AGRICULTURAL AND FOREST METEOROLOGY 148 (2008) 1280–1292

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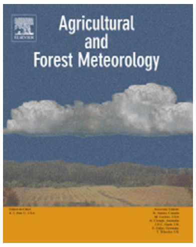

Meta-analysis of the effects of management factors on *Miscanthus* × *giganteus* growth and biomass production

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ARTICLE INFO

Article history:
Received 29 October 2007
Received in revised form
10 March 2008
Accepted 15 March 2008

Keywords:
Miscanthus × *giganteus*
Miscanthus
Meta-analysis
Biofuels
Non-linear mixed models

ABSTRACT

Biomass crops in the U.S. have the potential to reduce the dependence on foreign energy supply, to lower net greenhouse emissions, and to diversify agroecosystems. *Miscanthus* × *giganteus* has been extensively researched in Europe but the response to key agronomic management factors has not been summarized. In this study we have collected most of the relevant and up to date European literature on the response of dry biomass production to planting density and nitrogen (N) fertilizer and we provide quantitative estimates of the effect of these practices. The data were analyzed through non-linear mixed models which take into account the hierarchical structure of the data due to variability among countries, locations and years. *M.* × *giganteus* responded to N fertilizer only after the third growing season and planting density only had a significant effect on the second growing season. The similarity among growth curves, when dry biomass production was analyzed as function of thermal time, shows the stability of the cropping system against other environmental factors.

1. Introduction

Biofuels can be used to address three important societal concerns: energy supply security, lower net greenhouse emissions, and support for agriculture (Koonin, 2006). The urgent need for a transition from nonrenewable carbon (C) sources to renewable biosources can be realized, in part, by dedicated biomass crops (Ragauskas et al., 2006). *Miscanthus* × *giganteus* is an ideal biomass crop which can be used to generate heat, power and fuel, and alleviate carbon dioxide (CO_2) emissions (Heaton et al., 2004a). It is a perennial C_4 grass with high yield potential (Heaton et al., 2004b), efficient conversion of radiation to biomass, efficient use of nitrogen (N) and water, and good pest and disease resistance (Beale and Long, 1995). These are desirable characteristics for sustainable production which can also provide environmental services such as improved soil quality and reduced nitrate leaching (Lewandowski et al., 2000).

Maximizing *M.* × *giganteus* biomass productivity requires consideration of climate, soil, genetics, and management factors (Heaton et al., 2004a; Lewandowski et al., 2003). The most critical phase of *M.* × *giganteus* production is planting and establishment of the crop (Christian and Haase, 2001). *M.* × *giganteus* is a naturally occurring sterile hybrid that must be propagated vegetatively by either rhizomes or plantlets and this requirement makes establishment costly (Lewandowski, 1998). Improvements in propagation and planting technology such as storage and mechanization of rhizome establishment are in progress (Lewandowski et al., 2003). In addition, appropriate temperatures and timely water supply are critical for successful establishment of the crop (Lewandowski et al., 2000).

Growth patterns of *M.* × *giganteus* can be evaluated based on growth across seasons and within a growing season (Fig. 1).

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doi:10.1016/j.agrformet.2008.03.010

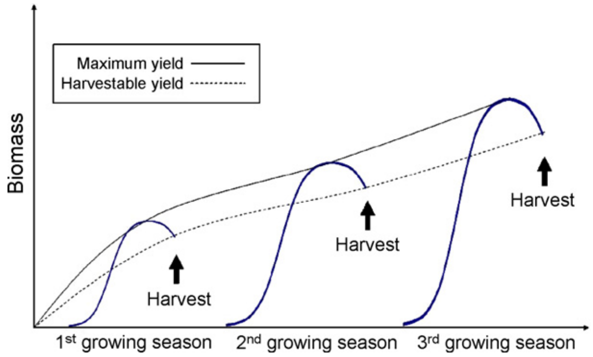

Fig. 1 – Schematic representation of *M.* × *giganteus* growth across three growing seasons. The arrows indicate harvest, the solid line indicates maximum yield and the dashed line indicates harvestable yield.

Yearly maximum biomass yields in the fall or harvestable biomass yields in the late fall or winter are measured to describe growth across growing seasons. Models of growth across seasons of *M.* × *giganteus* potential annual dry biomass (Clifton-brown et al., 2004; Price et al., 2004), have commonly ignored the establishment phase of the crop. As a perennial crop, *M.* × *giganteus* produces less biomass the first growing season and annual biomass production is expected to increase during the first 3–5 years (Fig. 1). The rate of growth depends on environmental conditions such as soil type, precipitation, and temperature, as well as management practices such as harvest time, planting density, and N fertilizer application. Describing and predicting growth patterns until ceiling yields are realized is particularly important from the economical standpoint since the initial investment in planting will not be recovered immediately.

Within a growing season, *M.* × *giganteus* biomass accumulation normally peaks between August and October and decreases thereafter mainly due to translocation of assimilates and leaf detachment (Beale et al., 1996). Yield losses during this period range from 10 to 30% of total biomass production (Clifton-Brown et al., 2001a) and are accompanied by lower moisture and mineral content which are desirable characteristics (Lewandowski et al., 2000).

Common agronomical decisions in *M.* × *giganteus* production include N fertilization, initial planting density and harvest time. Reports indicate that the response of *M.* × *giganteus* to N fertilization is small. For example, there was a small difference in N uptake in Rothamsted (Christian and Haase, 2001), UK during two seasons among three N rates (i.e. 0, 60, 120 kg ha^{-1}). Conversely, a significant response to N fertilizer was observed with irrigation at the University of Essex, UK (Christian and Haase, 2001) and similarly in Italy and Greece, the highest recorded yields were obtained when the highest rate of fertilizer was applied with irrigation (Danalatos et al., 1998; Foti et al., 1996).

Nitrogen fertilizer requirements of *M.* × *giganteus* are low when compared to row crops (Lewandowski et al., 2000). The high N use efficiency is mostly a result of the ability of the crop to recycle N (Christian et al., 2006), and of the C_4 photosynthetic pathway (Beale and Long, 1997). This high N use efficiency results in material low in N concentration, which is highly desirable for direct combustion in order to minimize pollution. Nevertheless, the high biomass yield achieved by the crop results in nutrient off-take, which needs to be compensated with applications of N fertilizer (Beale and Long, 1997; Himken et al., 1997).

High planting density benefits the crop by improving competition for resources with weeds, and achieving high yields faster than when using low planting densities, yet it also increases costs (Christian and Haase, 2001). Initial benefits of high densities are expected to wane once the crop is mature; the maximum dry biomass production will be the same regardless of the initial planting density (Clifton-Brown and Lewandowski, 2002). Additionally, experiments in Italy (Foti et al., 1996) and Greece (Danalatos et al., 1998) showed that for high initial planting densities (4 plant m^{-2}) a large number of shoots died back as a result of severe competition for nutrients and light. This highlights the importance of choosing an optimal planting density from both economical and agronomical points of view.

M. × *giganteus* harvestable dry biomass yield depends strongly on harvest date (Beale and Long, 1995). The harvesting window in *M.* × *giganteus* production is determined by the first frost in the fall and the time of regrowth in the spring. At the point of maximum biological yield the crop is green and the moisture content is high. Delaying harvest after this point improves burning quality but there are also losses of biomass due to leaf detachment and even lodging (Lewandowski and Heinz, 2003) thus choosing a harvest date represents a compromise between harvestable yield and quality. In addition, optimal harvest dates might vary among regions depending on weather conditions (Lewandowski and Kicherer, 1997). Lewandowski and Heinz (2003) showed that snow precipitation before harvest caused breaking of the upper stems and ice rain and snow caused heavy lodging.

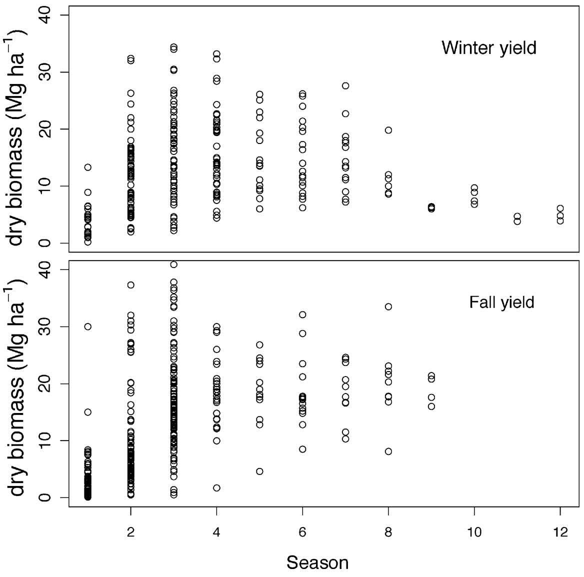

Fig. 2 – Scatter plots of *M.* × *giganteus* dry biomass and season for Winter yield (above) and Fall yield (below). Fall yield was harvested before December 21st and Winter harvest after this date. Season represents the specific growing season for the crop.

In the literature there are several statements about how many years it is expected that $M. \times$ *giganteus* would maintain its ceiling yield (Ercoli et al., 1999; Lewandowski et al., 2003; Schwarz et al., 1994) but there are no studies which include "long-term" yield data (i.e. the longest reported study has 12 seasons). Additionally, Jorgensen (1996) suggested that $M. \times$ *giganteus* dry biomass production declines after only eight seasons (Fig. 2). However, a closer look at this study revealed that the lower yields were a result of extreme weather (i.e. dry and cold) and not because the crop was deteriorating. For this reason, the analysis did not include this study's later years (8–12). The lack of long-term experiments implies that it is not possible to provide an accurate estimate of how long $M. \times$ *giganteus* will maintain its ceiling yields. We speculate that this will depend on soil type, climate, and management.

If $M. \times$ *giganteus* will become the carbon-neutral, renewable source of heat, power and fuel of the future (Hall and Scrase, 1998) it will be beneficial to quantitatively summarize the effects of the most relevant management practices. Also, a quantitative description of the growth patterns should help identify the fundamental constrains on productivity as well as providing predictions for potential biomass yield where this information is lacking.

A large number of studies have focused on different aspects of $M. \times$ *giganteus* production yet this information has not been summarized. The limitation lies in part, in that many of these studies have not been published in peer-reviewed literature. On the other hand, most modeling efforts have ignored the initial phase of the crop which typically lasts 2–5 years (Price et al., 2004). Still, much of these data can provide valuable information if appropriate methods are used to analyze it. The general framework of meta-analysis (Gurevitch and Hedges, 2001) and non-linear mixed models are used here to distill the relevant information from the vast amount of published literature.

The objectives of this analysis are: (1) to describe $M. \times$ *giganteus* growth across seasons in different environments as affected by N fertilization and planting density, and (2) to describe $M. \times$ *giganteus* growth within a growing season and the factors affecting it.

2. Materials and methods

2.1. *Database compilation*

A literature search of primary research was conducted with Silver-Platter (Ovid Technologies, New York, NY) and Web of Science (ISI, Philadelphia, PA) electronic databases and through location of studies included in the references. The criteria for including studies in the analysis were different for the different aspects of $M. \times$ *giganteus* growth. The literature search was divided into those studies which included multiple biomass measurements within a growing season and those which reported only final biomass. Studies that reported final biomass only were further divided in those studies which reported biomass before December 21st (Fall yield) and after this date (Winter yield). It would be important to establish a relationship between harvest time and dry biomass production, however few studies reported both harvest times. For the analysis of dry biomass production across seasons no attempt of establishing this relationship was carried out. A description of the studies used in the analysis across seasons and within seasons can be found in Tables 1 and 2, respectively. The variables included in the database were: location, country, year, experiment, growing season, planting date, number of stems, Fall yield, Fall moisture, day of harvest (fall), Winter yield, Winter moisture, day of harvest (winter), plant height, planting density, mean air temperature, total precipitation, and N fertilizer rate.

In some studies the initial planting density was higher than the effective final density because weather conditions during the winter months caused loss of plants. The planting density used in the analysis is always the final effective planting density. Unless information is provided regarding winter kill, it was assumed that the planting density reported is the final effective planting density.

2.2. *Statistical analysis*

The first step in the statistical analysis was to define conceptually the structure of the data and the experimental unit. The clustering structure was organized with the *country* as the first level, the second level was the *location* and the third level was the *experiment*. This error structure was modeled as random effects, because the interest was not to predict yield for specific locations or countries but rather to study the effect of management variables on the growth patterns of $M. \times$ *giganteus*. Some studies reported data from different countries, and it was considered safe to regard data coming from different countries, although reported in the same study, as being independent (Gates, 2002; Miguez and Bollero, 2005).

The database used to investigate the growth patterns of $M. \times$ *giganteus* within a growing season included multiple biomass harvests within a growing season rather than just one harvest (Table 2). In this case the covariate used was thermal time. Accumulated thermal time (TT) was calculated by accumulation of daily average air temperature (T^a) over the growing season, from the first (d_1) to last day (d_n), $TT = \sum_{i=d_1}^{d_n} T_i^a$. The weather data reported in each study were used or it was estimated from weather records from the closest weather station available.

2.2.1. *Description of the database and preliminary analysis*

There were 31 studies included in the analysis of dry biomass across seasons (Table 1). Many studies included data from several countries and multiple seasons. Therefore, there were 645 observations total. From all the studies included in the analysis, 55% were obtained from journal articles, 39% from conference proceedings, and 6% from book chapters. Before the statistical analysis was carried out, data obtained from Winter harvests were explored and some specific observations were not included in the subsequent analysis for the following reasons. One data point from an irrigated study in Catania, Italy, was removed from the analysis, because it was the only season that was not irrigated. Therefore, it was considered that this last data point does not reflect the potential productivity at this site (the biomass production was 32 Mg ha^{-1} in the fourth season and 19 Mg ha^{-1} in the fifth). Experimental units 116 and 117 (Fig. 3) did not provide planting density and N fertilizer rate data so they were not included in

Table 1 – Studies included in the database for the analysis of *M.* × *giganteus* growth across seasons

Author(s)	Category[a]	Country	Location(s)	Experiment
Acaroglu and Aksoy (2005)	1	Turkey	Konya	110
Bao Iglesias et al. (1996)	2	Spain	Rianxo	111
Beale and Long (1995)	1	England	Essex	158
Beale and Long (1997)	1	England	Essex	158
Bullard et al. (1995)	1	England	ADAS-Rosemound	156
Christian and Hasse (2001)	3	Austria	St. Florian, Atzenbrugg, Markgraf, Steinbrunn, Ilz	1–5
Christian et al. (2006)	1	England	Rothamsted	146
Clifton-Brown and Lewandowski (2002)	1	Germany	Ihinger Hof	164
Clifton-Brown et al. (2000)	1	Ireland	Tipperary	155
Clifton-Brown et al. (2001a)	3	Ireland	TCD-Dublin, Hyperion	6–23
		England	ADAS-A Rickwood, ADAS-B Rickwood, IACR-Oxford, Essex	
		Spain	Santiago de Compostela	
		Portugal	Lisboa	
		Netherlands	BTG- Enschede	
		Germany	BFH-Braunschweig, FAL-Grosshansdorf, LWG-Frankfurt	
		Belgium	SORGHAL- Brussels	
		Italy	ENEA-A Tresaia, ENEA-B Brasimone, Catania	
		Greece	Pikermi	
Clifton-Brown et al. (2001b)	1	Sweden		24–89
		Denmark		
		England		
		Germany		
		Portugal		
Dalianis et al. (1996)	2	Greece	Kefalonia	130
Danalatos et al. (1996)	2	Greece	Xanthi	129
Danalatos et al. (1998)	2	Greece	Lamia	127,128
Ercoli et al. (1999)	1	Italy	Pisa	90
Foti et al. (1996)	2	Italy	Catania	119
Himken et al. (1997)	1	Germany	Rhine valley	109
Hotz et al. (1996)	2	Germany	Veitshoechheim	140–142
Jorgensen (1997)	2	Denmark	Hornum	112–117
Jorgensen (1996)	1	Denmark	Hornum	118
Kahle et al. (2001)	1	Germany	Klein Markow Boitzenhagen Guntersleben	94–97
Kilpatrick et al. (1994, 1996)	2	England	ADAS Rickwood, ADAS Rosemound, ADAS Starcross	137–139
Lewandowski (1998)	1	Germany	Gutenzel, Hohenheim	159–163
Lewandowski and Heinz (2003)	1	Germany	Gutenzell, Ihinger Hof, Durmersheim	143–145
Lewandowski and Kicherer (1997)	1	Germany	Gutenzell, Ihinger Hof, Durmersheim	91–93
Petrini et al. (1996)	2	Italy	Cervia	126
Pignatelli et al. (1998)	2	Italy	ENEA-A Tresaia, ENEA-B Brasimone	120–125
Price et al. (2004)	1	England	Buckfast, ADAS-Bridget, ADAS-HighM, Boxworth, Gleadthorpe, ADAS-Rickwood, ADAS-Rosemound	147–154
Rohricht and Beier (1998)	2	Germany	Saxony	131–136
Schwartz et al. (1994)	2	Germany	Fitchel Mountain, Niedere Gest, Ehrendorf, Gauland, Ascherberg, Sulingen, Ehrendorf, Hallertau, Ampermoos, Donauried, Boitzenhagen	98–108
van der Werf et al. (1993)	1	Netherlands	ter Apel	157

[a] Category refers to the type of publication: 1, journal article; 2, conference proceedings; 3, book chapter.

the analysis which tested these effects. After this preliminary exploration of the data the statistical analysis was carried out. There were 51 studies that reported dry biomass sampled in the fall. A study by Clifton-Brown et al. (2001a) reported data for many genotypes and locations but here only *M.* × *giganteus* data were considered. In addition, the crop failed in Denmark and Sweden so data from these locations were not included. The total number of observations included in this analysis was 184. The database used for the description of *M.* × *giganteus* growth within a growing season included 11 studies. The details of this dataset can be found in Table 2. Bullard et al. (1995) was not used because it reported the first year of growth only and yields were low. Christian et al. (2006) was not used because it did not report data within the growing season.

Table 2 – Studies included in the database for the analysis of *M.* × *giganteus* growth within seasons

Author(s)	Category[a]	Country	Location(s)
Beale and Long (1995)	1	England	Essex[b]
Beale and Long (1997)	1	England	Essesx[b]
Bullard et al. (1995)	1	England	ADAS-Rosemound
Christian et al. (2006)	1	England	Rothamsted
Clifton-Brown et al. (2000)	1	Ireland	Tipperary
Danalatos et al. (1996)	2	Greece	Xanthi[b]
Danalatos et al. (1998)	2	Greece	Lamia[b]
Foti et al. (1996)	2	Italy	Catania[b]
Jorgensen (1996)	2	Denmark	Hornum
Lewandowski (1998)	1	Germany	Gutenzel, Hohenheim
Schwartz et al. (1994)	2	Germany	Fitchel Mountain, Niedere Gest, Ehrendorf, Gauland, Ascherberg, Sulingen, Ehrendorf, Hallertau, Ampermoos, Donauried, Boitzenhagen
van der Werf et al. (1993)	1	Netherlands	ter Apel

[a] Category refers to the type of publication: 1, journal article; 2, conference proceedings.

[b] Irrigated study.

2.3. *Statistical model*

The non-linear function used to describe the growth of *M.* × *giganteus* over the years (Fig. 1) was the logistic growth function (Thornley and Johnson, 2000),

$$f(x) = \frac{\phi_1}{1 + \exp((\phi_2 - x)/\phi_3)} \qquad (1)$$

In this case, $f(x)$ is *M.* × *giganteus* dry biomass and x is the input, more specifically, the *season* (year). To avoid confusion the time covariate was termed *season* and growing season was used when referring to growth in specific years. Also, *season* was used rather than year because all of the studies were not conducted at the same time. For this particular analysis the domain of the function was defined to be the natural numbers

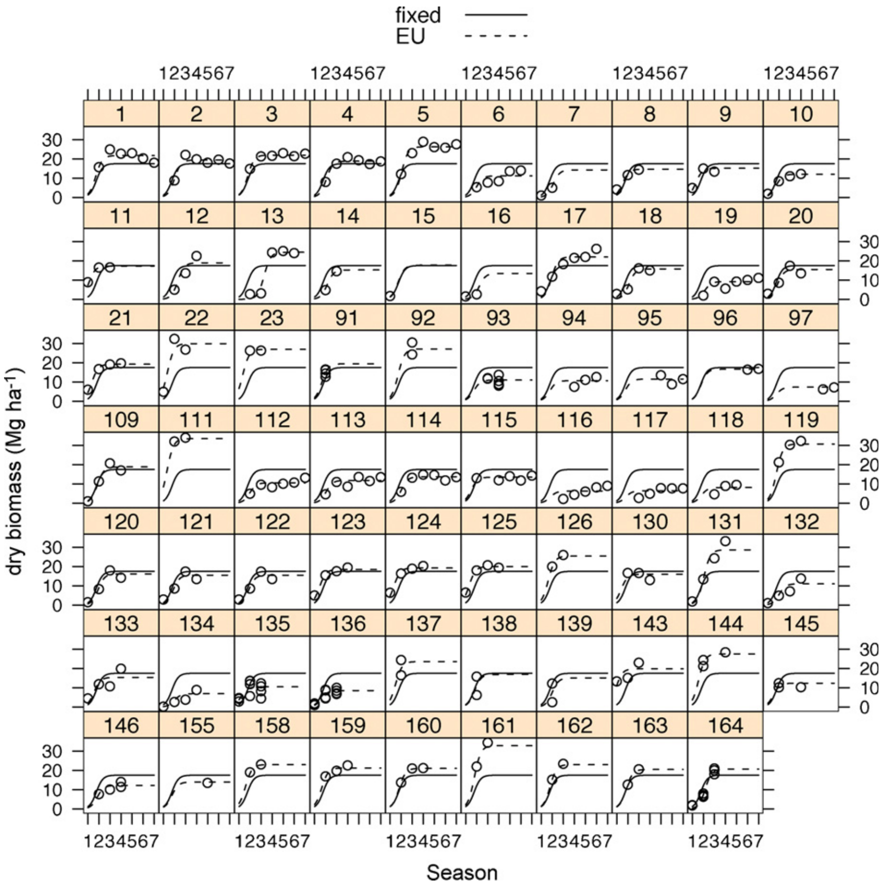

Fig. 3 – Fixed (solid line) and individual prediction, fixed plus random effect [experimental unit (EU), dashed line] from a logistic growth mixed model for *M.* × *giganteus*' Winter yield. This figure does not reflect the structure of the data or the effects of nitrogen and planting density. The number in each panel corresponds to the *experiment* in Table 1.

in the across growing season analysis (the predictions are only valid for whole numbers) and the positive real numbers for the within growing season analysis. The units were Mg ha^{-1} for *M.* × *giganteus* dry biomass and years for *season*. The first parameter (ϕ_1) had a meaningful interpretation and it was the asymptote (*Asym*), or the maximum dry biomass for a mature *M.* × *giganteus* crop. The second parameter (ϕ_2) represented the inflection point, or the point at which the crop achieved half of the maximum dry biomass (*xmid*). The third parameter (ϕ_3) was the scale (*scal*) and is normally an indication of the spread of the function, but it can also be interpreted as the time elapsed between the crop achieving half and approximately three quarters of the maximum dry biomass. Due to the aforementioned clustering hierarchy a mixed model was used to implement this approach. The mixed model was described as follows

$$
\begin{aligned}
&y_{ijkl} = f(\Phi_{ijkl}, \nu_{ijk}) + e_{ijkl}\\
&\Phi_{ijkl} = A_{ijkl}\beta + B_{i,jkl}b_i + B_{ij,kl}b_{ij} + B_{ijkl}b_{ijk},\\
&b_i \sim N(0, \Psi_1),\ b_{ij} \sim N(0, \Psi_2),\ b_{ijk} \sim N(0, \Psi_3)\ e_{ijkl} \sim N(0, \sigma^2)
\end{aligned}
$$

where *M.* × *giganteus* dry biomass (y_{ijkl}) depends on *country* (*i*), *location* within *country* (*j*), *experiment* within *location* within *country* (*k*), and the *season* (*l*). The function relating the covariate vector (ν_{ijk}) to the specific parameter vector (Φ_{ijkl}) was the non-linear function mentioned above (Eq. (1)). The group-specific parameter vector (Φ_{ijkl}) was modeled through the design matrix (A_{ijkl}) for the fixed component where the parameters were collected in the fixed parameters vector (β), plus the design matrices for random effects with the corresponding random effects vectors. In this formulation the *experiment* were nested within *location*, which were nested within *country*, so there are three levels of grouping. The model assumed that the three random vectors have zero mean and a general variance–covariance matrix, (i.e. Ψ_1 for *country* random effects, Ψ_2 for *location* within *country* random effects, and Ψ_3 for *experiment* within *location* within *country* random effects). A major advantage of this mixed model was the flexibility in the modeling of these variance–covariance matrices. The errors, (e_{ijkl}), were assumed to have zero mean and common variance. Additionally, the random effects and the error were assumed to follow a normal distribution. The random effects were assumed to be independent for different countries, locations and experiments and the within errors were assumed to be independent for different *ijkl* and to be independent of the random effects. The non-linear mixed model was fitted using the methods described in Pinheiro and Bates (2000), and it was implemented with the R software (R Core, 2006). The modeling process followed the principles in Pinheiro and Bates (2000) and the nlme (Pinheiro et al., 2007) and lattice (Sarkar, 2007) packages in **R** were used. Statistical modeling consisted of first fitting a preliminary model with fixed components for the three parameters of the logistic equation (Eq. (1)) and random effects for the lower level of clustering which in this case is the experimental unit or *experiment*. Later the N fertilization and planting density factor were added and the clustering structure with *country* and *location*. This process also involved the simplification of the variance–covariance matrix of the random effects. Thus, the final model has the clustering structure as well as the N fertilization and planting density factors. Residuals were checked for patterns, and autocorrelation. Nested models were compared using likelihood ratio tests and not nested models were compared using Bayesian Information Criteria (BIC) (Pinheiro and Bates, 2000).

3. Results and discussion

3.1. *Biomass accumulation across growing seasons*

3.1.1. *Growth curve modeling and model selection for Winter and Fall yield*

The ceiling dry biomass yield attained by *M.* × *giganteus* in some European countries was close to 40 Mg ha^{-1} (Fig. 2). However, this yield was not realized in the first season and it might take between 3 and 5 years until ceiling yields are attained (Fig. 3). In addition, the growth pattern of *M.* × *giganteus* across seasons differed among studies, which represent different locations and countries (Fig. 3). Although the general growth pattern was similar there was significant variation in maximum yield potential and time elapsed until ceiling yields were reached. A preliminary model was fitted for Winter yield (Fig. 3) and Fall yield (Fig. 4), which did not account for the clustering structure of the data or the effect of other variables, such as N fertilization and planting density. Biomass yield variability and differences in number of seasons per study (i.e. unbalanced data) can be assessed with the results of this preliminary model (Figs. 3 and 4). This model assumed a general variance–covariance matrix for the random effects and did not converge. A simpler model with a diagonal variance–covariance matrix which included the *Asym*, *xmid* and *scal* random effects was fitted instead. Figs. 3 and 4 are useful in the modeling process because they provide a visual evaluation of the potential of the model at explaining the data as well as the agreement between observed and fitted values identifying possible outliers. The next step was to incorporate the effects of planting density and N fertilizer. These were included in the models considering a linear dependency of the parameters of the non-linear model (i.e. *Asym*, *xmid* and *scal*) on the effects of planting density and N fertilizer. For Fall yield these were not statistically significant. For Winter yield incorporating the clustering structure lead to two candidate models and the simplest one was chosen based on a likelihood ratio test. The test comparing a model with random effects for *xmid* at the level of *country* and *location* and one without these random effects favored the more parsimonious model (p-value = 0.66 and BIC 1576 vs. 1565). A test against a simpler model without the random effect on the *xmid* at the level of experiment was significant (p-value < 0.0001) so the model was not further simplified. Thus, the final variance–covariance structure for *country* and *location* were simplified and a random effect for *Asym* was included. For the *experiment* level a random effects for *Asym* and *xmid* were included (Table 3).

3.1.2. *Effects of planting density and N fertilization*

Further analysis of the model included planting density and N fertilizer and these effects were significant for Winter yield. These variables were reported in most studies and they were also considered to be relevant management practices. Clearly, a higher planting density results in higher establishment cost and application of fertilizer also results in additional costs and

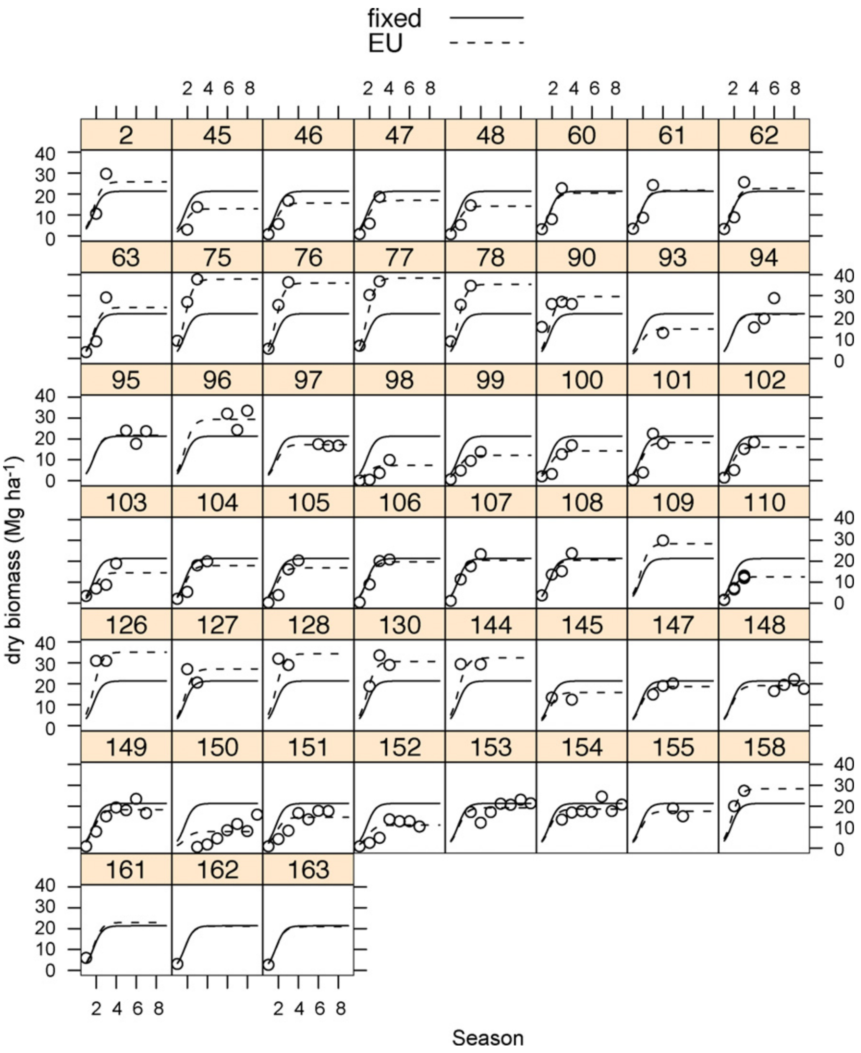

Fig. 4 – Fixed (solid line) and individual prediction, fixed plus random effect [experimental unit (EU), dashed line] from a logistic growth mixed model for *M.* × *giganteus*' Fall yield. This figure does not reflect the structure of the data or the effects of nitrogen and planting density. The number in each panel corresponds to the *experiment* in Table 1.

the residual N can lead to high levels of N in drinking water or contribute to pollution of surface waters (Dinnes et al., 2002). The literature suggests that initial planting density does not affect the ceiling yield but it does affect how fast the crop achieves maximum biomass (Clifton-Brown et al., 2001b; Jorgensen, 1996). In this case the hypothesis is that initial planting density will affect *xmid* (time, in years, until half maximum biomass) but not *Asym*. The results show that there

Table 3 – Description of the non-linear mixed model which incorporated the clustering structure but did not model possible effects of other factors on the fixed part of the model

Fixed terms	Winter yield			Fall yield		
	Asym	*xmid*	*scal*	*Asym*	*xmid*	*scal*
Estimate	18.4	1.77	0.301	24.9	1.79	0.48
Random effects						
Country (S.D.)	2.42			8.13		
Location (S.D.)	4.58			5.04		
Experiment (S.D.)	4.53	0.426		2.61		
Residual	2.44			4.02		

S.D. = standard deviation.
Asym = asymptote, *xmid* = half time until max yield, *scal* = time between half yield and 3/4 yield.

Table 4 – ANOVA table including the effect of initial planting density and nitrogen effects on the parameters of the logistic regression

Source	d.f.	Error d.f.	*F*-value	*p*-value
Asym.(Intercept)	1	191	187.9	<0.0001
Asym.NRate	1	191	9.29	0.0026
Asym.PlantDensity	1	191	0.75	0.3877
xmid.(Intercept)	1	191	799	<0.0001
xmid.Nrate	1	191	0.13	0.7156
xmid.PlantDensity	1	191	5.11	0.0249
scal.(Intercept)	1	191	65.28	<0.0001
scal.Nrate	1	191	0.37	0.5424
scal.PlantDensity	1	191	0.06	0.8081

Asym = asymptote, *xmid* = half time until max yield, *scal* = time between half yield and 3/4 yield. Nrate = Nitrogen fertilizer rate (kg N ha^{-1}), PlantDensity = plant density (plant m^{-2}).

was no linear effect of initial planting density on the *Asym* parameter estimate (*p*-value = 0.39) or the *scal* (*p*-value = 0.81) but it significantly affected *xmid* (Table 4). The estimated effect of plant density on *xmid* was −0.13 years/plant m^{-2} (lower: −0.212, upper: −0.050 years/plant m^{-2}). This effect should be interpreted as a decrease in the time elapsed until half of the dry biomass as the plant density increases.

Predictions of dry biomass production across seven seasons, four planting densities (1and 4 plant m^{-2}), and four N rates (0 and 100 kg N ha^{-1}) are shown in Figs. 5 and 6. Nitrogen fertilizer did not have an important effect for the first three growing seasons but it did seem to have a relatively small effect on the maximum dry biomass in the long term (i.e. *Asym*). These results suggest that the lack of N effect reported in many studies could be due to the short length of experimentation and the prevalence of studies conducted during the first years of *M.* × *giganteus* growth. In fact, Beale and Long (1997) estimated a fertilizer requirement for a dry biomass production of 15 Mg ha^{-1} to be 92 kg N ha^{-1}, 13 kg P ha^{-1}, and 204 kg K ha^{-1} based on yearly crop off-take. Ercoli et al. (1999) described an interaction between irrigation and N fertilization on *M.* × *giganteus* biomass production. In

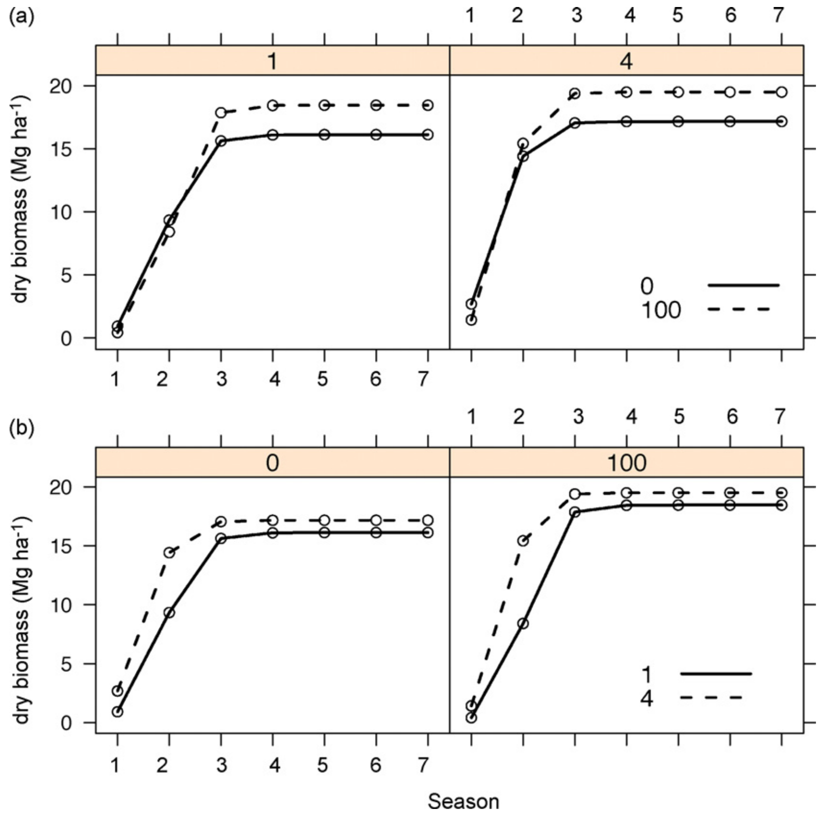

Fig. 5 – Dry biomass of *M.* × *giganteus* sampled in the Winter (after December 21st). (a) The two lines within a panel represent the effect of N fertilizer rate (0 and 100 kg N ha^{-1}) each panel represents a planting density (1 and 4 plant m^{-2}). (b) The two panels represent the effect of N fertilizer rate (0 and 100 kg N ha^{-1}) and the two lines within a panel represent two planting densities (1 and 4 plant m^{-2}).

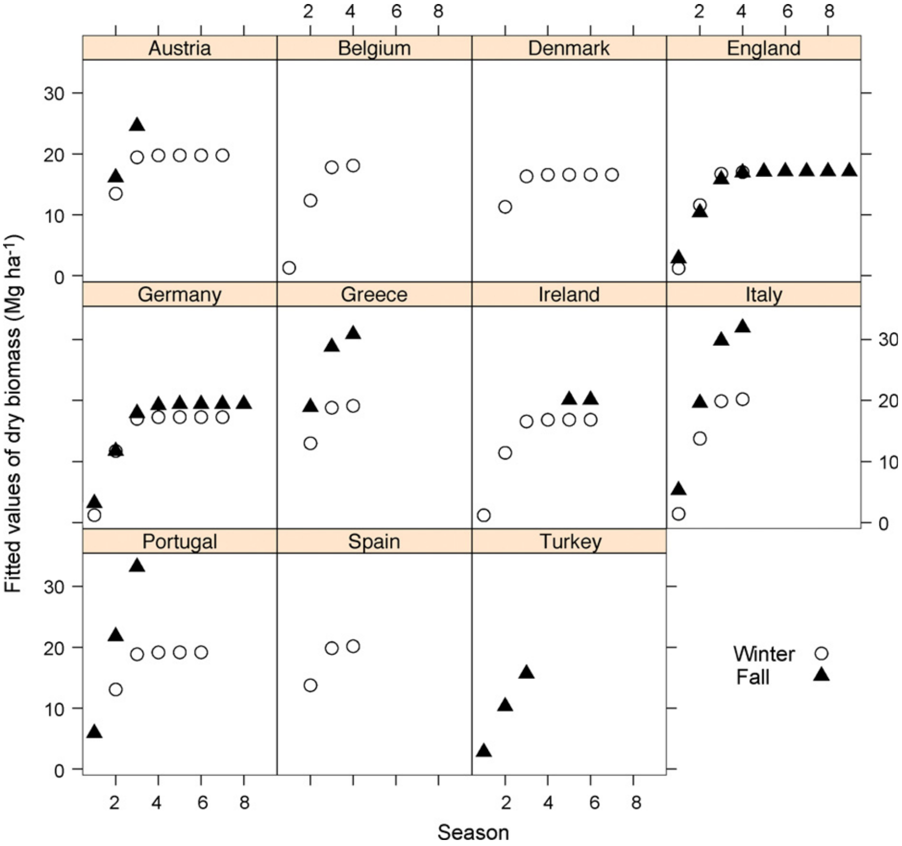

Fig. 6 – Fitted values of *M.* × *giganteus* dry biomass production from two separate non-linear mixed models. One fitted to the Winter yield and the other fitted to Fall yield.

this study, there was almost no difference between rainfed and irrigated *M.* × *giganteus* when no N fertilizer was applied, however there was an increase in biomass of approximately 10 Mg ha^{-1} on irrigated fields that received 200 kg N ha^{-1}. Additionally, the authors provided dry biomass for the four growing seasons, averaged over N fertilizer and irrigation treatments of the study (15, 26, 27, and 26 Mg ha^{-1}). The data reported, however, were averaged over the four studied seasons, so it was not possible to evaluate the interaction with season. The large effect of N fertilization on *M.* × *giganteus* dry biomass production at this location was likely due to the high yields attained by the crop and consequently the large removal of N from the system. This result seems to point to the relatively simple interpretation that if no N fertilizer is added to the cropping system, there will be a decrease in dry biomass production in the long run. Clearly, when *M.* × *giganteus* is harvested there is an off-take of N from the soil that should be replaced by an external source of N.

M. × *giganteus* is very effective in nutrient translocation back to the rhizomes (Beale and Long, 1997). Yet, if the continual removal of N in the dry biomass from the cropping system is not replaced, the potential growth of the crop will not be attained. The dry biomass production for the first growing seasons is relatively low and thus the N requirements will not be high. Christian et al. (2006) recovered 37.9% of labeled N in 1-year-old plants, but were able to recover 55.4% in 2-year-old plants. This study showed that *M.* × *giganteus* becomes more efficient at utilizing N fertilizer when the crop is at least 2- or 3-years old compared to 1-year-old crop. This information suggests that it is more efficient to apply fertilizer to a *M.* × *giganteus* crop after the second growing season since the requirements of the crop will be higher and the root system will be well developed allowing for a high efficiency of N fertilizer use.

Our analysis also suggests that considerably high yields can be obtained with very little N fertilizer, because the difference in the response to N fertilizer between 0 and 100 kg N ha^{-1} was small compared with more typical row crops responses (Gastal and Lemaire, 2002). Again, this is evidence of the high N use efficiency of *M.* × *giganteus* and its ability to recycle nutrients through the rhizome (Christian et al., 2006). As shown in Table 4, the estimated effect of N fertilization on *Asym* was 0.0232 Mg ha^{-1}/kg N ha^{-1} (lower: 0.0077, upper: 0.0386 Mg ha^{-1}/kg N ha^{-1}). This effect should be interpreted as an increase of 0.0232 Mg ha^{-1} in the ceiling yield for every kg N ha^{-1} applied. A simple economic scenario, assuming a cost of 1 US$ per N kg (including application), would indicate that 1 Mg of *M.* × *giganteus* should be US$ 43 to recover the cost of N application. However, fertilizer prices have increased in recent years (http://www.ers.usda.gov/Data/FertilizerUse/) and, for example, a projected price of 1.27 US$ per N kg would require US$ 55 per Mg of *M.* × *giganteus*. A more responsive site (0.0386 Mg ha^{-1}/kg N ha^{-1}) and 1 US$ per N kg would require a break-even value of US$ 26 per Mg of

M. × *giganteus* or 33 US$ if 1.27 per N kg are assumed. This analysis suggests a range of prices needed for M. × *giganteus* biomass to justify the application of N fertilizer, but it should also be considered that N prices are likely to increase and the response to N fertilizer will strongly depend on soil attributes as well as weather conditions. Additionally, the response of crops to N fertilizer is usually better described by a non-linear asymptotic response (Gastal and Lemaire, 2002; Thornley and Johnson, 2000). This reflects the fact that each additional unit increase in N fertilizer application produces a diminishing return in dry biomass production. In the range of N rates studied here, it was not possible to detect a non-linear relationship between N fertilizer application and ceiling yields (i.e. *Asym*).

Our results agree with Clifton-Brown et al. (2007). For a 15 years of M. × *giganteus* biomass yields in Ireland, they described an initial phase where biomass yields increased during the first four growing seasons up to 16 Mg ha^{-1}, followed by a stable period with average biomass yields of 17 Mg ha^{-1}, and finally a decline phase with lower biomass yields (11 Mg ha^{-1}). This is in agreement with the results of our analysis compiled in Fig. 2. Although our evidence does not allow for a definite statement about a deterioration of the cropping system, a decline after approximately 10 years is both suggested by our analysis and in agreement with Clifton-Brown et al. (2007). Additionally, Clifton-Brown et al. (2007) found that M. × *giganteus* responded significantly to N fertilization after 9 years of cultivation. In our analysis a small effect of N fertilization was observed after only 3 years possibly because of the greater statistical power since observations from 31 studies were included.

3.2. *Environmental factors: harvest time (Fall yield vs. Winter yield)*

In general, Fall yields were higher than Winter yields because the dry biomass sampled in the winter had losses due to leaf drop and lodging (Fig. 2). The analysis of Fall yield did not suggest an effect of N fertilizer rate or plant density. To allow for a comparison between Fall and Winter harvests, a model without the effect of N fertilizer or plant density was fitted to both datasets and their fitted values presented in Fig. 6. M. × *giganteus*' yield normally peaks in August–October and decreases thereafter due mostly to leaf detachment. Therefore the dry biomass recorded in the fall is higher than the dry biomass recorded in the winter. The comparison further reveals that countries at lower latitudes which have higher yield potential (i.e. Portugal, Greece, and Italy) also have the largest proportional decrease in dry biomass. A likely explanation is that the colder climate at more northern locations induces senescence before December 21st, which separates fall from winter, and thus yield recorded in the fall at

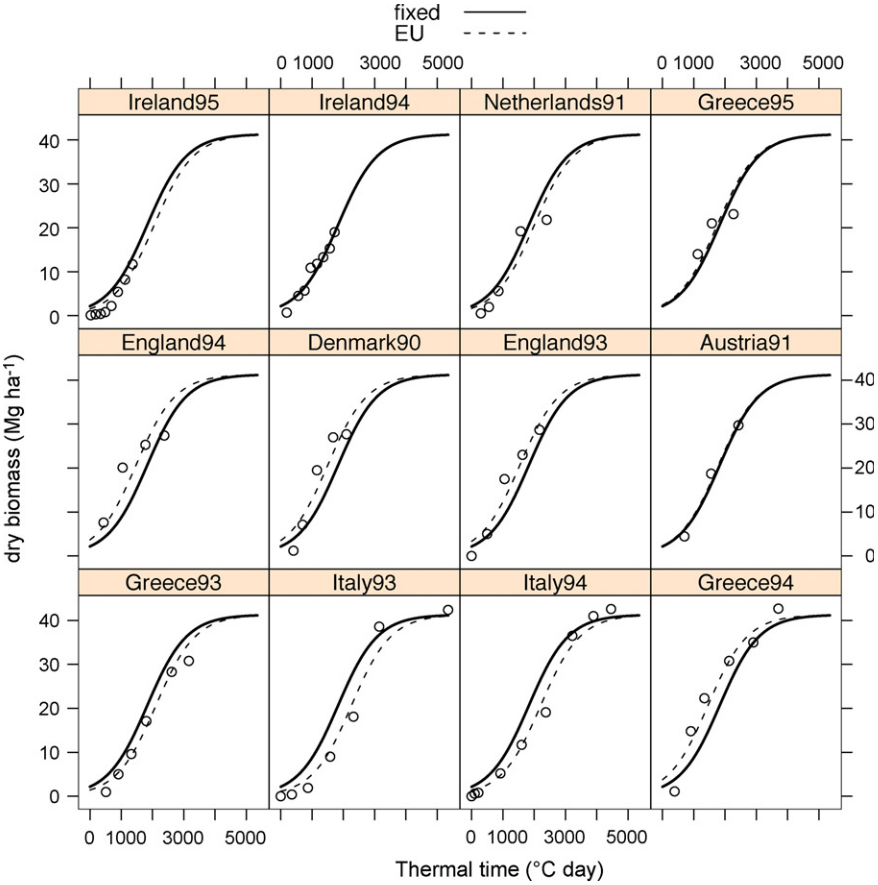

Fig. 7 – M × *giganteus* dry biomass accumulation during a growing season in different locations and years. The solid line is the fixed logistic function of the model and the dashed line is the prediction based on the mixed model for each individual location/year. The estimates for the fixed component can be found in Table 5.

northern locations (i.e. England and Germany) has already suffered reductions in yield from leaf drop. Conversely, yield recorded before December 21st at southern locations (i.e. Greece, Italy and Portugal) has not been affected by leaf drop as much as the northern locations. Fig. 1 shows conceptually that harvests at northern locations (in fall and winter) are better represented by the dashed line while harvests at southern locations are better represented by the difference between the solid and dashed lines. The estimated fixed effect for *Asym* (18.4 Mg ha^{-1} vs. 24.9 Mg ha^{-1}) and the estimate of the standard deviation (2.4 Mg ha^{-1} for winter and 8.1 Mg ha^{-1} for fall) associated with the *Asym* random effects in both models also illustrate the larger variability in ceiling yields in the fall. These results have implications for decisions regarding harvest date since delaying harvests at northern locations do not represent substantial biomass losses and therefore nutrient recycling and lower moisture in the biomass can be improved by delaying harvest as suggested by Lewandowski et al. (2003). At southern locations harvesting in the fall represents a much higher dry biomass production and thus would be preferable albeit considering drying costs and mineral content in the harvested biomass. Additionally, commercial production of *M.* × *giganteus* should consider other relevant economic and logistic factors such as storage, transportation, suitable climatic conditions for harvesting, and long-term agronomic consequences.

3.3. *Biomass accumulation within a growing season*

3.3.1. *Growth pattern within a growing season*

The data included in this analysis show almost identical growth patterns among different locations and years when dry biomass accumulation is plotted against thermal time (Fig. 7). Due to the lack of significant random effects no further effects on the parameters of the model were investigated (Eq. (1)). The parameter estimates and the 95% confidence intervals are shown in Table 5. Countries at lower latitudes (Greece and Italy) achieved higher yields as a consequence of the longer growing season and higher temperatures. Conversely, countries at higher latitudes (Denmark, England, Germany, Ireland and Netherlands) achieved similar growth rates but lower yields due to the shorter growing season. Although this result is expected, the agreement is enhanced by uniformity of the genetic material grown in these different locations. For example, in Ireland, Clifton-Brown et al. (2000) found a clear relationship between thermal time and dry biomass accumulation. Although *M.* × *giganteus* biomass production is determined in part by management practices, soil type, and other environmental factors, the accumulated thermal time is crucial for estimating potential production. The horizontal shift among the growth curves (Fig. 7) might be revealing that thermal time for these experiments was estimated rather than recorded. Using the logistic growth function (Eq. (1)) and the accumulated thermal time from the last frost in the winter until the last frost in the Fall as input data, we obtain the simplest statistical model used to predict *M.* × *giganteus* potential dry biomass. The simplicity of the model is both the most attractive feature of the function and the major limitation (Thornley and Johnson, 2000).

Table 5 – 95% Confidence intervals for the parameter estimates in the within season growth analysis

Fixed term	Lower	Estimate	Upper	Units
Asym	38	41.3	44.6	Mg ha^{-1}
xmid	1600	1831	2062	°C
scal	537	632	727	°C

Asym = asymptote, *xmid* = half time until max yield, *scal* = time between half yield and 3/4 yield.

4. Conclusions

The recent emphasis in biomass derived ethanol production (Farrell et al., 2006; U.S.DOE, 2006) highlights the importance of investigating the agronomic practices that will determine the success of *M.* × *giganteus* as a renewable source of energy. A quantitative relationship was given between the effect of initial planting density and N fertilizer on the production of dry biomass of *M.* × *giganteus*. It was shown that the delay of harvest time has the most marked impact on locations where the dry biomass yield potential is high. The analysis of the growth within a season provides one of the simplest models for predicting potential biomass production, based only on the thermal units accumulated during the growing season. The within growing season analysis shows that for the experiments being analyzed, a logistic growth function using thermal time as the independent variable provides an excellent fit. Naturally, these data were obtained from experimental plots which were well managed in terms of typical agronomic practices such as weed control, baseline soil nutrient availability, water availability, etc. We do not argue that these variables are less important than temperature patterns and the length of the growing season. The data suggest that once these agronomic practices are in place, temperature seems to account for most of the variation in growth patterns and it seems to be quite robust in many different environments across Europe

The needed aggregation of data from multiple trials in different countries and locations obtained with meta-analysis enhances the precision and accuracy of the pooled results. The information generated in this manuscript will help improve current management practices and the assessment of their economical and environmental outcomes.

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