, 60 and 120 kg N  $ha^{-1} y^{-1}$ . In the first year of their observations, they reported an increase in soil water nitrate with increasing N application rate, although



mean concentrations were relatively low, ranging from 3.1 to 4.5 mg N l<sup>-1</sup>. During the second year of observations, concentrations were lower than in the first year and there was no statistical difference among the three original treatments. However, a fourth treatment was added (180 kg N ha<sup>-1</sup>) and the mean concentration in this treatment was statistically greater than the others (4.8 mg N l<sup>-1</sup>) (Curley *et al.*, 2009). Curley *et al.* (2009) did not report on actual leaching losses or whether biomass yields were influenced by the different N treatments, nor did they indicate the portion of the manure N that was in an inorganic form when applied. If the quantity of drainage water was not affected by the treatments, then the quantity of nitrate leached would be proportional to the soil water nitrate concentrations.

### C. SUMMARY AND IMPLICATIONS FOR RESEARCH NEEDS

Empirical evidence from the central US, Germany and the UK indicates that ET from *M. × giganteus* is greater than from typical annual crops and thus has the potential to reduce surface water flows and groundwater recharge where it replaces these annual crops on a large scale. A simulation study of the southern UK suggests that large-scale plantings of *M. × giganteus* may result in a reduction of ET (and thus more groundwater recharge and surface flows) if it were to replace C<sub>3</sub> grasses and annual crops. More work needs to be done to quantify water use of *M. × giganteus* under a wide variety of conditions in order to establish relationships that will be useful for modelling the impacts in areas where production appears most economically feasible. It would also be useful to monitor the hydrology of small watersheds with substantial *M. × giganteus* plantings, to verify the scaling up of models based on plot and field observations.

Where *M. × giganteus* can be grown with little or no N fertilizer, nitrate leaching losses will likely be low compared to crops such as maize that have a high N requirement. However, because *M. × giganteus* is slow to establish, large losses of nitrate to leaching are possible during the establishment year, although the quantity will likely depend on weather and prior land use. More research is needed to quantify the N leaching and N<sub>2</sub>O emissions for a variety of N fertilizer rates and timings in settings where *M. × giganteus* responds to N fertilizer application. Additionally, the possibility of using cover crops in the establishment year to minimize N losses without inhibiting *M. × giganteus* establishment deserves investigation (Fig. 9).

*Miscanthus × giganteus* is also likely to sequester carbon, alter wildlife habitats and have other environmental consequences. Although *M. × giganteus* provides an economic advantage by producing high biomass per unit area and per unit of water transpired, the environmental costs and

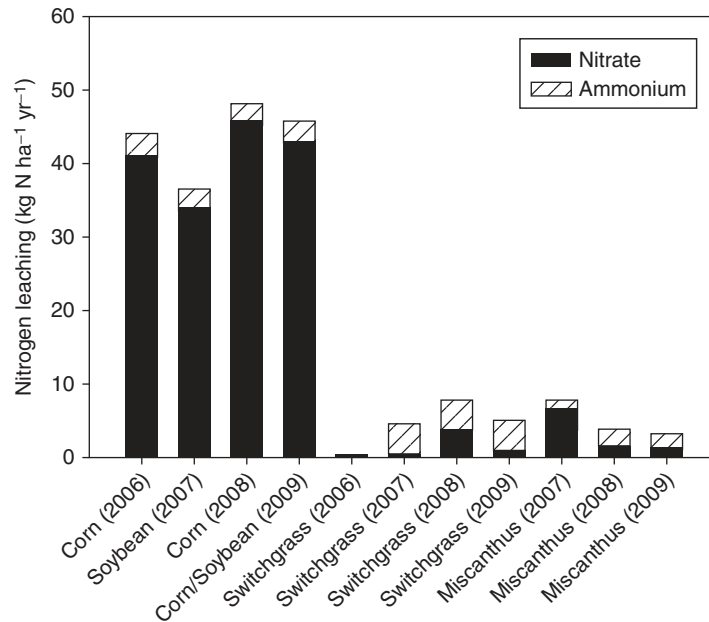


Fig. 9. Annual leaching fluxes of nitrate and ammonium in ion exchange resin lysimeters at 50 cm depth under corn-soybean, switchgrass, and Miscanthus grown on central Illinois, USA Mollisols. Adapted from McIsaac *et al.* (in press).

benefits of large-scale plantings will depend on local conditions and the fraction of the landscape planted to *M. × giganteus*. The relative costs and benefits of reduced surface water flows or nitrate leaching, or other effects will vary in different contexts. Landowners, policy makers and citizens need reliable and locally relevant information about these impacts in order to make informed decisions about land management and policy alternatives. Since these consequences may occur over decades, mechanistic models based on empirical research are needed to provide reasonable projections of the impacts in a wide range of settings.

## VI. TECHNICAL CHALLENGES TO COMMERCIAL PRODUCTION

Even though European researchers have studied *M. × giganteus* as a biomass feedstock since the early 1980s, and Illinois researchers have studied its use since the early 2000s, barriers remain to the commercial production of the grass. Given that the biomass potential of *M. × giganteus* is great for

some temperate areas in North America, it is important that these hurdles be overcome in a timely fashion in order to avoid being unprepared should an energy crisis occur. These challenges occur in the grass' propagation and establishment, agronomy, pest and pest controls, and genetics.

#### A. PROPAGATION, RHIZOME STORAGE AND ESTABLISHMENT

##### 1. Propagation

Because it is sterile, asexual propagation, such as rhizome divisions or micropropagation, must be used to multiply *M. × giganteus* into commercial quantities. Overall, propagating this grass is a relatively simple horticultural exercise; conversely, propagating large commercial quantities is less so.

Propagating *M. × giganteus* using rhizome divisions entails separating a rhizome mass into small pieces for replanting. This can be done with potted plants growing in greenhouses and can also be conducted using field-grown plants. University of Illinois experience has determined that the potted *M. × giganteus* can often be divided every 4–8 weeks (Pyter *et al.*, 2009) when grown in greenhouses under 12 h per day artificial light during winter using 10 cm square pots and an artificial, soil-less potting mix.

Small rhizome segments can also be used to produce plantlets or plugs by dividing rhizomes into very small, two-to-three node segments, potting the segments and growing these rhizome segments into small plants. This technique is being used commercially with success to produce large numbers of plants using relatively small amounts of rhizomes. This is a likely method for planting commercial acreages where irrigation or reliable natural precipitation is available to ensure that the small plants will become established (Fig. 10).

*Miscanthus × giganteus* rhizomes can also be field-planted, grown into established plants and harvested after one or two growing seasons. Mechanical rhizome lifters are available (e.g. Tomax, Ltd., Waterford, Ireland) which can be used to improve harvest efficiency and yields. By observation, most of the mechanically harvested rhizomes pieces are 10–25 g. Pyter *et al.* (in press) found that 20–25 g rhizome pieces produced statistically similar end-of-season biomass as rhizomes pieces of 40 g. Pyter *et al.* (2009) reported hand-harvested yields of 7–10 rhizome pieces from 1-year clumps and 25–30 rhizome pieces from 2-year clumps. Mechanical harvesting likely yields greater numbers of the smaller rhizome pieces. In 2010, a mechanically harvested 0.4 ha field of 1-year plants yielded enough rhizomes to replant approximately 3.6 ha, representing a ninefold multiplication factor (Pyter *et al.*, in press).

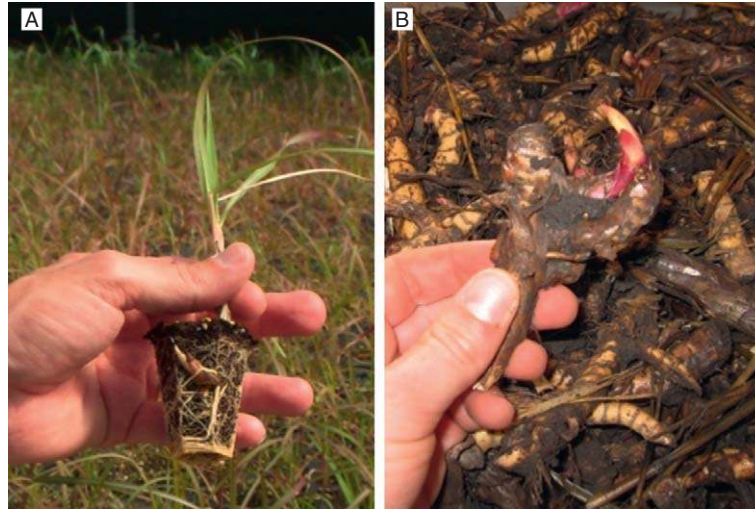


Fig. 10. (A) Greenhouse grown “plug” of *M. × giganteus*. (B) Field dug *M. × giganteus* rhizome. Photo credits N. Boersma and E. Heaton, respectively.

Micropropagated *M. × giganteus* plants are commercially available in the US, but are expensive. In addition, in Denmark and Clifton-Brown *et al.* (2007) in Ireland found that rhizome-produced *M. × giganteus* plants survived the first winter, while plants directly regenerated using micropropagation died the first winter after planting (Clifton-Brown, 1997).

### 2. Rhizome storage

Currently, research is being conducted in Illinois in an attempt to identify the physiological condition of rhizomes necessary for survival. In one Illinois study, Pyter *et al.* (in press) found that rhizomes can be successfully stored at 4 °C for up to 4 months in moist sand. In an ongoing 2010 study, rhizomes have been shown to require a moisture content of at least 50% to regrow after planting. When improperly stored, rhizomes can dry below this level and not survive planting. Additional work is being conducted to measure carbohydrate levels within rhizomes harvested at different times of the year to identify conditions that can limit establishment success.

### 3. Planting and establishment

Mechanical nursery or vegetable transplanters have been successfully used to plant *M. × giganteus* rhizomes and plugs. Rhizomes planted to 10 cm produced the greatest amount of biomass at the end of the first season’s growth

in Illinois (Pyter *et al.*, in press). Planting densities have varied, with successful plantings occurring at densities of 10,000–12,000 ha<sup>-1</sup> (Pyter *et al.*, 2009). As small rhizome segments and plugs are used, planting densities may increase to 20,000–25,000 ha<sup>-1</sup> to reduce gaps in plantings due to low rhizome emergence rates. Emergence rates have ranged from as low as 50% to 98% depending on rhizome size and storage conditions (Huisman and Kortleve, 1994) in Europe. Pyter *et al.* (2009) reported 60–70% of the rhizomes sprouting in an Illinois planting.

Plugs comprising small plants, growing in a small amount of horticultural potting mix can be produced using micropropagated plantlets, rhizome pieces or rooted stem cuttings and can be an outstanding substitute for planting rhizomes (Atkinson, 2009). The main limitation to planting plugs is the probable need for irrigation during establishment. Plugs can be well established and relatively drought-tolerant in less than a growing season, but during the establishment period, a prolonged period of droughty weather can compromise plug survival without the availability of water to supplement natural precipitation.

While established *M. × giganteus* have survived winter air temperatures as low as –20° C in Illinois (Pyter *et al.*, 2009), there have been situations where first year *M. × giganteus* crops have been damaged or killed during the first winter after planting. Clifton-Brown and Lewandowski (2000a) and Clifton-Brown *et al.* (2001a) reported that *M. × giganteus*, especially first year plantings, is at risk when soil temperatures at 5 cm drop below –3 °C. During the 2008–2009 winter, 2008 plantings in portions of the US Midwest were severely damaged; 6.5 ha in Illinois were thinned during that winter to the degree that the replanting was necessary. Concerns over first year winter damage can obviously limit where *M. × giganteus* can be commercially produced.

In December 2009, in a Decatur, Illinois, US demonstration planting, daily soil temperatures at a 5-cm depth were as much as 4 °C colder under first year plants than under third year plants. A small, little-developed rhizome mass, along with virtually no insulating leaf litter on the soil surface may combine to explain the susceptibility of winter damage in first year plants. Also, Lewandowski (1998) speculated that *M. × giganteus* is more tolerant of cold when the temperature decline is slow and steady, rather than sudden. She also indicated that cold damage may occur when temperatures vary above and below freezing, writing that *M. × giganteus* may sprout during warm stretches, only to be damaged when temperatures drop, because it is susceptible to cold damage (Lewandowski, 1998). These hypotheses require further study for confirmation.

Commercial barriers caused by a lack of propagation and planting information or by inconsistent research findings require that additional work be

completed prior to successful large-scale planting. Research that identifies efficient methods and equipment designed for low-cost propagation and planting with guaranteed establishment are necessary prior to commercial acceptance of *M. × giganteus* as a biomass feedstock.

## B. AGRONOMY

### 1. Fertility

It is not clear whether fertilizer applications will increase the harvestable yields of *M. × giganteus* (Heaton *et al.*, 2010; Miguez *et al.*, 2008). Experiments in Europe have shown contrasting responses of *M. × giganteus* to N fertilizer. For example, in Austria, Schwarz *et al.* (1994) reported an increase of only 1 Mg ha<sup>-1</sup> in dry biomass (from 20.6 to 21.6 Mg ha<sup>-1</sup>) with an increase in N level from 0 to 180 kg N ha<sup>-1</sup> in a 3-year-old crop. However, the authors hypothesize that because the soil had a high capacity for nutrient delivery, the lack of N response can be attributed, at least in this case, to the combined effect of optimal initial nutrient supply and the fact that only the second and third growing seasons were reported (Schwarz *et al.*, 1994). Similarly, in Germany, Himken *et al.* (1997) did not observe a significant N fertilization effect on above-ground or rhizome dry biomass, possibly because the soil supplied the N needed for the yield levels at this location. Moreover, this study measured above-ground biomass as well as below-ground biomass and showed that the rhizome biomass can exceed 15 Mg ha<sup>-1</sup> for a crop that achieved a peak dry biomass of 30 Mg ha<sup>-1</sup> in September (Himken *et al.*, 1997). Finally, at Rothamsted Research Farm, in England, biomass yields from a 14-year planting of *M. × giganteus* did not respond to N application (Christian *et al.*, 2008). The authors wrote that this was likely the result of the soil type at the research site, the management of the previous crop produced on the research site and the efficient resource utilization of the C<sub>4</sub> grass including the natural recycling of N and other minerals within the plant.

Conversely, increasing levels of nitrogen increased yields in an Italian study (Ercoli *et al.*, 1999). These authors found an interaction between irrigation and N fertilization. With 100 kg N ha<sup>-1</sup> of fertilizer, irrigation increased dry biomass by 3.7 Mg ha<sup>-1</sup>, and with 200 kg N ha<sup>-1</sup>, irrigation increased dry biomass by 9.8 Mg ha<sup>-1</sup>. Other minerals may play a role in *M. × giganteus* biomass yields. In a 15-year *M. × giganteus* study in Ireland, Clifton-Brown *et al.* (2007) showed an increased yield with nitrogen in some years, but not in others. This study also showed a yield increase with an application of K, indicating the need to study fertilization regimes beyond nitrogen.

Given the inconsistent results of these studies, a simplistic explanation of *M. × giganteus* response to fertilizer remains difficult. It is likely that the response of *M. × giganteus* to fertilization is due to the interactions of weather conditions, soil type and agronomic management. Thus, yield response to fertilization may change from field to field or even change within the same field from year to year. Moreover, predicting an exact response of *M. × giganteus* to fertilization is also difficult, making it problematical for agronomists to make accurate fertilization recommendations when managing *M. × giganteus*.

## 2. Weeds, insects and diseases in *M. × giganteus*

*a. Weed control.* Weed control during the first year, and sometimes the second year, is necessary to produce a successful *M. × giganteus* crop (Christian and Haase, 2001; Lewandowski *et al.*, 1995, 2000). Several researchers have evaluated post-planting tillage and cover crops as weed controls with varying success (Buhler *et al.*, 1998; Bullard *et al.*, 1995; Schwarz *et al.*, 1994; Venturi *et al.*, 1999). More consistent weed control, however, is likely to be accomplished through the use of herbicides. Unfortunately, the herbicides registered for *M. × giganteus* are limited to applications to landscape ornamentals.

Both pre-emergence and post-emergence herbicides have been used in Europe; researchers generally found that herbicides that are safe for application to corn can be safely applied to *M. × giganteus* (Bullard *et al.*, 1995; Serafin and Ammon 1995, as cited in Lewandowski *et al.*, 2000). Buhler *et al.* (1998) reported that metolachlor appeared to be safe on various warm-season perennial grasses, and Huisman *et al.* (1997) recommended applications of atrazine to control weeds. In Illinois experiments that applied herbicides at typical corn rates, Pyter *et al.* (2009) reported that *M. × giganteus* was tolerant of pre-emergence applications of pendimethalin, pendimethalin + atrazine, S-metolachlor and S-metolachlor + atrazine, and post-emergence applications of 2,4-D ester and dicamba. More recently, Anderson *et al.* (in press) found in field studies that herbicides, in general, that are applied to control weeds in maize were safe to apply to *M. × giganteus* and included atrazine, pendimethalin and S-metolachlor applied as pre-emergence herbicides, and bromoxynil, dicamba and mesotrione + atrazine applied as post-emergence herbicides.

*b. Insect and disease control.* Huggett *et al.* (1999) found that the corn leaf aphid (*Rhopalosiphum maidis*) survived, was highly fecund and able to transmit barley yellow dwarf virus (BYDV) which is a concern because *Miscanthus* spp. can carry the virus with or without showing symptoms.



In the US, Bradshaw *et al.* (2010) also reported corn leaf aphid and yellow sugarcane aphid (*Sipha flava*) on field-grown *M. × giganteus* in four states. Christian *et al.* (1997) observed larvae of the common rustic moth (*Mesapamea secalis*) feeding on *M. × giganteus* tissues in the spring, but harvestable stem density did not appear to be affected. In Illinois, Spencer and Raghu (2009) reported *M. × giganteus* to be a site of oviposition and emergence of the Western Corn Rootworm (*Diabrotica virgifera virgifera*), a major pest of maize. Finally, Prasifka *et al.* (2009) noted that fall armyworm (*Spodoptera frugiperda*) infested field plots of *M. × giganteus* and fed on its leaves in laboratory studies.

While Christian and Haase (2001) report that no diseases greatly affect *M. × giganteus* production, several pathogens have been found on the grass. *Fusarium* spp. (Thinggaard 1997, as cited in Lewandowski *et al.*, 2000), BYDV (Bullard *et al.*, 1995; Christian *et al.*, 1994) and *Miscanthus* blight (*Leptosphaeria* spp.) (O'Neill and Farr, 1996) can affect *M. × giganteus*. At several Midwestern US sites, nematodes, including two species of *Xiphinema* and one species of *Longidorus* (*Longidorus breviannulatus*), were found in soils surrounding *M. × giganteus* roots (Mekete *et al.*, 2009). They also reported that great numbers of *L. breviannulatus* appeared to destroy fibrous roots and stunt lateral roots (Mekete *et al.*, 2009). Ahonsi *et al.* (2010) reported occurrences of the leaf blight, *Pithomyces chartarum*, in Kentucky.

### 3. Harvesting technology

At the conclusion of the growing season, *M. × giganteus* usually drops most of its leaves as it senesces, and the senesced stems are typically harvested during the winter, from November through the end of March in temperate areas, depending on snow cover and access to fields. US companies are evaluating different equipment for cutting, conditioning, windrowing and baling the stems to determine efficient and effective methods. Traditional hay equipment works, but it is a slow process given the toughness and large number of harvestable stems. The harvest goal is to cut at 5–10 cm, but in past evaluations, some ill-suited equipment left *M. × giganteus* stems of more than 30 cm. Leaving biomass in the field unharvested is a concern that equipment manufacturers must consider; more than 2 t ha<sup>-1</sup> of biomass remained in an Illinois field following a 2010 harvest.

Baling machines have successfully produced variously sized round and rectangular bales of *M. × giganteus* in the past. Moisture levels of the biomass tend to vary with harvest time. Heaton (2006) reports moisture levels from 50% in an October harvest down to less than 10% in a February harvest. Under cover, the stored bales have remained intact and in good

condition for at least 3 years without excessive breakdown or attracting rodent or insect pests.

Existing technology allows *M. × giganteus* to be cut, baled and stored for later use. At present, however, the challenges to commercial production are the inefficiencies of equipment designed for hay and straw crops, not for heavier-stemmed biomass crops. It is very likely that technology that combines woody plant harvest with hay and straw crop harvest will be suitable for biomass crops.

#### 4. Eradicating *M. × Giganteus*

Eradicating *M. × giganteus* in order to convert a planting to another biomass feedstock or to a row crop has also been studied. Anderson (2010) found that tillage combined with glyphosate applications can control mature *M. × giganteus*, but treatments will likely need to be repeated in a second growing season for complete eradication. In another experiment, Anderson *et al.* (2010) examined planting glyphosate-resistant soybeans directly into a mature stand of *M. × giganteus* and found that soybean yield was not reduced when either one or two sequential glyphosate applications were made in-crop compared with a weed-free control. The following year, this field was rotated into glyphosate-resistant corn, and corn yields were similar to the weed-free control following one or two sequential applications of glyphosate. While glyphosate applications kept the *M. × giganteus* from reducing yields, Anderson (2010) speculated that it will likely take more than two growing seasons to completely eradicate the biomass grass.

Overcoming agronomic challenges are paramount to successful commercial production of *M. × giganteus*. Some of this research will be, by necessity, local and continuous, given the probability of local responses to fertility and pest problems, along with the evolution of pests and *Miscanthus* genetics. Improved harvesting technologies, however, are likely to be useful throughout the entire geographic area where *M. × giganteus* is commercially grown.

### C. NEW VARIETIES

The *M. × giganteus* clone used in University of Illinois feedstock research originated from rhizomes obtained from the Chicago Botanic Gardens (Glencoe, Illinois) in 1988 (Pyter *et al.*, 2009) and has been part of a landscape demonstration planting at the University since that time. In addition to this common landscape clone, there are other *M. × giganteus* types being developed and marketed specifically for biomass production. For example, 'Freedom' Giant *Miscanthus* was developed at Mississippi State University and is being produced for commercial planting by SunBelt Biofuels (<http://>

[www.extension.org/pages/“Freedom”\\_Giant\\_Miscanthus\\_is\\_Viable\\_Biofuel\\_Feedstock](http://www.extension.org/pages/“Freedom”_Giant_Miscanthus_is_Viable_Biofuel_Feedstock); [http://www.biomassmagazine.com/article.jsp?article\\_id=3536](http://www.biomassmagazine.com/article.jsp?article_id=3536)). New Energy Farms of Canada (<http://www.newenergyfarms.net/pricing.aspx>) lists ‘Amuri’ and ‘Nagara’ as very cold-tolerant, high-yielding *Miscanthus*. Biotechnology firms such as Ceres, Inc. and Mendel Bioenergy Seeds are evaluating additional forms of *Miscanthus* to determine biomass potential.

Additional *M. × giganteus* genotypes present growers with options when producing this crop that will allow for selection from a pallet of grasses for different locations and environments in order to produce the most productive and least input-dependent grass for an area. Moreover, it is likely that additional genotypes will be developed in the future, which offers additional opportunities to fine-tune planting choices such as disease and/or insect resistance. The barrier to commercial production of these new genotypes is the need to conduct research so that agronomists can direct growers to make the best choices for a biomass production scheme.

## ACKNOWLEDGEMENTS

This work was primarily supported by the Illinois Council on Food and Agriculture Research (Award 04-SRI-036). Additional support was provided by the Energy Biosciences Institute at the University of Illinois and the Iowa State University Department of Agronomy. The authors thank Dustin Schau for reviewing this manuscript.

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