The Agronomy of the Energy Crops *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea* in Wales.

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A thesis submitted to Cardiff University for the higher degree of Doctor of Philosophy

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Summary

For this study, the energy crops *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea* were planted at sites across Wales. Non-destructive methods of estimating crop yields were developed; the most significant relationship for *Miscanthus* was between mean shoot height and mean shoot dry weight, whereas for *Arundo* it was between mean shoot volume and mean shoot dry weight; although these estimates were over-estimates of actual crop yield when scaled up to field size. Yield estimates were obtained from destructive sampling for *Phalaris* and these were shown to be under estimates of whole crop yield. Crop growth data were compared in relation to soil type, soil chemistry and climatic conditions. Soil clay content and soil preparation were identified as of utmost importance to *Miscanthus* and *Arundo* success, but had no effect on *Phalaris* crops. Both *Arundo* and *Miscanthus* showed sensitivity to air temperatures during the growing season. Delaying harvest of both crops produced material with decreased moisture and mineral content, although results were not significant in all cases. The nitrogen, phosphorus and potassium content of leaves were higher than that in the cane, and harvest following leaf abscission was recommended. *Arundo* did not senesce completely during the winter period, and produced harvested material with higher mineral content than *Miscanthus*. Both crops required further drying to meet moisture content requirements for combustion crops. *Phalaris* was the only crop to meet moisture content and mineral content threshold levels, although the results were not consistent across sites. Leaf chlorophyll content was significantly correlated to above ground plant mineral content. Organic and inorganic fertilisers were applied to the three crops, and produced no response in *Phalaris*. Both *Miscanthus* and *Arundo* increased growth in response to high phosphorus levels, and generally in response to high fertiliser applications. At recommended application rates cattle manure showed most effect.

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Contents

Chapter 1: Introduction

Chapter 2: Site Descriptions

Chapter 3
Methods of Estimating Crop Yields from Trial Plots of the Energy Grasses *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea*.

Chapter 4
Site Conditions in Wales and Their Influence on the Success of *Miscanthus* x *giganteus* and *Arundo donax* Crops.

Chapter 5
The Effect of Harvest Date on the Quantity and Quality of *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea* Crops in the Welsh Climate.

Chapter 6
Determination of Plant Senescence Status, Nutrient Content and Predicted Crop Yield in *Miscanthus* and *Arundo donax*, using a Hand-held Chlorophyll Content Meter.

Chapter 7
The Effects of Organic and Inorganic Fertiliser Applications on Pot Trials and a Field Trial of *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea*.

Chapter 8: Discussion

List of figures

List of tables

Chapter 1: Introduction

1.1 Climate change

The aggregation of greenhouse gases (carbon dioxide, methane, nitrous oxide, hydrofluorocarbons, perfluorocarbons, and sulphur hexafluoride) in the Earth's atmosphere restricts the return of long-wave radiation from the Earth's surface and reflects heat back to the Earth. This is a natural process which maintains the Earth's surface at 33°C warmer than it would be in their absence (BERR, 2008b). However, the accumulation of greenhouse gases in the atmosphere is increasing as a result of anthropogenic activities, and this is causing the "enhanced greenhouse effect" or global warming. Carbon dioxide accumulation is responsible for about 70% of the enhanced greenhouse effect, for which fossil fuel combustion is the primary cause (BERR, 2008b). The Intergovernmental Panel on Climate Change have predicted that greenhouse gas emissions will continue to increase and will result in global warming of about 0.2°C per decade over the next two decades (IPCC, 2007). This will cause sea ice melt, thus raising the sea level and causing coastal flooding, and mountain snow melt which will cause flooding of river plains. It is considered "virtually certain" that land areas will experience an increased frequency of hot weather, and will "very likely" experience heat waves and increased heavy precipitation (IPCC, 2007). The global effects of this warming will include plant and animal species extinctions and increased human malnutrition and diseases (IPCC, 2007). Wales is forecast to experience increased storminess and subsequent flooding, and high summer temperatures (WAG, 2006), which will continue to increase for the next 50-100 years, even in the absence of increased greenhouse gas emissions (WAG, 2002).

The immediate consequence of fossil fuel combustion is air pollution, mostly in the form of oxides of sulphur and nitrogen. Sulphur dioxide is a major factor in asthma and chronic lung disease, whereas nitrogen dioxide contributes to respiratory illnesses, all of which are forecast to increase with global warming (IPCC, 2007). These gases also combine with atmospheric water vapour to produce acid rain, which has severe detrimental effects upon freshwater fauna and flora.

1.2 Security of energy supply

The UK currently obtains 90% of its energy requirements from fossil fuels, but population increases and higher energy consumption are quickly depleting the world's finite sources of these fuels. Oil and gas production in the UK has reached its peak and is now in decline, leading our country to increasing dependence on imports (DTI, 2007) from the Middle East. We are already a net importer of coal, and are predicted to be a net importer of oil by 2010, and to import 40% of our gas requirements by 2010. This dependency on imports has implications for security of supply, energy prices and fuel availability. It has been recognised that alternative sources of energy for heat and electricity production must be identified to meet future energy demands, and that a large proportion of our energy must be supplied from renewable sources.

1.3 Government response – Europe, UK and Wales

1.3.1 Europe

In their climate change and renewable energy package, the European Union committed to a reduction in greenhouse gas emissions of 20% by 2020, compared to 1990 levels (European Commission, 2008). This will be achieved partly by the production of 20% of energy from renewable sources (European Commission, 2008). Currently, the EU produces 13.9% of its total energy consumption from renewable energy sources, of which 10.7% are from hydro power (WAG, 2002).

1.3.2 UK

The Kyoto Protocol agreement (United Nations, 1998) required a 12.5% reduction in UK emissions of greenhouse gases by 2008-2012. The British government further promised to reduce carbon dioxide emissions to 20% below 1990 levels by 2010, and to a 60% reduction by 2050, with real progress by 2020 (WAG, 2008a; BERR, 2008a). Total greenhouse gas emissions fell by 17% between 1990 and 2007, but this included a decrease of only 6.4% of carbon dioxide emissions (BERR, 2008b).

In order to achieve its 2020 target, the UK government aims to produce approximately 32% of total electricity, 14% of total heat and 10% of its total transport fuel requirement from renewable sources (BERR, 2008a). This will result in 15% of all energy consumption being produced from renewable sources by 2020 (BERR, 2008a). In response, the contribution of renewable energy to total electricity production has slowly increased during the past 6 years, from 1.8% in 2002 (DTI, 2007), to 3.3% in 2004 (RPA, 2004), to 4.5% in 2006 and 5% in 2007 (BERR, 2008b). However, despite heat production being the largest single proportion (49%) of UK energy, and contributing the largest proportion (47%) of carbon emissions, only 0.6% of heat production is currently supplied from renewable sources (BERR, 2008a). UK energy production from renewable sources currently accounts for 1.7% of total energy production, of which 0.8% is obtained from hydro power (WAG, 2002).

Biomass production has been identified as a major future contributor to renewable energy, and is expected to provide 45% of renewable heat by 2020, with a smaller (8.5%) contribution to renewable electricity (BERR, 2008a). This contribution includes combustion of energy crops, wood, manures, slurries, organic waste and waste wood (BERR, 2008). Purpose grown energy crops, to include perennial rhizomatous grasses and willow coppice, are estimated to contribute about 17.8% of the total biomass contribution to future energy production in the UK (DEFRA, 2007). This will be achieved by increasing the amount of land used for production of perennial crops by 350,000 hectares (6.5% total UK arable land), to bring the total land used for energy and biofuel production to about 1 million hectares (17% total UK arable land) (DEFRA, 2007), as recommended by the Biomass Task Force (Biomass Task Force, 2005). However, reports have suggested that this area could be increased to 5.5 million hectares (30% agricultural land) with the potential to generate more than half of total UK energy consumption (House of Commons, 2006), including two thirds of electricity consumption (RCEP, 2000). In response, the Rural Development Programme for England has introduced a new Energy Crops Scheme providing establishment grants for *Miscanthus* and short rotation coppice (Natural England, 2008).

Successful existing biomass power generation within the UK include the Stevens Croft Biomass Power station in Lockerbie, which generates sufficient electricity (44MW) to power 70,000 homes. It is the largest dedicated biomass plant in the UK, and is fuelled by sawmill co-products, recycled fibre and 20% willow from a total of 4,000 hectares of local short rotation coppice (EPSRC, 2008). In Teesside, the Wilton wood burning power station (30MW) is supplied by recycled wood, sawmill off-cuts, managed forests in North East England, and a total of 7,500 acres of willow coppice (EPSRC, 2008).

1.3.3 Wales

In Wales, the Economic Development Committee has recommended the progression towards a zero carbon economy over the next 20 – 50 years (WAG, 2002). The Welsh Assembly Government is committed to an annual 3% reduction in greenhouse gas emissions from 2011 onwards (WAG, 2008a). This includes a commitment to the production of 20% of total energy consumption from renewable energy sources by 2020. In 1997, 1.5% of total energy consumption was produced from renewable sources, of which 0.7% was from hydro power (WAG, 2002). Proposals for future Welsh energy production include an increase to 137% of electricity consumption from renewable sources, of which half will be obtained from marine sources, a third from wind and the remainder from both indigenous and imported biomass sources (WAG, 2008a).

The Economic Development Committee proposal included the recommendation that 10% of Welsh agricultural land should be used for energy crop production by 2020, which currently accounts for less than 0.05% of land use (WAG, 2002). However approval has been granted for the construction of the "Prenergy" 350MW power station at Port Talbot, which is expected to be the world's largest biomass electricity plant, and will import biomass from overseas. Thus it is presently unclear if Welsh Assembly policy will promote further energy crop production, or if future recommendations will promote Welsh food production (WAG, 2008a). At the time of writing, we are still awaiting publication of the Welsh Biomass Energy Strategy from the Welsh Assembly Government, and information regarding the possible inclusion of an energy crops grant scheme in the Rural Development Plan for Wales (DEFRA, 2007; BERR, 2008a). However, the latest report from the Welsh Assembly (WAG, 2008b) presents a less than optimistic view of this proposal, and is currently awaiting consultation.

1.4 Current biomass production in Wales

Successful biomass energy production is currently showcased at the Bluestone Holiday Village in Pembrokeshire, which has a biomass energy centre fuelled by *Miscanthus* and willow coppice grown by local farmers. This provides all heat requirements for the Blue Lagoon leisure pool, sports centre and offices; a heat demand of 12,000 MW hours per year. This requires the supply of 6,000 tonnes of wood and energy crop fuels, which could be produced from 400 hectares of *Miscanthus* (Anon, 2008a). The energy supply company PBESCO currently has 100 hectares of *Miscanthus* planted in the area (Anon, 2008b).

Co-firing biomass fuels in efficient coal powered stations can decrease emissions by about 10% (DTI, 2007). Subsequently, Aberthaw power station in the Vale of Glamorgan is committed to combust 200,000 tonnes of biomass crops per year, which requires the production of around 10,000 hectares of energy crops, including willow coppice and *Miscanthus* (Anon, 2008b).

The "Prenergy" renewable energy plant at Port Talbot is expected to be the world's largest biomass electricity plant and will fulfil almost 70% of Wales' 2010 renewable energy target. It will produce sufficient energy to power nearly half (587,100) of all Welsh homes throughout the year (Prenergy Power, 2006). It will be supplied by sustainable wood chip sources (Prenergy Power, 2006) from the US and Canada (BERR, 2008) in order to take advantage of a variety of species with rapid growth rates and lower delivered moisture content due to rapid post harvesting drying achievable in more southerly latitudes (Prenergy Power, 2006).

1.5 Energy grass crops

Combustion of biomass releases carbon dioxide into the atmosphere, but the amount released is less than or equal to the amount absorbed during growth, so energy crops are generally regarded to be carbon neutral. Rhizomatous energy grasses, (*Miscanthus, Arundo donax, Phalaris arundinacea*), senesce during the winter during which time the minerals contained in the above ground plant matter are recycled by nutrient translocation and leaf abscission, thus replenishing the rhizome and soil reserves. In theory therefore these crops have very low or no fertiliser requirements, and produce a biomass fuel with low mineral content. Energy grasses have very few natural pests and therefore have little or no pesticide requirement (Lewandowski *et al.,* 2000). The leaf fall during senescence and the canopy closure as the crop matures suppress upcoming weeds, and therefore the crop has no herbicide requirement after the first year. Leaf litter accumulation on the ground improves the soil organic matter and encourages both vertebrate and invertebrate communities. The crops often have deep root systems, which increase water and nutrient use efficiency, and also increase soil carbon (Metcalfe & Bullard, 2001). These factors of energy grass production enable not only the negation of carbon dioxide emissions, but also a decrease in nitrogen and sulphur oxide pollutants. In contrast, traditional arable farming practice recommends the use of up to seven different pesticide applications per year, including two or three herbicides, three fungicides and one insecticide (Semere & Slater, 2005). Energy grasses have advantages over willow coppice in that existing farming machinery can be used for planting and harvesting, and can potentially provide an opportunity for agricultural diversification and for sustaining rural communities.

The crops that have been identified as potential energy crops within the UK are *Miscanthus* spp., *Panicum virgatum* and *Phalaris arundinacea*. *Panicum virgatum* (Switchgrass) originates from North America. It is a perennial grass sown from seed with a lifespan of 8 years. It can reach heights of three metres in its native prairie lands, with annual yields reaching 22 tonnes dry matter per hectare (t DM ha^{-1}) (Lewandowski *et al.,* 2003a). The Herbaceous Energy Crops Research Program in the US identified switchgrass to be the most promising biomass crop for US energy production, and concentrated their research on obtaining its maximum yields (Lewandowski *et al.,* 2003a). European studies have reported yields of 11-21 t DM ha^{-1} in Greece and 7-26 t DM ha^{-1} in Italy (Alexopoulou *et al.*, 2001). However, several trials have been attempted within the UK with little success of establishing the crop. Trials in Herefordshire found switchgrass to compete poorly against grass and broad-leaved weeds, and the crop did not become established (Semere & Slater, 2005). Similarly, Christian *et al.* (1999) found growth to be very slow during its first year, which necessitated weed control. Switchgrass was established in 2003 at one of the sites featured in this study (Cae Clovers) but establishment was only achieved using 1 year old greenhouse grown plants and it was decided not to include this crop in the study. This crop uses the C_4 photosynthetic pathway, and is likely to require high summer temperatures during its establishment period.

European research is continuing to identify new crops with the potential to act as a renewable energy source. Studies in The Netherlands identified Hemp (*Cannabis* sp.) as the best option for energy crops in that region (Hanegraaf *et al.,* 1998). Other studies have identified kenaf (*Hibiscus cannabinus*) in Greece (Alexopoulou & Christou, 2001), with yields of 9-12 t DM ha^{-1}. The Wales Biomass Centre at Cardiff University is conducting trials of *Arundo donax* and *Cyanara cardunculus* as novel energy crops for Wales. The *Cyanara* trial was not replicated at sites other than at Llysdinam, where initial growth measurements were not promising. Therefore the data from that trial are not included in this study. The results from the *Arundo* trials are included in this study.

The three crops with which this project is primarily concerned are *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea. Miscanthus* is a C_4 plant and both *Arundo donax* and *Phalaris arundinacea* are C_3 plants. These terms refer to the photosynthetic pathways adopted within the plant. Photosynthesis is the process by which light energy is converted to chemical energy, during which carbon dioxide is used to produce plant matter. In C_3 plants, photosynthesis is accompanied by photorespiration, a wasteful process in which oxygen is used and carbon dioxide is generated. In C_4 plants this process is suppressed by increased concentration of carbon dioxide within the plant cells (Jones, 1992). Subsequently, C_4 plants use a highly efficient light-use pathway, estimated to be 40% greater than that of C_3 plants, which is accompanied by enhanced water-use and nitrogen-use efficiencies (Heaton *et al.*, 2004). This enables C_4 plants to be most suited to warm and temperate climates (Lewandowski *et al.,* 2003a). However, the C_4 plant *Miscanthus* has been shown to be unusually cold tolerant (Heaton *et al.*, 2004). C_3 photosynthesis is more suited to low winter temperatures and a short growing season (Lewandowski *et al.,* 2003a), although the C_3 plant *Arundo donax* has a high photosynthetic efficiency similar to C_4 plants (Cosentino *et al.*, 2006).

1.5.1 *Miscanthus*

Miscanthus (Asian Elephant Grass) originates from Southeast Asia, and can now be sourced from within the UK. It is a perennial rhizomatous grass that produces erect woody stems which can achieve heights of up to four metres after 3-5 years from planting. The amount of biomass produced per rhizome increases annually during its establishment period as the plant produces more shoots each year. The oldest reported *Miscanthus* stand in Europe is 20 years old, and its productive lifespan is estimated to be 20-25 years (Lewandowski *et al.,* 2003a). It has produced annual yields of 20-24 oven dry tonnes per hectare (odt ha^{-1}) in the UK (Bullard, 2000; Metcalfe & Bullard, 2001; Nixon *et al.*, 2001), and European trials have revealed annual yields of up to 30 t DM ha^{-1} (Acaroglu & Aksoy, 1998; Schweiger & Stolzenburg, 1993). The average annual yield targets for *Miscanthus* in the UK, as determined by DEFRA, are 16 odt ha^{-1} (Viegas, 2005). It is the most widely researched of all the energy crops within central and southern Europe.

Miscanthus x *giganteus* is the most commonly researched Elephant Grass taxon. It is believed to be a natural hybrid of *Miscanthus sacchariflorus* and *Miscanthus sinensis* (Lewandowski *et al.,* 2003a). Breeding programmes have been set up in Europe to create new hybrids with higher biomass potentials, and long term studies exist that investigate the relative success of different genotypes in various European climates (Clifton-Brown & Lewandowski, 2000; Lewandowski *et al.*, 2003b).

1.5.2 *Phalaris arundinacea*

Phalaris arundinacea (Reed Canary Grass) is commonly found growing on riverbanks within the UK. It originates from cool temperate regions in the Northern hemisphere. It is a perennial rhizomatous grass that can be sown from seed, and has been shown to reach heights of up to three metres for a productive lifespan of five to ten years. Studies to date have shown lower yields than for *Miscanthus* and *Arundo*, producing a maximum of 12 odt ha^{-1} (Lewandowski *et al.,* 2003a). However its establishment period is shorter and maximum heights can be achieved in the second year. It is also the only existing energy grass most suited to regions with cold winters and short summers (Lewandowski *et al.,* 2003a). As a native species it is subject to pests and diseases (Christian *et al.*, 1999), which may confer advantages to the biodiversity of the crop.

1.5.3 *Arundo donax*

Arundo donax (Giant Reed) is native to the Mediterranean and is traditionally the reed used for wind instruments. It is a perennial rhizomatous reed with erect hollow stems that are able to reach heights of up to 8-9 metres (Perdue, 1958) within the Mediterranean region, where it grows naturally on marsh land or river banks (IENICA, 2002). It takes 3-5 years to achieve maximum biomass (Christou *et al.*, 2001). It has been previously studied in Europe, and has been shown to produce substantially higher yields than *Miscanthus*. Studies in Spain have produced annual yields of up to 63 t DM ha^{-1} (Hidalgo & Fernandez, 2001), and research in Greece has revealed annual yields of up to 39 t DM ha^{-1} (Lewandowski *et al.*, 2003a).

1.6 Project aims

In anticipation of a new Energy Crop Scheme in the Rural Development Plan for Wales, this project has studied the three energy crops with most potential for successful energy production in Wales; *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea*. Two sites in Wales (Llysdinam and Llwynprenteg) were planted with these three crops side by side; which is the only study of its kind in the peer-reviewed literature. Additional sites were planted with one or more of the crops to provide further replication of studies. This is the only extensive study of several different potential energy crops in Wales, and the results of these studies are presented from a Welsh perspective. There are several existing models which predict the productivity of *Miscanthus* and other selected crops in Europe (Clifton-Brown *et al.*, 2004; Tuck *et al.*, 2006), Britain (Price *et al.*, 2004), England (DEFRA, 2007b) and Ireland (Clifton-Brown *et al.*, 2000), but no models to date have included data from Welsh sites.

In Chapter 3, the lack of consistency between methods of estimating yields used by studies in the peer-reviewed literature was identified. This study aimed to determine if relationships exist between simple non-destructive field measurements and *Miscanthus* and *Arundo* crop yields. It also investigated different methods of calculating yield estimates from destructive sampling methods, where a proportion of the crop was removed and weighed. The aim of this study was to discover the most suitable and accurate method of estimating yields from both destructive and non-destructive sampling methods of energy grass crops.

Chapter 4 was a study of the success of *Miscanthus* and *Arundo* crops with specific reference to sites across Wales. The aim of the study was to determine which site factors and climatic factors were important to the success of the two crops.

The optimum harvest date of *Miscanthus* (Himken *et al.*, 1997; Clifton-Brown and Jones, 2001; Clifton-Brown *et al.*, 2001; Lewandowski and Heinz, 2003; Lewandowski *et al.*, 2003) and *Phalaris* (Landstrom *et al.*, 1996; Burvall, 1997; Hadders and Olsson, 1997; Yates and Christian, 2001; Christian *et al.*, 2006) crops have been investigated in other studies, but similar studies have not been reported for *Arundo* crops. Chapter 5 determined the differences between an early autumn harvest date and a spring harvest of the three energy crops *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea*, in terms of quantity and quality. Crop quality was defined in terms of moisture content and mineral content, specifically nitrogen, phosphorus and potassium, of harvested plant material. The three crops were studied at sites across Wales and their suitability as combustion crops were discussed.

In order for energy crops to be suitable for combustion, moisture content and mineral content at harvest should both be low. In Chapter 5 these factors were studied through the winter period to investigate their decline as the crops senesced. The need for a reliable non-destructive method for determination of leaf nutrient content was identified, and the use of a chlorophyll content meter for this purpose was investigated in Chapter 6. The study investigated chlorophyll degradation during senescence of *Miscanthus* and *Arundo* crops, and determined the relationship between leaf chlorophyll content and the nutrient content of above ground plant matter. It also investigated the relationship between leaf chlorophyll content and *Miscanthus* and *Arundo* crop yields, and how this relationship can be used as an indicator of site suitability in future studies.

An important feature of energy crops is their low fertiliser requirement. However, studies in the literature have reported conflicting results regarding the response of *Miscanthus* (Himken *et al.*, 1997; Christian *et al.*, 1999; Ercoli *et al.*, 1999; Boehmel & Claupein, 2007; Danalatos *et al.*, 2007; Christian *et al.*, 2008), *Arundo* (El Bassam, 1998; Christou *et al.*, 2001; Angelini *et al.*, 2005) and *Phalaris* (Katterer *et al.*, 1998; Christian *et al.*, 1999; Lewandowski & Schmidt, 2006) to fertiliser applications. This study investigated the effects of a range of organic manure and inorganic fertiliser applications to all three crops in both pot and field situations. The aims of all trials were to determine the effect of each fertiliser application in terms of crop growth and final yield, and to investigate whether different levels of nitrogen, phosphorus and potassium (N:P:K) in the applications had an effect on the N:P:K content of the leaves and canes at harvest. The field trial also included a study of the N:P:K content of the rhizomes at harvest.

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Chapter 2: Site Descriptions.

All studies within this project were carried out at one or more of the following sites: Llysdinam, Llwynprenteg, Bluestone 1, Bluestone 2, Cae Clovers and Coleg Sir Gâr. The location, soil type and former land use of each site is detailed below (Table 2.1). Soil type and association have been described for each site in accordance with Rudeforth *et al.* (1984). See Appendix 2 for site photographs.

2.1 Planting materials

Miscanthus x *giganteus* crops were planted from rhizomes obtained from Rebecca Heaton, ADAS, UK. *Arundo donax* crops were planted from rhizomes obtained from Rudi Toneatti's farm in Lestans, Italy. *Phalaris arundinacea* crops were planted from seed, of unknown variety, obtained from Countrywide, Worcester, UK.

2.2 Site preparation

As the project developed at different sites over time, site preparation methods and planting dates varied. For the majority of *Miscanthus* and *Arundo* crops, the ground had one herbicide application and was then ploughed, rotivated and harrowed. For *Phalaris* crops, the general method was harrowing followed by rolling the ground after planting. Specific ground preparation details relevant to each site and crop are displayed in Tables 2.2 and 2.3.

2.3 Planting method

Miscanthus and *Arundo* rhizomes were planted by hand at 0.5m spacing, which equates to 40,000 plants per hectare. *Phalaris* seed was broadcast at 3kg/acre.

2.4 Site management

Weed control was necessary during the first year of *Miscanthus* growth at Llysdinam, Llwynprenteg, Bluestone and Cae Clovers. The *Arundo* and *Phalaris* crops at Cae Clovers and Coleg Sir Gâr were also weeded by hand during early crop growth.

(a) Llysdinam

Location	Newbridge-on-Wye, Llandrindod Wells, Powys
Grid reference	SO 005 581
Altitude (m)	190
Soil type	Cambic stagnogley soils
Soil association	Cegin
Soil association description	Seasonally waterlogged, loamy and clayey
Previous land use	Rough grazed pasture
Surrounding land use	Rough grazed pasture / woodland

(b) Llwynprenteg

Location	Llanafan, Nr. Pont-Rhyd-y-Groes, Ceredigion
Grid reference	SN 687 716
Altitude (m)	90
Soil type	Typical brown earths
Soil association	Denbigh 1
Soil association description	Brown, stony, well drained soils
Previous land use	Rough grazed pasture
Surrounding land use	Rough grazed pasture

(c) Bluestone 1

Location	Narberth, Pembrokeshire
Grid reference	SN 063 129
Altitude (m)	75
Soil type	Typical brown earths
Soil association	Milford
Soil association description	Reddish, fine, loamy soils
Previous land use	Rough grazed pasture
Surrounding land use	Rough grazed pasture / woodland

(d) Bluestone 2

Location	Slebech, Pembrokeshire
Grid reference	SN 032 149
Altitude (m)	60
Soil type	Typical brown earths
Soil association	Denbigh 1
Soil association description	Brown, stony, well drained soils
Previous land use	Rough grazed pasture
Surrounding land use	Rough grazed pasture / energy crops

(e) Cae Clovers

Location	Cwmystwyth, Ceredigion
Grid reference	SN 776 744
Altitude (m)	280
Soil type	Typical brown podzolic soils
Soil association	Manod
Soil association description	Free draining, fine, loamy soils
Previous land use	Rough grazed pasture
Surrounding land use	Rough grazed pasture

(f) Coleg Sir Gâr

Location	Pibwrlwyd campus, Carmarthen, Carmarthenshire
Grid reference	SN 410 181
Altitude (m)	15
Soil type	Typical alluvial gley soils
Soil association	Conway
Soil association description	Deep, stoneless, fine, silty soils
Previous land use	Unimproved grassland
Surrounding land use	Unimproved grassland

Table 2.1: Site descriptions and soil descriptions for all sites in this study.

Site and crop	Llysdinam *Miscanthus*	Llysdinam *Phalaris*	Llysdinam *Arundo*	Llwyn Prenteg *Miscanthus*	Llwyn Prenteg *Phalaris*	Llwyn Prenteg *Arundo*	Cae Clovers *Miscanthus*	Cae Clovers *Phalaris*	Cae Clovers *Arundo*
Pre-planting preparation dates	Mar-Apr 2004	Mar-Apr 2004	Mar 2005	Mar-Apr 2004	Mar-Apr 2004	Mar-Apr 2004	Mar-Apr 2003	Mar-Apr 2003	Mar-Apr 2003
Herbicide	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha	Weedol 57g in 4.5l/17m²	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha re-applied 3/05	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha
Ploughed	√	√	x	√	√	√	√	√	√
Limed @1.6t/acre	x	x	x	√	√	√	x	x	x
Rotivated	√	√	x	Rolled	Rolled	√	Rolled	Rolled	Rolled
Harrowed	√	√	x	√	√	√	√	√	√
Planting date	4/04	5/04 re-seeded 9/04	4/05	4/04	4/04 re-seeded 9/04	4/05	4/03	4/03	4/03
Post-planting maintenance									
Harrowed	x	√	x	x	√	x	x	x	x
Rolled	x	√	x	x	√	x	x	x	x
Fertilisers applied (4/04)	x	x	x	N:P:K 60:50:50	N:P:K 60:50:50	N:P:K 60:50:50	x	x	x
Weeded	√	x	x	√	x	x	√	√	√

Table 2.2: Site preparation details for Llysdinam, Llwynprenteg and Cae Clovers.

Site and crop	Bluestone 1 *Miscanthus*	Bluestone 2 *Arundo*	Coleg Sir Gâr *Miscanthus*	Coleg Sir Gâr *Phalaris*	Coleg Sir Gâr *Arundo*
Pre-planting preparation dates	3/03	3/05	3/04	3/04	3/04
Herbicide	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha	Glyphosphate 360 @ 5l/ha
Ploughed	√	√	x	x	x
Limed @1.6t/acre	x	x	x	x	x
Rotivated	√	√	√	√	√
Harrowed	x	x	x	x	x
Planting date	4/03	4/05	4/04	5/04	4/04
Post-planting maintenance					
Harrowed	x	x	x	x	x
Rolled	x	x	x	√	x
Fertilisers applied	Sewage cake	x	x	x	x
Weeded	√	x	√	√	√

Table 2.3: Site preparation details for Bluestone and Coleg Sir Gâr.

2.5 References

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Chapter 3

Methods of Estimating Crop Yields from Trial Plots of the Energy Grasses *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea*.

3.1 Introduction

The majority of energy grass crop studies in the literature have been carried out on trial size plots, from as small as 12 square metres (Lewandowski & Heinz, 2003). Yield estimates are then generally determined from a sub-sample of the trial plot, either by harvesting an area of the plot or single stems from the plot. The lack of consistency in yield estimation methods creates difficulty in comparing results between studies.

Miscanthus studies have calculated yield estimates from an area as small as 1m^2, which equated to two *Miscanthus* plants per plot (Lewandowski & Heinz, 2003), or from a larger 9m^2 area (Nixon & Bullard, 2003). Potter *et al.* (1995) harvested 4m^2 of the grass *Spartina* and sedge *Cyperus* to obtain yield estimations. Estimations based on stem samples include Riche (2005), who estimated yields from a sub-sample of 20 stems per plot. *Arundo* studies have sub-sampled 10m^2 from 400m^2 trial plots (Angelini *et al.*, 2005). Yield estimations of *Phalaris* have been calculated from small sub-plot sizes of 0.75m^2 (Riche, 2005) and1.25m^2 (Sahramaa & Jauiainen, 2003).

It is generally understood that these yield estimates tend to be vast overestimates of the actual yield achieved from field scale crop trials. Indeed, Riche (2005) provided both estimated yields and final plot yields and found that, for *Phalaris* and *Miscanthus*, estimated yields were approximately double the actual plot yield. Most studies, however, fail to provide whole crop final yields, either because the trial plot size is too small or because the final harvest is not measured.

Studies of short rotation willow coppice and poplar coppice have shown that yields can be successfully estimated from stem diameter (Heaton, 1999; Arevalo *et al.*, 2007), although there is some discrepancy regarding the height of the stem at which the diameter is measured. Bergkvist and Ledin (1998) measured the diameter of 30 shoots at 55cm above ground level, and were able to calculate a relationship to predict shoot dry weight using non-linear regression. Sage (1999) confirmed a relationship between the dry weight and the stem diameter at half stem length (providing the stem is taller than 1m and the diameter is wider than 5mm). Bell *et al.* (2006) showed that only stem diameter (at a height of 1m) could be used as an indicator of growth, whereas Bullard *et al.* (2002) showed that only cylindrical stem volume was related to final yield. However some short rotation coppice studies calculate estimates from harvesting a sub-sample of the trial plot. McCracken *et al.* (2001) calculated yields by harvesting 40 willow plants from the central plot area; Armstrong *et al.* (1999) harvested 36 poplar plants from their central plot, whereas Labrecque & Teodorescu (2005) based their estimates on a sample of 8 stems per plot.

Despite the structural differences between willow crops and energy grass crops, this study aimed to determine if similar relationships exist between simple non-destructive field measurements and crop yield. It also investigated different methods of calculating yield estimates from destructive sampling methods, where a proportion of the crop was removed and weighed. The aim of this study was to discover the most suitable and accurate method of estimating yields from both destructive and non-destructive sampling methods of energy grass crops.

3.2 Methods

3.2.1 Site details

Full site details are provided in Chapter 2.

From November 2006 to February / March 2007, the crops at Llysdinam, Llwynprenteg and Bluestone were visited every two weeks. At Llysdinam and Llwynprenteg, *Miscanthus* and *Arundo* were sub-sampled every 2 weeks and *Phalaris* was sub-sampled every 4 weeks. At Bluestone, *Miscanthus* and *Arundo* were sub-sampled every two weeks, although access problems caused disruption to *Miscanthus* sub-sampling during January 2007 and cessation of the study on 2 February 2007.

3.2.2 *Miscanthus* and *Arundo* measurements

3.2.2.1 Single shoot measurements

Length

33 shoots were sampled at random from the whole crop area. Length was measured from the ground to the topmost leaf ligule.

Diameter

For the 33 random shoots, stem diameter was measured using mechanical callipers at a height of 40cm from the base of the stem.

Calculation of stem volume

Stem volume was determined as

pi x (stem diameter/2)^2 x shoot length (as defined above)

Shoot count

The number of shoots per plant was counted for 10 randomly chosen plants from the whole crop area.

3.2.2.2 Whole plant measurements

5 randomly selected plants were used to determine whole plant yield estimates for each *Miscanthus* and *Arundo* crop at each site. All shoots per plant were counted, and heights and stem diameters were measured for 10 shoots per plant. All shoots from each plant were cut at the base and collected. Fresh weight of each plant was measured immediately upon return to the research centre. 5 shoots were randomly selected from each plant, weighed fresh, and oven dried at 80°C to constant weight, to obtain dry weight values.

3.2.2.3 Sub-plot measurements

Three randomly selected sub-plots were measured within each *Miscanthus* and *Arundo* crop at each site. Within the *Miscanthus* crops at Llysdinam and Llwynprenteg only, the area measured was 4 square metres. Due to access problems, it was not possible to obtain sub-plot measurements from the *Miscanthus* crop at the Bluestone site. Due to the smaller size of the *Arundo* plots at Llysdinam, Llwynprenteg and Bluestone, the 3 replicate sub-plot measurements were obtained from 2 square metre areas.

For each sub-plot, the total number of plants within the area was counted, although this sometimes proved difficult due to spreading of the plants. The total number of shoots within the area was also counted.

All shoots within each area were cut at the base, collected and weighed fresh immediately upon return to the research centre. Ten shoots from each sub-plot were selected at random, weighed fresh, and oven dried at 80°C to constant weight, to obtain dry weight values.

3.2.3 *Phalaris* sub-plot measurements

The *Phalaris* crops at Llysdinam and Llwynprenteg were visited every 4 weeks and yield estimates were obtained from 5 randomly selected 1m^2 quadrats. All shoots within the quadrat were cut at the base and collected. The fresh weight of each sub-plot was determined immediately upon arrival at the research centre, and a sub-sample of approximately 500g was accurately weighed fresh, then dried at 80°C to constant weight, to obtain a dry weight value.

3.2.4 Whole crop yields

Only at Llwynprenteg was it possible to obtain whole crop yields for *Miscanthus*, *Arundo* and *Phalaris*. The *Phalaris* crop was harvested in the first week of February, and the *Miscanthus* and *Arundo* crops were harvested in the last week of March. The crops were mown using an 8 foot Kuhn mower (model GMD66) pulled by a standard Massey Ferguson 390 tractor, then baled using a Krone baler (model KR125). Bales were weighed in the field for fresh weight. A sub sample was taken from the bales and oven-dried at 98°C for 48 hours and re-weighed to obtain dry weight. All whole crop harvesting and subsequent yield measurements were carried out by ADAS.

3.2.5 Yield estimate calculations from non-destructive sampling methods

3.2.5.1 *Miscanthus* and *Arundo* calculations and statistical analysis

The *Miscanthus* and *Arundo* crops were analysed separately and for each crop the following analyses were performed:

a. Correlation of shoot volume (pi x (stem diameter/2)^2 x shoot length) and shoot dry weight on each sampling date

b. Correlation of shoot length and shoot dry weight on each sampling date

c. Correlation of total plant height and whole plant dry weight on each sampling date. Total plant height was calculated as the multiple of the mean shoot height (calculated from 10 shoots sampled) and the total number of shoots in the plant.

Correlation analysis was performed on each subset of data (a, b, c as above) at each site. Data were transformed as necessary to meet the assumptions of the test. If data transformation was not successful the analysis was performed using Spearman's Rank tests.

The data from each sampling date were combined within each subset of data (a, b, c as above), and regression analyses were performed to investigate the relationship between the parameters at each site. Data were transformed as necessary to meet the assumptions of the test. Where regression analysis showed a significant relationship between the parameters, analysis of covariance was used to determine significant differences between the relationships at all sites. Regression analysis including a fitted line plot was produced from data from all sampling dates at all sites if analysis of covariance showed no significant differences between the sites.

3.2.6 Yield estimate calculations from destructive sampling methods

3.2.6.1 *Miscanthus* and *Arundo* calculations

The samples collected from the field were used to estimate crop yields using 4 methods, as described in Table 3.1.

Method	Estimated dry weight (DW) of sub-plot =
1a	Mean dry weight per shoot x mean number of shoots per plant x mean number of plants per sub-plot
1b	Mean dry weight per shoot x mean number of shoots per sub-plot
2	Mean dry weight per plant x mean number of plants per sub-plot
3	Sub-plot dry weight as removed directly from field.

Table 3.1: Methods of estimating the dry weight of a sub-plot using destructive sampling.

All estimates were scaled up to oven dried tonnes per hectare (odt/ha) for comparison with field scale yields.

3.2.6.2 *Phalaris* calculations

It was not possible to analyse *Phalaris* crops using the non-destructive sampling methods, as it was not possible to distinguish individual plants within the dense crops. Destructive sampling was therefore only possible using method 3, as above.

3.3 Results

3.3.1 *Miscanthus* results

3.3.1.1 Correlation of mean shoot volume and mean shoot dry weight

Mean shoot volume for each date was calculated from the data recorded for the 5 replicate whole plant datasets. For practical reasons, shoot volumes were calculated for November and December data only. Two datasets showed a significant strong positive correlation between mean shoot volume and mean shoot dry weight (Figure 3.1 and Table 3.2). These were the November datasets at Llwynprenteg (P=0.006) and Llysdinam (P=0.035). The Bluestone datasets showed positive relationships that were not significant.

November and December data combined

The data from the two dates were combined and regression analyses were performed to investigate the relationship between the two variables at each of the sites (Figures 3.2 and 3.3). Regression analyses showed significant relationships between the combined mean shoot volume and mean shoot dry weight data at Llwynprenteg (P=0.01) and Llysdinam (P=0.02). The Bluestone data did not reveal a significant relationship (Table 3.3).

As the Bluestone dataset did not show a significant relationship, analysis of covariance was performed for the regression analyses from Llwynprenteg and Llysdinam only. This showed no significant differences between the intercepts or slopes of the two lines. Regression analysis of the data from Llysdinam and Llwynprenteg combined showed a highly significant positive relationship between mean shoot volume and mean shoot dry weight (P=0.000, R^2=0.844, n=20).

3.3.1.2 Correlation of mean shoot height and mean shoot dry weight

At Bluestone, mean shoot height had a significant strong positive correlation with mean shoot dry weight on two out of five occasions, in early December (P=0.043) and

January (P=0.004), although the later December data were close to significance (P=0.06; Figure 3.4a and Table 3.4). At Llwynprenteg, in late November, all January dates and in late February (Figure 3.4b), the mean shoot height showed a significant (Table 3.4) strong positive correlation with mean shoot dry weight on five out of eight occasions. At Llysdinam the mean shoot height showed a significant (P=0.023) strong positive correlation with mean shoot dry weight only in mid December, out of eight sampling dates. The data from late November (P=0.058) and mid January (P=0.059) were close to significance (Figure 3.4c and Table 3.4).

In summary, the mean shoot height showed a strong significant positive correlation with mean shoot dry weight at Llwynprenteg and Bluestone on roughly half of all sampling dates, whereas there were close to no significant relationships at Llysdinam.

All dates combined

The data from all sampling dates from each site were combined and regression analyses were performed to further investigate the relationships between the two variables (Figure 3.5). The combined data from all sampling dates showed highly significant (P<0.0001) linear relationships between mean shoot height and mean shoot dry weight at all sites (Table 3.5).

Analysis of covariance determined there to be no significant differences between the intercepts or the slopes of the regression lines from the different sites. Further regression analysis of data from all sites combined showed a highly significant relationship between mean shoot height and mean shoot dry weight (P=0.000; Figure 3.8).

3.3.1.3 Correlation of total plant height and plant dry weight

At Llwynprenteg the total plant height showed a significant (Table 3.6) strong positive correlation with plant dry weight on all dates except the 20 November (Figure 3.6a). At Llysdinam the total plant height showed a significant (Table 3.6) strong positive correlation with plant dry weight on all eight sampling dates (Figure

3.6a). At Bluestone the total plant height and plant dry weight showed a significant strong positive correlation on all five sampling dates (Figure 3.6c and Table 3.6).

All dates combined

Figure 3.7 shows regression analyses of total plant height and plant dry weight from all dates at each of the *Miscanthus* sites studied. On combining all data from all sampling dates, it was shown that total plant height had a highly significant positive linear relationship with plant dry weight at all sites (Table 3.7).

Analysis of covariance determined there to be no significant differences between the intercepts or the slopes of the regression lines from the different sites. Further regression analysis of data from all sites combined showed a highly significant relationship between mean total plant height and mean plant dry weight (P=0.000; Figure 3.9).

Site and date	Abbreviation	R	P value	Significance
Llwynprenteg 20 Nov 06	Lp nov 06	0.972	0.006	**
Llwynprenteg 4 Dec 06	Lp 4 dec 06	x	x	n.s.
Llysdinam 22 Nov 06	Llys nov 06	0.905	0.035	*
Llysdinam 6 Dec 06	Llys 6dec 06	x	x	n.s.
Bluestone 23 Nov 06	Blu nov 06	x	x	n.s.
Bluestone 7 Dec 06	Blu 7dec 06	x	x	n.s.

Table 3.2: Correlation analysis of mean *Miscanthus* shoot volume and mean shoot dry weight at all sites for November and December dates. Correlation statistic (R) and P value are shown for significant results. Abbreviations are as used in Figure 3.1.

Site	R^2	R	P value	Significance
Llwynprenteg	0.5829	0.764	0.01	**
Llysdinam	0.5114	0.715	0.02	*
Bluestone	x	x	x	n.s.

Table 3.3: Regression analysis of mean *Miscanthus* shoot volume and mean shoot dry weight for November and December data combined at all sites. Regression statistic (R^2), correlation statistic (R) and P value are shown for significant results.

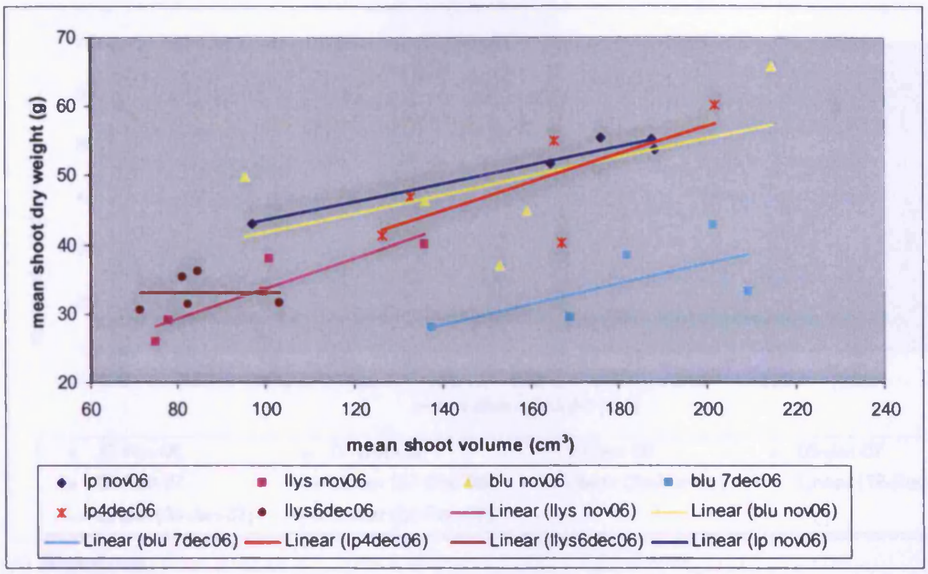


Figure 3.1: Correlation of mean *Miscanthus* shoot volume (cm^3) and mean shoot dry weight (g) at all sites for November and December dates (see Table 3.2, p. 34 for abbreviations).

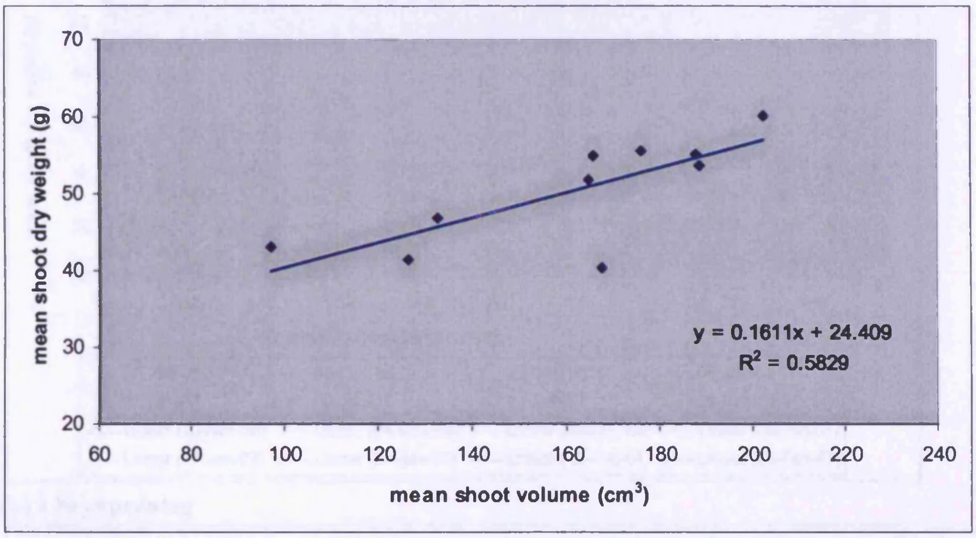


Figure 3.2: Regression of mean *Miscanthus* shoot volume and mean shoot dry weight for November and December data combined at Llwynprenteg.

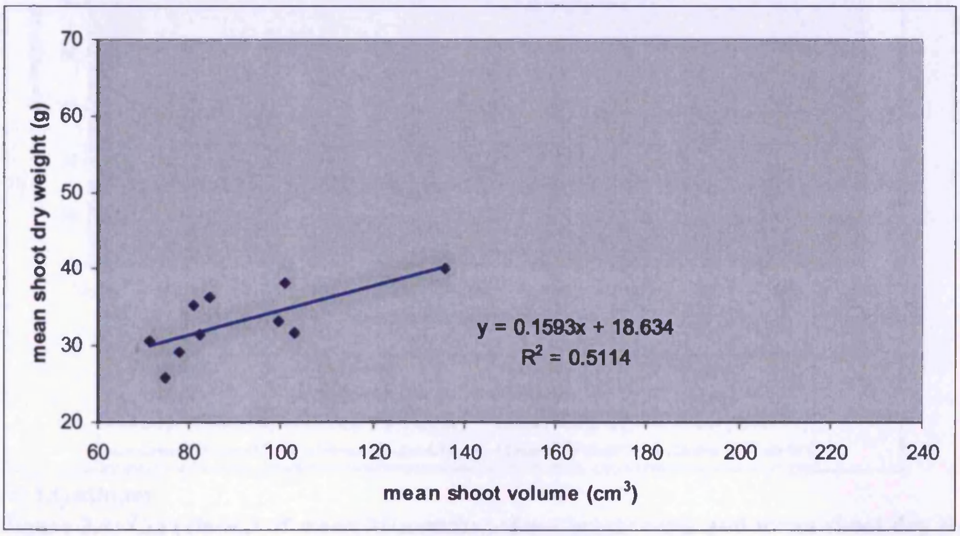
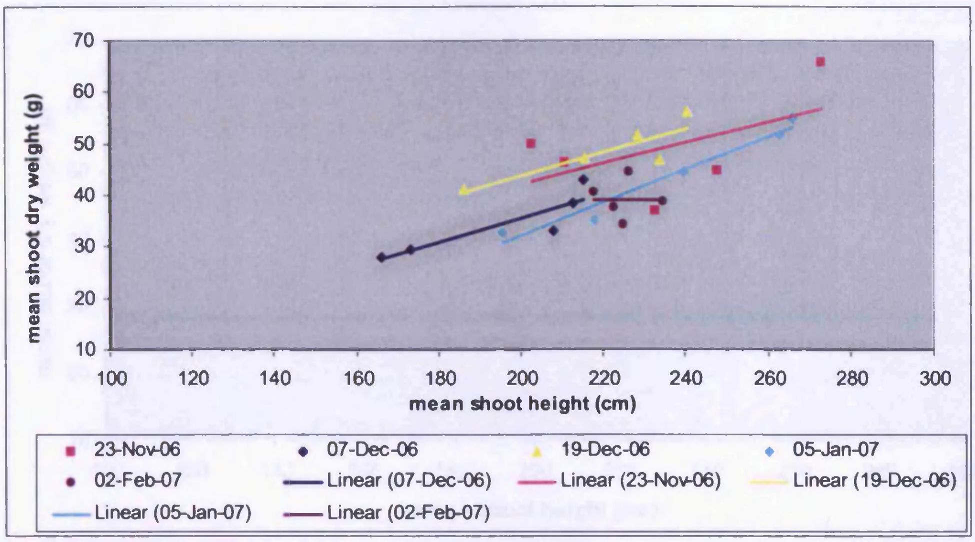
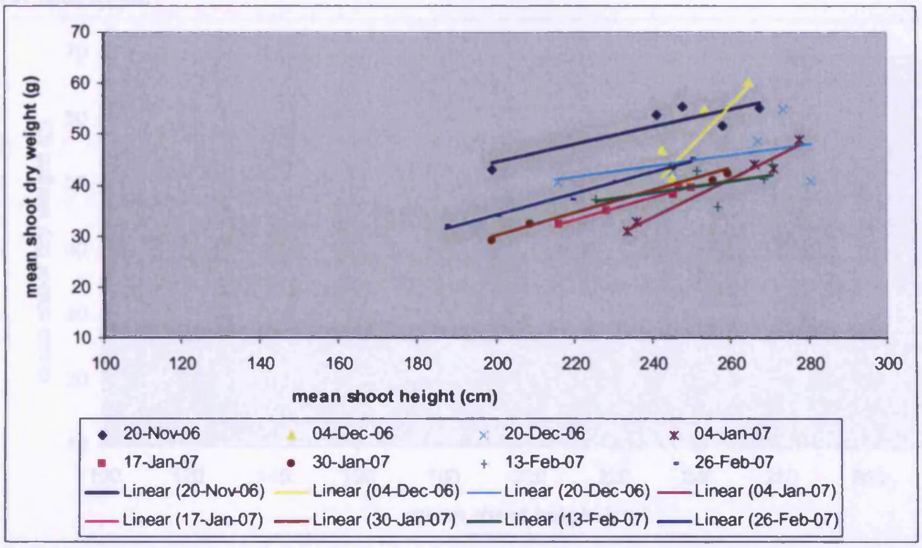


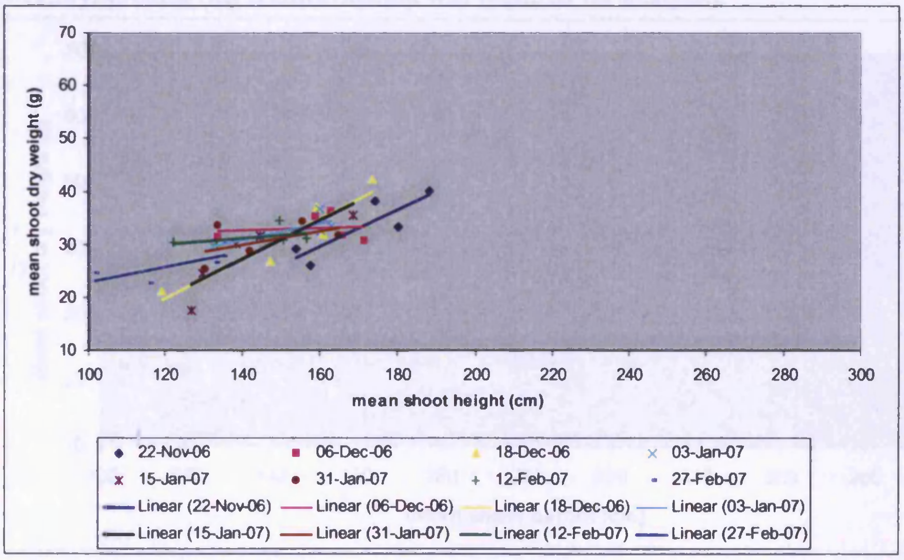
Figure 3.3: Regression of mean *Miscanthus* shoot volume and mean shoot dry weight for November and December data combined at Llysdinam.



(a) Bluestone.

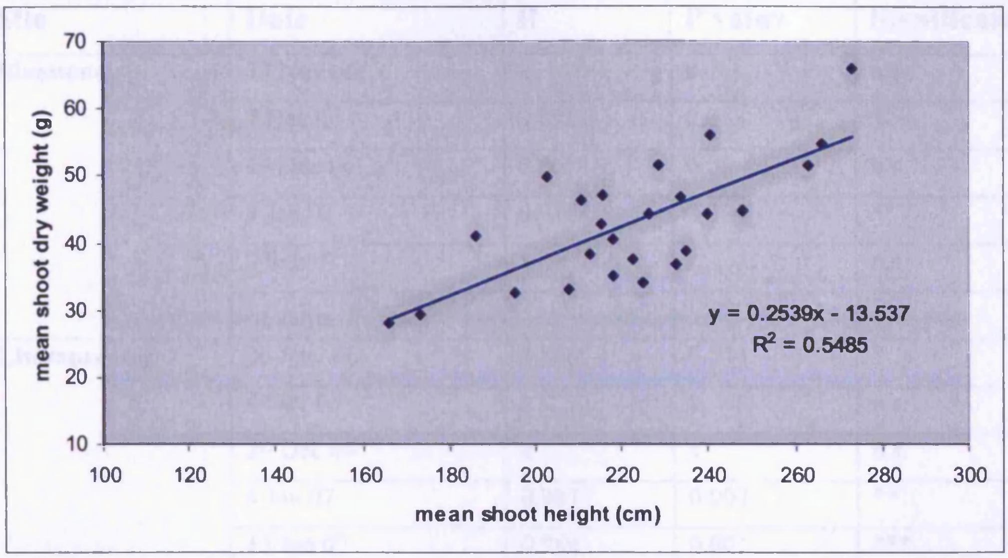


(b) Llwynprenteg

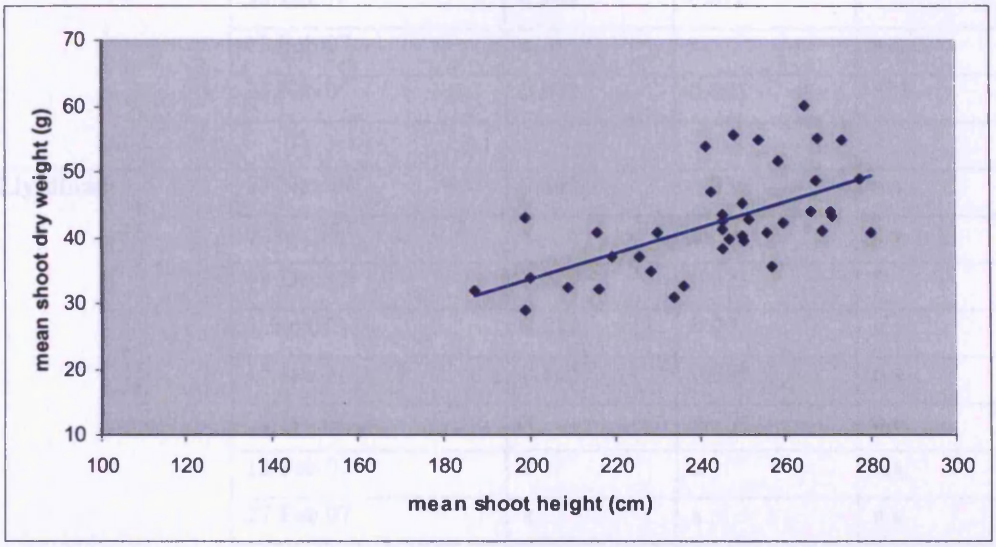


(c) Llysdinam

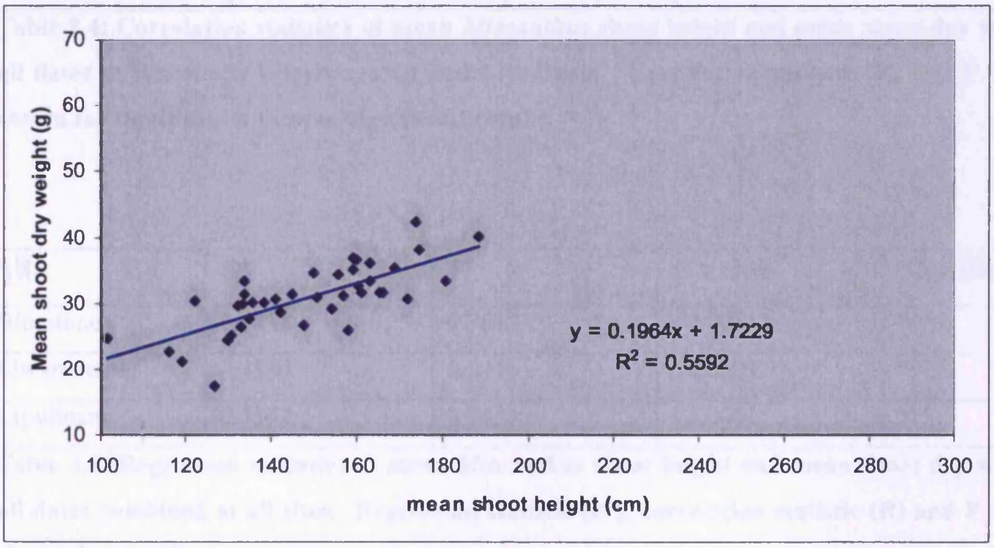
Figure 3.4: Correlation of mean *Miscanthus* shoot height (cm) and mean shoot dry weight (g) for all dates at (a) Bluestone, (b) Llwynprenteg and (c) Llysdinam.



(a) Bluestone.



(b) Llwynprenteg (log transformation was required for analysis).



(c) Llysdinam

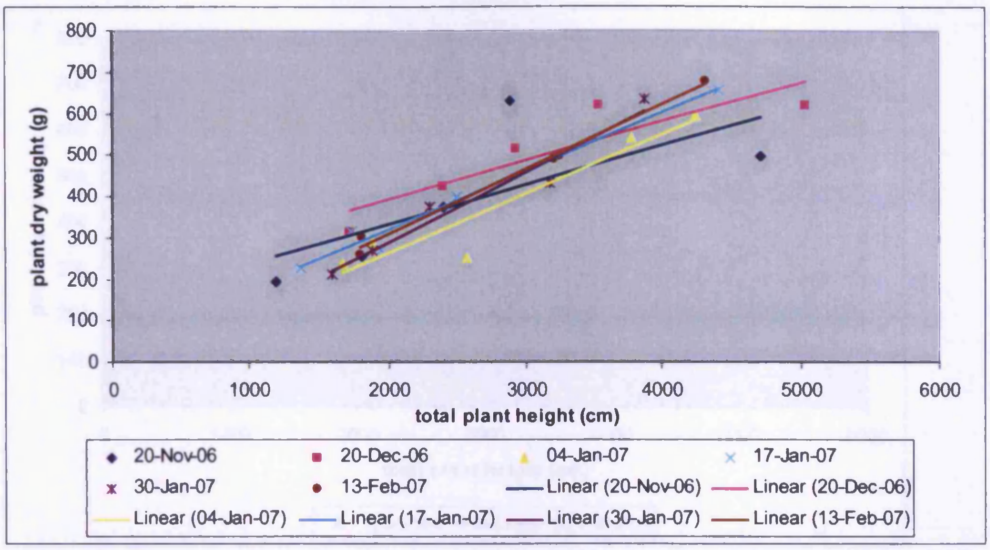
Figure 3.5: Regression of mean *Miscanthus* shoot height and mean shoot dry weight for all dates combined at (a) Bluestone, (b) Llwynprenteg and (c) Llysdinam.

Site	Date	R	P value	Significance
Bluestone	23 Nov 06	x	x	n.s.
	7 Dec 06	0.891	0.043	*
	19 Dec 06	0.861	0.06	n.s.
	5 Jan 07	0.979	0.004	**
	2 Feb 07	x	x	n.s.
Llwynprenteg	20 Nov 06	0.888	0.044	*
	4 Dec 06	x	x	n.s.
	20 Dec 06	x	x	n.s.
	4 Jan 07	0.987	0.002	**
	17 Jan 07	0.989	0.001	***
	30 Jan 07	0.954	0.012	*
	13 Feb 07	x	x	n.s.
	26 Feb 07	0.993	0.001	***
Llysdinam	22 Nov 06	0.865	0.058	n.s.
	6 Dec 06	x	x	n.s.
	18 Dec 06	0.929	0.023	*
	3 Jan 07	0.811	0.09	n.s.
	15 Jan 07	0.865	0.059	n.s.
	31 Jan 07	x	x	n.s.
	12 Feb 07	x	x	n.s.
	27 Feb 07	x	x	n.s.

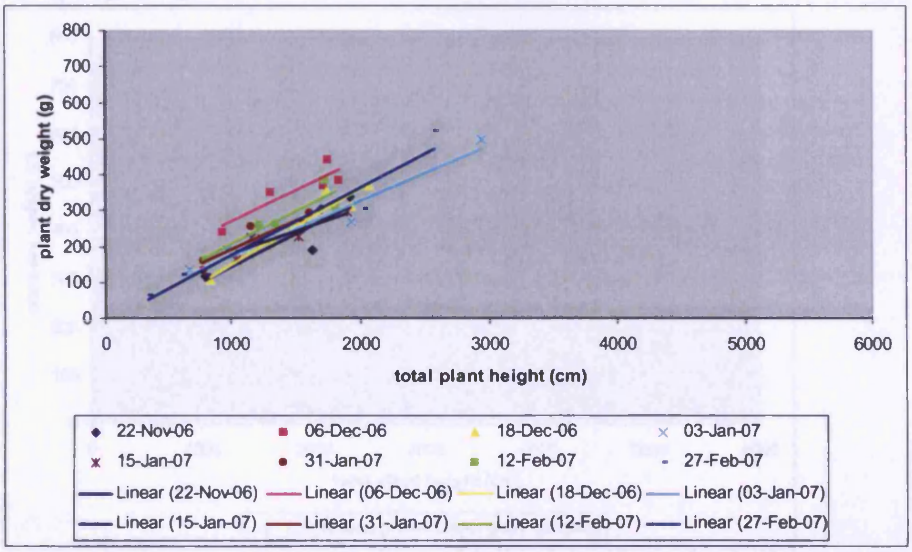
Table 3.4: Correlation statistics of mean *Miscanthus* shoot height and mean shoot dry weight for all dates at Bluestone, Llwynprenteg and Llysdinam. Correlation statistic (R) and P value are shown for significant and near-significant results.

Site	R^2	R	P value	Significance
Bluestone	0.5485	0.741	<0.0001	***
Llwynprenteg	0.4104	0.641	<0.0001	***
Llysdinam	0.5592	0.748	<0.0001	***

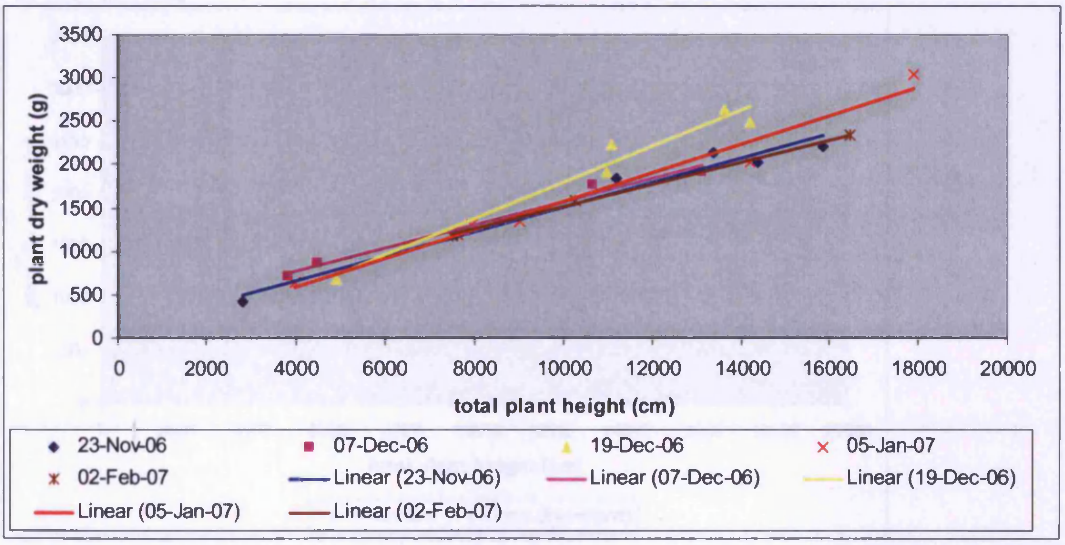
Table 3.5: Regression statistics of mean *Miscanthus* shoot height and mean shoot dry weight for all dates combined at all sites. Regression statistic (R^2), correlation statistic (R) and P value are shown for significant results.



(a) Llwynprenteg.

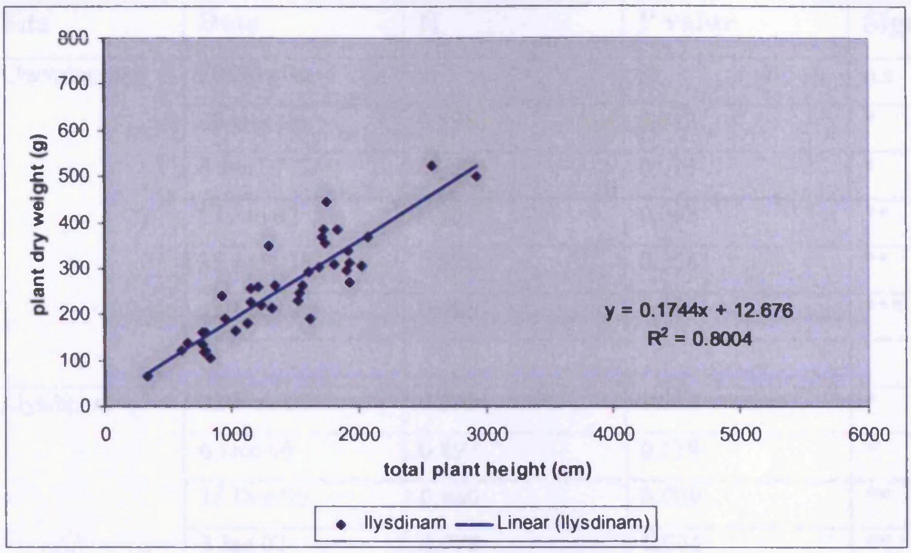


(b) Llysdinam.

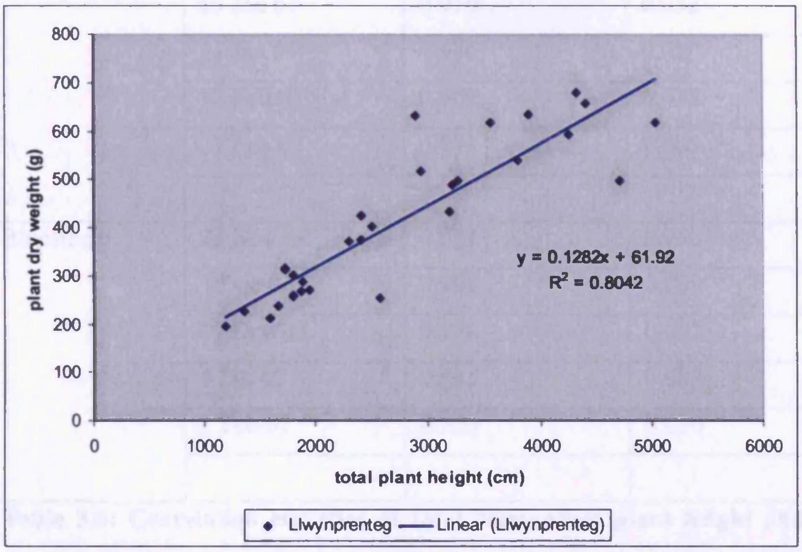


(c) Bluestone

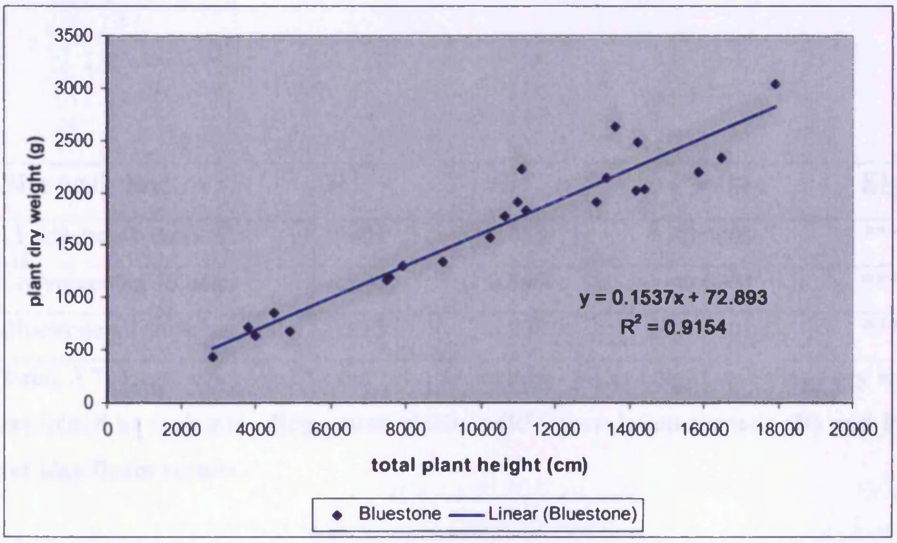
Figure 3.6: Correlation of total *Miscanthus* plant height (cm) and plant dry weight (g) for all dates at (a) Llwynprenteg, (b) Llysdinam and (c) Bluestone. Note the different scale for (c).



(a) Llysdinam



(b) Llwynprenteg



(c) Bluestone

Figure 3.7: Regression of total *Miscanthus* plant height and plant dry weight for all dates combined at (a) Llysdinam, (b) Llwynprenteg and (c) Bluestone. Note the different scale for (c).

Site	Date	R	P value	Significance
Llwynprenteg	20 Nov 06	x	x	n.s.
	20 Dec 06	0.891	0.042	*
	4 Jan 07	0.952	0.013	*
	17 Jan 07	0.987	0.002	**
	30 Jan 07	0.976	0.004	**
	13 Feb 07	0.995	0.000	***
Llysdinam	22 Nov 06	0.890	0.043	*
	6 Dec 06	0.897	0.039	*
	18 Dec 06	0.960	0.009	**
	3 Jan 07	0.977	0.004	**
	15 Jan 07	0.910	0.032	*
	31 Jan 07	0.936	0.02	*
	12 Feb 07	0.909	0.032	*
	27 Feb 07	0.973	0.005	**
Bluestone	23 Nov 06	0.981	0.05	*
	7 Dec 06	0.991	0.009	**
	19 Dec 06	0.976	0.003	**
	5 Jan 07	0.985	0.002	**
	2 Feb 07	0.999	0.016	*

Table 3.6: Correlation statistics of total *Miscanthus* plant height and plant dry weight for all dates at all sites. Correlation statistic (R) and P value are shown for significant results.

Site and date	R^2	R	P value	Significance
Llysdinam all dates	0.801	0.895	<0.0001	***
Llwynprenteg all dates	0.804	0.897	<0.0001	***
Bluestone all dates	0.915	0.957	<0.0001	***

Table 3.7: Regression statistics of total *Miscanthus* plant height and plant dry weight for all dates combined at each site. Regression statistic (R^2), correlation statistic (R) and P value are shown for significant results.

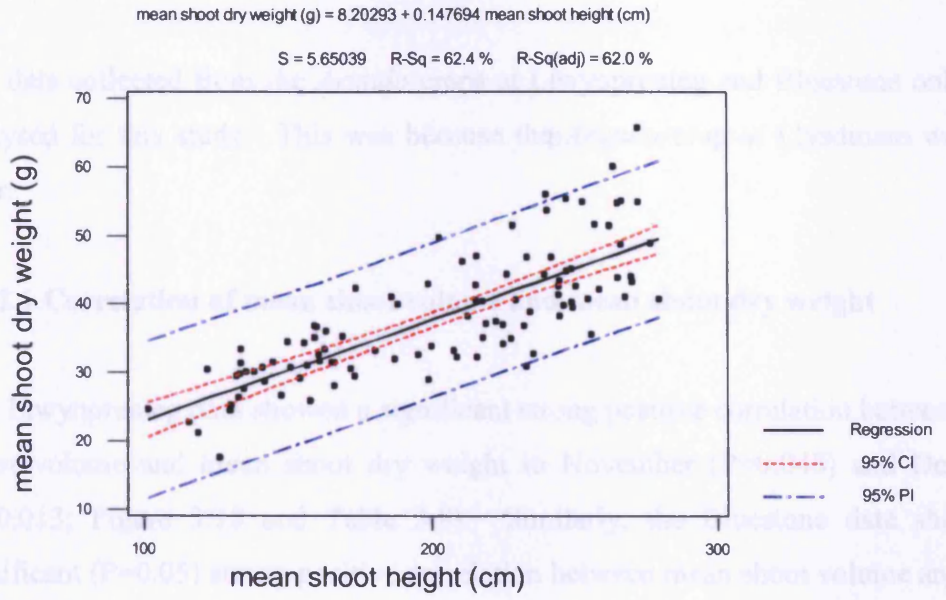


Figure 3.8: Fitted line plot for regression analysis of mean *Miscanthus* shoot height and mean shoot dry weight for all dates at all sites

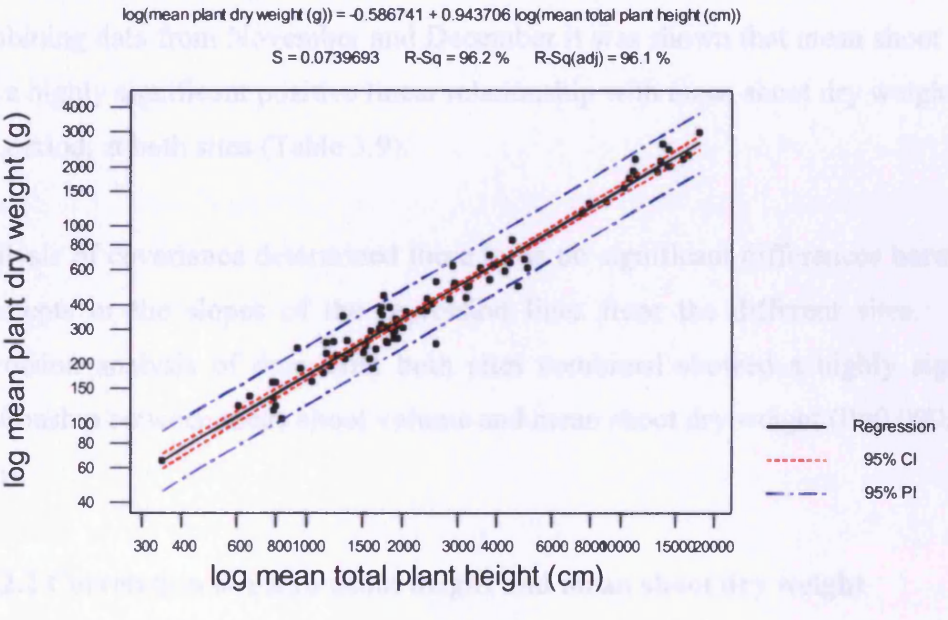


Figure 3.9: Fitted line plot for regression analysis of $\log_{(10)}$ data for mean total *Miscanthus* plant height and mean plant dry weight for all dates at all sites

3.3.2 *Arundo* Results

The data collected from the *Arundo* crops at Llwynprenteg and Bluestone only were analysed for this study. This was because the *Arundo* crop at Llysdinam was very poor.

3.3.2.1 Correlation of mean shoot volume and mean shoot dry weight

The Llwynprenteg data showed a significant strong positive correlation between mean shoot volume and mean shoot dry weight in November (P=0.048) and December (P=0.013; Figure 3.10 and Table 3.8). Similarly, the Bluestone data showed a significant (P=0.05) strong positive correlation between mean shoot volume and mean shoot dry weight in November, but the December data did not exhibit a significant relationship (Figure 3.10 and Table 3.8).

Both dates combined

Figure 3.11 shows regressions of the data from the two dates combined. On combining data from November and December it was shown that mean shoot volume had a highly significant positive linear relationship with mean shoot dry weight during this period, at both sites (Table 3.9).

Analysis of covariance determined there to be no significant differences between the intercepts or the slopes of the regression lines from the different sites. Further regression analysis of data from both sites combined showed a highly significant relationship between mean shoot volume and mean shoot dry weight (P=0.000; Figure 3.16).

3.3.2.2 Correlation of mean shoot height and mean shoot dry weight

At Llwynprenteg, mean shoot height showed a significant strong positive correlation with mean shoot dry weight on four out of eight occasions, on dates during December and January. The data from late November (P=0.094) and late January (P=0.099) exhibited relationships which were near to significance (Figure 3.12a and Table 3.10).

At Bluestone, mean shoot height showed a significant strong positive correlation with mean shoot dry weight on three out of eight occasions, in mid December (P=0.03), mid January (P=0.041) and early February (P=0.042). The data from early January (P=0.067) and late February (P=0.06) were close to significance (Figure 3.12b and Table 3.10).

All dates combined

On combining data from all dates it was shown that mean shoot height had a highly significant (P<0.0001) positive linear relationship with mean shoot dry weight at both sites (Figure 3.13 and Table 3.11).

Analysis of covariance determined there to be no significant differences between the intercepts or the slopes of the regression lines from the different sites. Further regression analysis of data from both sites combined showed a highly significant relationship between mean shoot height and mean shoot dry weight (P=0.000; Figure 3.17).

3.3.2.3 Correlation of total plant height and plant dry weight

As all *Arundo* plants had fewer than ten shoots, total plant height was calculated as the sum of all shoot heights. This analysis was performed for all dates at both sites.

At Llwynprenteg, the total plant height showed a significant (P=0.037) strong positive correlation with plant dry weight only in late November (Figure 3.14a and Table 3.12). At Bluestone, the total plant height showed a significant strong positive correlation with plant dry weight on five out of eight occasions, including all December and January dates, and late February (Figure 3.14b and Table 3.12).

All dates combined

On combining data from all dates it was shown that total plant height had a highly significant (P<0.0001) positive linear relationship with plant dry weight at both sites (Figure 3.15 and Table 3.13).

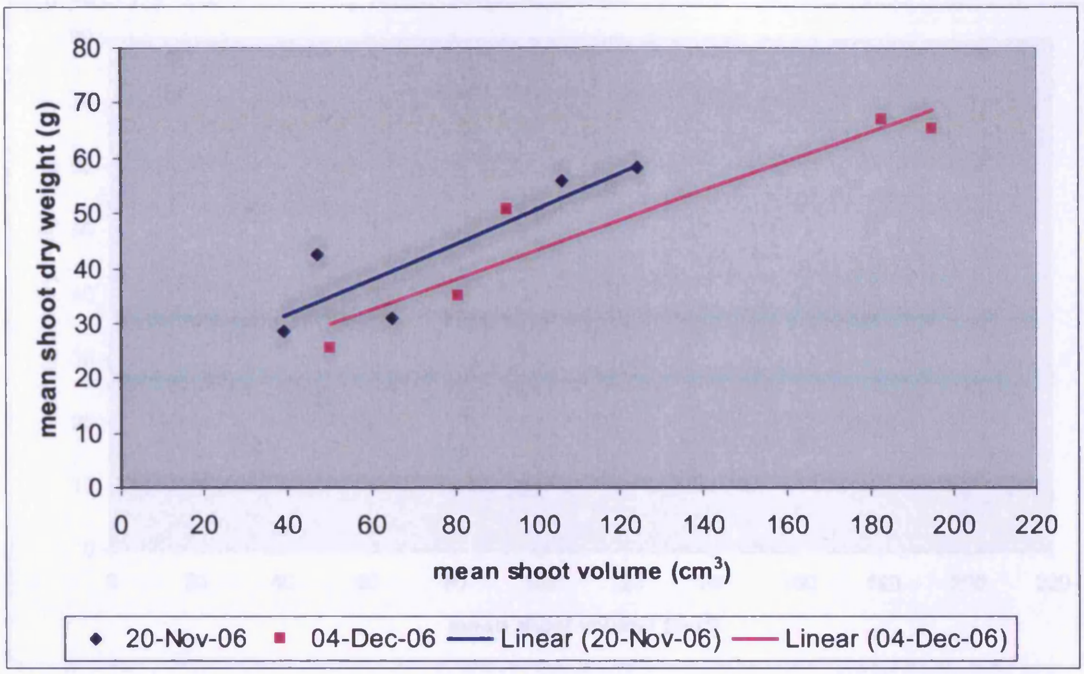
Analysis of covariance determined there to be no significant differences between the intercepts or the slopes of the regression lines from the different sites. Further regression analysis of data from both sites combined showed a highly significant relationship between mean total plant height and mean plant dry weight (P=0.000; Figure 3.18).

Site and date	**Date**	**R**	**P value**	**Significance**
Llwynprenteg	20 Nov 06	0.899	0.048	*
	4 Dec 06	0.950	0.013	*
Bluestone	23 Nov 06	0.899	0.05	*
	7 Dec 06	x	x	n.s.

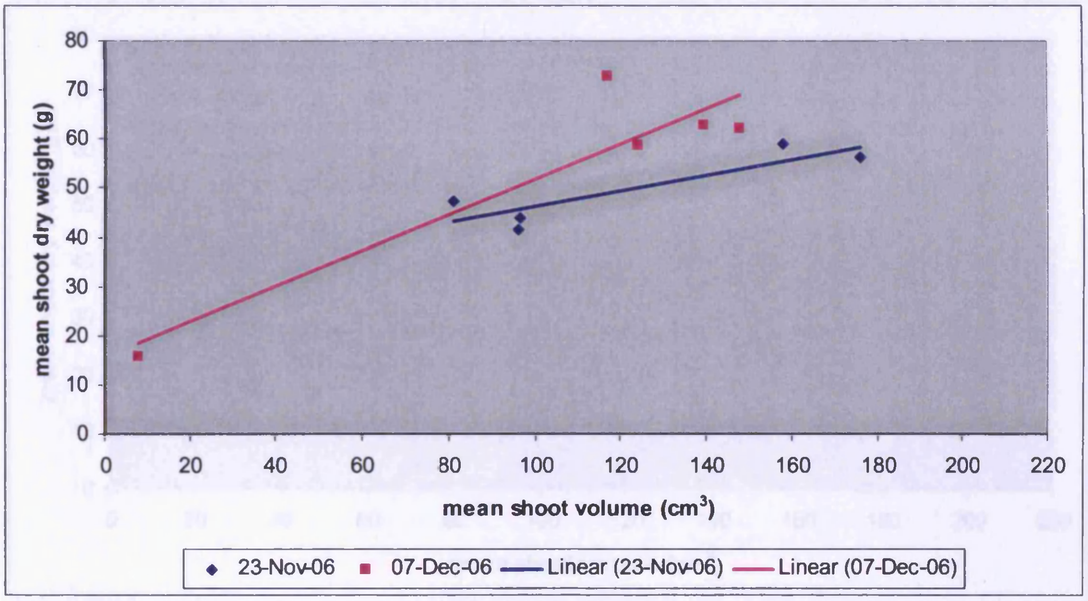
Table 3.8: Correlation statistics of mean *Arundo* shoot volume and mean shoot dry weight for November and December dates at Llwynprenteg and Bluestone. Correlation statistic (R) and P value are shown for significant results.

Site	**R^2**	**R**	**P value**	**Significance**
Llwynprenteg	0.813	0.902	0.0003	***
Bluestone	0.678	0.824	0.003	**

Table 3.9: Regression statistics of mean *Arundo* shoot volume and mean shoot dry weight for November and December dates combined. Regression statistic (R^2), correlation statistic (R) and P value are shown for significant results.

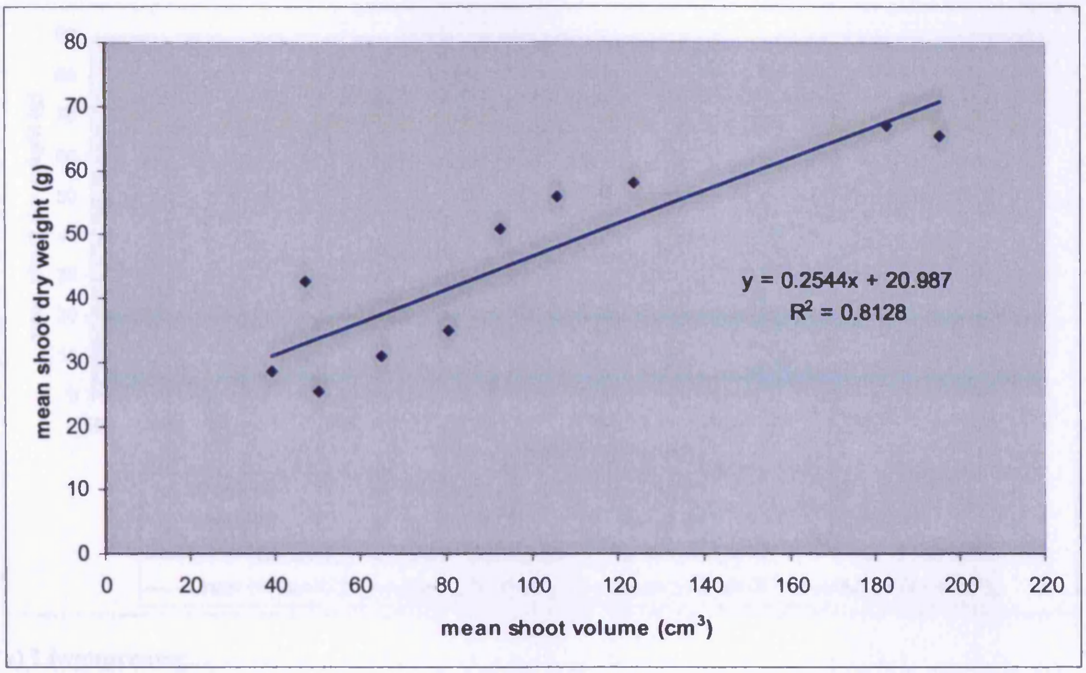


(a) Llwynprenteg.

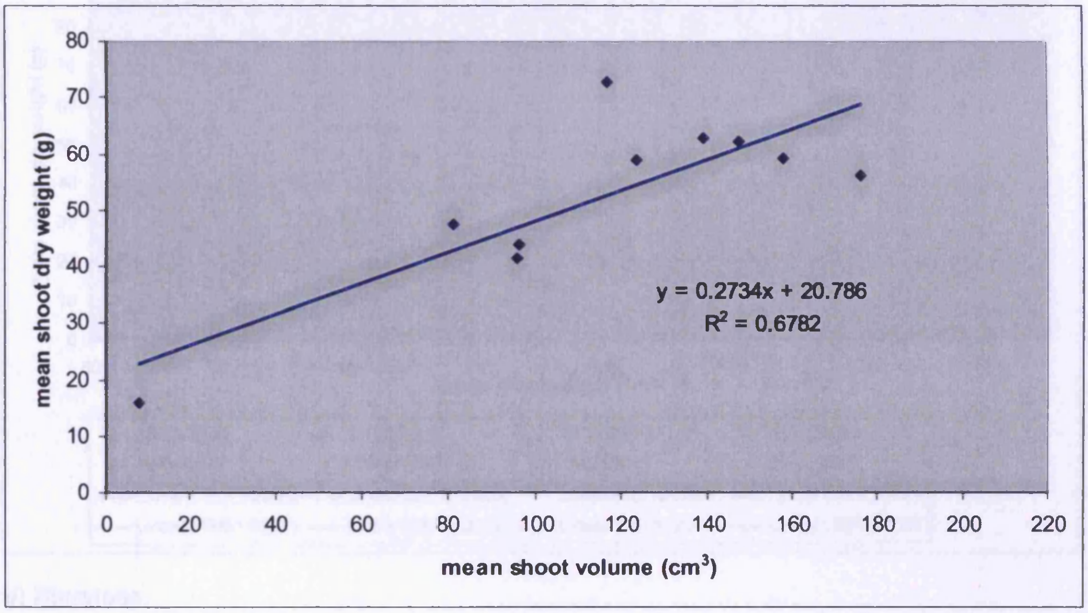


(b) Bluestone

Figure 3.10: Correlation of mean *Arundo* shoot volume (cm^3) and mean shoot dry weight (g) for November and December dates at (a) Llwynprenteg and (b) Bluestone.

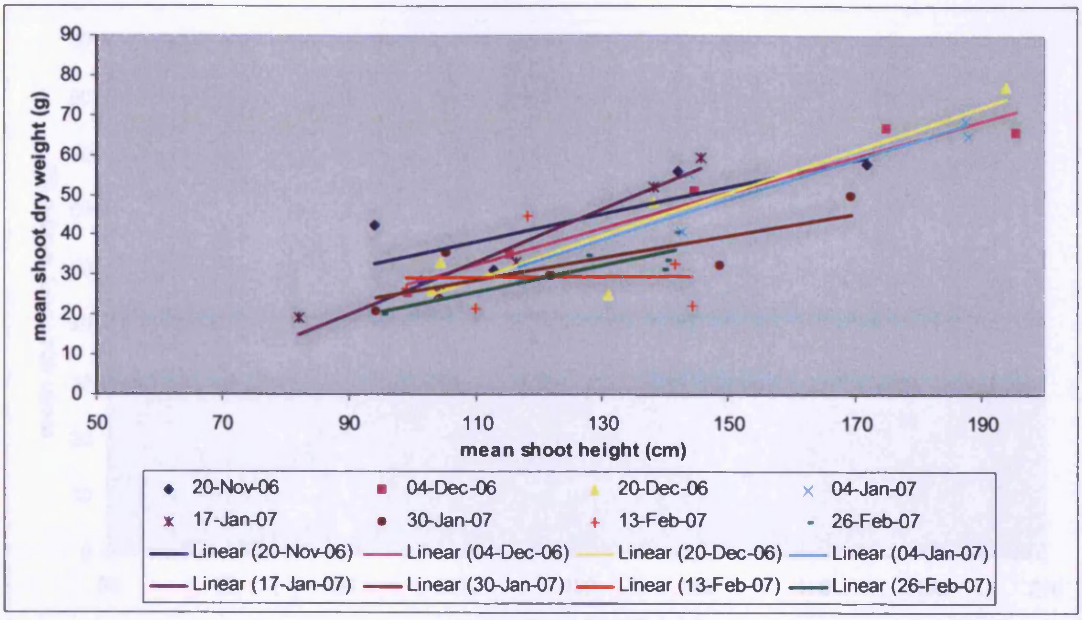


(a) Llwynprenteg.

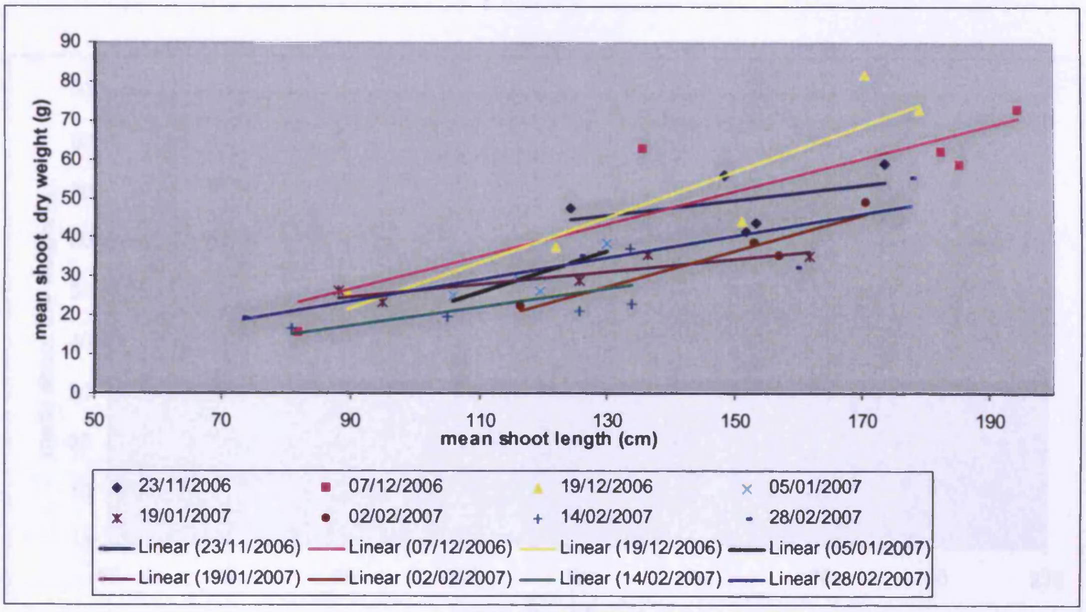


(b) Bluestone

Figure 3.11: Regression of mean *Arundo* shoot volume and mean shoot dry weight for November and December data combined, at (a) Llwynprenteg and (b) Bluestone.

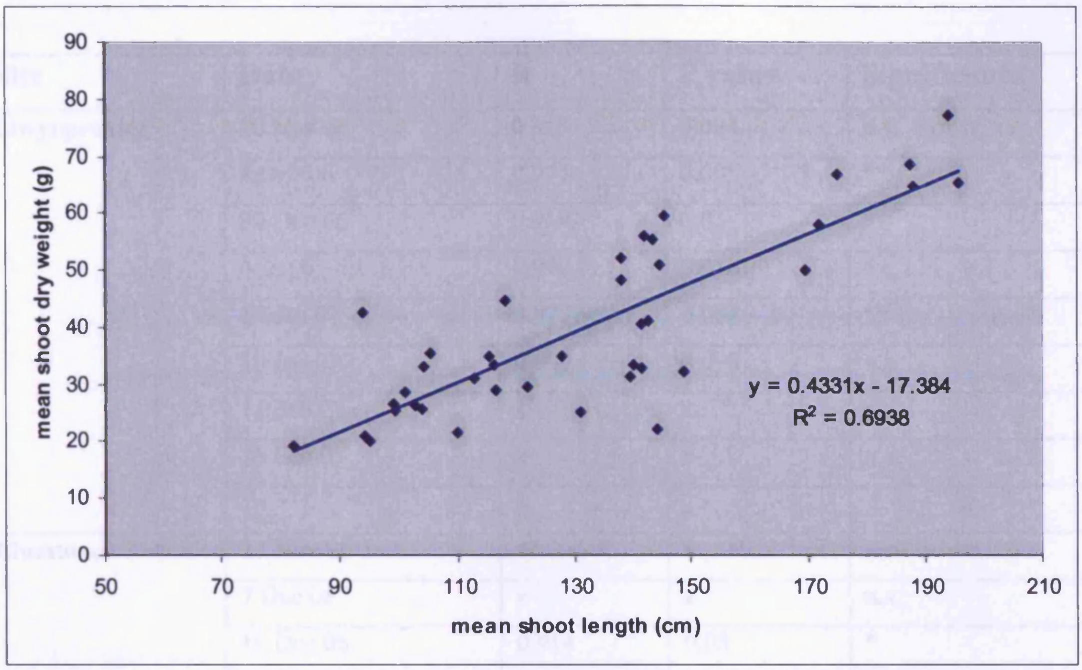


(a) Llwynprenteg.

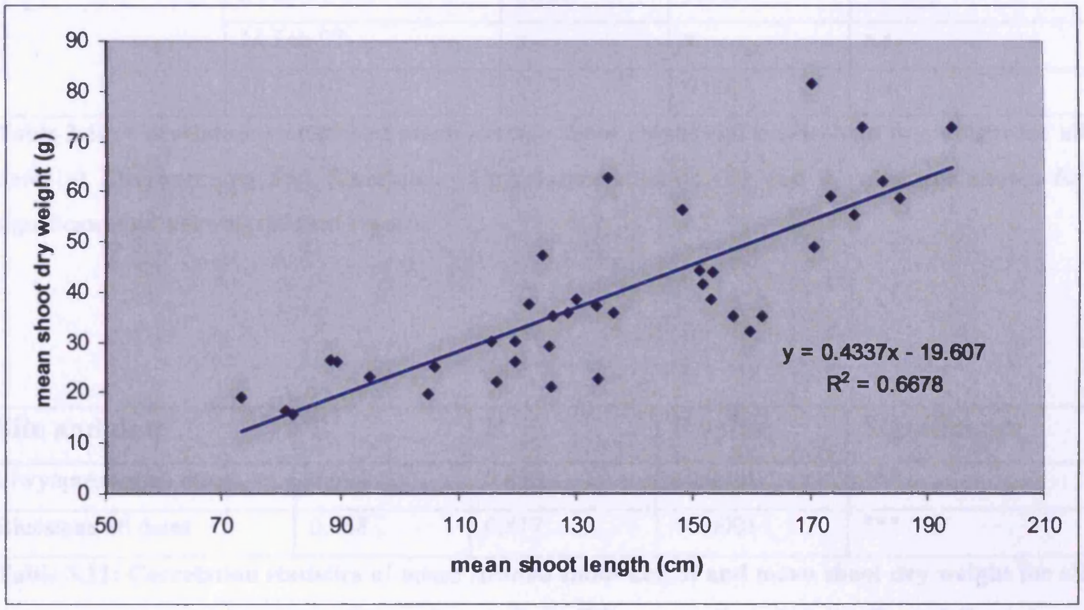


(b) Bluestone

Figure 3.12: Correlation of mean *Arundo* shoot height (cm) and mean shoot dry weight (g) for all dates at (a) Llwynprenteg and (b) Bluestone.



(a) Llwynprenteg



(b) Bluestone

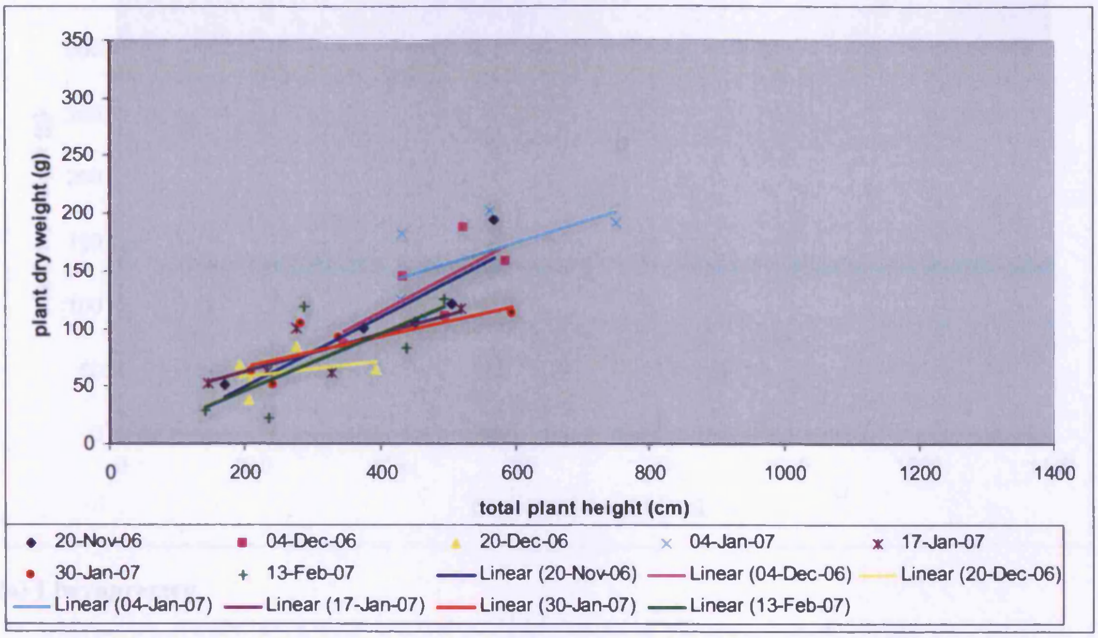
Figure 3.13: Regression of mean *Arundo* shoot height (cm) and mean shoot dry weight (g) for all dates combined at (a) Llwynprenteg and (b) Bluestone.

Site	Date	R	P value	Significance
Llwynprenteg	20 Nov 06	0.813	0.094	n.s.
	4 Dec 06	0.974	0.005	**
	20 Dec 06	0.914	0.03	*
	4 Jan 07	0.940	0.018	*
	17 Jan 07	0.977	0.004	**
	30 Jan 07	0.807	0.099	n.s.
	13 Feb 07	x	x	n.s.
	26 Feb 07	x	x	n.s.
Bluestone	23 Nov 06	x	x	n.s.
	7 Dec 06	x	x	n.s.
	19 Dec 06	0.914	0.03	*
	5 Jan 07	0.852	0.067	n.s.
	19 Jan 07	0.894	0.041	*
	2 Feb 07	0.958	0.042	*
	14 Feb 07	x	x	n.s.
	28 Feb 07	0.858	0.06	n.s.

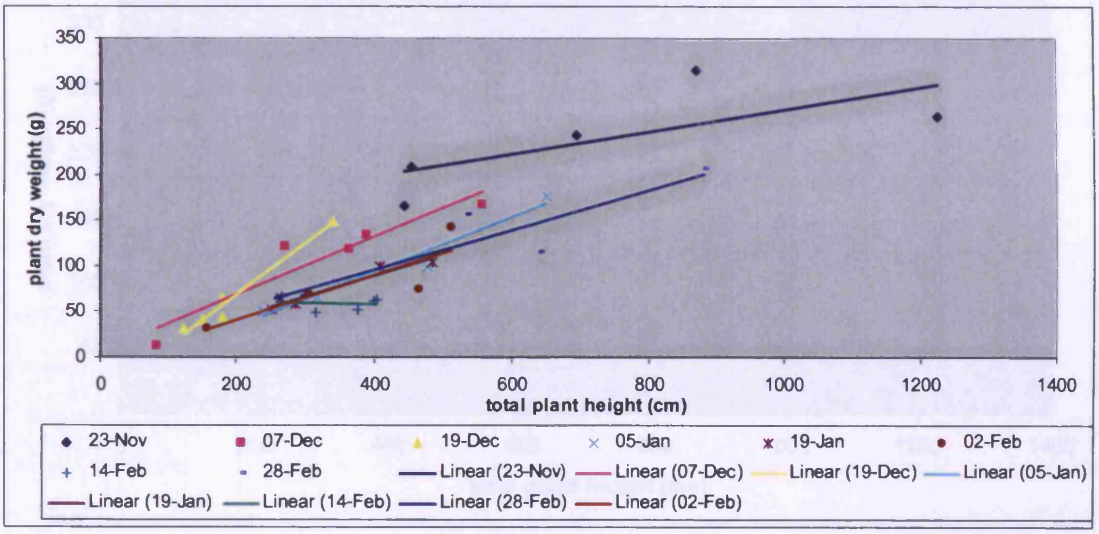
Table 3.10: Correlation statistics of mean *Arundo* shoot height and mean shoot dry weight for all dates at Llwynprenteg and Bluestone. Correlation statistic (R) and P value are shown for significant and near-significant results.

Site and date	R^2	R	P value	Significance
Llwynprenteg all dates	0.694	0.833	<0.0001	***
Bluestone all dates	0.668	0.817	<0.0001	***

Table 3.11: Correlation statistics of mean *Arundo* shoot height and mean shoot dry weight for all dates combined at Bluestone. Regression statistic (R^2), correlation statistic (R) and P value are shown for significant results.

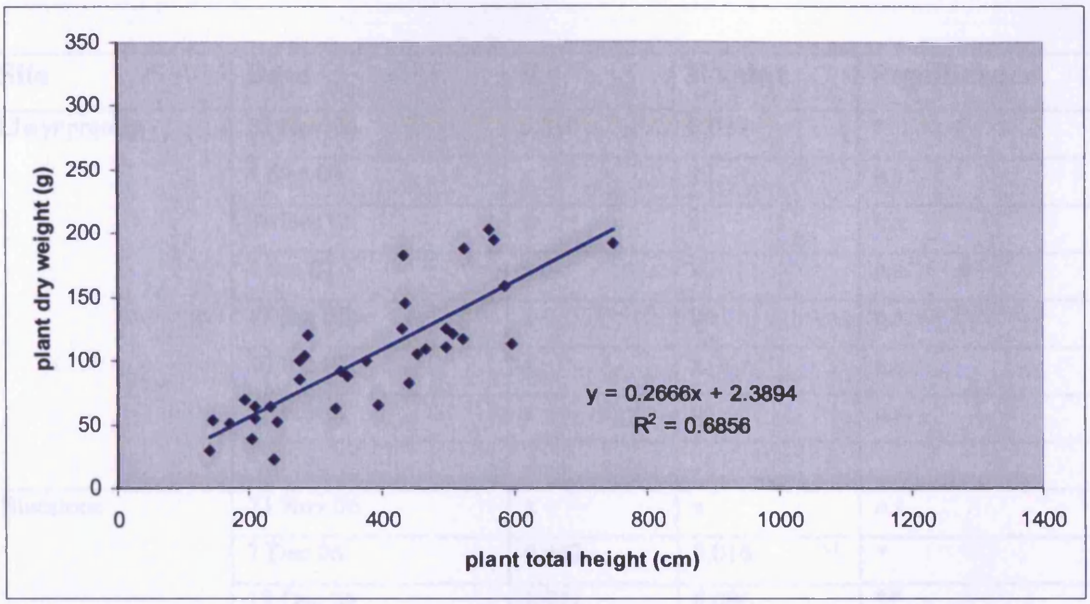


(a) Llwynprenteg

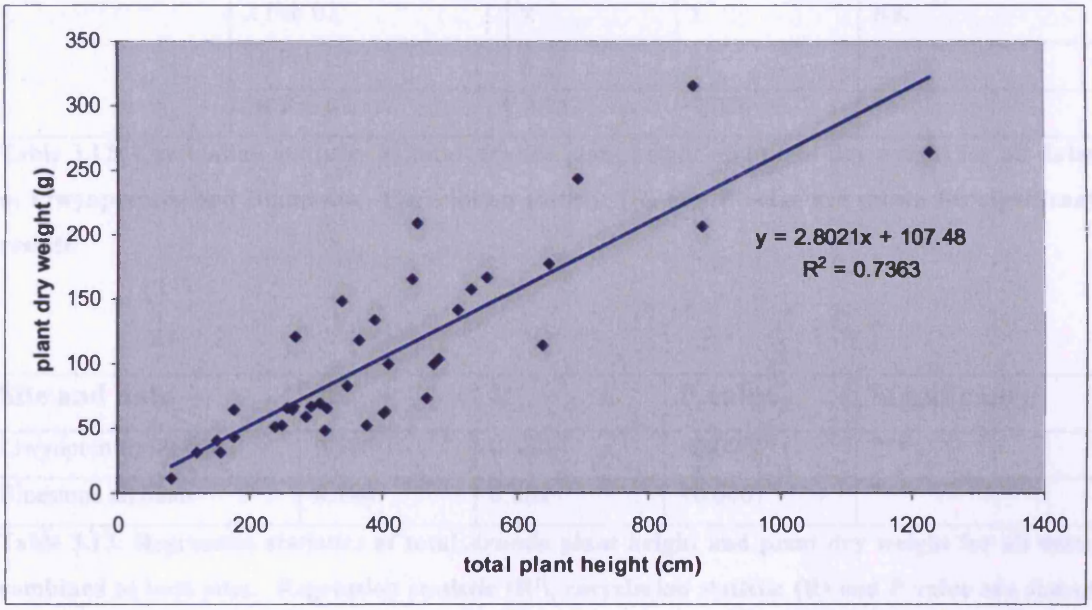


(b) Bluestone

Figure 3.14: Correlation of total *Arundo* plant height (cm) and plant dry weight (g) for all dates at (a) Llwynprenteg and (b) Bluestone.



(a) Llwynprenteg



(b) Bluestone

Figure 3.15: Regression of total *Arundo* plant height and plant dry weight for all dates combined at (a) Llwynprenteg and (b) Bluestone.

Site	Date	R	P value	Significance
Llwynprenteg	20 Nov 06	0.810	0.037	*
	4 Dec 06	x	x	n.s.
	20 Dec 06	x	x	n.s.
	4 Jan 07	x	x	n.s.
	17 Jan 07	x	x	n.s.
	30 Jan 07	x	x	n.s.
	13 Feb 07	x	x	n.s.
Bluestone	23 Nov 06	x	x	n.s.
	7 Dec 06	0.942	0.016	*
	19 Dec 06	0.971	0.006	**
	5 Jan 07	0.978	0.004	**
	19 Jan 07	0.966	0.007	**
	2 Feb 07	x	x	n.s.
	14 Feb 07	x	x	n.s.
	28 Feb 07	0.922	0.026	*

Table 3.12: Correlation statistics of total *Arundo* plant height and plant dry weight for all dates at Llwynprenteg and Bluestone. Correlation statistic (R) and P value are shown for significant results.

Site and date	R^2	R	P value	Significance
Llwynprenteg all dates	0.686	0.828	<0.0001	***
Bluestone all dates	0.790	0.889	<0.0001	***

Table 3.13: Regression statistics of total *Arundo* plant height and plant dry weight for all dates combined at both sites. Regression statistic (R^2), correlation statistic (R) and P value are shown for significant results.

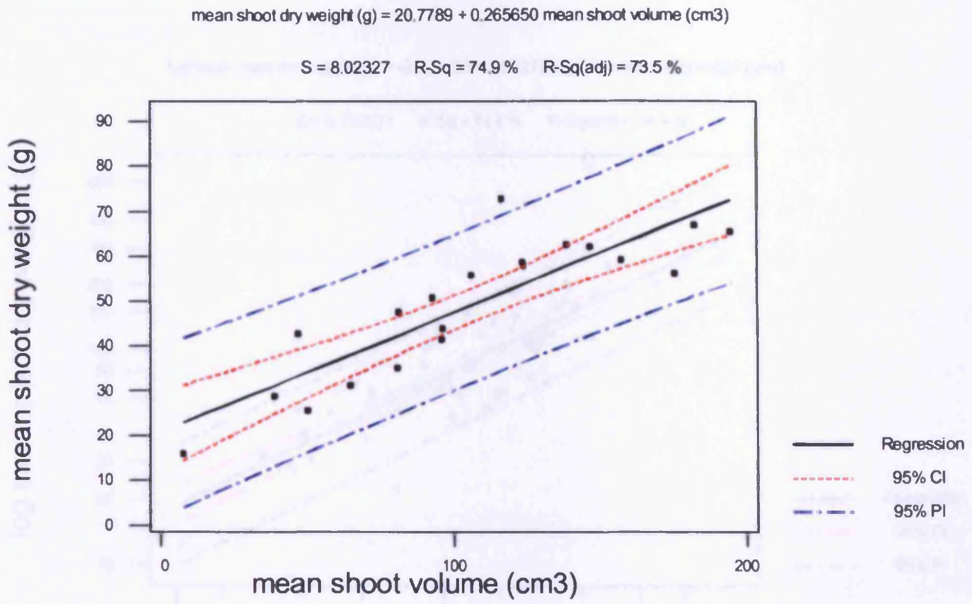


Figure 3.16: Fitted line plot for regression analysis of mean *Arundo* shoot volume and mean shoot dry weight for all dates at both sites

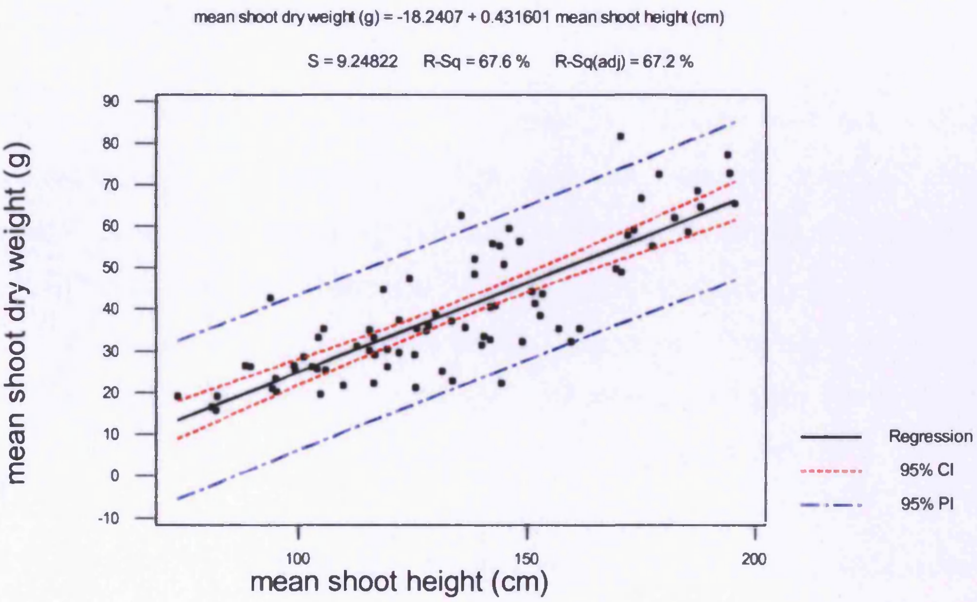


Figure 3.17: Fitted line plot for regression analysis of mean *Arundo* shoot height and mean shoot dry weight for all dates at both sites

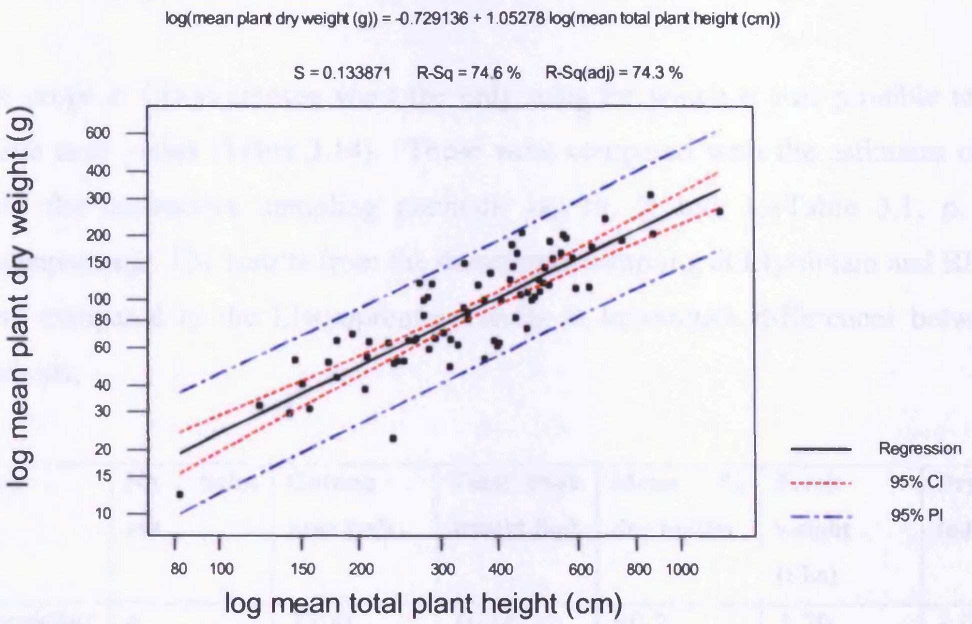


Figure 3.18: Fitted line plot for regression analysis of log data for mean total *Arundo* plant height and mean plant dry weight for all dates at both sites

3.3.3 Destructive sampling

3.3.3.1 Llwynprenteg whole crop results

The crops at Llwynprenteg were the only ones for which it was possible to obtain whole crop yields (Table 3.14). These were compared with the estimates obtained from the destructive sampling methods 1a, 1b, 2 and 3 (Table 3.1, p. 31) at Llwynprenteg. The results from the destructive sampling at Llysdinam and Bluestone were compared to the Llwynprenteg results to investigate differences between the methods.

Crop	No bales cut	Cutting area (m^2)	Total fresh weight (kg)	Mean % dry matter	Fresh weight (t/ha)	Dry weight (odt/ha)
Miscanthus	6	2700	1024	80.2	3.79	3.04
Arundo	1	254.6	57.5	48.2	2.26	1.09
Phalaris	9	3752	1819	72.5	4.85	3.51

Table 3.14: Whole crop harvested yields obtained from Llwynprenteg (A. Clarke, ADAS).

3.3.3.2 *Miscanthus* results

For practical reasons, the destructive sampling methods were not trialled at the *Miscanthus* crop at Bluestone. The estimates obtained from all *Miscanthus* destructive sampling methods at Llwynprenteg were over double the actual crop yield (3.0 odt/ha) for both November and February sampling dates (Figure 3.19). Estimates based on the November figures were slightly more accurate using mean dry weight per plant x plants per sub-plot (method 2), whereas in February it was slightly more accurate to estimate yields based on the mean dry weight of a shoot.

The yield estimates obtained from the destructive sampling of the *Miscanthus* crop at Llysdinam (Figure 3.20) showed little variation between methods. Overall the estimates obtained from this crop were lower than the *Miscanthus* at Llwynprenteg. Estimates were higher in November than in February using all methods, at both sites (Figures 3.19 and 3.20).

3.3.3.3 *Arundo* results

Destructive sampling was not carried out on the Llysdinam *Arundo* crop due to poor growth. All *Arundo* yield estimates at Llwynprenteg (Figure 3.21) were over double the actual crop yield (1.1 odt/ha). In both November and February slightly more accurate estimates were achieved from sub-sampling a $2m^2$ area of the crop (method 3).

The yield estimates obtained from the *Arundo* at Bluestone (Figure 3.22) were higher than those obtained at Llwynprenteg, although the methods showed a similar pattern of results. Highest estimates were obtained from methods 1a and 1b, thus making these the least accurate methodologies. All February estimates were higher than their corresponding November estimates at each site, with one exception at Bluestone (Figures 3.21 and 3.22).

3.3.3.4 *Phalaris* results

For the data at Llwynprenteg, *Phalaris* estimates for both November and February were under-estimates of the actual crop yield (3.5 odt/ha). The estimates obtained at Llwynprenteg were higher during November than February, whereas at Llysdinam estimates were higher in February than in November (Figure 3.23).

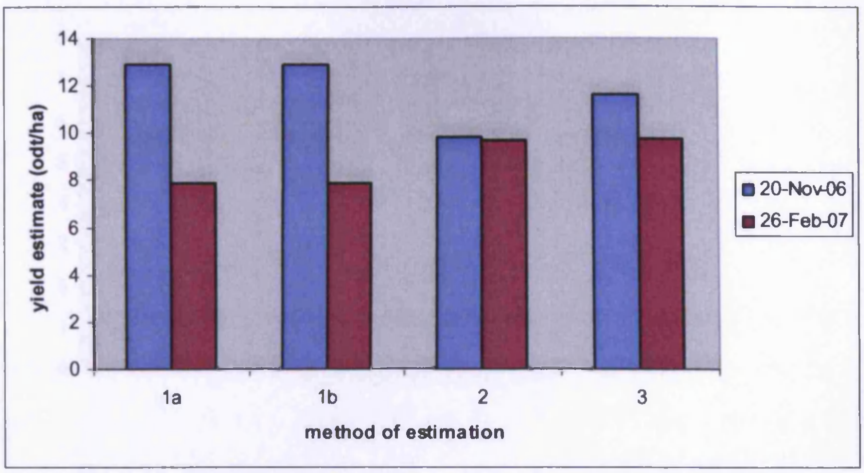


Figure 3.19: Comparison of destructive sampling methods for estimating *Miscanthus* yields at Llwynprenteg (see Table 3.1, p. 31, for key to methods).

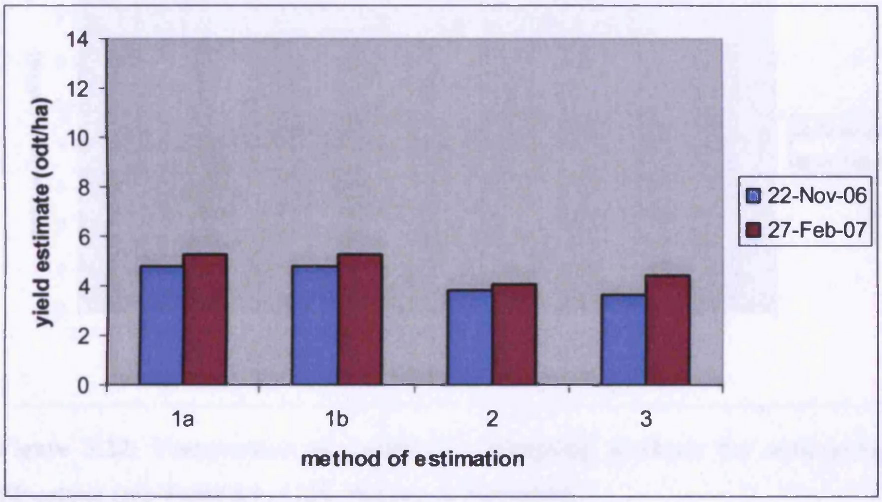


Figure 3.20: Comparison of destructive sampling methods for estimating *Miscanthus* yields at Llysdinam (see Table 3.1, p. 31, for key to methods).

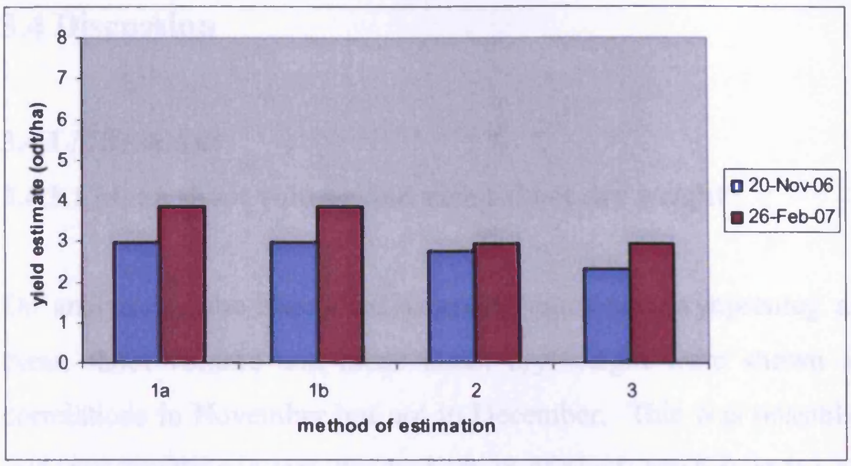


Figure 3.21: Comparison of destructive sampling methods for estimating *Arundo* yields at Llwynprenteg (see Table 3.1, p. 31, for key to methods).

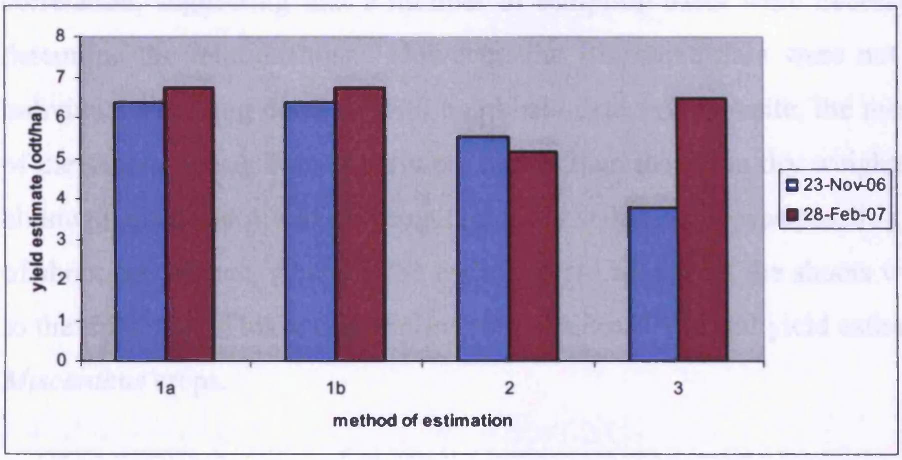


Figure 3.22: Comparison of destructive sampling methods for estimating *Arundo* yields at Bluestone (see Table 3.1, p. 31, for key to methods).

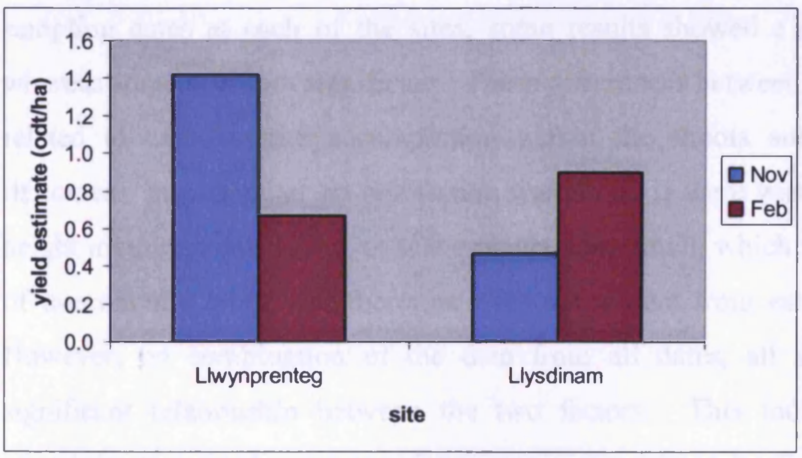


Figure 3.23: Yield estimates obtained from destructive sampling of *Phalaris* crops at Llwynprenteg and Llysdinam, using method 3 (see Table 3.1, p. 31, for key to methods).

3.4 Discussion

3.4.1 *Miscanthus*

3.4.1.1 Mean shoot volume and mean shoot dry weight

On analysis of the individual sampling dates at Llwynprenteg and Llysdinam, the mean shoot volume and mean shoot dry weight were shown to have significant correlations in November but not in December. This was possibly a consequence of leaf loss, resulting in less standardisation of plant structure as the plants gradually lost their leaves, and the possibility of shrinkage of shoot width during senescence. The combined November and December data for these sites showed significant correlation, suggesting that a number of sampling dates were necessary in order to determine the relationships. However, the Bluestone data were not significant on individual sampling dates or with combined data. At this site, the mean dry weights of the shoots during November were higher than the mean dry weights in December, although mean shoot volume remained fairly stable. This was possibly a consequence of shoot senescence, whereby the carbohydrate content of the shoots was translocated to the rhizomes. This was therefore not considered a useful yield estimate method for *Miscanthus* crops.

3.4.1.2 Mean shoot height and mean shoot dry weight

On analysis of the mean shoot height and mean shoot dry weight from individual sampling dates at each of the sites, some results showed a significant correlation, whereas some were not significant. These differences between dates might have been related to carbohydrate accumulation within the shoots and translocation to the rhizomes. In particular, no correlation was found if there was a very small range of height measurements taken, or if the plants were small, which was probably a feature of uneconomic crops and therefore may not detract from estimates of good crops. However, on combination of the data from all dates, all sites showed a highly significant relationship between the two factors. This indicates that it may be necessary to record a range of measurements over a period of time to produce accurate correlations.

The results from Bluestone showed a cluster of measurements at a height of approximately 210-230cm and 40-45g dry weight. Llwynprenteg measurements clustered at approximately 245-270cm in height and 40-45g dry weight. Therefore the crop at Llwynprenteg achieved a greater mean shoot height at a similar dry weight to Bluestone. This would suggest that shoot width was important, although the mean shoot volume correlations did not suggest a relationship at Bluestone. It is therefore possible that the shoots at Bluestone contained thicker stem walls than at Llwynprenteg. This may be a symptom of age, as the Bluestone crop was one year older than the Llwynprenteg crop.

The Llysdinam results clearly showed a less successful crop than at the other sites, with the majority of measurements falling between 130 and 165cm mean shoot height at 25-35g dry weight. Analysis of covariance showed no differences between the three sites, but that the less successful crop at Llysdinam simply displayed a lower range of measurements than the more successful crops at Llwynprenteg and Bluestone. This was therefore a good method of estimating shoot yield at all sites, and regression of all data produced a highly significant result.

3.4.1.3 Total plant height and plant dry weight

All individual sampling dates at Llysdinam and Bluestone, and all except one sampling date at Llwynprenteg, showed a significant correlation between the total plant height (mean shoot height multiplied by the number of shoots) and plant dry weight. All sites showed a highly significant relationship on combination of data from all dates. This indicates that only one sampling date during this period was necessary to determine the relationship between the two factors, providing a range of values were recorded on that occasion.

Again, the analysis of covariance showed there to be no differences between the sites, and that the range of values from each site reflected the success of that site's crop. This method has therefore been shown to be a highly effective method of estimating plant dry weight at individual dates throughout the season, which produced a highly significant relationship on combination of data from all sites.

3.4.1.4 *Miscanthus* summary

The most successful non-destructive sampling method for the *Miscanthus* crops studies were therefore; the measurement of mean shoot height to calculate estimated shoot dry weight, or; the measurement of mean total plant height to calculate estimated plant dry weight. The destructive sampling methods studied revealed that a yield estimate based on plant dry weight was more accurate than one based on shoot weight. This was most probably because, at the sites in this study, it proved fairly easy to determine individual plants. At more dense or irregularly planted sites, however, it can be difficult to distinguish individual plants. At these sites it may be more effective to calculate yield estimates from shoot measurements.

3.4.2 *Arundo*

3.4.2.1 Mean shoot volume and mean shoot dry weight

On analysis of the individual sampling dates at Llwynprenteg and Bluestone, the mean shoot volume and mean shoot dry weight were shown to have significant correlation at both dates at Llwynprenteg but only in November at Bluestone. The December dataset contained a cluster of heavy shoots and one light shoot. This may have confounded the correlation analysis, which is more suited to a range of values. The combined November and December data for these sites showed a highly significant relationship, suggesting there was indeed a useful relationship between the two. This was substantiated by the highly significant relationship between the data from both sites combined.

3.4.2.2 Mean shoot height and mean shoot dry weight

On analysis of the mean shoot height and mean shoot dry weight from individual sampling dates at each of the sites, half of the dates at each site showed a significant correlation. These occurred during December and January at both sites. This would therefore be the optimum time period to obtain height measurements for yield estimate purposes, which may be caused by less variation in shoot widths during this period. This requires further investigation.

On combination of the data from all dates, both sites showed a highly significant relationship between the two factors. This indicates that it may be necessary to record a range of measurements over a period of time to produce accurate correlations, or that measurements should only be made during the optimal time period of December to January. Analysis of covariance showed there to be no significant differences between the two sites, and regression analysis of all results combined showed a highly significant relationship. Spencer *et al.* (2006) showed a significant relationship between *Arundo* shoot dry weight and height, but suggested that, in contrast to this study, this was not well-described by a straight line. Increased significance was shown between shoot dry weight and height^2. Cosentino *et al.* (2006) reported a significant correlation between the shoot height and dry weight in the first year of growth but not in the second, but did show significant correlation between shoot height and whole crop dry weight in both years.

3.4.2.3 Total plant height and plant dry weight

On examination of the relationship between total plant height (sum of all shoot heights) and plant dry weight, a significant correlation was shown on one occasion only at Llwynprenteg. At Bluestone, 5 out of 8 correlations were significant. This result differed from the *Miscanthus* results, which was probably due to the increased variation in shoot widths displayed by *Arundo* crops. It is therefore possible that, unlike *Miscanthus*, sampling of *Arundo* crops necessitates shoot width measurements in addition to height measurements in order to ascertain accurate estimates of plant yield. The significant Bluestone correlations occurred with data from December and January which supports the idea that *Arundo* shoots exhibited less width variation during this time period. This may be related to physiological activity during the winter period, and storage of carbohydrates in the stems to enable continued growth. Personal observations have suggested that *Arundo* does not lose its green-ness during this period and therefore the above-ground section of the plant was not yet senescing.

Both sites showed a highly significant relationship on combination of data from all dates, and on combination of all data from both sites. Therefore if the crop is sampled successively over a period of time, it was possible to estimate plant dry weight from height measurements alone.

3.4.2.4 *Arundo* summary

All non-destructive sampling methods produced a highly significant relationship between on-site measurements and crop dry weight. The most consistent results were shown by measurement of mean shoot height during December and January, although the highest R^2 regression statistic was obtained from calculation of the shoot volume in November and December. It has therefore been suggested that shoot width is important to yield estimates of *Arundo* crops, although this measurement has less impact during December and January, probably due to standardisation of the shoot width during this period.

The differences between the destructive sampling methods of *Arundo* crops in this study were smaller than those obtained from the *Miscanthus* crops. This was because the *Arundo* crops were not fully developed and all plants contained fewer than ten shoots so were not encroaching upon each other's space. This enabled easy identification of individual plants. Future research of well established older crops is necessary to determine if and how this situation changes.

3.4.3 Destructive sampling

Miscanthus yield estimates obtained from February data were more accurate than those obtained from November data. This was to be expected, as yield losses occurred during the period from November to February due to leaf loss and stem breakage, which resulted in non-harvestable litter remaining in the field. Therefore November estimates of *Miscanthus* will always be over-estimates of the actual yield obtained during the following spring.

The yield estimates for the *Arundo* crops in this study increased between November and February. This suggests that the crop was still growing so yield losses did not occur over the winter period. As stated previously, *Arundo* crops in this trial did not lose their green-ness during the winter period (personal observations).

All *Miscanthus* and *Arundo* yield estimates were more than double the actual yield obtained. This is common when sub-sampling field scale trials (A. Clarke, pers. comm.). Indeed, Riche (2005) provided both estimated yields and final plot yields and found that, for *Phalaris* and *Miscanthus*, estimated yields were approximately double the actual plot yield. During sub-sampling of crops it is possible to cut each stem at ground level and to accurately collect all stems from a particular area or plant. Mechanical harvesting results in losses due to failure to collect all shoots from the site and failure to cut shoots at ground level. Studies have shown that 20% of alfalfa crops and up to 40% of sweet sorghum crops were lost during the harvesting process and that 5.6% of the total switchgrass crop was lost during baling (Sanderson *et al.*, 1997). Kahle *et al.* (2001) showed that between 23 and 51% of the total *Miscanthus* crop was not harvested due to pre-harvest losses (leaf loss and stem breakage) and harvested residues. It is also likely that small errors in measuring sub-plots will be greatly exaggerated when the result is scaled up to t/ha, and that small sub-plots will not reflect the variation in crop density and patchiness displayed in the whole crop. Also, if the crops have been allowed to dry further before actual crop harvesting, as was the case with the Llwynprenteg crops, then more losses would have occurred subsequent to February sampling due to stem breakage, and further breakage would occur during the mechanical harvesting process.

In contrast to *Arundo* and *Miscanthus*, mechanical harvesting is likely to collect more of the *Phalaris* crop than sub-sampling a small area. This is because *Phalaris* crops fall over within the field during late summer, which creates huge difficulty in determining which stems to include within a small harvestable quadrat. Losses from sub-samples are likely to occur as many fallen stems lay outside the harvestable quadrat. Lodging of *Phalaris* crops also causes problems during mechanical harvesting of the crop. Therefore this crop may be more suited to an earlier summer harvest, or a two-cut system involving an early and late harvest to avoid lodging problems.

3.5 Conclusions

This study showed that yield estimates of *Miscanthus* and *Arundo* crops were obtained from non-destructive measurements within the field, but that the results greatly over-estimated the actual crop yield. Crop losses occur at harvest mainly due to mechanical harvesting inadequacies. Further research is needed to quantify these losses, which could lead to suggestions on how to minimise them. For research trial plots, it would be useful to standardise yield estimate methodology to enable comparisons between studies. The results from this trial indicate that total plant height is the most useful on-site measurement of *Miscanthus* crops, and that shoot volume during November is the most accurate measurement for *Arundo* crops. Further research is needed to document the changes and differences in *Arundo* shoot widths, and how this reflects its growth and physiology throughout the season. *Phalaris* has proven most difficult to determine yield estimate methodology and further research on this crop is needed to determine how to avoid the problems encountered during this trial.

3.6 References

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Chapter 4

Site Conditions in Wales and Their Influence on the Success of *Miscanthus* x *giganteus* and *Arundo donax* Crops.

4.1 Introduction

Miscanthus originates from South-east Asia and has been widely introduced to Europe, originally as an ornamental species and more recently as an energy crop. *Arundo donax* naturally grows wild in the Mediterranean region, in addition to China and southern USA (El Bassam, 1998). It has been introduced to some non-native European countries for energy crop purposes, for example the UK and Germany. In this study, both species have been grown side by side at sites in Wales, to determine their suitability as energy crops within this region. The sites have been compared in terms of soil and climatic conditions, in an attempt to determine the factors that have an important influence on crop success.

Soil types most suitable for *Miscanthus* growth include sands and sandy loams with up to 10% clay content, and clay content greater than 25% is considered unsuitable (El Bassam, 1998). Establishment is successful on sandy and stony soils with sufficient rainfall. Optimum soil pH is considered to be 5.5-7.5 (El Bassam, 1998). There are no data available for optimum soil pH of *Arundo*. *Arundo* is usually found growing wild on fertile, moist soils along river banks, but has also successfully established on dry, infertile soils along fields or roadsides (El Bassam, 1998). Soil types suitable for *Arundo* growth vary from heavy clays to loose sands to gravelly soil (Christou *et al.*, 2001).

Many studies have investigated the effect of temperature and precipitation or irrigation on *Miscanthus* yield. Some studies have discovered rainfall to be the most important factor to affect yield, including Clifton-Brown *et al.* (2001) who suggested a requirement of 700mm y^{-1} for maximum yields in Germany, whereas Long & Beale

(2001) concluded a minimum of 500mm precipitation during the growing season is required in eastern England, and an Austrian study recorded the site receiving most rainfall (844 mm y^{-1}) to consistently produce higher yields than those receiving less (Christian & Haase, 2001).

Arundo studies agree that irrigation can be important to *Arundo* growth, although irrigation rates are inconclusive. El Bassam (1998) reports no significant differences between the yields obtained from 300 mm y^{-1} and 700 mm y^{-1} irrigated crops. Studies in Greece concluded that irrigated plants produced taller, thicker shoots with more leaves than non-irrigated plants (Christou *et al.*, 2001). Similarly, Varnvuka *et al.* (2007) showed irrigation to increase *Arundo* yield, and also that only irrigated crops produced flowers.

Site temperature affects the length of the crops' growing seasons, and their growth potential with regard to height, number of shoots produced and subsequent yield produced. *Miscanthus* studies have used several methods to define the length of the growing season. There is evidence that the crop may start to grow when air temperatures reach 10°C (Lewandowski & Heinz, 2003), or alternatively when soil temperatures reach 10°C (El Bassam & Huisman, 2001). However, the majority of studies regard the start of the growing season to be the day after the last air frost (Clifton-Brown *et al.*, 2000; Christian & Haase, 2001; Nixon & Bullard, 2001; Heaton *et al.*, 2004; Price *et al.*, 2004). All of the aforementioned studies regard the season to end on the last air frost-free day; although El Bassam & Huisman (2001) suggest that this differs for crops grown in southern Europe, which cease growing at the time of flowering in August or September. The productivity model MISCANMOD defines the end of the season to be either the time of flowering or when air temperatures fall below 10°C, whichever occurs sooner (Clifton-Brown *et al.*, 2004). Our study implied that, in Wales, many plants produce flowers during the late autumn/early winter period. The precise timing of flower emergence was not recorded, but generally it occurred after summer during September at the sites in this study (personal observations). Once the crop has successfully started growth, multiple shoots have been shown to emerge once accumulated daytime temperatures exceed 200-400°C (Bullard & Nixon, 1999).

Similar studies to determine the growing season or temperature requirements of *Arundo* have not been reported in the literature, although one study has shown that new shoots are produced from *Arundo* rhizomes at soil temperatures between 9-14°C (Spencer & Ksander, 2006).

There are several existing models of *Miscanthus* productivity which are based on different parameters, including geographic location, temperature and rainfall, and aim to predict *Miscanthus* productivity in Europe (Clifton-Brown *et al.*, 2004; Tuck *et al.*, 2006), Britain (Price *et al.*, 2004), England (Defra, 2007) and Ireland (Clifton-Brown *et al.*, 2000). No models to date have included data from sites in Wales, or have included side-by-side comparisons of *Miscanthus* and *Arundo* crops.

This study was not an additional attempt to map European success of energy crops, but was a small scale study investigating the success of *Miscanthus* and *Arundo* crops in Wales. The aim of the study was to determine which site factors and climatic factors are important to the success of the two crops in a Welsh setting.

4.2 Methods

4.2.1 Sites

The *Miscanthus* and *Arundo* crops at Llysdinam, Llwynprenteg, Cae Clovers and Bluestone were used for this study. General site details are listed in Table 4.1 (see Chapter 2 for detailed site information).

Site	Llysdinam	Llwynprenteg	Cae Clovers	Bluestone 1	Bluestone 2
Grid reference	SO 005 581	SN 687 716	SN 776 744	SN 063 129	SN 032 149
Altitude (m)	190	90	280	75	60
Soil type	Cambic stagnogley	Brown earth	Brown podzolic	Brown earth	Brown earth
Soil description	Loamy, clayey, seasonally waterlogged	Brown, stony, well-drained	Fine, loamy, free-draining	Reddish, fine, loamy	Brown, stony, well-drained

Table 4.1: Site details for all sites (soil type and description from Rudeforth *et al.*, 1984).

4.2.2 Soil sampling

Soil was collected from each site on one occasion between January and March of each year. The area beneath each crop was paced along a W-shaped path and a total of eight soil cores were collected at random intervals using a soil corer. The samples were pooled and mixed, from which a single 500g sample was removed and sent to Eurofins laboratories, Wolverhampton for analysis of pH, phosphorus, potassium, magnesium and total nitrogen content.

4.2.3 Climate data

Weather data from the Met Office MIDAS weather station nearest to each site holding daily temperature and precipitation records were obtained from the British Atmospheric Data Centre. Details of each weather station are provided in Table 4.2.

Site	Llysdinam	Llwynprenteg	Cae Clovers	Bluestone 1	Bluestone 2
Station name	Llysdinam	Trawsgoed	Cwmystwyth	Scolton Country Park	Scolton Country Park
Grid reference	SO 009584	SN 673735	SN 773749	SM 989219	SM 989219
Altitude (m)	196	63	301	75	75

Table 4.2: Details of weather station with appropriate records nearest to each site.

The minimum air temperatures for each weather station dataset were searched for the dates of the beginning and end of the mid year frost-free period, which was determined as the day after the last Spring air temperature below 0°C until the day before the first Autumn air temperature below 0°C. This period was deemed to be the growing season for *Miscanthus*. The daily maximum air temperatures within this period were accumulated and deemed cumulative maximum air temperature. Daily precipitation was totalled for each month. Cumulative rainfall within the *Miscanthus* growing season was also determined. For investigation of the *Arundo* yields it was not possible to determine growing season, as there are no studies in the literature to report the specific climatic factors that affect the timing of the season. At Llwynprenteg and Bluestone, *Arundo* did not senesce during the winter period, and therefore is likely to continue growth through a prolonged period (personal observations). Therefore the daily maximum air temperatures were averaged to produce figures for mean monthly temperature.

4.2.4 Crop sampling

4.2.4.1 Survival

During years 1 and 2, crop survival was calculated by counting the number of plants present along 30 random rows. This was expressed as a percentage of the mean number of plants planted along each row. In year 3 crop survival was calculated by counting the number of plants present within three replicate $2m^2$ areas, and the mean value was expressed as a percentage of the mean number of plants originally planted within an area of this size. These values give an estimate of crop survival but the methodology is very inaccurate, as there was variation between the sites in terms of how the crops were planted and size of plot. There were also unavoidable differences between the rhizomes planted at each site, in terms of size of rhizome, number of buds per rhizome, length of time from rhizome harvest to planting and storage of rhizomes prior to planting.

4.2.4.2 Shoot count

The number of shoots per plant was counted for 30 random plants, excluding plants at the crop edge.

4.2.4.3 Shoot dry weight

A sub-sample of 30 random shoots, excluding plants at the crop edge, were chopped at the stem base within the field and returned to the laboratory. Fresh weight of each shoot was recorded immediately upon return to the research centre, and all shoots were individually oven dried at 80°C to constant weight, and this dry weight was recorded. All sites were sampled within the same week on one occasion during the winter period each year.

4.2.4.4 Plant dry weight

All shoots from five random plants were cut at the base and removed from the site in 2006-2007 and 2007-2008 seasons, excluding plants from the crop edge. Fresh weight of each plant was recorded, and five random shoots from each plant were weighed fresh then oven dried at 80°C to constant weight, and this dry weight was recorded. The mean percentage dry weight of all shoots was determined, in order to calculate the dry weight of each plant. All sites were sampled once during the last week of November or first week of December in each year.

4.2.5 Statistical analysis

Analysis of variance (ANOVA) was performed on the collected data for each year to investigate differences between sites. Data were transformed as necessary to meet the assumptions of the test, and Kruskal-Wallis tests were used for data which did not meet the assumptions of ANOVA after transformation. Significant ANOVA results were further tested with pair-wise Tukey-Kramer tests. Significant results obtained from the non-parametric Kruskal-Wallis test were further tested for pair-wise differences using Mann-Whitney tests. For all pair-wise comparisons the significance level was corrected using the Bonferroni method.

4.3 Results

4.3.1 Soil samples

The texture, soil composition and overall soil classification for each site are shown in Table 4.3. "General purpose grade" classification, as defined by Eurofins laboratories' specifications, includes natural topsoil and premium grade topsoil that has deteriorated and is suitable for general good quality agriculture. "Economy grade" classification is divided into 'low clay' and 'high clay' sub-grades, and is suitable for low production agricultural land.

	Llysdinam		**Llwynprenteg**		**Cae Clovers**	**Bluestone**	
	Miscanthus	***Arundo***	***Miscanthus***	***Arundo***	***Miscanthus***	***Miscanthus***	***Arundo***
Texture	Clay	Silty clay	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam
	Soil composition (%)						
Coarse sand	5	3	14	16	20	15	12
Medium sand	5	3	6	5	4	11	10
Fine sand	6	4	5	4	6	15	7
Coarse silt	12	12	7	8	7	17	17
Silt	30	38	37	36	37	24	27
Clay	42	40	31	31	26	18	27
Classification	Economy: high clay	Economy: high clay	General purpose	General purpose	General purpose	Economy: low clay	Economy: low clay

Table 4.3: Soil analysis results at each site.

4.3.2 Crop Survival

The percentage survival of both *Miscanthus* and *Arundo* at each site are displayed in Table 4.4. The different values obtained between years, for example, Llysdinam *Miscanthus* in years 1, 2 and 3, are likely to reflect inaccuracies in the method rather than actual fluctuations in crop success between these years. However the data appear to show an overall lower survival rate of *Miscanthus* at Bluestone in comparison to all other sites. *Arundo* showed an overall increased survival at Bluestone, in comparison to both Llysdinam and Llwynprenteg, the latter of which showed a decrease in survival between years 1 and 2, suggesting that plants did not survive the first winter.

	Survival (%)				
	Miscanthus			***Arundo***	
year	**1**	**2**	**3**	**1**	**2**
Llysdinam	74.4	57.2	77.1	56.7	57.1
Llwynprenteg		71	85.4	92.3	64.6
Bluestone		55	53.4	86.9	85.4
Cae Clovers		78.4			

Table 4.4: Mean percentage survival at all sites. Data were not collected for all years at all sites.

4.3.3 Crop dry weight

4.3.3.1 *Miscanthus*

As the *Miscanthus* crops were not all planted in the same year, different years' sampling is summarised in Table 4.5. Consequently there were not always the same data available for all sites. Where no replicate whole plant samples were collected, mean dry weight of a plant was determined as the multiple of mean dry weight per shoot and mean number of shoots per plant. For these calculations, no error bars are displayed on the chart (Figure 4.3).

Year of growth	**1**	**2**	**3**	**4**
Llysdinam	2004	2005	2006	2007
Llwynprenteg	2004	2005	2006	2007
Bluestone	2003	2004	2005	2006
Cae Clovers	2003	2004	2005	2006

Table 4.5: Calendar year relating to year of growth for *Miscanthus* crops at each site.

For first year *Miscanthus* growth, data were only available for Llysdinam and Llwynprenteg. Statistical analysis revealed the mean dry weight of a shoot at Llwynprenteg to be significantly greater than that at Llysdinam (P=0.000), but there was no difference between the number of shoots per plant (Figures 4.1 and 4.2). The calculated dry weight of a plant at Llwynprenteg (53.1g) was subsequently more than double that calculated from Llysdinam data (20g).

In the second year of *Miscanthus* growth, the crops at both Llwynprenteg and Bluestone achieved a greater mean dry weight per shoot than the crop at Cae Clovers (P=0.000). There were no significant differences between the Llysdinam crop and all other sites (Figure 4.1). The difference between the number of shoots per plant at each site was highly significant (P=0.000). There were less shoots per plant at Llysdinam in comparison to Llwynprenteg, Bluestone and Cae Clovers, and Bluestone plants also had more shoots than both Llwynprenteg and Cae Clovers (Figure 4.2). No replicate plant samples were removed for dry weight determination, but the calculated mean plant dry weight was four times greater at Bluestone (803.3g) than Llwynprenteg (201.3g), which was itself more than double that at Cae Clovers (80.6g). Cae Clovers (80.6g) and Llysdinam (74.5g) had similar calculated values for plant dry weight.

In the third year the increased *Miscanthus* growth at Bluestone and Llwynprenteg exceeded that at Llysdinam, and similarly Llysdinam growth exceeded Cae Clovers. Analysis of the shoot dry weight data revealed highly significant (P=0.000) differences between the groups. No significant differences were shown between the mean shoot dry weight at Bluestone and at Llwynprenteg. Bluestone shoots weighed significantly more than the *Miscanthus* crops at both Llysdinam (P=0.0014) and Cae Clovers (P=0.0016), as did Llwynprenteg shoots (P=0.000 for both), and Llysdinam shoot dry weight was significantly greater than at Cae Clovers (P=0.001) (Figure 4.1). Analysis of the number of shoots per plant showed no significant differences between the *Miscanthus* crops at Llwynprenteg, Bluestone or Llysdinam (Figure 4.2). There was no shoot count data available for the third year at Cae Clovers. The calculated mean plant dry weight at Bluestone (1007.4g) was over two and a half times the mean plant dry weight at Llwynprenteg and over four and a half times greater than that at Llysdinam. The latter two sites had replicate data for plant samples, and analysis of these data revealed no significant differences between the two sites (Figure 4.3).

During the fourth year of growth, the *Miscanthus* crop at Cae Clovers increased in shoot dry weight to achieve similar results to Llysdinam. Subsequently it was no longer significantly lower than the crop at Bluestone, but remained significantly lower than Llwynprenteg (P=0.000). The Llysdinam crop was lower than both Bluestone and Llwynprenteg (P=0.000) (Figure 4.1). Bluestone contained a significantly greater number of shoots per plant than both Llwynprenteg and Cae Clovers (P=0.001), but not Llysdinam (Figure 4.2). Replicate plant samples were collected from Llysdinam, Llwynprenteg and Bluestone for fourth year *Miscanthus* growth, and analysis of these data showed Bluestone to be significantly greater than Llysdinam (P=0.012) only (Figure 4.3). The calculated mean plant dry weight at Cae Clovers (489.8g) was almost identical to the mean plant weight at Llysdinam (488.2g).

Lines of best fit for the mean plant dry weight of each year were plotted for each site (Figure 4.4). The crops at Cae Clovers, Llysdinam and Llwynprenteg all showed a very poor growth in year 1, which resulted in the line of best fit originating at zero in year 1. The slope of the Llwynprenteg line then rose more steeply than the other two sites, at a similar rate of increase to the crop at Bluestone. Bluestone produced a much larger crop mostly because of its success during the first year which resulted in the line of best fit originating at over 300g in year 1.

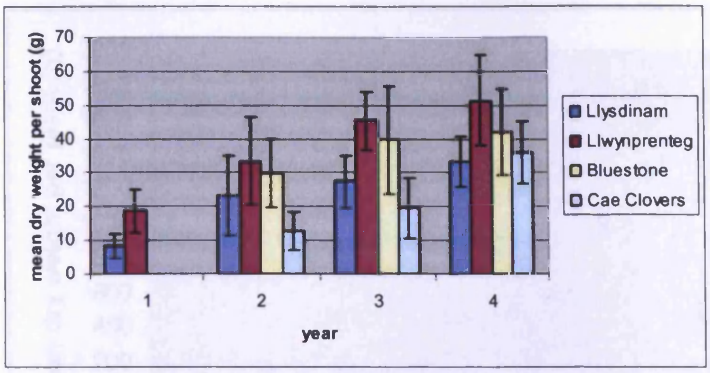

Figure 4.1: Mean ± 1 standard deviation shoot dry weight (g) for *Miscanthus* crops at all sites in years 1, 2, 3 and 4. See text for significant differences.

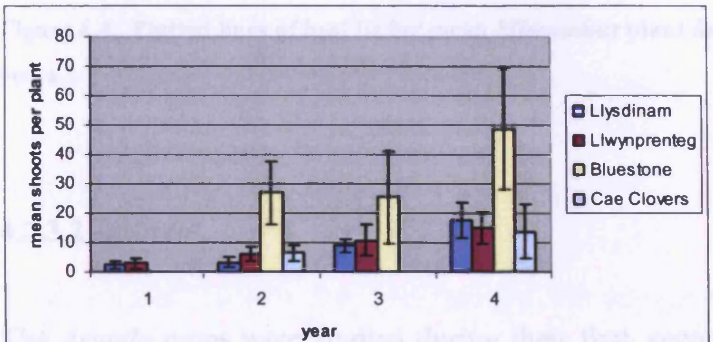

Figure 4.2: Mean ± 1 standard deviation number of shoots per plant for *Miscanthus* crops at all sites in years 1, 2, 3 and 4. See text for significant differences.

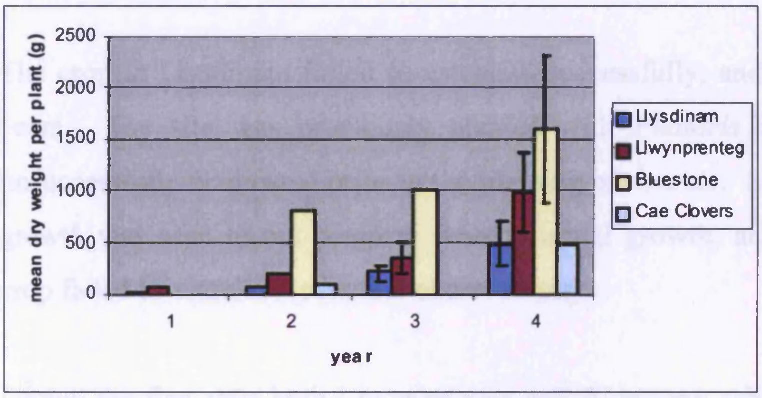

Figure 4.3: Mean ± 1 standard deviation plant dry weight (g) for *Miscanthus* crops at all sites in years 1, 2, 3 and 4. Blocks with no error bars are calculated mean plant dry weight, which is the multiple of mean shoot dry weight and mean number of shoots per plant. See text for significant differences.

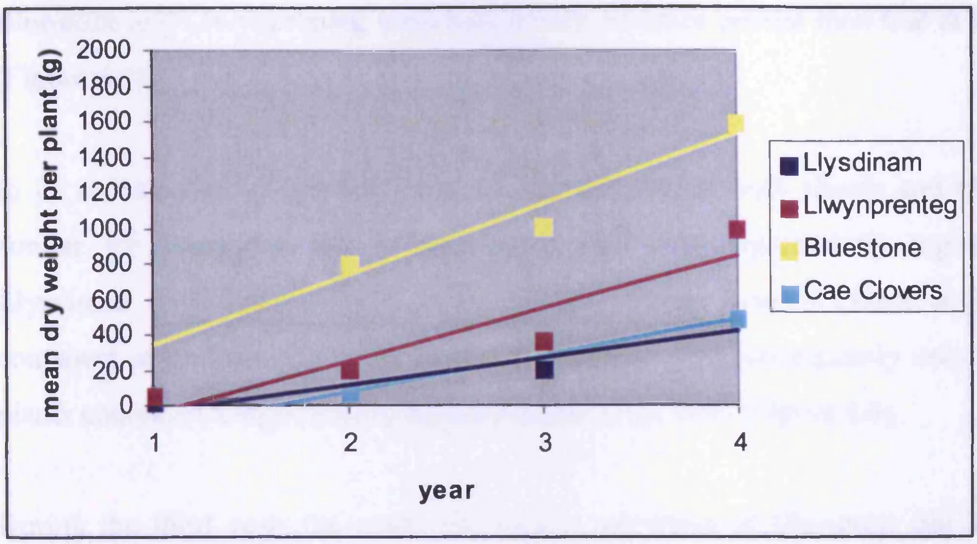

Figure 4.4: Plotted lines of best fit for mean *Miscanthus* plant dry weight (g) at all sites over the 4 years.

4.3.3.2 *Arundo*

The *Arundo* crops were studied during their first, second and third years of growth. All crops were planted during 2005, and subsequently the same data were collected from each site during each year. *Arundo* was planted at Llysdinam, Llwynprenteg and Bluestone only.

The crop at Llysdinam failed to establish successfully, and growth was poor for all years. The site was previously planted with *Phalaris arundinacea*, which was unsuccessfully eradicated prior to the planting of *Arundo*. Subsequently, *Phalaris* re-growth was seen to out-compete *Arundo* initial growth, and consequently the latter crop failed to establish (personal observations).

During the first year both Llwynprenteg and Bluestone achieved a greater shoot dry weight than Llysdinam (P=0.000), although there were no significant differences between the former two sites (Figure 4.5). The *Arundo* crop at Llysdinam had no more than one shoot per plant for all plants. The Llwynprenteg and Bluestone crops both had more than one shoot per plant, but there were no significant differences between the two sites (Figure 4.6). The calculated mean dry weight per plant at

Bluestone and Llwynprenteg were both nearly 30 times greater than that at Llysdinam (Figure 4.7).

In its second year of growth the mean dry weights of both shoots and plants were similar for Bluestone and Llwynprenteg, and were significantly higher than at Llysdinam (P=0.000) (Figure 4.5 and 4.7). Some *Arundo* plants at Llysdinam contained more than one shoot in the second year, and subsequently only Bluestone plants contained a significantly higher number (P=0.009) (Figure 4.6).

During the third year, the mean dry weight per shoot at Bluestone did not exceed Llwynprenteg, and there was no significant difference between the two. *Arundo* crops at both sites had significantly greater shoot weights than at Llysdinam (P=0.000) (Figure 4.5). Statistical analysis revealed that Bluestone plants carried significantly more shoots than both Llwynprenteg and Llysdinam, and that Llwynprenteg plants carried significantly more shoots than Llysdinam plants (P=0.000; Figure 4.6). Bluestone and Llwynprenteg both had significantly greater dry weights per plant than Llysdinam (P=0.000), although there were no differences between the first two (Figure 4.7).

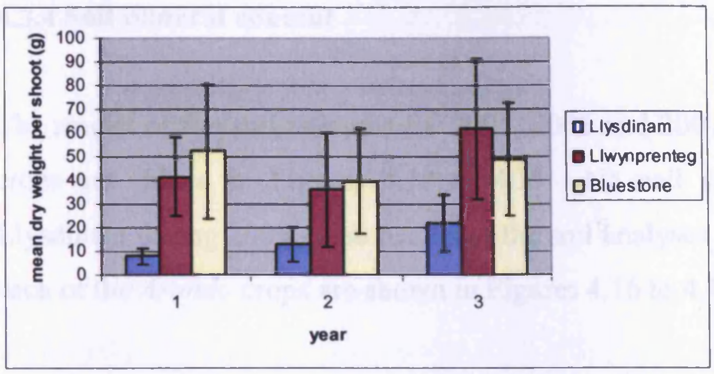

Figure 4.5: Mean ± 1 standard deviation shoot dry weight (g) for *Arundo* crops at all sites in years 1, 2 and 3. See text for significant differences.

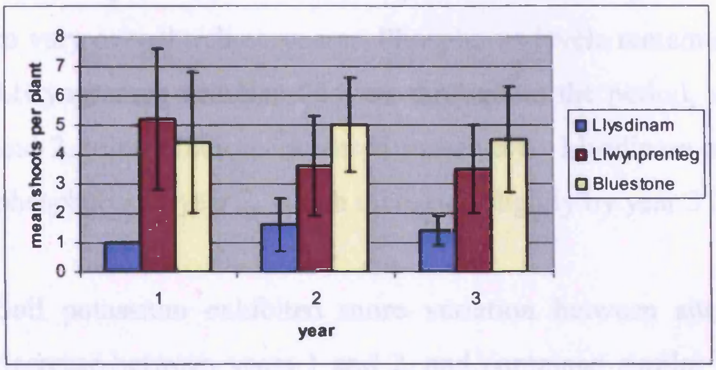

Figure 4.6: Mean ± 1 standard deviation number of shoots per plant for *Arundo* crops at all sites in years 1, 2 and 3. See text for significant differences.

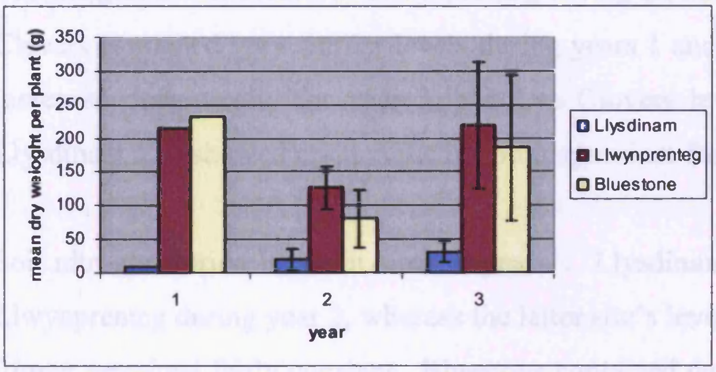

Figure 4.7: Mean ± 1 standard deviation plant dry weight (g) for *Arundo* crops at all sites in years 1, 2 and 3. Blocks with no error bars are calculated mean plant dry weight, which is the multiple of mean shoot dry weight and mean number of shoots per plant. See text for significant differences.

4.3.4 Soil mineral content

The results of the soil analyses for 2005, 2006 and 2007 for each of the *Miscanthus* crops are shown in Figures 4.12 to 4.15. No soil samples were obtained from Llysdinam during 2005. The results of the soil analyses for 2005, 2006 and 2007 for each of the *Arundo* crops are shown in Figures 4.16 to 4.19.

4.3.4.1 Annual fluctuations of *Miscanthus* soil mineral content

The phosphorus, potassium, magnesium and nitrogen content of the soil were shown to vary over the three years. Phosphorus levels remained between 10 and 20mg/l at Llwynprenteg and Cae Clovers throughout the period, and at Bluestone for years 1 and 2, after which it increased massively. Llysdinam soil contained a low level of phosphorus in year 2, which increased slightly by year 3 (Figure 4.10).

Soil potassium exhibited more variation between sites, although all exhibited a decrease between years 1 and 2, and contained similar levels in year 3. Llysdinam potassium levels were the same as Llwynprenteg levels in years 2 and 3 (Figure 4.11).

Soil magnesium increased at Llwynprenteg during all three years. Bluestone and Cae Clovers contained very similar levels during years 1 and 2, whereas Bluestone levels increased dramatically for year 3, and Cae Clovers levels increased very slightly. Llysdinam also showed an increase in soil magnesium from years 2 to 3 (Figure 4.12).

Soil nitrogen varied between sites in year 1. Llysdinam contained similar levels to Llwynprenteg during year 2, whereas the latter site's levels decreased slightly, and the former remained fairly constant. Bluestone contained decreased levels in years 1 and 2, but increased by year 3 to the same level as Llysdinam (Figure 4.13).

The pH of the soil was fairly consistent at all sites during years 1 and 2, whereas all sites showed a slight increase in pH by year 3. All sites remained slightly acidic throughout the period, with a pH of between 5 and 6.5 (Figure 4.8).

4.3.4.2 Annual fluctuations to *Arundo* soil mineral content

For practical reasons, the soil from the Bluestone 2 site was not sampled during year 1.

The soil phosphorus, potassium, magnesium and nitrogen under the *Arundo* crops varied considerably over the 3 years at all sites. Soil phosphorus fluctuated between 15 and 20 mg/l at Llwynprenteg and fluctuated around 10 mg/l at Llysdinam. At Bluestone it increased from 9 to 19 mg/l between years 2 and 3 (Figure 4.14).

Soil potassium increased between years 1, 2 and 3 at Llysdinam and years 2 and 3 at Bluestone to reach a similar level in year 3. At Llwynprenteg soil potassium was high in year 1, rapidly decreased in year 2 and then increased again to its original level in year 3 (Figure 4.15).

At Llwynprenteg soil magnesium fluctuated around 120 mg/l, whereby at Llysdinam it fluctuated around 100 mg/l over the 3 years. At Bluestone there was a steeper increase between years 2 and 3 to reach a lower year 3 level of 89 mg/l (Figure 4.16).

Soil nitrogen at all sites fluctuated between 0.3 and 0.5% (Figure 4.17).

The pH of the soil varied little over the years. All sites showed a slightly reduced pH in year 2 and a slightly increased pH in year 3. All sites over the three years were slightly acidic, with a pH of between 5.7 and 7 (Figure 4.9).

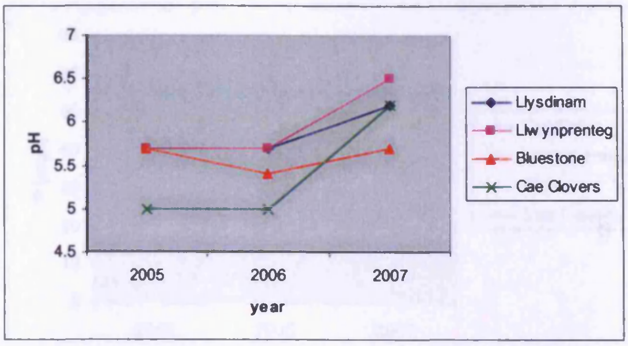

Figure 4.8: Annual soil pH at all *Miscanthus* sites.

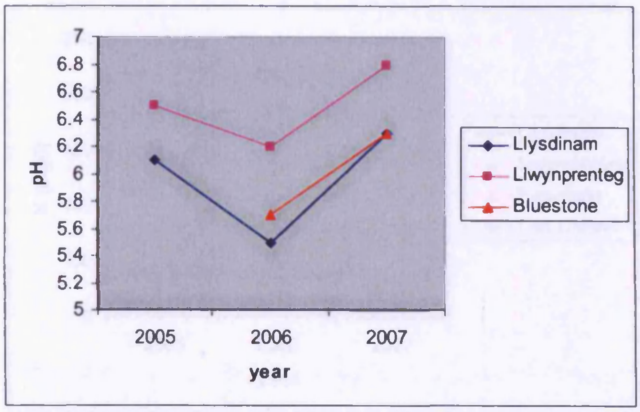

Figure 4.9: Annual soil pH at all *Arundo* sites.

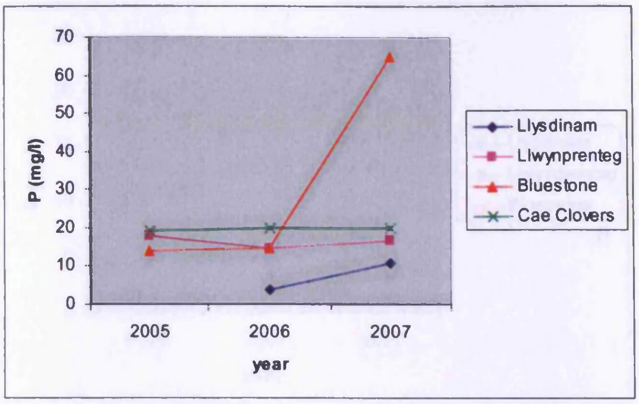

Figure 4.10: Annual soil phosphorus content at all *Miscanthus* sites.

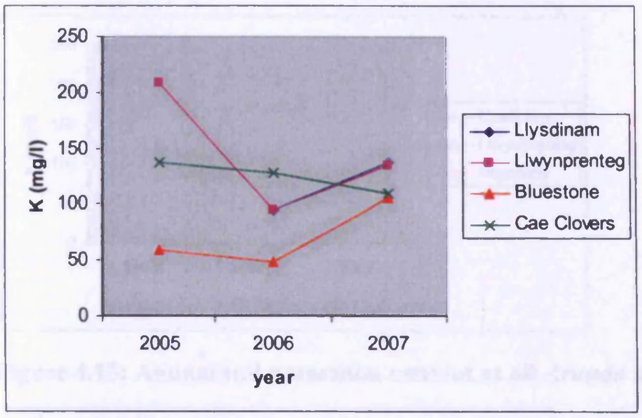

Figure 4.11: Annual soil potassium content at all *Miscanthus* sites.

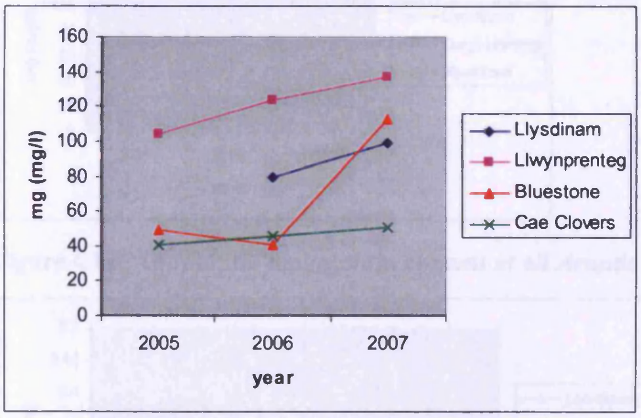

Figure 4.12: Annual soil magnesium content at all *Miscanthus* sites.

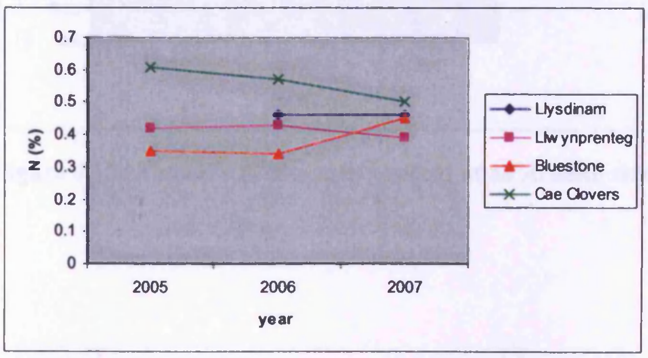

Figure 4.13: Annual soil nitrogen content at all *Miscanthus* sites.

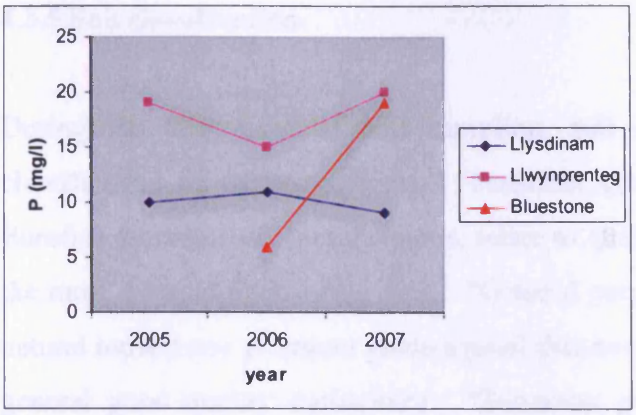

Figure 4.14: Annual soil phosphorus content at all *Arundo* sites.

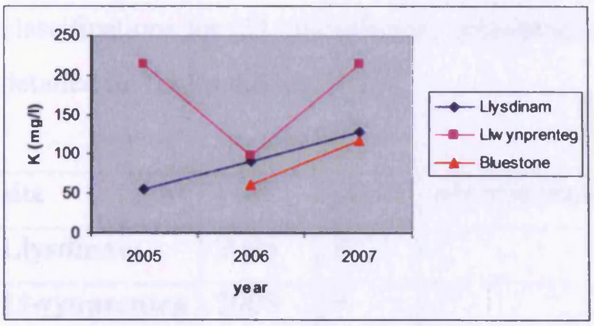

Figure 4.15: Annual soil potassium content at all *Arundo* sites.

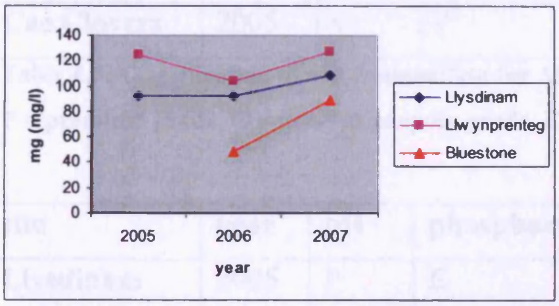

Figure 4.16: Annual soil magnesium content at all *Arundo* sites.

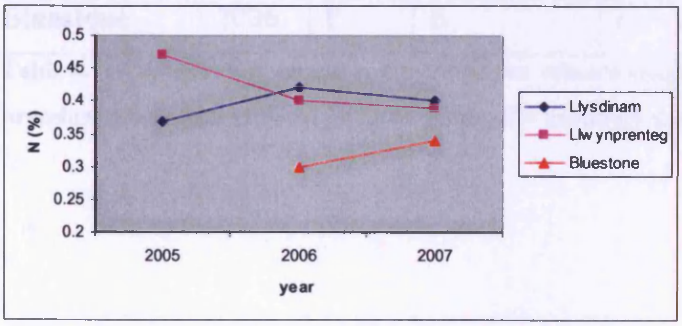

Figure 4.17: Annual soil nitrogen content at all *Arundo* sites.

4.3.5 Soil classification

During the first year of soil sampling, soil analysis included an agricultural classification of its components. "Premium grade" classifications, as defined by Eurofins laboratories' specifications, relate to qualities of natural topsoil, suitable for the most demanding growing uses. "General purpose grade" classifications relate to natural topsoil and premium grade topsoil that has deteriorated, which are suitable for general good quality agriculture. "Economy grade" classifications relate to low quality soil, which is useful for low productivity agricultural uses. Details of the soil classifications for pH, phosphorus, potassium, magnesium and nitrogen at all sites are detailed in Tables 4.6 and 4.7.

site	year	pH	phosphorus	potassium	magnesium	nitrogen
Llysdinam	2005	P	E	E	P	P
Llwynprenteg	2005	P	P	P	P	P
Bluestone	2005	P	E	E	P	P
Cae Clovers	2005	G	P	P	P	P

Table 4.6: Classification of soil composition for *Miscanthus* crops at all sites. Classifications are; P = premium grade, G = general purpose grade, E = economy grade.

site	year	pH	phosphorus	potassium	magnesium	nitrogen
Llysdinam	2005	P	E	E	P	P
Llwynprenteg	2005	P	P	P	P	P
Bluestone	2006	P	E	E	P	P

Table 4.7: Classification of soil composition for *Arundo* crops at all sites. Classifications are; P = premium grade, G = general purpose grade, E = economy grade.

4.3.5.1 Comparisons between the two crops

Phosphorus levels under *Miscanthus* and *Arundo* crops showed little difference, with all sites containing roughly between 10 and 20 mg/l, with the exception of Bluestone. The *Arundo* soil at Bluestone increased steeply from 6 to 19 mg/l, whereas in the *Miscanthus* crop the increase was from 14 to 65. Only at Llwynprenteg and Cae Clovers were the phosphorus and potassium levels at premium grade in 2005 (Tables 4.6 & 4.7).

Soil potassium under both *Arundo* and *Miscanthus* crops started at different levels in year one. All sites showed a decrease in year 2, with the exception of the *Arundo* at Llysdinam which showed an increase. By year 3 levels were similar at all *Miscanthus* sites and ranged from 106 to 137 mg/l., and from 117 to 128 mg/l at all *Arundo* sites except Llwynprenteg. At Llwynprenteg the soil potassium under the *Arundo* crop increased sharply to reach a year 3 level of 215 mg/l.

Soil magnesium differed between sites but showed a similar pattern and was at similar values under both the *Miscanthus* and the *Arundo* crops. All sites achieved premium grade classifications for both magnesium and nitrogen, so these were not limiting nutrients within this study. Soil nitrogen fluctuated between roughly 0.3 and 0.6% under the *Miscanthus* crops at all sites, and between 0.25 and 0.5% under the *Arundo* crops.

Soil pH was acidic under both crops at all sites throughout the study period and achieved premium grade classification at all sites except Cae Clovers.

4.3.6 Correlation analyses of soil nutrients and crop yields

4.3.6.1 *Miscanthus* results

No soil samples were analysed in year four. Therefore, plant dry weight results for year four *Miscanthus* growth were plotted against year three soil phosphorus, potassium, magnesium and nitrogen, and data were tested for correlations. A non-significant (P=0.106) strong correlation was shown between *Miscanthus* plant weight and soil phosphorus (Figure 4.18). However, this relationship existed only as a result of the considerable increase in Bluestone soil phosphorus in year three. No relationship existed when Bluestone data were removed.

No correlations existed between plant weight and soil potassium, magnesium or nitrogen (Figures 4.18 and 4.19).

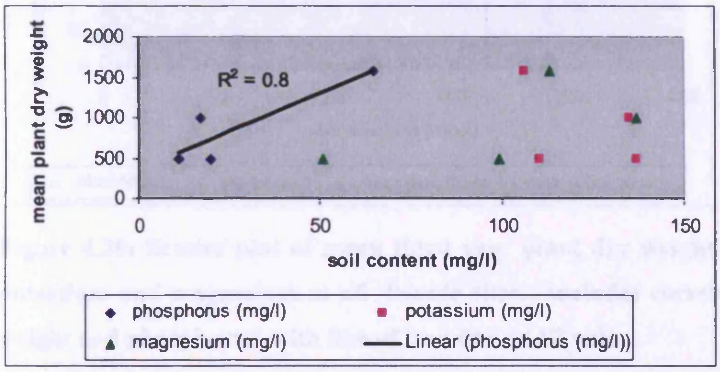

Figure 4.18: Scatter plot of mean fourth year plant dry weight and third year soil phosphorus, potassium and magnesium at all *Miscanthus* sites. Includes correlation analysis of mean plant dry weight and phosphorus, with line of best fit and R^2 value.

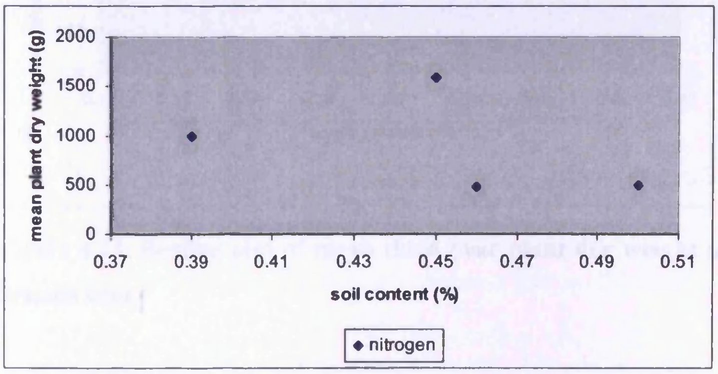

Figure 4.19: Scatter plot of mean fourth year plant dry weight and third year soil nitrogen at all *Miscanthus* sites.

4.3.6.2 *Arundo* results

Plant dry weight results for year three *Arundo* growth were plotted against year 3 soil phosphorus, potassium, magnesium and nitrogen, and data were tested for correlations. A very strong correlation was shown between soil phosphorus and plant dry weight (Figure 4.20), but this was marginally non-significant (P=0.056).

No correlations existed between plant weight and soil potassium, magnesium or nitrogen (Figures 4.20 and 4.21).

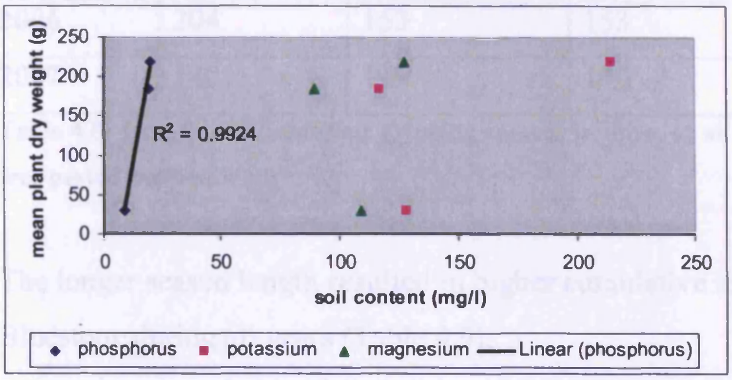

Figure 4.20: Scatter plot of mean third year plant dry weight and third year soil phosphorus, potassium and magnesium at all *Arundo* sites. Includes correlation analysis of mean plant dry weight and phosphorus, with line of best fit and R² value.

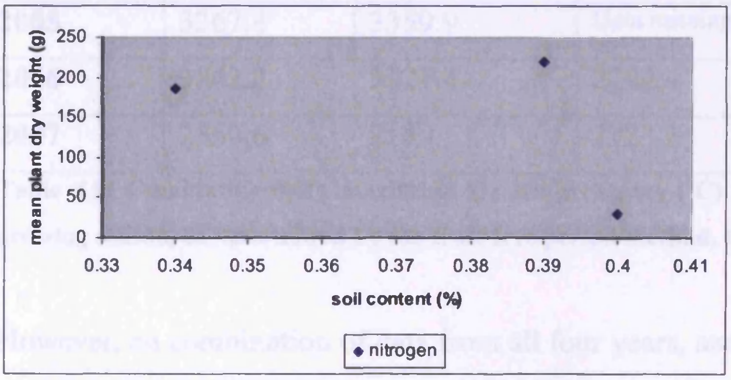

Figure 4.21: Scatter plot of mean third year plant dry weight and third year soil nitrogen at all *Arundo* sites.

4.3.7 *Miscanthus* growing season and temperatures

The *Miscanthus* growing season, as determined by the frost free period method, started consistently earlier at Bluestone over the four year period. The season at Bluestone also tended to finish later (Figures 4.22 to 4.25). This resulted in a longer growing season at Bluestone in all years (Table 4.8).

	Llysdinam	Llwynprenteg	Cae Clovers	Bluestone
2004	189	217	217	267
2005	179	186	Data missing	224
2006	204	153	153	205
2007	140	119	140	206

Table 4.8: Length of *Miscanthus* growing season, in days, at all sites, as determined by the frost free period method.

The longer season length resulted in higher cumulative temperatures being obtained at Bluestone during all years (Table 4.9).

	Llysdinam	Llwynprenteg	Cae Clovers	Bluestone
2004	3368.8	3617.2	3281.4	4223.4
2005	3267.8	3359.9	Data missing	3757.2
2006	3842.2	3021.4	2792.4	3676.1
2007	2550.6	2189	2323.3	3530.6

Table 4.9: Cumulative daily maximum air temperatures (°C) achieved during the *Miscanthus* growing season, as determined by the frost free period method, for all sites.

However, on combination of data from all four years, analysis of variance revealed no significant differences between the mean growing season lengths (P=0.06) or the mean cumulative temperatures achieved (P=0.08) between sites. Figures 4.22 to 4.25 display the cumulative temperatures for each of the sites during the *Miscanthus* growing season for 2004-2007. The growing season was defined as the frost-free period following the last spring air frost and preceding the first autumn air frost.

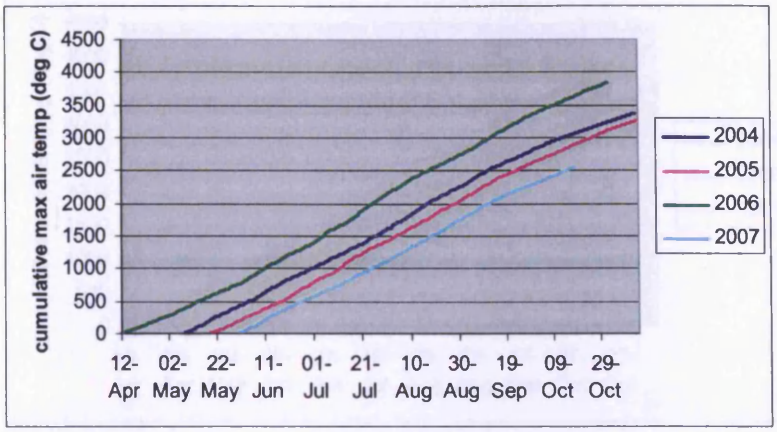

Figure 4.22: Annual cumulative maximum air temperatures obtained during the *Miscanthus* growing seasons, defined as the frost-free periods, at Llysdinam.

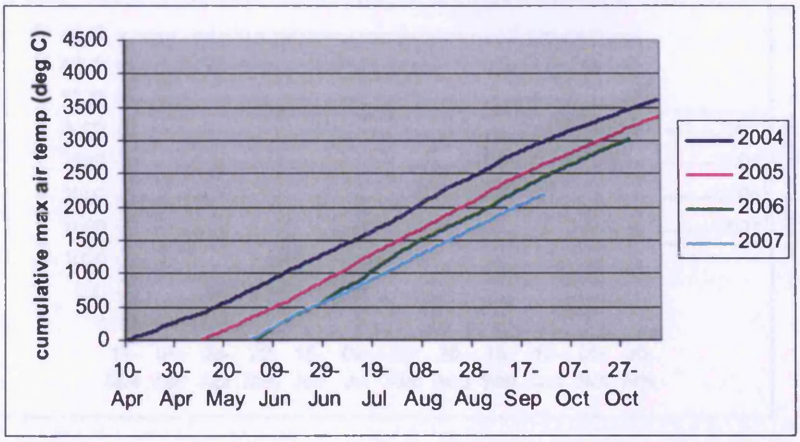

Figure 4.23: Annual cumulative maximum air temperatures obtained during the *Miscanthus* growing seasons, defined as the frost-free periods, at Llwynprenteg.

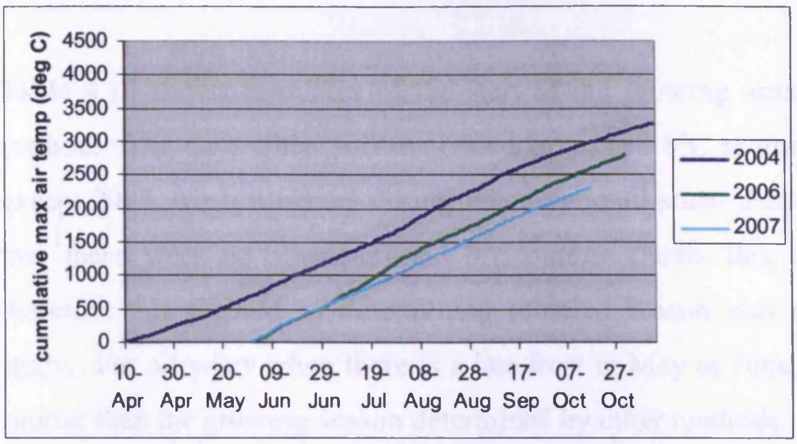

Figure 4.24: Annual cumulative maximum air temperatures obtained during the *Miscanthus* growing seasons, defined as the frost-free periods, at Cae Clovers.

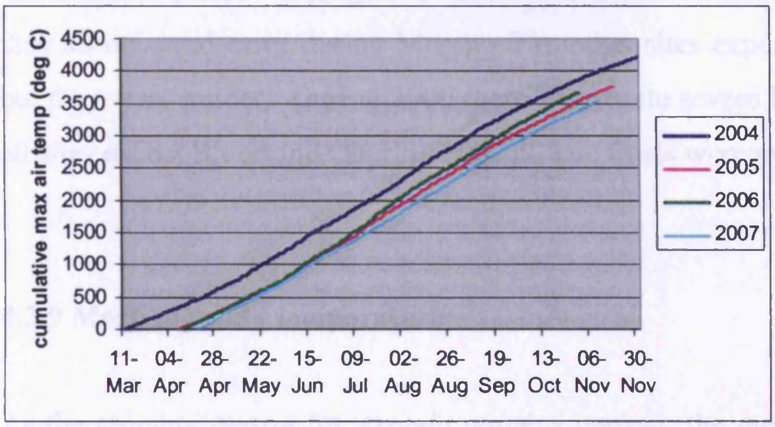

Figure 4.25: Annual cumulative maximum air temperatures obtained during the *Miscanthus* growing seasons, defined as the frost-free periods, at Bluestone.

4.3.8 Growing season determination

Table 4.10 shows the dates of the start of the growing season as defined by each method. The date when soil temperatures exceed 6°C is during March in all years except 2007, when it occurs during February at all sites. Personal observations were that there were no above-ground *Miscanthus* shoots this early in the year, and therefore this method of determining growing season was not considered for this study. For all years when there is a late frost in May or June, the frost free period is shorter than the growing season determined by other methods.

Table 4.11 gives dates and temperatures of the five air frosts immediately preceding the frost free period. During 2004 Bluestone experienced the most severe frosts, but they all occurred early during March. The other sites experienced much later frosts but they were milder. During 2006 there were quite severe late frosts during April at all sites except Bluestone. During 2007 all late frosts were mild.

4.3.9 Mean monthly temperature

As the growing season for *Arundo* remains unclear, the mean monthly temperatures for each of the sites are displayed in Figures 4.26 to 4.29. In California, the *Arundo* growing season was shown to start in February or March and to end in November or December (Spencer & Ksander, 2006).

	Date of the first day of *Miscanthus* growing season			
2004	**Frost free**	**Soil > 6 °C**	**Soil > 10 °C**	**Air > 10 °C**
Llysdinam	8 May	16 March	23 April	18 April
Llwynprenteg	10 April	14 March	13 April	19 April
Cae Clovers	10 April	Missing data	Missing data	10 May
Bluestone	11 March	Missing data	Missing data	18 April
2005				
Llysdinam	19 May	16 March	25 April	22 April
Llwynprenteg	11 May	11 March	24 March	17 April
Cae Clovers	11 May	Missing data	Missing data	10 May
Bluestone	10 April	Missing data	Missing data	10 April
2006				
Llysdinam	12 April	26 March	19 April	12 April
Llwynprenteg	1 June	25 March	15 April	11 April
Cae Clovers	1 June	Missing data	Missing data	3 May
Bluestone	11 April	Missing data	Missing data	11 April
2007				
Llysdinam	31 May	20 February	7 April	5 April
Llwynprenteg	31 May	13 February	9 April	30 March
Cae Clovers	31 May	Missing data	Missing data	1 April
Bluestone	20 April	Missing data	Missing data	23 March

Table 4.10: Date of the first day of the *Miscanthus* growing season at all sites in all years, as determined by (a) the first day of the period when minimum air temperatures remain over 0°C (b) the first day of the period when soil temperatures at 30cm depth remain over 6°C (c) the first day of the period when soil temperatures at 30cm depth remain over 10°C (d) the first day of the period when maximum air temperatures exceed 10°C.

	Last frost		Previous air frosts							
2004	**Date**	**Temp**	**Date**	**Temp**	**Date**	**Temp**	**Date**	**Temp**	**Date**	**Temp**
Llysdinam	7/5	-0.8	26/3	-1	12/3	-0.3	10/3	-0.2	9/3	-2.1
Llwynprenteg	9/4	-2.2	8/4	-0.6	10/3	-0.4	9/3	-1.5	8/3	-0.4
Cae Clovers	9/4	-1.6	8/4	-0.4	29/3	-0.3	26/3	-1.2	25/3	-1.4
Bluestone	10/3	-4.4	9/3	-4.5	2/3	-3.8	1/3	-4.1	29/2	-3.6
2005										
Llysdinam	18/5	-1.2	11/5	-1.7	10/5	-1	17/4	-2.9	15/4	-2.2
Llwynprenteg	10/5	-1.3	17/4	-2.5	15/4	-2	9/4	-4	8/4	-0.4
Cae Clovers	10/5	-0.6	17/4	-2.1	15/4	-1.5	9/4	-2.7	14/3	-2.9
Bluestone	9/4	-0.5	14/3	-1.4	13/3	-1.9	7/3	-3.1	6/3	-2.9
2006										
Llysdinam	11/4	-2.6	6/4	-1.5	5/4	-3.6	4/4	-1	23/3	-1
Llwynprenteg	31/5	-0.5	11/4	-0.3	10/4	-3.7	6/4	-2.3	5/4	-1.2
Cae Clovers	31/5	-0.6	11/4	-1.5	10/4	-3.2	6/4	-2.3	5/4	-4.5
Bluestone	10/4	-0.1	6/4	-0.4	5/4	-2.6	23/3	-2.2	22/3	-0.2
2007										
Llysdinam	30/5	-0.6	29/5	-0.5	19/4	-0.9	11/4	-0.6	8/4	-0.1
Llwynprenteg	30/5	-0.2	20/4	-0.7	19/4	-0.9	7/4	-0.2	5/4	-0.8
Cae Clovers	30/5	-1.3	29/5	-1	19/4	-1.6	6/4	-0.5	5/4	-1.5
Bluestone	19/4	-0.1	4/4	-0.1	21/3	-1.9	2/3	-0.8	10/2	-0.8

Table 4.11: Date and temperatures of the last five air frosts preceding each year's frost free period at each site. Cells are colour coded for temperature as follows; 0>x>-1, -1>x>-2, -2>x>-3, -3>x>-4, -4>x>-5.

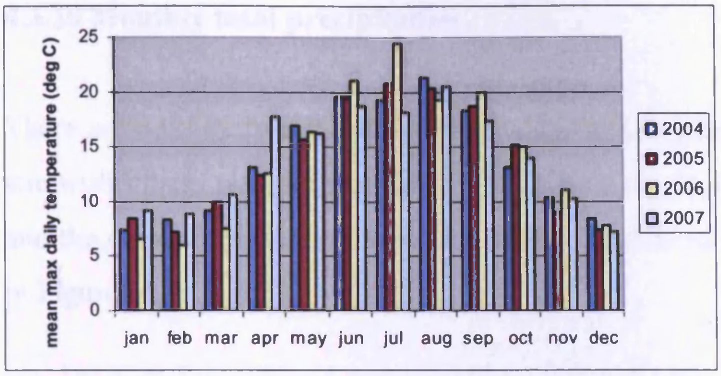

Figure 4.26: Monthly mean maximum daily temperatures for all years at Llysdinam.

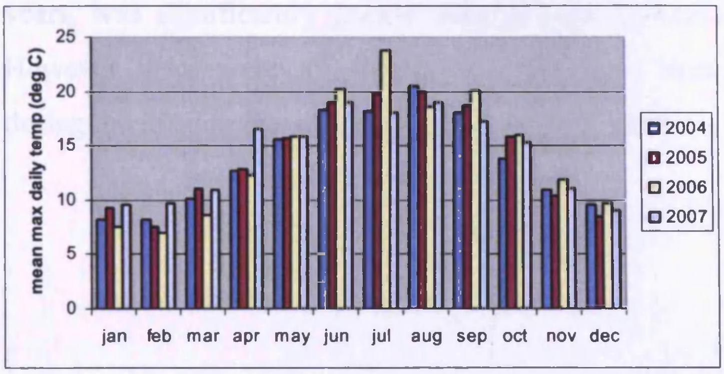

Figure 4.27: Monthly mean maximum daily temperatures for all years at Llwynprenteg.

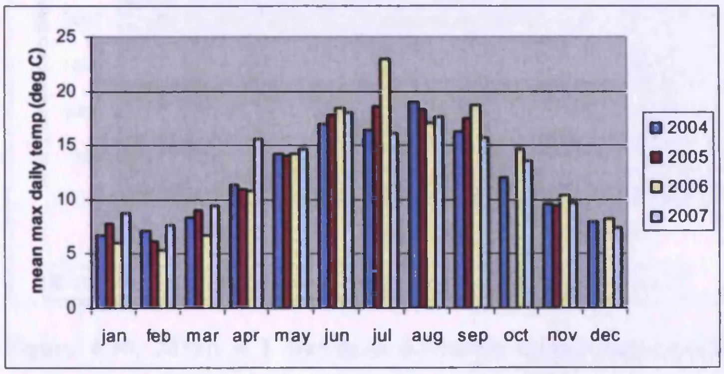

Figure 4.28: Monthly mean maximum daily temperatures for all years at Cae Clovers.

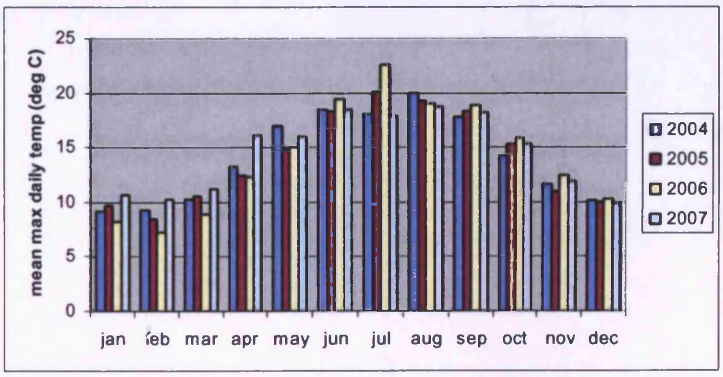

Figure 4.29: Monthly mean maximum daily temperatures for all years at Bluestone.

4.3.10 Monthly total precipitation

There were no daily rainfall data available for Llwynprenteg, so comparison of this site with others was not possible. The nearest site to Llwynprenteg is Cae Clovers, and the monthly precipitation at Llysdinam, Cae Clovers and Bluestone are presented in Figures 4.31 to 4.33.

The mean total annual rainfall at Cae Clovers, after combination of data from all four years, was significantly greater than at both Llysdinam and Bluestone (P=0.000). However there were no significant differences between the cumulative rainfalls during the *Miscanthus* growing season (Figure 4.30).

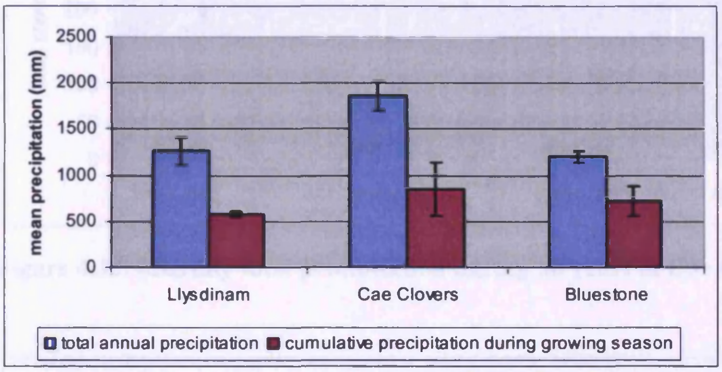

Figure 4.30: Mean ± 1 standard deviation annual precipitation and cumulative precipitation during the *Miscanthus* growing season.

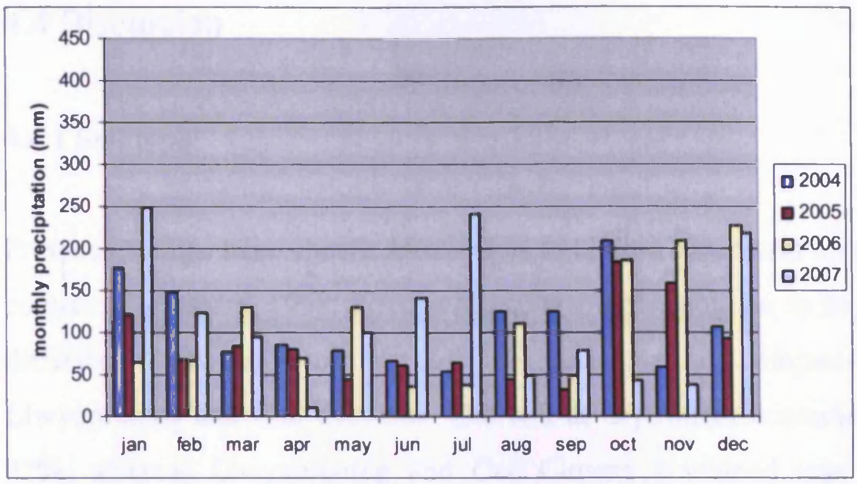

Figure 4.31: Monthly total precipitation during all years at Llysdinam.

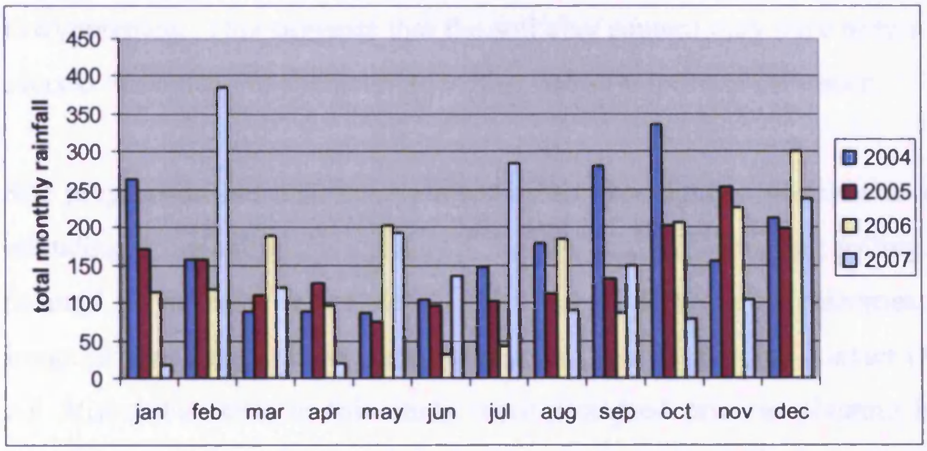

Figure 4.32: Monthly total precipitation during all years at Cae Clovers.

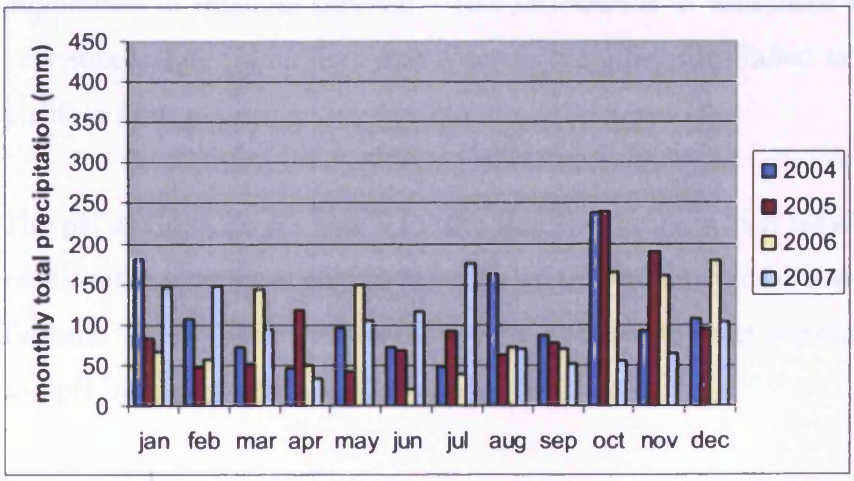

Figure 4.33: Monthly total precipitation during all years at Bluestone.

4.4 Discussion

4.4.1 Soil type

Previous studies have shown *Miscanthus* to be less successful on soils with high clay content (El Bassam, 1998; Price *et al.*, 2004). This could be an important factor in the difference between crop success at Bluestone in comparison to Llysdinam, Llwynprenteg and Cae Clovers. The soil at Llysdinam contained the most clay, at 42%, whereas Llwynprenteg and Cae Clovers contained less, at 31% and 26% respectively, and Bluestone contained the least at 18%. However the yields at these three sites were generally similar at Llysdinam and Cae Clovers, and higher at Llwynprenteg. This suggests that the soil clay content may have been a factor in crop success but other site characteristics also had an important influence.

Site preparation has also been shown to be important to *Miscanthus* establishment, including pre-planting ploughing of the site and post-planting rolling. These were deemed particularly crucial to the survival of *Miscanthus* rhizomes because their irregular shape may otherwise prevent good soil to rhizome contact (ADAS, 2006). All *Miscanthus* sites in this study were ploughed prior to planting but none were rolled afterwards. The soil clay content may have had most effect on crop establishment success during the first year when soil to rhizome contact was of utmost importance to rhizome survival. The *Miscanthus* at Bluestone was shown to grow very successfully in its first year whereas the other sites failed to produce substantial yields at first.

The pH at Cae Clovers was 5 for the first two years, which may have had an impact on its initial growth, as studies have shown optimum pH to lie between 5.5 and 7.5 (El Bassam, 1998). This was the only site that failed to meet premium classification for soil pH in its first year of soil analysis.

The failure of the *Arundo* crop at Llysdinam with respect to out-competition from *Phalaris* has been discussed. However its lack of competitive advantage may have been due to the high clay content of the Llysdinam soil, which as discussed for

Miscanthus, may have prevented good soil to rhizome contact after planting. It is also likely that the field site was exposed to colder weather conditions than the weather station data, which were recorded from a sheltered walled garden location.

4.4.2 Crop survival

The figures for crop survival can only be regarded as a guide to crop success, as there were many unavoidable flaws in experimental design which prevented an accurate calculation. Some crops were planted by machinery, which prevented accurate distribution of an equal number of rhizomes along each row. Therefore a calculation based on the mean number of rhizomes originally planted per row can be inaccurate. Also each site was a different size, and therefore was subject to varying degrees of edge effects, whereby a very small site would be affected for a large proportion of the crop and a large site would be affected for a small proportion. Plant size also has an effect on percentage survival, as large plants shade out smaller plants, resulting in patchy distribution within the crop. The crop at Bluestone, in particular, was subject to this patchiness (personal observations).

The rhizomes themselves can also vary greatly in terms of size and number of buds. To ensure establishment success rhizomes should be planted within four hours of harvest, otherwise cold storage is essential to avoid rhizome desiccation, which can cause the rhizomes to die (ADAS, 2006). There were no records available for this study regarding time from rhizome harvest to planting or interval storage procedure.

Clifton-Brown (1997) showed that *Miscanthus* plants surviving the first winter period following planting continued to survive subsequent winters, and concluded that rhizome reserves were crucial. Overall the *Miscanthus* at Bluestone showed a lower percentage survival than the other sites, although it had been shown that this crop achieved much higher yields. It is likely that the increased plant growth during the first year at this site created its observed patchiness whereby strong plants shaded out weaker growth.

Arundo showed an overall increased survival at Bluestone, in comparison to both Llysdinam and Llwynprenteg, the latter of which showed a decrease in survival between years 1 and 2, suggesting that plants did not survive the first winter. Llwynprenteg received slightly lower mean temperatures during the winter period and earlier autumn frosts. It is possible that the crop is very sensitive to decreased temperatures during the first winter. This may be caused by the continued presence of green shoots during this period, which may have been killed or damaged by frosts or lowered temperatures, and thus prevented sufficient translocation of nutrients to the rhizomes to enable a second year's growth. It has been shown that insufficient below-ground stored carbohydrates causes poor winter survival in grass crops (Smith, 1972).

4.4.3 Dry weights

4.4.3.1 *Miscanthus*

The *Miscanthus* plants at Bluestone were consistently larger than at the other sites. The increased mean dry weight per plant was reflected less in the dry weight per shoot but more significantly in the number of shoots per plant. This suggests that the rhizomes at Bluestone had greater energy reserves to produce more shoots, or were larger rhizomes with more buds, or that the earlier start to the growing season allowed increased shoot production during the early growing season. There was no difference between mean shoot dry weight at Bluestone and Llwynprenteg until year four, when the Bluestone crop also contained more shoots. However, the differences between plant dry weights were not significant, which reflects the large variation within the crop, which consisted of both large and small plants.

The *Miscanthus* crop at Llysdinam was similar to Cae Clovers in year 2, but achieved higher shoot weights in year 3, whereas Cae Clovers achieved higher shoot weights in year 4. However these sites were planted in different years, so that both year 3 at Llysdinam and year 4 at Cae Clovers were 2006. It is highly likely therefore that the increased shoot dry weight during this year was a result of climatic conditions. During 2006 Llysdinam experienced a longer than average growing season, and subsequent higher cumulative temperature. Cae Clovers' growing season, as determined by the frost free period, was shortened considerably by a late mild air frost of -0.6 at the end of May. The previous period of air frosts occurred during mid April, and therefore the calculated growing season was reduced by 49 days by this method. It is very likely that this late mild frost had little effect on the crop's growth.

If we presume this to be true then we can conclude that Cae Clovers also experienced a longer than average growing season during 2006, and a higher cumulative temperature during this period. Both sites also experienced higher than average rainfall during April 2006, which is when the crops would have been starting to grow. Therefore annual fluctuations in crop success were affected by specific climate conditions experienced that year.

4.4.3.2 *Arundo*

Within the three years of study there were no significant differences between the *Arundo* crops at Bluestone and Llwynprenteg, except for an increased number of shoots at the former site during year 3. This statistic however resulted in no significant increase in mean plant dry weight. The *Arundo* crop at Llysdinam was consistently very poor and significantly lower than the other two sites in all aspects. However we cannot conclude that these differences were the result of differences in site characteristics, because the *Arundo* at Llysdinam was annually out-competed by re-growth of *Phalaris arundinacea* at the site. However, the lack of differences between *Arundo* success at Llwynprenteg and Bluestone suggests the differences in site characteristics between the two sites were not important to crop growth. This supports the idea that *Arundo* utilises a longer growing season than *Miscanthus* and therefore is less affected by frost dates at the two sites. However the crop was shown to perform differently in years 1, 2 and 3, whereby year 2 (2006) produced lower plant dry weights at all sites. The mean daily maximum temperature in June, July and September in 2006 was higher than in 2005 and 2007 (Figures 4.26 to 4.29). However 2006 also received consistently less rainfall in June and July at all sites (Figures 4.31 to 4.33). The annual differences in crop success therefore were likely to be related to the combined effect of high temperatures and low precipitation during the summer months.

4.4.4 Annual changes in soil properties

During senescence of the crops the plants lose their leaves and this falls as leaf litter and potentially returns nutrients to the soil, although translocation of nutrients during plant senescence results in a low level of minerals in litter. The soil nutrients are taken up and used by the plants during growth, although there is evidence from this study that the plants store sufficient nutrients within the rhizomes for the following year's growth. The nutrient levels within the soil fluctuated over the three years of sampling, but fluctuations were relatively small, and were likely to be a result of natural fluctuations and/or sampling errors in most cases.

There was very little variation in soil nitrogen between the sites or years with regard to both the *Miscanthus* and *Arundo* crops. This element has a crucial role in plant growth, in protein and chlorophyll production, and is generally considered to be the key factor in crop yield (Yara International, 2008). Although percentages were low, soil analyses from all sites achieved the premium classification for nitrogen content, and therefore contained sufficient amounts for optimal crop growth.

Phosphorus is also a key element in plant growth and root development and insufficient levels have a direct effect on plant establishment and development (Yara International, 2008). Only the soil at Llwynprenteg and Cae Clovers achieved the premium grade classification for phosphorus. As these sites were previously managed as ADAS research sites the premium grade reflects a history of good agricultural practice. Llysdinam and Bluestone attained economy classification, suggesting a deficiency in phosphorus that could prevent high productivity. The *Miscanthus* soil at Bluestone increased in phosphorus in 2007, but unfortunately the crop was removed from the site during this period so further analysis of its effect was impossible. There is no obvious explanation for this increase, as there are no records of phosphorus application to the site. Bluestone soil at the *Arundo* sites also increased in phosphorus content during 2007, although no marked increase in productivity was observed during this year. It is likely therefore that soil phosphorus is not a limiting nutrient for *Miscanthus* or *Arundo* growth, as a result of sufficient phosphorus reserves being present within the rhizomes.

Insufficient soil potassium affects crop yield and longevity, and increases crop susceptibility to stresses such as drought or disease, as it has a key role in the regulation of water loss from leaves (Yara International, 2008). As with phosphorus, only the crops at Llwynprenteg and Cae Clovers achieved the premium grade classification for soil potassium. Llysdinam and Bluestone attained economy grade, which suggests a deficiency that could prevent high crop productivity. Soil potassium increased at both sites, but remained at economy grade for all crops except the *Miscanthus* at Llysdinam, which attained premium grade in year 3. The reason for this increase is unclear, although potassium is a very mobile element that is leached out of crops during periods of rainfall. Fluctuations occur therefore in response to rainfall levels. It is likely that soil potassium was not a limiting factor on crop growth, as a result of sufficient reserves being present within the rhizomes.

Soil magnesium at all sites achieved premium grade classification for both crops, and was therefore sufficiently present to have no detrimental effect upon crop growth.

4.4.5 Correlations of soil nutrients and crop yields

The strong correlation between *Miscanthus* plant weight and soil phosphorus was shown to be non-significant, and to only exist as a result of a very large increase in Bluestone soil P in year three, for which there is no obvious explanation. Therefore this result can be discounted. However, the lack of relationships between soil nutrients and plant yield may be caused by a lack of variation in the soil conditions between sites, as correlation analysis is most useful when analysing a range of values.

The strong correlation between *Arundo* plant weight and soil phosphorus was very close to significance. In this study there were only three data points available for analysis, and therefore this result would require further analysis with the inclusion of more *Arundo* sites in order to reach reliable conclusions. Similarly, it is possible that relationships exist between other soil variables and plant yield, if it were possible to include a greater number of sites and a greater variation in soil properties. This study used all sites in Wales so is a valuable indicator of future research requirements.

4.4.6 *Miscanthus* growing season and temperatures

Combining the data from the four years of study, there were no significant differences between the length of the *Miscanthus* growing season or cumulative temperature achieved at different sites. This suggests that the effect of climate in Wales is reflected in annual fluctuations of plant yield, and that therefore a long term study is needed to cancel out annual differences.

4.4.7 Growing season determination

In this study the growing season for *Miscanthus* was defined as the frost-free period following the last spring air temperature below 0 °C until the day preceding the first autumn air temperature below 0 °C, as emerging *Miscanthus* shoots have been shown to be sensitive to late frosts (Clifton-Brown, 1997; Bullard & Nixon, 1999; Price *et al.*, 2004). However, the crop growing season within the UK is generally referred to as the period that soil temperatures at a 30cm depth do not fall below 6 °C for a period of 5 days (Met Office, 2008). The date when soil temperatures exceed 6°C is during March in all years except 2007, when it occurs during February at all sites. Personal observations were that there were no above-ground *Miscanthus* shoots this early in the year, which is consistent with another UK study where shoots appeared in late April (Christian *et al.*, 1999), and therefore this method of determining growing season was not considered for this study. *Miscanthus* studies elsewhere have regarded the start of the *Miscanthus* growing season to be when the soil temperature reaches 10 °C (El Bassam & Huisman, 2001) or when air temperatures reach 10 °C (Lewandowski & Heinz, 2003). There is therefore some discrepancy between studies regarding the start of the *Miscanthus* growing season. The definition used in this study was regarded to be most appropriate to the sites.

However, for all years when there was a late frost in May or June, the frost free period was shorter than the growing season determined by other methods. At Llysdinam this occured in three out of four years and the season was subsequently shorter than the period during which the soil was over 10°C by as much as 54 days, and was shorter than the period when the air temperature was over 10°C by as much as 56 days. At Llwynprenteg the last frost was in May or June in three out of four years, with a loss of as much as 52 days in comparison to soil temperatures and as much as 62 days in comparison to air temperatures over 10°C. Cae Clovers experienced a late frost in three out of four years, with a season reduced by up to 61 days in comparison to air temperature over 10°C. Bluestone had a growing season shortened by a late frost in 2007 only, although the frost was on 20 April, and only 28 days were lost in comparison to air temperature determination.

It may be useful therefore to look at severity of frost, and frequency of frosts to determine the *Miscanthus* growing season (Table 4.11). Further study is required to determine the air temperature that relates to a "significant" frost which can kill or stunt *Miscanthus* growth. Grass temperature can be approximately 5°C lower than the air temperature at 1.2m (Clifton-Brown, 1997), and therefore the effect of air frosts also differs depending on the size of above-ground shoots. Leaf litter from the previous season was present on the ground during spring and this would have offered some protection to new shoot growth.

The temperature requirements of growth of *Miscanthus* crops are regarded as between 11°C and 40°C between the months of April and September (Tuck *et al.*, 2006). All Welsh sites in this study achieved these temperatures during all years. Although there were no significant differences between the cumulative temperatures during the growing seasons at the sites, Bluestone consistently achieved the highest value and Cae Clovers the lowest. This was reflected in the success of the *Miscanthus* crops at these sites.

4.4.8 Rainfall

Rainfall has been shown to be important to previous *Miscanthus* studies, and minimum levels have been determined in Germany (700mm y^{-1}; Clifton Brown *et al.*, 2001) and Eastern England (500mm during growing season; Long and Beale, 2001). A European prediction model considered *Miscanthus* to require annual precipitation of between 600mm and 1500mm (Tuck *et al.*, 2006). All sites in this study received higher precipitation levels than these figures in all four years.

Average annual rainfall was higher at Cae Clovers than at both Bluestone and Llysdinam, yet this site failed to achieve the highest yields. However there was not an increased amount of rainfall during the *Miscanthus* growing season in comparison to the other sites. It may therefore be more useful to refer only to rainfall during the growing season when comparing site specific precipitation and its effect on *Miscanthus* yield. The precipitation levels during 2004-2007 at these Welsh sites were sufficient for both *Miscanthus* and *Arundo* growth, and therefore the yield differences shown between the sites were a consequence of other factors.

It is likely that both crops have a deep root system which enables use of deep water reserves, as has been shown for *Miscanthus* (Neukirchen *et al.*, 1999), and are thus less dependant on precipitation than other climatic factors.

4.5 Conclusions

It is likely that soil type and texture are most important to both crops' success during the establishment year, and this can also subsequently affect its future success. High soil clay content is considered to have a detrimental effect on *Miscanthus* establishment (El Bassam, 1998; Price *et al.*, 2004). This study showed that the greatest *Miscanthus* success was shown at the site with the lowest clay content (Bluestone) and that the lowest yielding crop was grown at the site with the highest clay content (Llysdinam). The least successful *Arundo* crop was also grown at Llysdinam, which may also be partly due to poor soil to rhizome contact after planting, as a result of high soil clay content. However it is likely that the field site at Llysdinam was exposed to colder weather than the location of the weather station, and this may have effected crop growth. The agricultural grade of the soil and its nutrient content was shown to have little effect on *Miscanthus* and *Arundo* success. This was most probably because the rhizomes contained sufficient nutrient reserves for crop growth.

The *Miscanthus* crops at Bluestone and Llwynprenteg were shown to grow at a similar rate, although the former site produced a greatly increased crop yield in the first year. The highly successful establishment year at Bluestone may have been caused by a lower soil clay content, but is most likely to be relative to differences in the rhizomes planted. No records were made of the rhizome size, number of buds, time from harvest to planting, or storage method, which are all important to crop success. However the mean number of shoots per plant at Bluestone more than doubled that shown at Llwynprenteg, which supports the theory that the rhizomes were bigger and/or contained more buds.

The *Arundo* crops at Bluestone and Llwynprenteg showed similar growth patterns, and were shown to fluctuate in response to annual climatic differences. It is likely that the combination of temperature and precipitation was important to *Arundo* growth, and that during the early years the crop was sensitive to warm, dry conditions. It is possible that the root system had not fully developed during this period and therefore that the crop could not utilise deep water sources. This effect may therefore change over time. Decruyenaere & Holt (2001) showed no response of *Arundo* growth rate or number of shoots produced to temperature or precipitation in Southern California.

All Welsh sites studied were shown to receive sufficient rainfall (>500mm) during the *Miscanthus* growing season, and increased annual rainfall at Cae Clovers had no effect on crop success. Price *et al.* (2004) showed cumulative temperature during the growing season and limited precipitation to both affect *Miscanthus* yield, and Heaton *et al.* (2004) showed rainfall but not cumulative temperature during the growing season to affect *Miscanthus* yield. However, during this study, rainfall was not limited, and it is likely therefore that the differences between the crops' successes were more dependant on cumulative temperatures during the growing season. Bluestone was consistently the warmest site and Cae Clovers the coldest site, and this was reflected in the differences between crop successes. However the *Miscanthus* crops at Bluestone and Llwynprenteg were shown to grow at a similar rate after the first year. The lower cumulative temperature achieved during the Llwynprenteg growing season was a result of later frosts, which were frequently mild, and therefore may have had little effect on the new shoot growth. This study suggests that the *Miscanthus* growing season requires some re-definition to account for severity and timing of frosts during early shoot growth.

It is clear that a long-term study of both crops is required to facilitate clear interpretation of the factors which most influence the success of *Miscanthus* and *Arundo* crops in the Welsh climate.

4.6 References

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Chapter 5

The Effect of Harvest Date on the Quantity and Quality of *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea* Crops in the Welsh Climate.

5.1 Introduction

There has been much debate surrounding the optimum date of harvest for energy crops. Traditionally, grass crops are harvested in summer. However, studies have shown that the harvest date of energy crops can be successfully delayed until late winter or early spring. This allows translocation of the nutrients from the above-ground plant matter to the rhizomes, where they are stored until the following spring. Himken *et al.* (1997) showed that phosphorus, potassium and magnesium concentrations in *Miscanthus* rhizomes decreased during May and June to coincide with shoot elongation, which suggests that the stored nutrients were remobilized during plant growth. The storage of nutrients in the rhizome reduces the requirement for fertiliser addition, thus reducing both financial and environmental costs. It has also been shown that insufficient below-ground stored carbohydrates causes poor winter survival in grass crops (Smith, 1972).

Subsequently, a delayed harvest generates harvested material containing minimal nutrients. This improves the combustion quality of the crop by reducing corrosion, slagging, fouling and environmentally harmful emissions (Miles *et al.*, 1996). Slagging is the formation of a glassy material and is caused by a low ash melting point, and fouling is the accumulation of materials on the equipment which causes a corrosive reaction. High concentrations of ash, potassium and chlorine within the plant material decrease the ash melting point (Hadders and Olsson, 1997; Jorgensen and Sander, 1997; Jorgensen, 1997), thus reducing the quality of the crop as a fuel. To a lesser degree, calcium content contributes to these problems, although the calcium/potassium ratio is important, as a high ratio, i.e. high calcium combined with low potassium, has been shown to reduce slagging (Monti *et al.*, 2008). Nitrogen content is important when considering crop quality, as NO_x emissions need to be minimised during combustion. They can be reduced during the combustion process, but this creates increased costs (Lewandowski & Kicherer, 1997).

Some studies have subsequently estimated the threshold levels of nitrogen content (Lewandowski & Kicherer, 1997; Kauter *et al.*, 2003; Yates & Riche, 2007; Monti *et al.*, 2008) and potassium content (Jorgensen, 1997; Lewandowski & Kicherer, 1997) of harvested material for use as a combustion crop, although these are often lower than the levels found in straw and wood chip (from Christian *et al.*, 2006), which are currently successfully used as combustion material (Table 5.1). There are no references to phosphorus threshold levels in the scientific literature.

Mineral	**Threshold (% dry matter)**	**Straw range (% dry matter)**	**Wood chip range (% dry matter)**
Nitrogen	0.6 - 1.0	0.3-1.5	0.1-0.7
Phosphorus	n/a	0.03-0.2	0.02-<0.1
Potassium	0.2 - 0.5	0.2-1.9	0.05-0.4

Table 5.1: Threshold levels for mineral content (% dry matter) of combustion crops (see text for references), and mineral content ranges sampled from straw and wood chip (Christian *et al.*, 2006).

A further benefit of a delayed harvest is reduced moisture content, caused by the plant matter drying out during senescence over the winter period. This reduces the need for crop drying prior to storage or combustion. Moisture content of less than 23% is necessary to mitigate the danger of self-ignition during straw storage (Clausen, 1994), although El Bassam and Huisman (2001) suggest that this occurs in *Miscanthus* crops at moisture contents over 50%, but that values between 15 and 50% allow fungal growth without natural ventilation. Straw bales with high moisture content can also cause inefficiency within the power station as they are too heavy for the conveyor belt (J. Ariss, Elean Power Station, pers. comm.). Elean Power Station in Cambridgeshire, UK, which is the world's largest straw burning power station, rejects all bales with moisture content higher than 25%.

The drawback of delaying harvest is the risk of winter biomass losses, caused by leaf loss and stem breakage during crop senescence. Studies in Germany showed *Miscanthus* yield losses to average 18% between December and February, and an additional 16% between February and March (Lewandowski and Heinz, 2003), or an overall average of 30% from September to March (Himken *et al.*, 1997). Trials in Hertfordshire, UK, showed *Miscanthus* yield losses of 17 to 34% from October to February (Clifton-Brown *et al.*, 2001), and losses of almost 40% were observed in Ireland between the date of the first autumn frost and a March harvest (Clifton-Brown and Jones, 2001). Studies on *Phalaris* in Hertfordshire, UK, showed this crop to also experience yield losses, of up to 37% within less than two months between December and February (Yates and Christian, 2001).

The general consensus to date is that *Miscanthus* crops should be harvested in early spring in Northern Europe, to enable moisture loss, but should be harvested in autumn in Southern Europe, to avoid biomass loss caused by the succession of dry and humid weather conditions and strong winds during the winter (El Bassam and Huisman, 2001). However, Swedish studies of *Phalaris* suggest that the cold and dry climatic conditions of northern Sweden preserve this crop during the winter in comparison to southern Sweden where it is warmer and wetter (Landstrom *et al.*, 1996), and increased winter losses occur due to an increased rate of decomposition (Hadders and Olsson, 1997).

The aims of this study were to determine the differences between an early autumn harvest date and a spring harvest in terms of quantity and quality of the three energy crops *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea*. Crop quality was defined in terms of moisture content and mineral content, specifically nitrogen, phosphorus and potassium, of harvested plant material. This study compared the three crops at sites across Wales and aimed to determine their suitability as combustion crops in a Welsh situation.

5.2 Methods

5.2.1 Sites

From October 2006 to February 2007, the crops at Llysdinam, Llwynprenteg and Bluestone were visited every two weeks (see Chapter 2 for full site details). At Llysdinam and Llwynprenteg, *Miscanthus* and *Arundo* were sub-sampled every 2 weeks and *Phalaris* was sub-sampled every 4 weeks. *Phalaris* sampling at Llwynprenteg ceased in January 2007, as the landowners were keen to harvest the crop. At Bluestone, *Miscanthus* and *Arundo* were sub-sampled every two weeks, although access problems caused disruption to *Miscanthus* sub-sampling during January and cessation of the *Miscanthus* study on 2 February 2007. The *Miscanthus* and *Arundo* crops at Coleg Sir Gâr were sampled once at the beginning of December 2006, and again at the end of February 2007.

During the winter of 2007 – 2008, the *Miscanthus* crops at Llysdinam, Llwynprenteg, Cae Clovers and Coleg Sir Gâr were sub-sampled once during the first week of December, and again during the last week of February. The *Arundo* crops at Llysdinam, Llwynprenteg, Bluestone and Coleg Sir Gâr were sampled on the same dates.

5.2.2 Crop sub-sampling

5.2.2.1 *Miscanthus* and *Arundo* measurements

Five plants were randomly selected from the crop, for which all shoots were counted, chopped at the base and collected. The fresh weight of each plant was determined immediately. Five shoots were randomly selected from each plant, weighed fresh, oven-dried at 80°C to constant weight, and re-weighed dry. Percentage moisture content was calculated as:
((fresh weight – dry weight)/fresh weight)*100.

5.2.2.2 *Phalaris* measurements

The *Phalaris* crops at Llysdinam and Llwynprenteg were visited every 4 weeks during 2006 – 2007, and yield estimates were obtained from 5 randomly selected 1m^2 quadrats. All shoots within the quadrat were cut at the base and collected. Fresh weight per $1m^2$ area was determined immediately upon arrival at the research centre, and a sub-sample of approximately 500g from each area was accurately weighed fresh, oven-dried at 80°C to constant weight, and re-weighed dry. Moisture content was calculated as above.

5.2.3 Yield estimate calculations

***Miscanthus* and *Arundo donax* calculations**

During this study, yield estimates were calculated for each plant sampled, as:
Mean shoot dry weight * number of shoots per plant.

***Phalaris* calculations**

Yield estimates were calculated for each quadrat sampled, as:
Fresh weight * % dry matter,
where % dry matter = 100 – moisture content.

5.2.4 Calorific value determination

Three oven-dried shoots sampled from each of the *Miscanthus*, *Arundo* and *Phalaris* crops at Llysdinam, Llwynprenteg and Bluestone during late November and late January, were milled to powder form and sent to the IGER laboratories for determination of calorific value.

5.2.5 Mineral content determination

During 2006 – 2007, the *Miscanthus* and *Arundo* crops at Llwynprenteg and Bluestone were analysed for mineral content. Three leaf samples, three cane samples, three rhizome samples and up to three flower samples, from each sampling date were sent to Natural Resource Management Ltd for laboratory analysis of total nitrogen, total phosphorus and total potassium content. Three samples of whole above-ground plant matter from the *Phalaris* crops at Llwynprenteg and Llysdinam, from each sampling date, were also sent for analysis.

5.2.6 Statistical analysis of results

5.2.6.1 Analysis of yield estimates, crop moisture content and calorific value data

At each site the datasets from the earliest sampling date (late November in 2006, early December in 2007) were tested for differences from the datasets from the latest sampling date (February 2007 and February 2008), using t-tests. Data were transformed as necessary in order to meet the assumptions of the test. Any datasets which did not meet the assumptions after transformation were tested using the non-parametric Mann-Whitney test.

5.2.6.2 Analysis of plant mineral content data

***Miscanthus* and *Arundo* data**

The leaves had dropped from the plants prior to the end of the sampling period. Subsequently, leaf datasets were analysed using t-tests for differences between the first sampling date and the last date that all plants sampled had leaves. Data were transformed as necessary in order to meet the assumptions of the test. Any datasets which did not meet the assumptions after transformation were tested using the non-parametric Mann-Whitney test.

For analysis of cane and rhizome, analysis of variance was performed on the data from the two sampling dates used for leaf analysis, in addition to data from the last date sampled. Data were transformed as necessary in order to meet the assumptions of the test. Any datasets which did not meet the assumptions after transformation were tested using the non-parametric Kruskal Wallis test. Pair-wise comparisons of significantly different datasets, as determined by ANOVA, were performed by Fishers tests.

***Phalaris* data**

Data from sampling dates during October, early December and late January at Llwynprenteg were used for ANOVA testing. At Llysdinam, these three dates were used in addition to a further date at the end of February. All tests as detailed above were performed.

5.3 Results

5.3.1 Yield estimates and crop moisture content

The plant fresh weights were determined using a hanging balance which proved to be very inaccurate when measuring light plants, whereas individual shoots were weighed accurately using a counter top balance. All statistical analyses were performed therefore using an estimate of plant weight, which was calculated as the mean shoot dry weight per plant multiplied by the number of shoots per plant.

5.3.1.1 *Miscanthus* yield estimates

2006-2007 data

The mean yield estimates for each sampling date during the period November 2006 to February 2007 are shown in Table 5.2. There were no significant differences between the earliest and latest sampling dates at Llysdinam, Bluestone or at Llwynprenteg. However, at Coleg Sir Gâr the mean plant yield in February was significantly lower than the mean plant yield in November (P=0.034).

2007 – 2008 data

The yield estimates for early December 2007 and late February 2008 are represented in Table 5.3. There were no significant differences between the two dates at all sites. At Llwynprenteg and Cae Clovers, the mean yield estimate was reduced during the period, but there was much variation within the crop which prevented the data from showing statistical significance. At Coleg Sir Gâr the February mean was greater than the December mean, although the variation within the dataset was very large. This site was not planted in a uniform arrangement, thus creating great difficulty in distinguishing individual plants within the crop. Therefore, I believe that these results can be discounted.

5.3.1.2 *Miscanthus* moisture content

The percentage moisture content of the *Miscanthus* crops from all sampling dates during the period November 2006 to February 2007 are displayed in Figure 5.1. All sites showed a highly significant reduction in moisture content between the first date sampled and the last date sampled (P=0.000).

The data from December 2007 and February 2008 are displayed in Table 5.4. The *Miscanthus* crops at all sites contained significantly less moisture in February than in December, except at Coleg Sir Gâr where the result was non-significant.

5.3.1.3 *Arundo* yield estimates

For both years 2006-2007 (Table 5.5) and 2007-2008 (Table 5.6), there were no significant differences between the yield estimates obtained from the first and last sampling dates at any site, although the mean plant yield was reduced at all sites except at Llysdinam in 2007-2008.

5.3.1.4 *Arundo* moisture content

The moisture content of the *Arundo* crops showed greater variation than that of the *Miscanthus* crops during 2006-2007. Despite this variation, all sites showed a significant reduction in moisture content from the first sampling date in November to the last sampling date in February (Figure 5.2). The P-values of the differences at Llysdinam, Llwynprenteg, Bluestone and Coleg Sir Gâr were 0.004, 0.000, 0.006 and 0.026 respectively.

During the 2007-2008 winter period, the *Arundo* crops showed less variation in shoot moisture content. The mean moisture content remained stable over the period, and there were no significant differences between that during early December 2007 and during late February 2008 at any site (Table 5.7).

Site	November mean plant yield (g) ± 1 standard deviation	February mean plant yield (g) ± 1 standard deviation	P value	Significance
Llysdinam	300 ± 108	272 ± 212	x	n.s.
Llwynprenteg	564 ± 311	386 ± 119	x	n.s.
Bluestone	3135 ± 1331	1787 ± 541	x	n.s.
Coleg Sir Gâr	695 ± 200	464 ± 119	P=0.034	*

Table 5.2: Mean ± 1 standard deviation *Miscanthus* yield estimates (g) as calculated by mean shoot dry weight multiplied by number of shoots per plant during November 2006 and February 2007. P values are not presented for non significant results.

Site	December mean plant yield (g) ± 1 standard deviation	February mean plant yield (g) ± 1 standard deviation	P value	Significance
Llysdinam	542 ± 110	541 ± 311	x	n.s.
Llwynprenteg	729 ± 266	492 ± 257	x	n.s.
Cae Clovers	388 ± 105	293 ± 194	x	n.s.
Coleg Sir Gâr	640 ± 148	1471 ± 935	x	n.s.

Table 5.3: Mean ± 1 standard deviation *Miscanthus* yield estimates (g) as calculated by mean shoot dry weight multiplied by number of shoots per plant during December 2007 and February 2008. P values are not presented for non significant results.

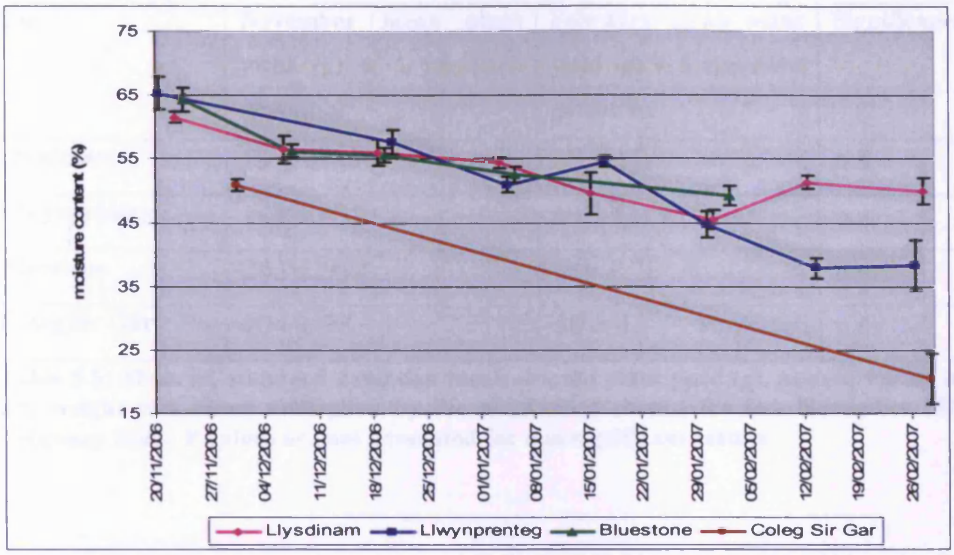

Figure 5.1: Mean ±1 standard deviation percentage moisture content of *Miscanthus* crops during the period November 2006 to February 2007.

Site	December mean % moisture content ± 1 standard deviation	February mean % moisture content ± 1 standard deviation	P value	Significance
Llysdinam	62 ± 2	47 ± 7	P=0.001	***
Llwynprenteg	55 ± 3	28 ± 5	P=0.000	***
Cae Clovers	53 ± 2	42 ± 7	P=0.006	**
Coleg Sir Gâr	40 ± 4	36 ± 2	P=0.066	n.s.

Table 5.4: Mean ±1 standard deviation percentage moisture content of *Miscanthus* crops during early December 2007 and late February 2008.

Site	November mean plant yield (g) ± 1 standard deviation	February mean plant yield (g) ± 1 standard deviation	Significance
Llysdinam	19 ± 15	15 ± 11	n.s.
Llwynprenteg	144 ± 61	43 ± 6	n.s.
Bluestone	243 ± 99	139 ± 72	n.s.
Coleg Sir Gâr	104 ± 98	58 ± 33	n.s.

Table 5.5: Mean ±1 standard deviation mean *Arundo* plant yield (g), as determined by the mean dry weight of a shoot multiplied by the number of shoots, for late November 2006 and late February 2007. P values are not presented for non significant results.

Site	December mean plant yield (g) ± 1 standard deviation	February mean plant yield (g) ± 1 standard deviation	Significance
Llysdinam	31 ± 15	33 ± 9	n.s.
Llwynprenteg	203 ± 62	143 ± 26	n.s.
Bluestone	228 ± 109	213 ± 125	n.s.
Coleg Sir Gâr	150 ± 83	86 ± 40	n.s.

Table 5.6: Mean ±1 standard deviation mean *Arundo* plant yield (g), as determined by the mean dry weight of a shoot multiplied by the number of shoots, for early December 2007 and late February 2008. P values are not presented for non significant results.

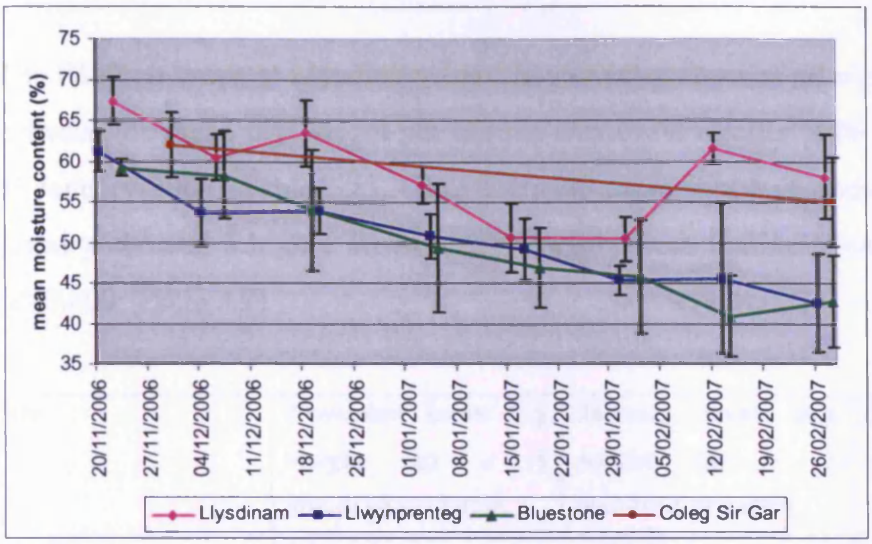

Figure 5.2: Mean ±1 standard deviation percentage moisture content of *Arundo* crops during the period November 2006 to February 2007.

Site	December mean % moisture content ± 1 standard deviation	February mean % moisture content ± 1 standard deviation	Significance
Llysdinam	55 ± 3	53 ± 8	n.s.
Llwynprenteg	59 ± 2	59 ± 6	n.s.
Bluestone	53 ± 4	54 ± 3	n.s.
Coleg Sir Gâr	48 ± 6	48 ± 7	n.s.

Table 5.7: Mean ±1 standard deviation percentage moisture content of *Arundo* crops during early December 2007 and late February 2008. P values are not presented for non significant results.

5.3.1.5 *Phalaris* results

The *Phalaris* crops at Llysdinam and Llwynprenteg showed no significant differences between the mean dry weight per sample area in November 2006 and that at the end of January 2007 (Table 5.8). However, the mean moisture content of the crops in January showed a highly significant reduction from that in November at both sites ($P=0.000$, Table 5.9).

Site	**November mean dry weight (g) ± 1 standard deviation**	**January mean dry weight (g) ± 1 standard deviation**	**Significance**
Llysdinam	93 ± 40	65 ± 9	n.s.
Llwynprenteg	110 ± 70	67 ± 6	n.s.

Table 5.8: Mean ±1 standard deviation dry weight (g) per 1m² of *Phalaris* crops during November 2006 and late January 2007. P values are not presented for non significant results.

Site	**November mean % moisture content ± 1 standard deviation**	**January mean % moisture content ± 1 standard deviation**	**P value and significance**
Llysdinam	70 ± 2	17 ± 2	P=0.000***
Llwynprenteg	73 ± 2	25 ± 3	P=0.000***

Table 5.9: Mean ±1 standard deviation percentage moisture content of *Phalaris* crops during November 2006 and late January 2007.

5.3.2 Calorific values of all crops

No differences in calorific values between November and January sampling dates were found at Llysdinam or Bluestone, although the data from Llwynprenteg were significantly greater in January than November (P=0.039). Similarly, the *Arundo* crops at Llysdinam, Bluestone and Llwynprenteg were analysed for differences in mean calorific value between late November sampling and late January sample dates, and no significant differences were found. The *Phalaris* crops at Llysdinam and Llwynprenteg were also analysed for differences between calorific values in late November and late January, and no significant differences were found.

The overall mean calorific values for each crop were; *Miscanthus*, 17.99 MJ/kg; *Arundo*, 17.59 MJ/kg; *Phalaris*, 17.94 MJ/kg.

5.3.3 Plant mineral content - *Miscanthus* results

The *Miscanthus* crops lost their leaves during late November / early December. At Llwynprenteg, the sampling dates used for analysis were 10 October 2006 and 4 December 2006 for the leaf data, with the inclusion of data from 26 February 2007 for cane and rhizome analysis. At Bluestone, the sampling dates used for leaf analysis were 12 October 2006 and 23 November 2006, with the inclusion of data from 2 February 2007 for cane and rhizome analysis.

5.3.3.1 Nitrogen

The mean nitrogen content of leaf, cane, flower and rhizome samples throughout the 2006 - 2007 winter period are displayed in Figures 5.3 and 5.4, which relate to data from Llwynprenteg and Bluestone respectively.

At Llwynprenteg, the mean nitrogen content of *Miscanthus* leaves fell rapidly during November prior to leaf abscission. There was significantly less nitrogen in the leaves during December than during October (P=0.005). However, at Bluestone leaf abscission occurred earlier, and no leaves were present on the December sampling date. There was no significant difference between mean leaf nitrogen content in October and in November.

The *Miscanthus* cane showed less variation in nitrogen content than the leaves, although the mean cane nitrogen during February was significantly less than that in October at Llwynprenteg (P=0.039), and than that in November at Bluestone (P=0.029).

There were insufficient flower samples for analysis, although all contained less nitrogen than leaf samples. At Bluestone, flower samples from all dates contained more nitrogen than *Miscanthus* cane, although at Llwynprenteg this was only true for one out of four sampling dates.

At Bluestone the mean rhizome nitrogen content remained fairly constant throughout the season, and showed no significant differences between the data obtained during October, November and February. At Llwynprenteg, however, the mean rhizome nitrogen content increased during the season, and was significantly greater in February than in October (P=0.041).

5.3.3.2 Phosphorus

The mean phosphorus content of leaf, cane, flower and rhizome samples throughout the 2006-2007 winter period are displayed in Figures 5.5 and 5.6, which relate to data from Llwynprenteg and Bluestone respectively.

Similarly to the nitrogen data, the mean phosphorus leaf content at Llwynprenteg fell during November prior to leaf drop, but varied little at Bluestone, where the leaves dropped earlier. At Llwynprenteg the leaf phosphorus reduction between October and December was significant (P=0.003), but there was no significant difference between the October and November sampling dates at Bluestone.

The mean phosphorus content of the cane reduced gradually throughout the winter period at both sites, and there was a significant reduction between the October and the February sampling dates at both Llwynprenteg and Bluestone (P=0.025 and P=0.021 respectively).

At Bluestone the mean phosphorus content of the rhizome remained fairly stable throughout the period, and there were no significant differences between the October, November and February sampling dates. At Llwynprenteg, however, the phosphorus content of the rhizome fluctuated throughout the season, with an overall increase which showed a significant difference between the February sampling dates and both the October and November dates (P=0.024).

5.3.3.3 Potassium

The mean potassium content of leaf, cane, flower and rhizome samples throughout the 2006-2007 winter period are displayed in Figures 5.7 and 5.8, which relate to data from Llwynprenteg and Bluestone respectively.

The mean potassium leaf content at Llwynprenteg fell gradually through October and November, which resulted in a significant decrease from the October sampling date to the December sampling date (P=0.005). At Bluestone, similar to the nitrogen and phosphorus results, the mean leaf potassium remained fairly stable and showed no significant difference between the October and November sampling dates.

The mean potassium content of the cane fell throughout the winter period at Llwynprenteg. There were significant differences between the October and December sampling dates and between the February date with both October and December dates (P=0.004). There were no significant differences between dates at Bluestone.

Within the rhizome, the mean crop potassium content fluctuated widely at Llwynprenteg although showed no significant differences between the October, December and February sampling dates. Likewise, at Bluestone, there were no significant differences between the sampling dates.

In summary, the *Miscanthus* at Llwynprenteg showed more variation over time than that at Bluestone. At Llwynprenteg, all above-ground parts of the plant showed a significant decrease in nitrogen, phosphorus and potassium content over the winter period, whereas the rhizome showed a significant increase in nitrogen and phosphorus. At Bluestone, values remained fairly constant throughout the period, with the exception of the nitrogen and phosphorus content of the cane, which decreased over the period.

5.3.4 Plant mineral content - *Arundo* results

The results of the mineral analysis for the *Arundo* data are displayed in Figures 5.9–5.14. The mean nitrogen content of the *Arundo* crops at Llwynprenteg and Bluestone are displayed in Figures 5.9 and 5.10 respectively. The mean phosphorus content is displayed in Figure 5.11 for Llwynprenteg and Figure 5.12 for Bluestone. The results for mean potassium are displayed in Figures 5.13 and 5.14 for the *Arundo* crops at Llwynprenteg and Bluestone respectively.

The *Arundo* crops lost their leaves during February. At Llwynprenteg, the sampling dates used for analysis were 10 October 2006, 20 November 2006 and 30 January 2007. At Bluestone, the sampling dates used for analysis were 12 October 2006, 23 November 2006 and 2 February 2007.

The leaf data showed some variation in both nitrogen and potassium content during October and November at Llwynprenteg only. However, overall there were no significant differences between the sampling dates for mean nitrogen, phosphorus or potassium content of leaves from either site.

The *Arundo* rhizome samples showed considerable variation through the period, but statistical analysis revealed no significant differences between the sampling dates for mean nitrogen, phosphorus or potassium at either site.

The *Arundo* cane was the only plant part to show significant variation within the winter period. At Llwynprenteg the mean nitrogen content of the cane increased steadily throughout the period, and showed significant differences between the October sampling date and both November and January, and between the November samples and those from January ($P=0.001$). At Bluestone there was a significant increase in mean cane nitrogen during November, in comparison to October data ($P=0.021$), although no other significant differences were found.

The mean phosphorus content of the cane showed no significant differences between the sampling dates at Bluestone. However, at Llwynprenteg, there was a significant increase in mean phosphorus in January in comparison to both October and November (P=0.006). There were no significant differences for mean cane potassium content between the sampling dates at either site.

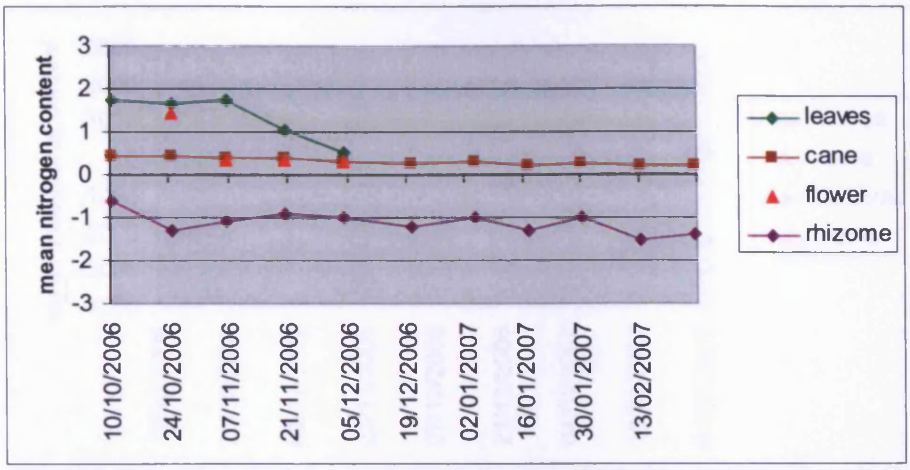

Figure 5.3: Mean nitrogen content (%w/w) of *Miscanthus* crop at Llwynprenteg during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

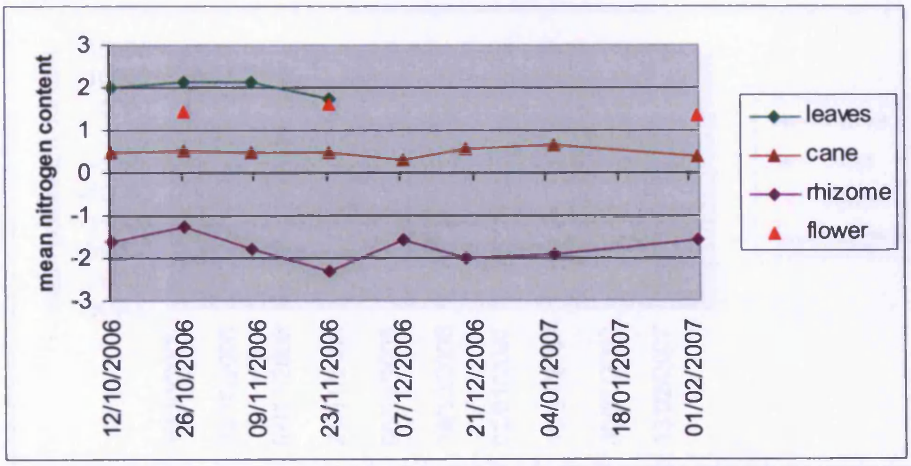

Figure 5.4: Mean nitrogen content (%w/w) of *Miscanthus* crop at Bluestone during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

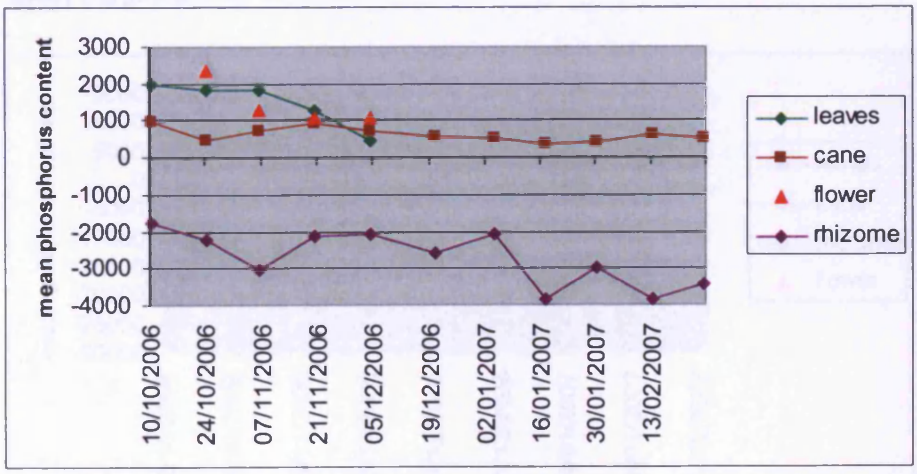

Figure 5.5: Mean phosphorus content (mg/kg) of *Miscanthus* crop at Llwynprenteg during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

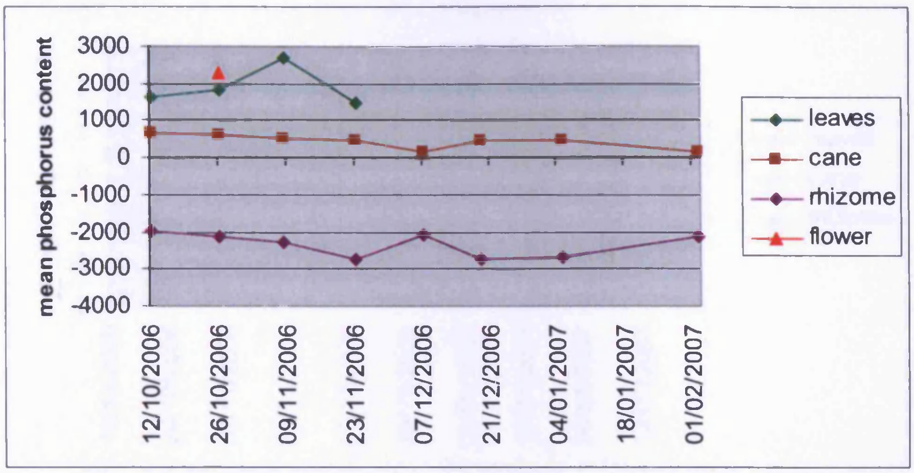

Figure 5.6: Mean phosphorus content (mg/kg) of *Miscanthus* crop at Bluestone during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

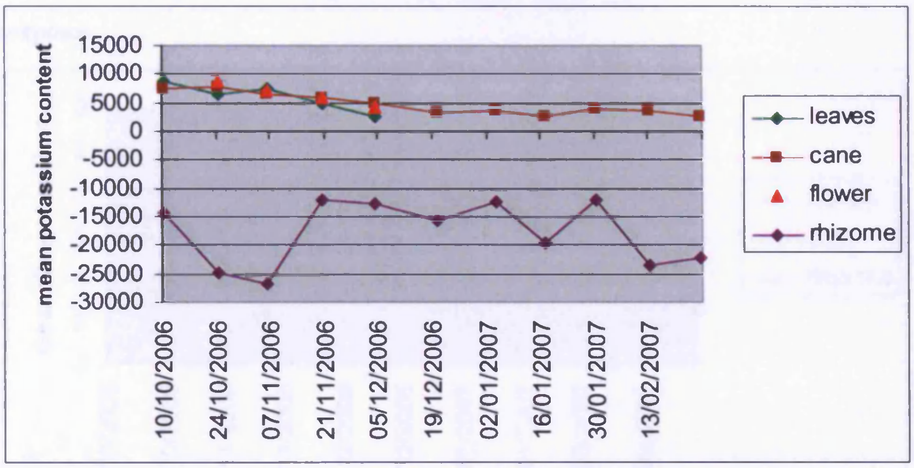

Figure 5.7: Mean potassium content (mg/kg) of *Miscanthus* crop at Llwynprenteg during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

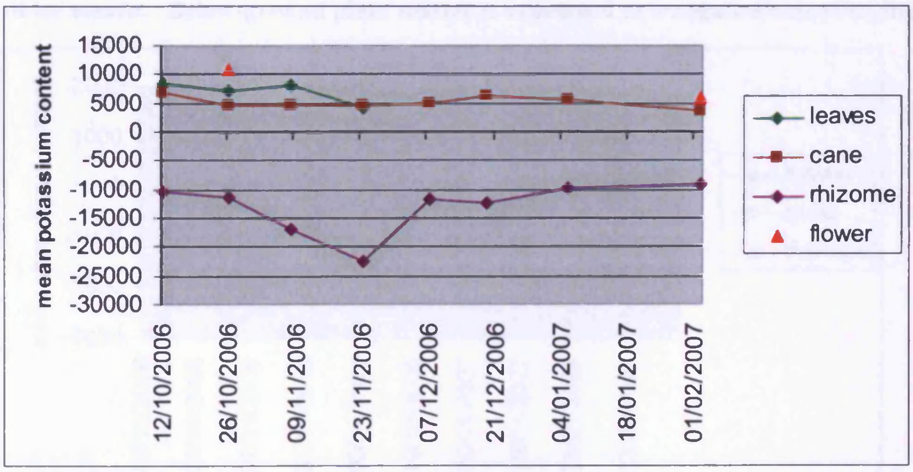

Figure 5.8: Mean potassium content (mg/kg) of *Miscanthus* crop at Bluestone during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

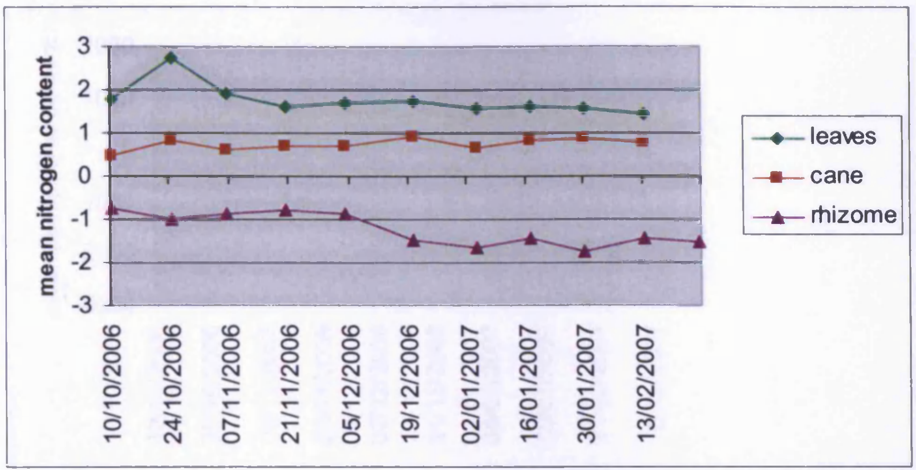

Figure 5.9: Mean nitrogen content (%w/w) of *Arundo* crop at Llwynprenteg during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

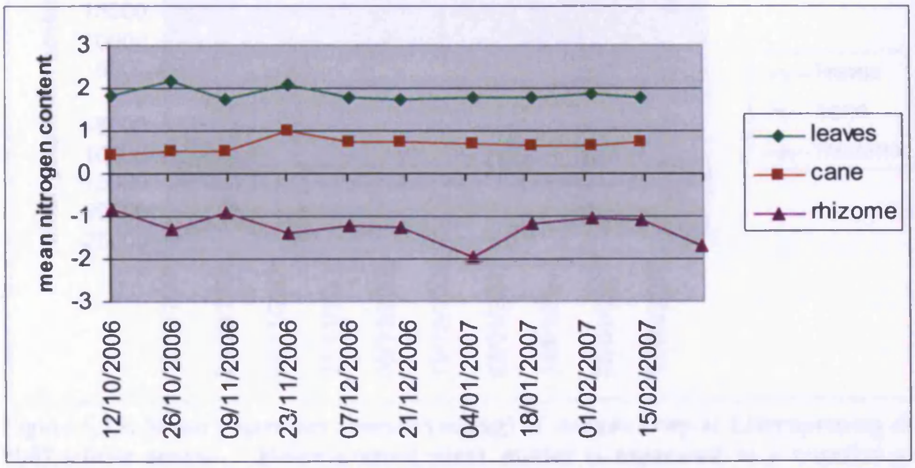

Figure 5.10: Mean nitrogen content (%w/w) of *Arundo* crop at Bluestone during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

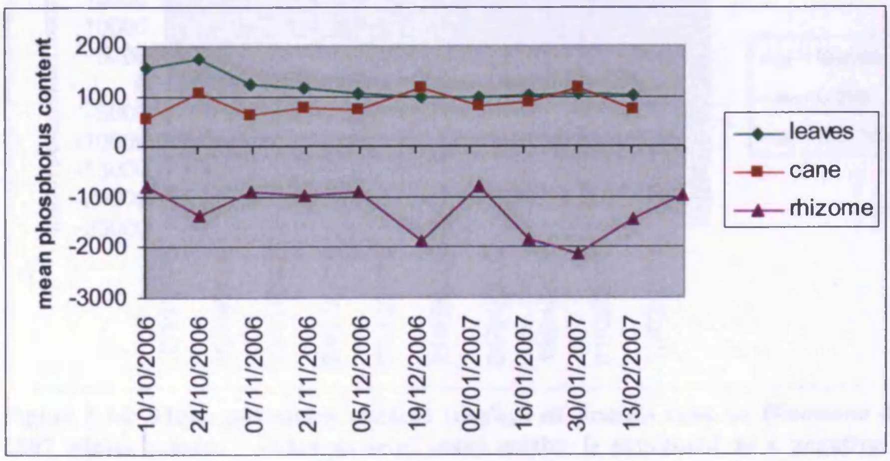

Figure 5.11: Mean phosphorus content (mg/kg) of *Arundo* crop at Llwynprenteg during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

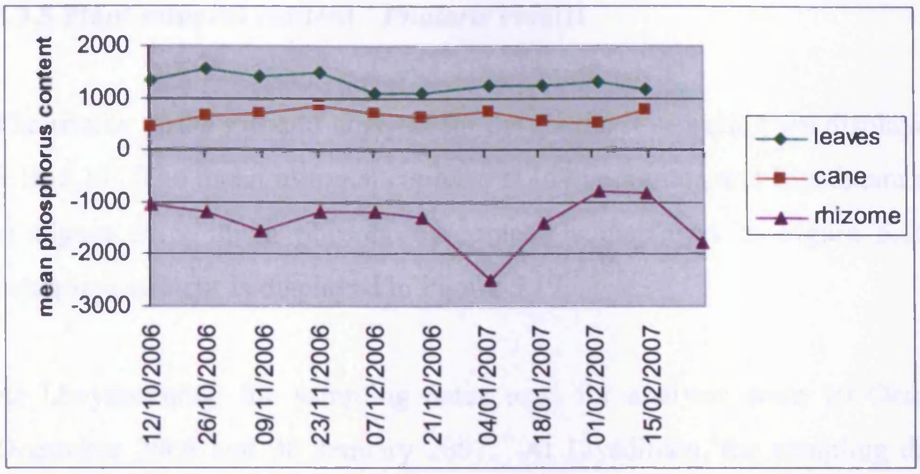

Figure 5.12: Mean phosphorus content (mg/kg) of *Arundo* crop at Bluestone during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

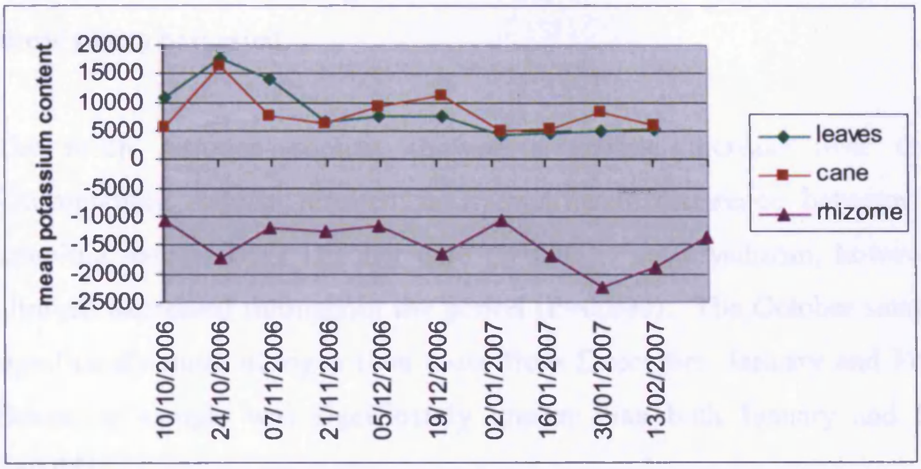

Figure 5.13: Mean potassium content (mg/kg) of *Arundo* crop at Llwynprenteg during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

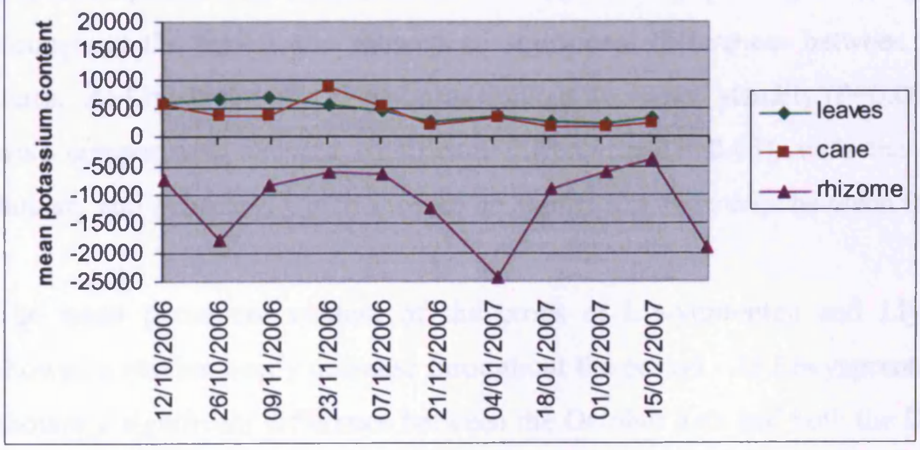

Figure 5.14: Mean potassium content (mg/kg) of *Arundo* crop at Bluestone during the 2006 – 2007 winter season. Below-ground plant matter is expressed as a negative value for display purposes.

5.3.5 Plant mineral content - *Phalaris* results

The results of the mineral analysis for the *Phalaris* sampling are displayed in Figures 5.15-5.17. The mean nitrogen contents at Llwynprenteg and Llysdinam are displayed in Figure 5.15, mean phosphorus content is displayed in Figure 5.16, and mean potassium content is displayed in Figure 5.17.

At Llwynprenteg, the sampling dates used for analysis were 10 October 2006, 4 December 2006 and 30 January 2007. At Llysdinam, the sampling dates used for analysis were 9 October 2006, 6 December 2006, 31 January 2007 and 27 February 2007. A February sampling date was not possible at Llwynprenteg, as the crop had already been harvested.

The mean nitrogen content showed a gradual increase over the period at Llwynprenteg, which resulted in a significant difference between the January sampling date and the October date ($P=0.05$). At Llysdinam, however, the mean nitrogen decreased throughout the period ($P=0.000$). The October sample contained significantly more nitrogen than those from December, January and February. The December sample was significantly greater than both January and February (all $P=0.05$).

The mean phosphorus content of the *Phalaris* at Llwynprenteg remained fairly stable throughout the period and showed no significant differences between the sampling dates. At Llysdinam, the phosphorus content decreased steadily ($P=0.000$). All pair-wise comparisons showed significant differences ($P=0.05$), with the exception of January and February, which showed no significant difference between the two.

The mean potassium content of the crops at Llwynprenteg and Llysdinam both showed a similar steady decrease throughout the period. At Llwynprenteg the results showed a significant difference between the October data and both the December and January data ($P=0.001$). At Llysdinam all dates were significantly different from each other ($P=0.000$).

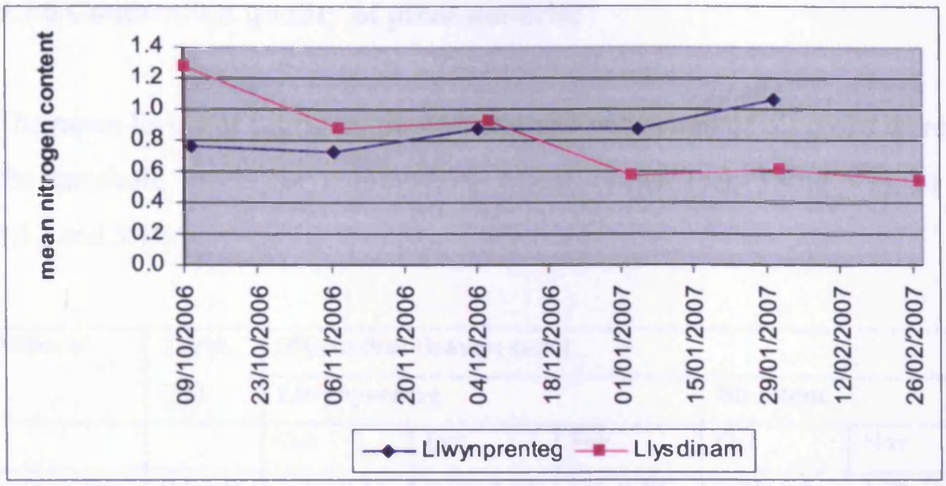

Figure 5.15: Mean nitrogen content (%w/w) of *Phalaris* crops at Llwynprenteg and Llysdinam during the 2006 – 2007 winter season.

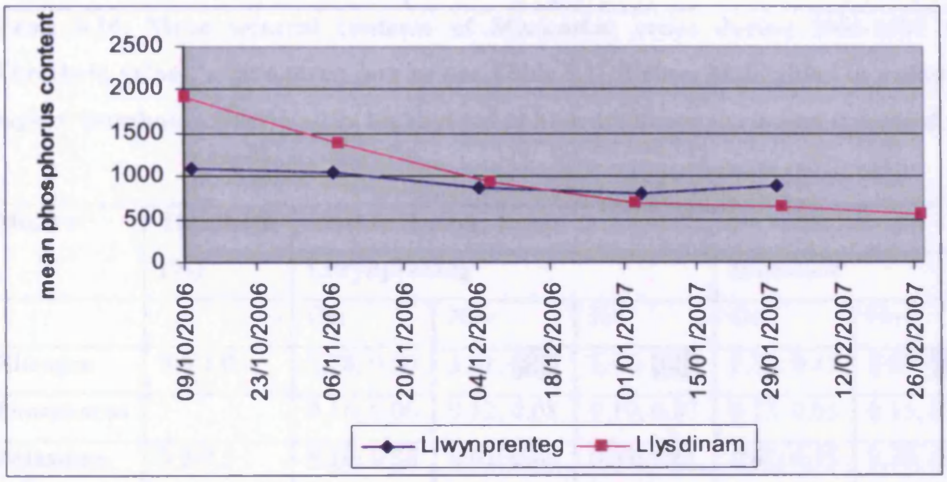

Figure 5.16: Mean phosphorus content (mg/kg) of *Phalaris* crops at Llwynprenteg and Llysdinam during the 2006 – 2007 winter season.

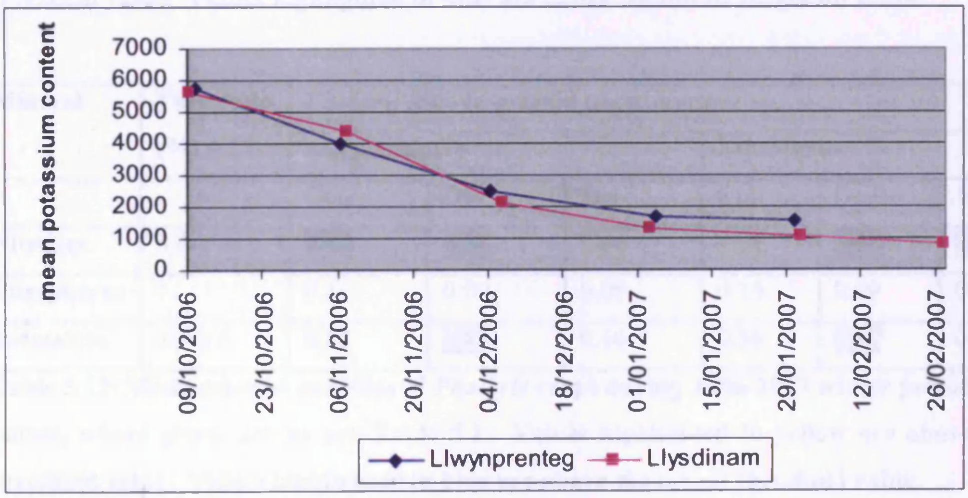

Figure 5.17: Mean potassium content (mg/kg) of *Phalaris* crops at Llwynprenteg and Llysdinam during the 2006 – 2007 winter season.

5.3.6 Combustion quality of plant material

The mean levels of nitrogen, phosphorus and potassium of all crops were compared to the threshold levels for combustion, where known (see Table 5.1), in Tables 5.10, 5.11 and 5.12.

Mineral	Thres. (%)	*Miscanthus* (leaves; cane)					
		Llwynprenteg			Bluestone		
		Oct	Dec	Feb	Oct	Nov	Feb
Nitrogen	0.6-1.0	1.76; 0.44	0.52; 0.32	n/a; 0.20	2.01; 0.46	1.76; 0.50	n/a; 0.37
Phosphorus	?	0.20; 0.10	0.05; 0.07	n/a; 0.05	0.16; 0.07	0.15; 0.05	n/a; 0.02
Potassium	0.2-0.5	0.89; 0.74	0.26; 0.50	n/a; 0.25	0.84; 0.68	0.41; 0.47	n/a; 0.37

Table 5.10: Mean mineral contents of *Miscanthus* crops during 2006-2007 winter period. Threshold values, where given, are as per Table 5.1. Values highlighted in yellow are above the highest threshold value. Values highlighted in blue are above the lowest threshold value.

Mineral	Threshold (%)	*Arundo* (leaves; cane)					
		Llwynprenteg			Bluestone		
		Oct	Nov	Jan	Oct	Nov	Feb
Nitrogen	0.6-1.0	1.78; 0.50	1.61; 0.70	1.42; 0.76	1.83; 0.43	2.09; 0.99	1.77; 0.73
Phosphorus	?	0.16; 0.06	0.12; 0.08	0.10; 0.07	0.13; 0.05	0.15; 0.08	0.12; 0.08
Potassium	0.2-0.5	1.10; 0.56	0.67; 0.64	0.52; 0.61	0.61; 0.55	0.57; 0.87	0.32; 0.25

Table 5.11: Mean mineral contents of *Arundo* crops during 2006-2007 winter period. Threshold values, where given, are as per Table 5.1. Values highlighted in yellow are above the highest threshold value. Values highlighted in blue are above the lowest threshold value.

Mineral	Threshold (%)	*Phalaris* (above-ground plant matter)						
		Llwynprenteg			Llysdinam			
		Oct	Dec	Jan	Oct	Dec	Jan	Feb
Nitrogen	0.6-1.0	0.76	0.88	1.06	1.27	0.93	0.61	0.54
Phosphorus	?	0.11	0.09	0.09	0.19	0.09	0.07	0.06
Potassium	0.2-0.5	0.58	0.25	0.16	0.56	0.21	0.11	0.08

Table 5.12: Mean mineral contents of *Phalaris* crops during 2006-2007 winter period. Threshold values, where given, are as per Table 5.1. Values highlighted in yellow are above the highest threshold value. Values highlighted in blue are above the lowest threshold value.

5.4 Discussion

5.4.1 Yield losses

The mean *Miscanthus* plant yields in both 2006-2007 and 2007-2008 were lower on the February sampling date than on the November / December sampling date at Llwynprenteg, Bluestone and Cae Clovers, although the differences were not significant. The losses occurred mainly as a result of leaf loss and stem breakage. At Coleg Sir Gâr there was a significant reduction in yield from November 2006 to February 2007. The greatest difference between yields was seen at Bluestone, where the February yield estimate was 43% less than its November value. At Llwynprenteg and Coleg Sir Gâr the February yield estimates were 32-33% less than the November values, and at Cae Clovers the difference was only 24%. The Llysdinam yield estimates did not differ between dates. These results paralleled the success of the crops in terms of plant yield, with the highest yield obtained at Bluestone and the lowest at Llysdinam and Cae Clovers. This suggests that, in the Welsh climate, a higher yielding *Miscanthus* crop suffers from a higher percentage winter biomass loss, which may be caused by the fact that higher yielding crops produce more leaves during the summer period and therefore lose more plant matter during senescence and leaf abscission.

The mean yield estimate at Llysdinam during 2007-2008 remained constant throughout the winter period, which may suggest that the plant had senesced prior to the initial sampling date. 2007 was a very short growing season for *Miscanthus* at Llysdinam due to early air frosts in October, so it is likely that the crop was killed at this point and had indeed senesced prior to the first sampling date of this study.

The results from the more successful crops at Bluestone, Llwynprenteg and Coleg Sir Gâr were similar to winter losses shown by the European *Miscanthus* Network Productivity trial, which showed spring yields to be on average 30-50% lower than their corresponding autumn yields (Clifton-Brown *et al.*, 2001). The greatest losses were observed in Ireland (up to 40%; Clifton-Brown and Jones, 2001), followed closely by trials in Hertfordshire, UK (35%; Yates and Riche, 2007) and Germany (up to 34%; Lewandowski and Heinz, 2003).

There were no significant differences between the February sampling dates and the November / December sampling dates for the *Arundo* data, although a reduction in mean plant yield was recorded at all sites except Llysdinam. The highest difference was 70% at Llwynprenteg in 2006-2007, although the difference in 2007-2008 was 30%. At Coleg Sir Gâr the difference between the two sampling dates was 43-44% in both years, whereas at Bluestone a difference of 43% was seen in 2006-2007 and 7% in 2007-2008. This provides evidence that leaf losses and/or stem breakage occurred within this crop.

The differences between the November and January sampling dates for the *Phalaris* crops were not significant, although the mean dry weight per area was lower for the later date at both sites. The yield loss was caused by leaf loss and stem breakage, as also shown in Hertfordshire, UK (Christian *et al.*, 2006). At Llysdinam a reduction of 30% mean dry weight was seen during the period, and at Llwynprenteg the reduction was 39%. These results are similar to those found at Hertfordshire, UK, which showed a 37% decrease in yield measured from December to February (Yates and Christian, 2001), and a 66% decrease from late July to February (Yates and Riche, 2007).

5.4.2 Moisture losses

The *Miscanthus* crops in 2006-2007 lost between 19 and 61% of their moisture content between the November and February sampling dates. The lowest moisture loss (19%) was recorded at Llysdinam, and was also recorded by a trial in Ireland between the date of the first frost (October) and a late March harvest (Clifton-Brown and Jones, 2001). However, the values at Llysdinam appeared to fall from November to January, and then to increase in February, and this is likely to be an effect of weather conditions. This site experienced very high precipitation (nearly 250mm, in comparison to nearly 150mm at Bluestone) during January 2007, which may have caused the senesced plant material to take up additional moisture. At Coleg Sir Gâr the moisture loss was 61%, and a loss of 42% was recorded at Llwynprenteg. These levels were similar to losses recorded in German studies, where moisture content reduced by 52% between December and February and a further 12% by March (Lewandowski and Heinz, 2003). Studies in Hertfordshire, UK, showed reductions of approximately 34% between October and February (Clifton-Brown *et al.*, 2001) and lower losses of 11-28% were reported from Austria (Clifton-Brown *et al.*, 2001).

The *Miscanthus* crop at Bluestone lost 23% of its moisture content between the end of November and the beginning of December, when access problems prevented further sampling. It is highly likely that the moisture would have continued to decrease through December. Extrapolation of the data to provide an estimated value for the end of February provides an estimated moisture loss of 33% between the November sampling date and the end of December. This was lower than the losses incurred at Llwynprenteg and Coleg Sir Gâr, which could be explained by the increased shoot number and uniformity of the crop at Bluestone, which may have provided protection from exposure to climate effects.

In the 2007-2008 sampling period, the moisture losses at Llysdinam (24%) and Llwynprenteg (49%) were similar to the previous year, but the crop at Coleg Sir Gâr experienced a much lower loss (10%). The *Miscanthus* at Cae Clovers was sampled during this period, and the moisture loss between December and February was 21% at this site. Moisture losses occur naturally throughout the winter period in *Miscanthus* crops as the plants senesce.

The *Arundo* crops showed a significant reduction in moisture content from late November to late February at all sites in 2006-2007. However, there were no differences in moisture content in 2007-2008. The losses calculated for 2006-2007 were 11, 13, 27 and 30% at Coleg Sir Gâr, Llysdinam, Bluestone and Llwynprenteg respectively. This crop therefore showed some evidence of senescence during the first sampling period, as the crop dried out slightly at all sites, but showed no evidence of senescence during the second year. This suggests that as the crop ages it is able to continue growing for a longer period, and shows no signs of winter senescence. This requires further study, as low moisture content is an essential requirement of energy crops. Luxton (pers. comm.) showed that harvested *Arundo* crops left to dry outdoors steadily lost moisture between March and June to August, by which time moisture content was below 25% (unpublished results), so this may be an inexpensive drying option for the crop.

The *Phalaris* crops at both sites experienced the largest moisture losses of all crops in this study. The moisture content differences from November 2006 to January 2007 were 66 and 76% at Llwynprenteg and Llysdinam respectively. These values are similar to those reported from Sweden, which were 75% moisture loss at northern sites and 71% at southern sites (Landstrom *et al.*, 1996), which were greater than the 52% losses shown in Hertfordshire, UK (Christian *et al.*, 2006). Studies have shown that *Phalaris* continues to grow during the winter period, resulting in new green stems present in the harvested material which increases its moisture content (Christian *et al.*, 1999; Christian *et al.*, 2006). Green shoots were also present in this study but they were very small and did not result in much increased moisture within the harvested material.

Phalaris was the only crop that consistently dried out sufficiently to meet power station moisture content standards. The *Miscanthus* and *Arundo* crops would both require drying prior to combustion, which would potentially create additional economic and energy costs, although Lewandowski *et al.* (2003) showed field drying of *Miscanthus* to reduce both moisture and nutrient content.

5.4.3 Calorific value differences

The calorific value of all crops was unaffected by harvest date, with the exception of an increased value for the *Miscanthus* crop at Llwynprenteg. This increase was minimal (from 17.74 to 18.27 MJ/kg), therefore calorific value does not need to be considered with regard to harvest date.

5.4.5 Plant mineral content

The leaf nitrogen, phosphorus and potassium contents of *Miscanthus* at Llwynprenteg fell rapidly prior to leaf abscission at Llwynprenteg, but not at Bluestone. The cane nitrogen and phosphorus contents fell gradually over the winter period at both sites, whereas the mean potassium only showed a significant decline at Llwynprenteg. At

Bluestone the rhizome nitrogen, phosphorus and potassium contents fluctuated over the period, but showed no significant changes, whereas at Llwynprenteg, mean nitrogen increased slightly over the period, mean phosphorus fluctuated more erratically whilst still showing an overall significant increase, and mean potassium fluctuated erratically but showed no significant differences.

The majority of studies in the literature report a decline in above-ground *Miscanthus* nutrient content over winter (Clifton-Brown and Jones, 2001; Clifton-Brown *et al.*, 2001; Himken *et al.*, 1997; Jorgensen, 1997; Lewandowski and Heinz, 2003). However, studies that included early sample dates have found that nutrient levels peak in spring and early summer, and decline thereafter (Beale and Long, 1997), with the most rapid decline occurring between June and August, in Denmark (Jorgensen, 1997). Hertfordshire studies have suggested that flowering indicates the timing of nutrient movement within the plant, although *Miscanthus* rarely flowers in that area (Yates and Riche, 2007). Their study showed little variation in *Miscanthus* nutrient composition between November and February, and suggest that translocation and leaching may have occurred earlier. This study has shown that, at these sites, many plants produced flowers throughout the winter period. The timing of flower emergence was not studied, but generally it occurred during September (personal observations). This would suggest that the plants had little time to translocate nutrients prior to the sampling period of this study; however a detailed study of the plants' nutrient composition through the year would be necessary to clarify the process. In this study sampling of the crop was carried out during the period October to February to coincide with visual signs of senescence, when the plants began to lose their green colouring.

Other studies have shown the decline in nitrogen within above-ground plant matter to be most likely due to translocation of the nutrients to the roots and rhizomes (Beale and Long, 1997) as it mostly occurs as a fixed element that is not leached during precipitation (Jorgensen, 1997). It is likely to occur during plant senescence following the breakdown of chlorophyll. This study showed a slight increase in rhizome nitrogen during the sampling period at Llwynprenteg, but no significant increase at Bluestone, which suggests that the nitrogen may have been lost in leaf litter at the latter site. Potassium mostly occurs in plants as soluble ions, which are easily leached by precipitation (Jorgensen, 1997), and this is likely to be the primary cause of winter loss (Chapin *et al.*, 1980). Unfortunately there are no rainfall data available for Llwynprenteg, but Bluestone received less rainfall than Llwynprenteg's nearby site at Cae Clovers during the winter of 2006-2007. Therefore, differing levels of precipitation may explain loss of potassium from plant matter at Llwynprenteg but not at Bluestone. Some translocation of potassium has been shown in other studies, as has translocation of phosphorus (Beale and Long, 1997), although studies of poplar suggest that phosphorus is mostly lost in leaf litter (Kauter *et al.*, 2003). This study showed an increase in rhizome potassium content prior to leaf abscission at both Bluestone and Llwynprenteg, which suggests that this mineral was translocated from the above-ground plant matter. However, the increase in rhizome phosphorus at Llwynprenteg occurred subsequent to leaf abscission, which suggests that, at this site, it was taken up from the leaf litter for rhizome storage.

Frost has been shown to kill the above-ground plant matter, and thereby to stop remobilisation of nutrients from leaves and stems (Christian *et al.*, 2008). Bluestone experienced successive frosts from 1 November to 3 November 2006, which reached -2.3°C. Llwynprenteg experienced a succession of frosts from 1 November to 5 November, which reached a minimum of -4.2°C. Both sites were thereafter frost-free until mid December. Therefore Llwynprenteg was exposed to the worst frost conditions during November, but at this site the leaves subsequently rapidly decreased in nitrogen and phosphorus content before dropping off the plant. At Bluestone nitrogen declined only slightly, and phosphorus, which had increased prior to the November frost, subsequently fell to a level no different to its October value. These data suggest that either the frost period did not kill the plants, but encouraged translocation, or that the minerals were leached rapidly out of the plant matter after it had been killed by frost. Himken *et al.* (1997) found 35-51% of nitrogen, phosphorus and potassium lost from the plant's leaves to be in senescent leaves on the ground, which indicates that nutrients were not all translocated.

The rhizome data showed an increase in nitrogen and phosphorus content at Llwynprenteg only. These results concur with other studies, which have shown an increase in both nitrogen and phosphorus over winter (Beale and Long, 1997). Himken *et al.* (1997) showed nitrogen to increase from September to November but not thereafter, and phosphorus to be constant prior to increasing from November to March. All increases were attributed to remobilisation of nutrients from the above-ground plant matter. The potassium content of the *Miscanthus* rhizomes varied substantially but showed no overall increase or decline. These results are in keeping with Beale and Long (1997).

The samples collected from the *Arundo* crops showed no differences in leaf or rhizome content of nitrogen, phosphorus or potassium throughout the winter period. The cane samples showed nitrogen content to increase at both sites, and phosphorus to increase at Llwynprenteg only. These results suggest that the crops continued to take up nutrients through the winter, and were not beginning to senesce. It has been shown in the Mediterranean that a proportion of this plant remains green during the winter period (El Bassam, 1998). Although the differences were not significant, the mean nitrogen and mean phosphorus content of the rhizomes from Llwynprenteg were higher in January than in October, which suggests that some translocation may have occurred during this period. The variation in the crop may have been the reason for the results to be non-significant, and therefore a larger study with more samples would be necessary to see clearer patterns. The apparent lack of senescence in *Arundo* crops would cause problems for its use as an energy crop, as its harvested plant material contained high levels of minerals.

The *Phalaris* crops at both Llwynprenteg and Llysdinam displayed a loss of potassium content in above-ground plant matter throughout the period from October until February. This was probably a result of leaching from the senescing crop, as discussed above, and was also found in other studies of *Phalaris* (Burvall, 1997; Christian *et al.*, 2006). The phosphorus content of the Llwynprenteg crop remained unchanged, but its nitrogen content increased over the period. At Llysdinam however the phosphorus and nitrogen content decreased during the winter. This suggests the two crops may have been at different stages of senescence, and that the Llwynprenteg *Phalaris* was still taking up nitrogen for further plant growth. However both crops showed a similar decrease in moisture during the period, which suggest that overall crops were indeed senescing. It is possible therefore that the Llwynprenteg crop continued to grow during the winter season which resulted in new shoots being included in the harvested material, as shown by other studies (Christian *et al.*, 1999;

Christian *et al.*, 2006). This re-growth was sufficient to increase mineral content levels above the threshold at Llwynprenteg. The differences between the two sites were likely to be caused by decreased winter temperatures at Llysdinam, which resulted in less new shoot growth. Therefore this crop may be better suited to colder temperatures for energy crop purposes.

Swedish studies have shown the mineral content of *Phalaris* to be related to the soil type, with increased minerals in clay soils (Landstrom *et al.*, 1996; Burvall, 1997). In this study, however, increased mineral content was shown at Llwynprenteg, which is a clay loam, in comparison to Llysdinam, which has high clay content. The majority of other studies have shown a decrease in *Phalaris* nutrient content during winter (Burvall, 1997; Hadders and Olsson, 1997; Yates and Christian, 2001; Christian *et al.*, 2006), although Landstrom *et al.* (1996) showed the nitrogen decline to occur from August to October but not thereafter, and Yates and Riche (2007) recorded an initial nitrogen decline from July to October, followed by a lesser decline from November to January. The latter study suggested remobilisation of nutrients probably occurred during late summer.

5.4.6 Combustion quality of plant material

The mean levels of nitrogen, phosphorus and potassium of all crops were compared to the threshold levels for combustion, where reported. The *Miscanthus* leaves contained greater mean nitrogen content than the threshold value in October, and both the cane and leaves had potassium values higher than the threshold. At Llwynprenteg, but not at Bluestone, the leaf nitrogen fell below the threshold prior to leaf fall. It therefore may be necessary to delay harvest of *Miscanthus* crops until after leaf abscission to avoid excessive nitrogen content in the harvested material. The potassium content fell below the highest threshold level in November/ December, but was still higher than the lowest threshold level reported in February. It may therefore be necessary to adjust power station operations to accommodate higher levels, in order to use *Miscanthus* as a combustion fuel, although Monti *et al.* (2008) showed *Miscanthus* stems to carry mineral contents below threshold levels. However field drying of both *Miscanthus* and *Arundo* crops may be sufficient to reduce nutrient content, as has been shown for the former crop (Lewandowski *et al.*, 2003).

The *Arundo* leaves had higher nitrogen contents than the threshold level on all sampling dates. Monti *et al.* (2008) showed *Arundo* to have increased leaf and cane nitrogen in comparison to *Miscanthus*. The fact that *Arundo* crops did not lose their leaves during the winter period may cause problems for its use as a fuel crop, as their high mineral content is unsuitable for combustion purposes. *Arundo* has also been shown to conserve live stems during the winter period (Monti *et al.*, 2008). The nitrogen content of the *Arundo* cane in this study fell below the highest threshold value, but remained higher than the lower value throughout the period. If it were possible to compensate for this lower level within the power station, then the canes may have a combustion use. It seems likely that the use of desiccants would be necessary to encourage leaf loss, which would increase the financial and energy costs of the crop. The leaves and canes also contained a higher potassium level than the threshold value on all dates, with the exception of February sampling at Bluestone. Further research is necessary to determine if the mineral levels decrease in later spring, in addition to study of the date of new shoot emergence, to determine if there is a time period more suited to *Arundo* harvest.

The *Phalaris* crops contained more potassium than the threshold level at both sites in October, and then fell below the lower level in January. Therefore, in terms of potassium, *Phalaris* was found to be the only crop suitable for combustion. At Llysdinam the nitrogen content fell likewise, and was below the lower threshold level in February. However, at Llwynprenteg, nitrogen levels increased throughout the winter period, and its January value was higher than the top threshold level. This crop clearly needs to be studied further to investigate its mineral cycling more fully. Other studies have shown *Phalaris* to have decreased potassium content at a January harvest in comparison to *Miscanthus*, but similar levels of nitrogen and phosphorus (Christian *et al.*, 1999).

5.5 Conclusions

All sites except Llysdinam experienced yield losses during the winter period, which were 24 to 43% for *Miscanthus*, 7 to 70% for *Arundo* and 30 to 39% for *Phalaris*. The moisture content of *Miscanthus* fell during the winter periods studied, although the amount of moisture lost varied from 10 to 61%. The *Arundo* crops lost between 11 and 30% moisture during the 2006 – 2007 winter period, but no moisture loss was observed in 2007 – 2008. The greatest moisture loss was recorded in the *Phalaris* crops, which lost 66 to 76%, and these were subsequently the only crops that achieved a final moisture content of less than 25%, thus being suitable for immediate combustion.

In terms of yield and moisture content, *Arundo* was clearly the least uniform of the crops, thus creating difficulty in assessing its potential as an energy crop in Wales. Further long term study of this crop is clearly needed. *Miscanthus* produced higher yields than *Phalaris* during this study, although moisture losses were not sufficient to produce a crop suitable for immediate combustion. This crop would therefore require drying and storage prior to combustion, when grown under Welsh climate conditions.

The most important factor influencing the optimum harvest date of energy crops will be the mineral content thresholds as determined by power stations. The thresholds referred to in this study suggest that *Miscanthus* crops should be harvested from December onwards, after leaf abscission has occurred. *Arundo* crops in this study did not meet these thresholds, and further investigation is required to determine if nutrient losses occur at a later date. The *Phalaris* at Llysdinam met the threshold levels from December onwards, but further study is required to determine the reason for the nitrogen increase recorded at Llwynprenteg. However this study relates to *Miscanthus* plants aged 3 – 5 years old, *Arundo* plants 2 – 3 years old and *Phalaris* plants 3 – 4 years old, and therefore the majority of these crops were not fully mature. Other studies have shown differences between mineral composition of young and mature plants (Monti *et al.*, 2008). Further long term studies are therefore required.

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Chapter 6

Determination of Plant Senescence Status, Nutrient Content and Predicted Crop Yield in *Miscanthus* and *Arundo Donax*, using a Hand-held Chlorophyll Content Meter.

6.1 Introduction

During senescence of *Miscanthus*, nutrients are translocated from the stem and leaves to the rhizome, where they are stored for the following year's re-growth. It is known that, in *Populus* spp, this process is important in particular for the recycling of nitrogen, magnesium and potassium, whereas the less mobile element phosphorus is recycled mostly by leaf litter decomposition into the soil (Kauter *et al.*, 2003). In *Miscanthus*, however, translocation of potassium, magnesium and phosphorus has been shown to occur during winter (Himken *et al.*, 1997), as well as translocation of nitrogen (Beale and Long, 1997). During senescence the chlorophyll molecules are broken down, resulting in the change of plant colour from green to its autumnal foliage colour. Breakdown of the chlorophyll molecules represents the plant's decrease of photosynthetic rate, thus the chlorophyll content of a plant is an indicator of the plant's productivity (Keskitalo, 2006). The chlorophyll molecule consists of 1 magnesium atom and 4 nitrogen atoms, which are released upon its catabolism. Chlorophyll content can thus be used as an estimate of nitrogen content within a plant. Shaahan *et al.* (1999) used chlorophyll content to successfully predict the nitrogen content of mango, mandarin, guava and grapevine. However, the chlorophyll measurements did not successfully predict magnesium content of all the plants. There are no specific senescence studies of *Arundo* in the literature, although it is generally accepted that this crop translocates nutrients from leaves to below ground structures in late summer or early autumn in the USA, and that this occurs prior to leaf senescence (Decruyenaere and Holt, 2001).

Many studies have shown chlorophyll meter readings to be correlated with nitrogen content of leaves (Parvizi *et al.*, 2004; Gaborcik, 2003; Madakadze *et al.*, 1999). Fewer studies have investigated relationships between leaf chlorophyll content and other nutrients. With energy grass crops it is important to understand the recycling of many elements during senescence of the crop. Recycling of elements is important for ongoing crop success, as nutrients are stored in the rhizomes during crop senescence or recycled via leaf litter decomposition for re-use during the following year's growth. This reduces the financial and environmental costs of fertiliser applications, and has also been shown to increase winter survival in grass crops (Smith, 1972).

Knowledge of nutrient translocation is also important in terms of fuel quality, as it is necessary to ensure nutrients are as low as possible if a crop is to be used for combustion. High mineral concentrations cause problems of slagging, fouling and corrosion within the power plant, as discussed in the previous chapter.

Several studies have investigated the usefulness of a chlorophyll content meter to determine the differences between nitrogen treatments (Girma *et al.*, 2006; Scharf *et al.*, 2006; Parvizi *et al.*, 2004; Cate and Perkins, 2003; Madakadze *et al.*, 1999). This type of study is useful when considering the uptake of nitrogen application by crops. The correlation between chlorophyll meter readings and nitrogen content has been used to optimise nitrogen fertilisation rates in winter wheat (Denuit *et al.*, 2002; Spaner *et al.*, 2005; Arregui *et al.*, 2006), potato crops (Denuit *et al.*, 2002), and spring barley (Spaner *et al.*, 2005), amongst others. However, not all studies have shown a strong correlation between chlorophyll content and plant nitrogen, due to the fact that chlorophyll molecules contain only 2% of the total leaf nitrogen (Lawler *et al.*, 1997).

Many studies have shown a correlation between leaf chlorophyll content and crop yield (Le Bail *et al.*, 2005; Girma *et al.*, 2006; Scharf *et al.*, 2006), which suggests that a chlorophyll content meter can be used as a yield prediction tool. This is because of the strong relationship between the plant's nitrogen content and its subsequent yield, which depends on the amount of photosynthesis achieved during the growing season (Clifton-Brown and Jones, 1997).

The SPAD 502 hand-held chlorophyll meter (Minolta) was used by Earl and Tollenaar (1997) to successfully estimate photosynthetic rates of maize. Chlorophyll meters have been used extensively in all areas of crop research over the past ten years, following their development in the 1960s.

This study uses the Opti-Sciences CCM-200 chlorophyll content meter. This device measures optical absorbency at two wavelengths; 665nm (red) and 940nm (infrared). This provides a chlorophyll content index (CCI) value, which is proportional to the amount of chlorophyll present in the plant material. The value accounts for bulk leaf absorbency, hence factoring in the effect of leaf thickness. Cate and Perkins (2003) showed that CCI values obtained from the CCM-200 chlorophyll content meter were strongly correlated with chlorophyll concentration values obtained from laboratory testing of the leaves of sugar maple (*Acer saccharum*).

The aims of this study were as follows:
To document the senescence of *Miscanthus* and *Arundo* crops in terms of chlorophyll degradation.
To determine if a chlorophyll content meter could be used to track nutrient translocation in *Miscanthus* and *Arundo* crops.
To determine if a chlorophyll content meter could be used to determine the uptake of nitrogen after fertilisation of *Miscanthus* and *Arundo* crops.
To determine if a chlorophyll content meter could be used to predict crop yields of *Miscanthus* and *Arundo*, and thereby be used further as an indicator of site suitability for growing the two crops.

6.2 Methods

6.2.1 Chlorophyll and mineral content measurement

Miscanthus and *Arundo donax* crops were studied at Llwynprenteg, Bluestone and Llysdinam (see Chapter 2 for full site descriptions).

Thirty random shoot height measurements were recorded within the crop. Shoot height was measured from the ground to the topmost leaf ligule. The interquartile range of shoot heights was calculated manually. Three random shoots whose height fell within the calculated interquartile range were used for further analysis. The chlorophyll content index (CCI) was recorded for the bottom 2 green leaves, middle 2 green leaves and the top 2 green leaves of the shoot using an Opti-Sciences CCM-200 chlorophyll content meter. Readings were taken at a distance of 5cm, 10cm and 15cm from the tip of each leaf, and the 3 measurements were averaged to obtain a mean leaf CCI. No brown leaves were used for CCI readings.

The shoot was then cut at the base and retained, the rhizome was dug up and a section was removed for chemical analysis. This procedure was repeated every two weeks from mid October to early February, although chlorophyll readings ceased when all leaves were brown.

The three shoots from each of the *Miscanthus* and *Arundo* crops at Llwynprenteg and Bluestone were dissected into the following sections: bottom 2 leaves, middle 2 leaves, top 2 leaves, cane with all leaves removed and rhizome sample. All sections were oven dried at 80°C to constant weight, then sent to Natural Resource Management Ltd, Berkshire for mineral content determination. Plant and rhizome samples were analysed for total nitrogen, total phosphorus, potassium, calcium and magnesium content. Financial constraints prevented analysis of a third site, so samples from the lowest yielding crop (Llysdinam) were not analysed.

6.2.2 Nitrogen fertiliser trial

A nitrogen fertiliser trial was set up within the *Miscanthus* crop at Llysdinam, as described in Chapter 7. This was used to determine the usefulness of a chlorophyll content meter in assessing the differences between nitrogen treatments. The crop was divided into 42 sub-plots measuring 2.5m^2, with a border of a minimum of 2.5m around each plot, and each sub-plot was randomly allocated a fertiliser treatment. For the purpose of this chlorophyll study, the 4 replicated sub-plots of each of the 3 inorganic nitrogen application levels were studied, in addition to the 4 replicated control sub-plots. The inorganic nitrogen application levels were low (37.5kg N/ha), medium (50kg N/ha) and high (87.5 kg N/ha), and were applied as ammonium nitrate during spring 2006. The control plots received no treatment.

During September, November and December 2006, chlorophyll readings were taken from the bottom 2 leaves, middle 2 leaves, and top 2 leaves of all shoots within a randomly placed 0.5m^2 quadrat within each sub-plot. All readings were taken at a distance of 5cm from the tip of the leaf. Shoot height measurements were recorded on the same occasions, measured from the ground to the topmost leaf ligule.

The sub-plots were harvested in March 2007. All shoots from within the sub-plot were chopped at the base, and the fresh weight of this bundle was measured. Ten shoots were removed from each bundle, weighed fresh and then oven-dried at 80°C to constant weight. Mean shoot moisture content was calculated, and applied to the sub-plot fresh weight to obtain an estimate of each sub-plot dry weight.

6.2.3 Data analysis

6.2.3.1 Chlorophyll content measurements

The two mean leaf CCI measurements for each bottom leaf, each middle leaf and each top leaf, were averaged to obtain a mean reading for bottom, middle and top leaves per shoot. The measurements from each shoot per site were averaged to obtain mean bottom, middle and top leaf CCI for each crop on each occasion. An average of all six mean leaf measurements was obtained for the purpose of "all leaves" calculations.

6.2.3.2 Statistical analysis

Correlation analysis was used to analyse relationships between leaf chlorophyll content and nitrogen, phosphorus, potassium, calcium and magnesium content. Correlation analysis was used to investigate relationships between the mean CCI of all leaves and the nitrogen, phosphorus, potassium and magnesium content of the rhizome sample at each date. Data from the two sites Llwynprenteg and Bluestone were combined for rhizome analysis.

Analysis of variance was used to determine differences between the treatment groups from the inorganic fertiliser trial. Data were transformed as necessary to meet the assumptions of the test. For data that did not fulfil ANOVA assumptions after transformation, the non-parametric Kruskal-Wallis test was used. Pair-wise differences were further determined by Fishers' a priori tests (for parametric data) or Mann Whitney tests (non-parametric data). Analysis was repeated during July, September and November.

6.2.3.3 Chlorophyll content and yield

The *Miscanthus* fertiliser trial was used to investigate relationships between leaf chlorophyll content and crop height and yield. All six leaf chlorophyll readings per shoot were averaged to obtain a mean chlorophyll index per shoot for each sub-plot. Mean shoot height was determined for each sub-plot, discounting any shoots =50cm. Total plant height was determined as the sum height of all shoots within a plant. July data were used, as these data showed the greatest variation in chlorophyll readings between groups. Correlation analysis was used to analyse relationships between mean shoot chlorophyll indexes and mean shoot height, mean total plant height and sub-plot dry weight. The relationships were tested with regression analysis to determine if chlorophyll content can be used as a tool for yield predictions.

6.3 Results

6.3.1 Chlorophyll content measurements

6.3.1.1 *Miscanthus*

In *Miscanthus* crops the chlorophyll content of leaves fell rapidly from October to early November then further declined to late November / early December, at which point all leaves were either brown or had fallen off (Figures 6.1 to 6.3). Prior to its autumnal decline, the chlorophyll content of middle leaves was higher than the chlorophyll content of top and bottom leaves at all sites.

6.3.1.2 *Arundo donax*

In *Arundo*, the chlorophyll content of leaves fluctuated throughout the season, although there was a general downward trend in chlorophyll content from August onwards (Figures 6.4 to 6.6). The chlorophyll content index did not fall as low as for *Miscanthus* and remained above 5 as late as February, whereas *Miscanthus* CCI dropped to below 10 by late November and below 3 by early December. The chlorophyll content of middle and top leaves was higher than the chlorophyll content of bottom leaves until very late senescence (January 2007).

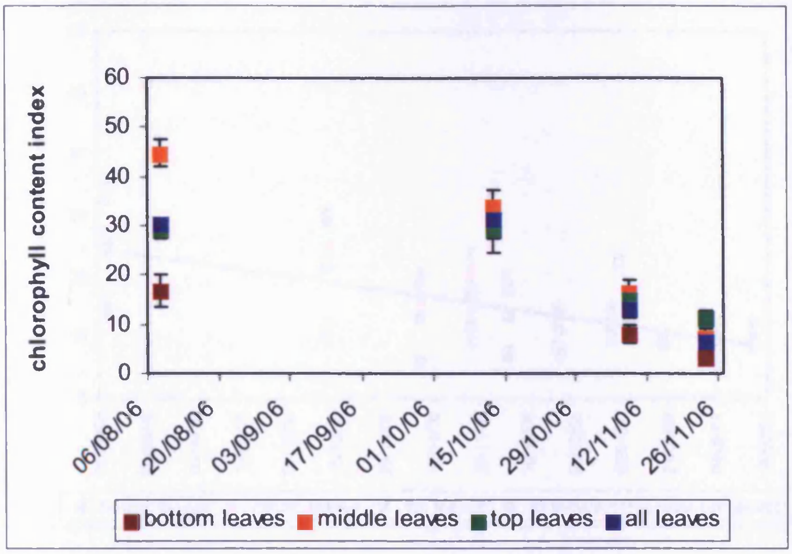

Figure 6.1: Mean chlorophyll content index ± 1 SEM for *Miscanthus* leaves at Bluestone during 2006.

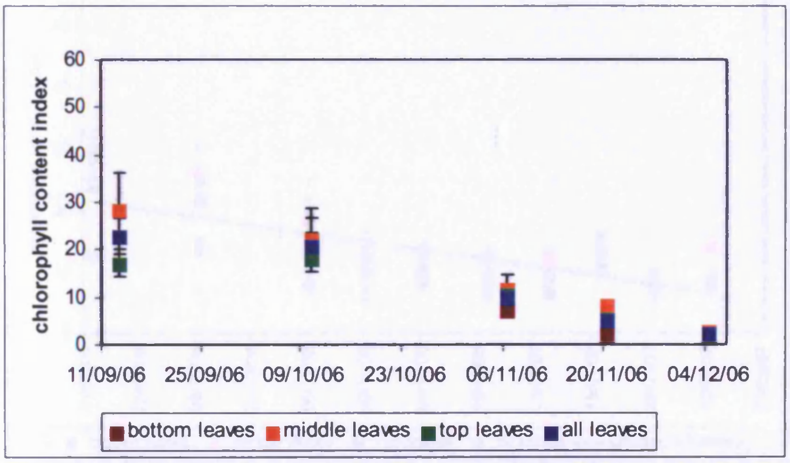

Figure 6.2: Mean chlorophyll content index ± 1 SEM for *Miscanthus* leaves at Llwynprenteg during 2006.

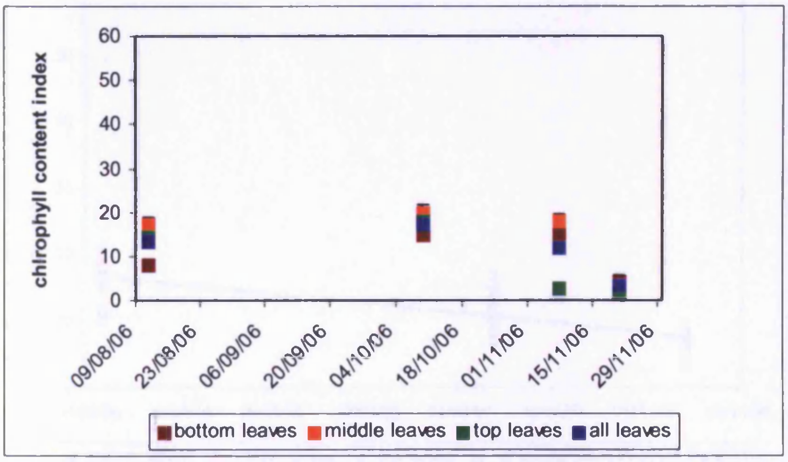

Figure 6.3: Mean chlorophyll content index ± 1 SEM for *Miscanthus* leaves at Llysdinam during 2006.

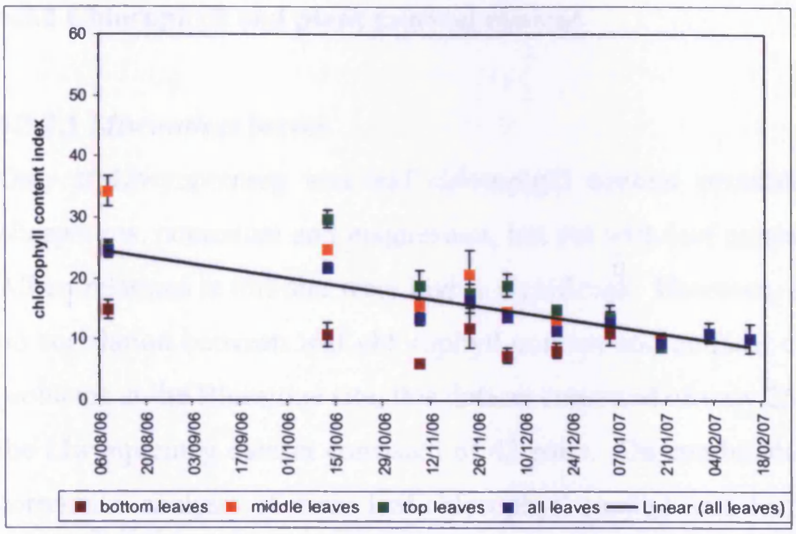

Figure 6.4: Mean chlorophyll content index ± 1 SEM for *Arundo* leaves at Bluestone during 2006.

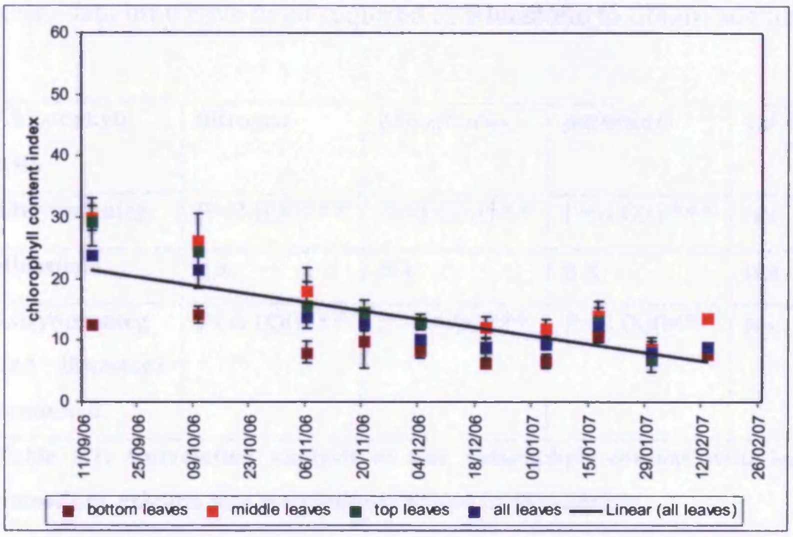

Figure 6.5: Mean chlorophyll content index ± 1 SEM for *Arundo* leaves at Llwynprenteg during 2006.

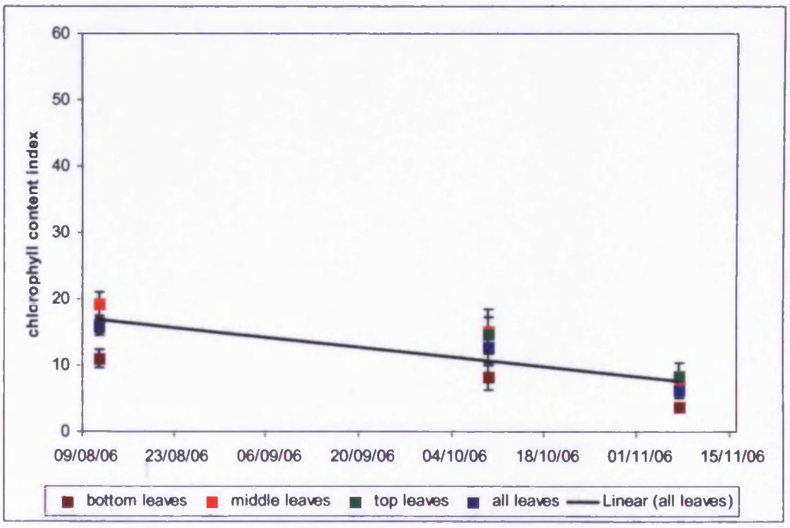

Figure 6.6: Mean chlorophyll content index ± 1 SEM for *Arundo* leaves at Llysdinam during 2006.

6.3.2 Chlorophyll and plant mineral content

6.3.2.1 *Miscanthus* leaves

Only at Llwynprenteg was leaf chlorophyll content correlated with leaf nitrogen, phosphorus, potassium and magnesium, but not with leaf calcium content (Table 6.1). All correlations at this site were highly significant. However, at Bluestone, there was no correlation between leaf chlorophyll content and nutrient content. Due to access problems at the Bluestone site, this dataset consisted of only 25 pairs of data, whereas the Llwynprenteg dataset consisted of 42 pairs. On combination of the two datasets, correlation analysis of mean leaf chlorophyll content with leaf nitrogen, phosphorus, potassium and magnesium was significant, but not with calcium. This suggests that more data may have been required at Bluestone to obtain accurate results.

Chlorophyll with	**nitrogen**	**phosphorus**	**potassium**	**calcium**	**magnesium**
Llwynprenteg	P=0.000***	P=0.000***	P=0.001***	n.s.	P=0.003**
Bluestone	n.s.	n.s.	n.s.	n.s.	n.s.
Llwynprenteg and Bluestone combined	P=0.000***	P=0.000***	P=0.000***	n.s.	P=0.006**

Table 6.1: Correlation analysis of leaf chlorophyll content with leaf nitrogen, phosphorus, potassium, calcium and magnesium content in *Miscanthus*.

6.3.2.2 *Arundo donax* leaves

Leaf chlorophyll content was correlated with nitrogen, phosphorus, potassium, calcium and magnesium content at both Bluestone and Llwynprenteg. Correlations with nitrogen, phosphorus and potassium were highly significant (Table 6.2).

Chlorophyll with	nitrogen	phosphorus	potassium	calcium	magnesium
Llwynprenteg	P=0.000***	P=0.000***	P=0.001***	P=0.05*	P=0.013*
Bluestone	P=0.000***	P=0.000***	P=0.001***	P=0.02*	P=0.016*

Table 6.2: Correlation analysis of leaf chlorophyll content with leaf nitrogen, phosphorus, potassium, calcium and magnesium content in *Arundo donax*.

6.3.2.3 *Miscanthus* rhizome

Mean chlorophyll content of all leaves was positively correlated with rhizome magnesium content (P=0.005), but not with rhizome nitrogen, phosphorus or potassium content.

6.3.2.4 *Arundo donax* rhizome

No correlations were found for mean chlorophyll content of all leaves with rhizome nitrogen, phosphorus, potassium or magnesium content.

6.3.3 Nitrogen fertiliser trial

6.3.3.1 July data

For the July data, there were significant differences (P=0.000) in chlorophyll content between the treatment groups for all parts of the plant (bottom leaves, middle leaves, top leaves and all leaves). Pair-wise comparisons revealed significant differences between the control sub-plots and all treatment groups, and between the high nitrogen application level with all groups, in all sections of the plant (Table 6.3).

6.3.3.2 September data

The results of the September data differed for the separate plant sections. For the bottom leaves, there were significant differences between the control sub-plots and the low nitrogen application rate and the high application rate, but not with the mid rate. The low application rate was significantly different to the high rate but not the mid rate. The mid application rate was only significantly different to the high rate (Table 6.4).

For the middle leaves, the control sub-plots were significantly different only to the high application rate. There were no differences between the groups for the top leaves data. The "all leaves" data showed significant differences between the high application rate and all other groups. All additional results are shown in Table 6.4.

6.3.3.3 November data

The results of the November data also differed for the separate plant sections. For the bottom leaves, there were significant differences between the control sub-plots and all nitrogen application rates, and between the high rate and all groups (Table 6.5). For the middle leaves, there were significant differences between the control sub-plots and all nitrogen application rates. There were no differences between the groups for the top leaves data.

For the "all leaves" data, there were significant differences between the control sub-plots and all nitrogen application rates, but no differences between any other groups (Table 6.5).

Bottom leaves	Low N	Mid N	High N	Middle leaves	Low N	Mid N	High N
Control	P<0.05*	P<0.05*	P<0.05*	Control	P<0.05*	P<0.05*	P<0.05*
Low N		n.s.	P<0.05*	Low N		n.s.	P<0.05*
Mid N			P<0.05*	Mid N			P<0.05*
Top leaves	Low N	Mid N	High N	All leaves	Low N	Mid N	High N
Control	P<0.05*	P<0.05*	P<0.05*	Control	P<0.05*	P<0.05*	P<0.05*
Low N		n.s.	P<0.05*	Low N		n.s.	P<0.05*
Mid N			P<0.05*	Mid N			P<0.05*

Table 6.3: Pairwise comparisons of leaf chlorophyll content for nitrogen treatment groups in *Miscanthus* fertiliser trial, July data.

Bottom leaves	Low N	Mid N	High N	Middle leaves	Low N	Mid N	High N
Control	P<0.05*	n.s.	P<0.05*	Control	n.s.	n.s.	P<0.05*
Low N		n.s.	P<0.05*	Low N		P<0.05*	n.s.
Mid N			P<0.05*	Mid N			P<0.05*
Top leaves	Low N	Mid N	High N	All leaves	Low N	Mid N	High N
Control	n.s.	n.s.	n.s.	Control	n.s.	n.s.	P<0.05*
Low N		n.s.	n.s.	Low N		n.s.	P<0.05*
Mid N			n.s.	Mid N			P<0.05*

Table 6.4: Pairwise comparisons of leaf chlorophyll content for nitrogen treatment groups in *Miscanthus* fertiliser trial, September data.

Bottom leaves	Low N	Mid N	High N	Middle leaves	Low N	Mid N	High N
Control	P<0.05*	P<0.05*	P<0.05*	Control	P<0.05*	P<0.05*	P<0.05*
Low N		n.s.	P<0.05*	Low N		n.s.	n.s.
Mid N			P<0.05*	Mid N			n.s.
Top leaves	**Low N**	**Mid N**	**High N**	**All leaves**	**Low N**	**Mid N**	**High N**
Control	n.s.	n.s.	n.s.	Control	P<0.05*	P<0.05*	P<0.05*
Low N		n.s.	n.s.	Low N		n.s.	n.s.
Mid N			n.s.	Mid N			n.s.

Table 6.5: Pairwise comparisons of leaf chlorophyll content for nitrogen treatment groups in *Miscanthus* fertiliser trial, November data.

6.3.4 Chlorophyll content and yield

The July data showed significant correlation of mean leaf chlorophyll per shoot with mean *Miscanthus* shoot height (P=0.03), but not with mean total plant height. Mean leaf chlorophyll per shoot in July also showed significant correlation with *Miscanthus* sub-plot dry weight at harvest (P=0.041). Regression analysis showed significant relationships between mean chlorophyll content and mean *Miscanthus* shoot height (P=0.03; Figure 6.7), and with sub-plot dry weight at harvest (P=0.027; Figure 6.8).

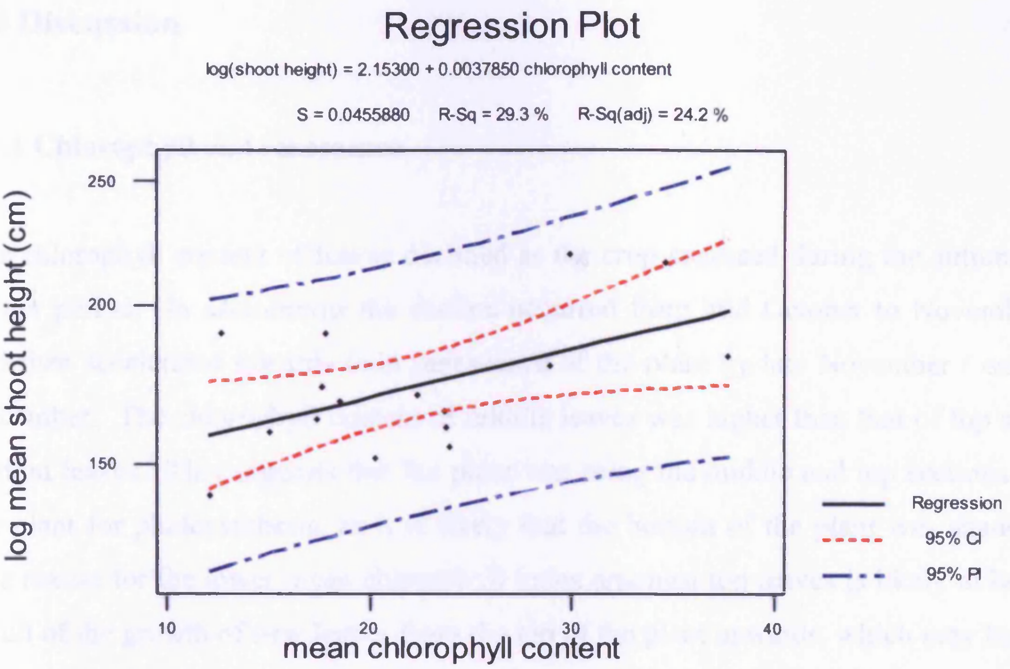

Figure 6.7: Regression analysis of mean chlorophyll content per *Miscanthus* shoot and log data of mean shoot height (cm).

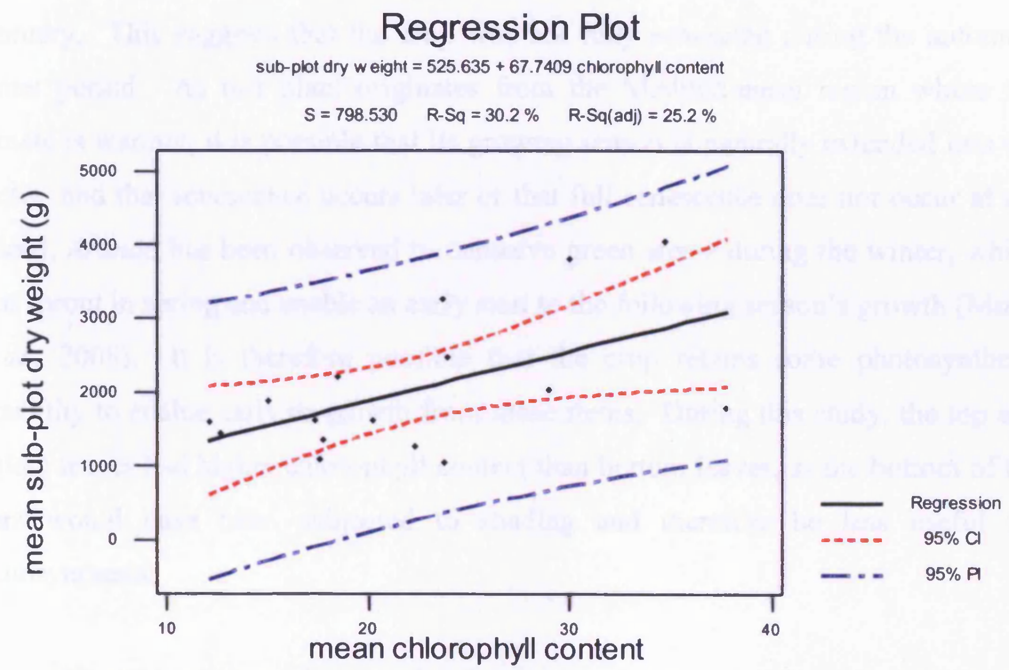

Figure 6.8: Regression analysis of mean chlorophyll content per *Miscanthus* shoot and mean dry weight per sub-plot (g).

6.4 Discussion

6.4.1 Chlorophyll and senescence

The chlorophyll content of leaves declined as the crop senesced during the autumn / winter period. In *Miscanthus* the decline occurred from mid October to November and then accelerated towards total senescence of the plant by late November / early December. The chlorophyll content of middle leaves was higher than that of top and bottom leaves. This suggests that the plant was using the middle and top sections of the plant for photosynthesis, as it is likely that the bottom of the plant was shaded. The reason for the lower mean chlorophyll index amongst top leaves is likely to be a result of the growth of new leaves from the top of the plant upwards, which may have not yet fully developed their chlorophyll content at the time of sampling.

In *Arundo donax*, there was much greater variation in chlorophyll content indices over the entire period, which probably reflected variation in shoot maturity. The leaves showed a general decline in chlorophyll content from August onwards, although they retained a higher level of chlorophyll than *Miscanthus* leaves, even as late as February. This suggests that the crop was not fully senescing during the autumn / winter period. As this plant originates from the Mediterranean region where the climate is warmer, it is possible that its growing season is naturally extended into the winter, and that senescence occurs later or that full senescence does not occur at all. Indeed, *Arundo* has been observed to conserve green stems during the winter, which then sprout in spring and enable an early start to the following season's growth (Monti *et al.*, 2008). It is therefore possible that the crop retains some photosynthetic capability to enable early re-growth from these stems. During this study, the top and middle leaves had higher chlorophyll content than bottom leaves, as the bottom of the plant would have been subjected to shading and therefore be less useful for photosynthesis.

The implications of these findings for *Arundo* as a biomass crop are as discussed in the previous chapter. This crop may not be suitable for the current biomass market, as senescence and translocation did not appear to occur during the winter, so nutrient levels remained high throughout the period.

6.4.2 Chlorophyll and nutrients

Leaf chlorophyll content was shown to have significant relationships with leaf nutrient content in both *Miscanthus* and *Arundo*. This agrees with studies on wheat which showed highly significant correlations between chlorophyll readings and nitrogen, manganese, iron, zinc and copper (Parvizi *et al.*, 2004). Studies on the grasses *Festuca* and *Lolium* showed highly significant relationships between leaf chlorophyll and nitrogen content (Gaborcik, 2003), as did studies of switchgrass (*Panicum virgatum*) (Madakadze *et al.*, 1999).

It is likely that the Bluestone data did not show correlations because the dataset was too small, due to access problems at the Bluestone site. The Llwynprenteg data showed that chlorophyll meter readings can be used as an indicator of the nitrogen, phosphorus, potassium and magnesium content of *Miscanthus* leaves. Repeated readings throughout the senescence period could be used to track the decline of these nutrients as they are translocated to the rhizomes or recycled via leaf litter to the soil. This could be useful to determine harvest date which should ideally take place when plant mineral content is low. Development of this methodology would enable crop sampling during senescence to determine harvest date to coincide with the decline of above ground plant matter mineral content to meet fuel quality recommendations, as discussed more fully in Chapter 5. In *Arundo donax*, chlorophyll readings can similarly be used to track the decline of nitrogen, phosphorus, potassium, calcium and magnesium content in leaves. However this crop failed to reach fuel quality recommendations at both sites, which was reflected in the lack of a total decline in chlorophyll. This was discussed further in Chapter 5.

The nutrient content of *Miscanthus* and *Arundo* rhizomes were not correlated with leaf chlorophyll, with the exception of rhizome magnesium content in *Miscanthus*. This relationship showed a positive correlation, which indicated that the rhizome magnesium increased with increased chlorophyll content within the plant. This does not indicate translocation, but that the rhizome was taking up additional magnesium from other sources. It is therefore likely that the rhizomes took up additional magnesium from the soil to meet the demands of increased photosynthesis, thereby increasing chlorophyll synthesis. This study did not show a relationship between chlorophyll degradation and the nitrogen, potassium and phosphorus contents of *Miscanthus* rhizomes, and was therefore not a useful method for translocation studies of the crop. In this study, a small section of the rhizome was removed for analysis of nutrient content, which was necessitated by a desire not to kill the entire plant on each sampling occasion, as this would have severely affected the results of other experiments occurring simultaneously within the same crop. This methodology assumed that translocation resulted in an increase in rhizome nutrient concentration and that minerals were uniformly distributed within the entire rhizome. However, it is likely that translocation results in increased rhizome growth and not necessarily an increased mineral concentration. It is possible that a larger portion of the rhizome is required for analysis in order to achieve significant results. Himken *et al.* (1997) removed a quarter of the rhizome plus attached roots for analysis during their study of *Miscanthus* translocation.

Due to logistic and financial reasons, this study was also limited by the analysis of samples from one year only. It must be noted that nutrient composition of crops, and therefore chlorophyll / mineral relationships are likely to change as the crop matures. Indeed, Monti *et al.* (2008) noted such an occurrence on comparison of first and second year growth with fourth year growth in several crops.

6.4.3 Chlorophyll and nitrogen

The *Miscanthus* nitrogen fertiliser trial showed consistently highly significant differences in chlorophyll content between the treatment groups during July sampling, which coincided with maximum productivity. All sections of the plant had significant differences between all treatment groups, with the exception of between the low and mid nitrogen application rates. These results show that leaf chlorophyll content can be used to demonstrate differences in uptake between nitrogen fertiliser treatments, but that chlorophyll measurements need to be timed at the plant's maximum productivity, i.e. during the summer period. The September and November datasets displayed much more variation amongst the pairwise comparisons, although they consistently showed there to be no differences of chlorophyll content of top leaves amongst the groups. This indicates that all groups utilised the top leaves for a similar rate of photosynthesis, and that, when subjected to varying levels of nitrogen fertilisation, differences in plant production occurred elsewhere in the plant.

6.4.4 Chlorophyll and shoot height and weight

The mean leaf chlorophyll content of a *Miscanthus* shoot was correlated with the plant's mean shoot height, which is a similar measure to canopy height, and is a useful indicator of crop growth and success. It was also correlated with sub-plot dry weight. This relationship exists because increased leaf chlorophyll indicates increased photosynthesis and therefore increased plant productivity and growth.

The regression analyses indicated that the mean leaf chlorophyll could successfully be used to predict shoot height and plot yield. This has been shown to be the case in studies of winter wheat (Girma *et al.*, 2006), corn (Scharf *et al.*, 2006), wheat (Parvizi *et al.*, 2004) and switchgrass (Madakadze *et al.*, 1999). However, many studies have shown discrepancies in this relationship, as a mean leaf chlorophyll value does not take into account the number of leaves on the plant. Adamsen *et al.* (1999) suggested that chlorophyll content indices are limited at the upper range of photosynthesis, and produce a less accurate relationship with wheat senescence than analysis of digital photography, and similarly, Houles *et al.* (2007) showed stronger relationships between chlorophyll and nitrogen on a canopy level than within a leaf. Olfs *et al.* (2005) reported that chlorophyll content readings can be affected by the growth stage of the crop, the age of the leaf and the point of measurement on the leaf. It must also be noted that taking single or repeated chlorophyll content readings per leaf does not account for size of leaves. Therefore the relationship between leaf chlorophyll and height or yield is limited by how much the leaves differ in size; as a sample from a large leaf may record the same chlorophyll index as a small leaf. In order to improve the success of this method, it may be preferable to calculate mean chlorophyll content per leaf, mean leaf area and mean number of leaves per plant, and thus create an equation to calculate the growth potential of the whole plant.

The relationship between mean leaf chlorophyll and shoot height and yield enables a further use for the chlorophyll meter, as an indicator of site suitability for crop growth. The chlorophyll contents of the middle leaves of both *Miscanthus* and *Arundo* were consistently higher than other leaves. These readings could therefore be measured and used for comparison of differences between sites, which would reflect the suitability of the sites for crop growth. In this study Bluestone was the highest yielding *Miscanthus* crop, and its middle leaf chlorophyll content was greater than that at Llwynprenteg, which was greater than that at Llysdinam; the lowest yielding crop. The *Arundo* crops at Bluestone and Llwynprenteg produced similar yields, which were reflected in the similarity between the lines of best fit produced for their "all leaves" data. The *Arundo* crop at Llysdinam was very poor, and its chlorophyll content of leaves was lower than the other two sites.

6.5 Conclusions

The senescence of *Miscanthus* occurred from October to November, and then more rapidly to complete senescence in December. *Arundo* did not senesce completely through the winter, and retained some photosynthetic capability as late as February. This may result in the crop being unsuitable as an energy crop in Wales, as leaf chlorophyll content is correlated with mineral content, which needs to be low prior to combustion.

The chlorophyll content of *Miscanthus* leaves can be used as an indicator of their nitrogen, phosphorus, potassium and magnesium content. In *Arundo donax*, chlorophyll readings are related to nitrogen, phosphorus, potassium, magnesium and calcium content of leaves. These relationships can be used to track the decline of minerals in leaves prior to harvest. No relationships between leaf chlorophyll content and the nitrogen, phosphorus or potassium content of the rhizomes were found for either *Miscanthus* or *Arundo* crops.

In *Miscanthus*, leaf chlorophyll content can be used to exhibit differences between the uptake of nitrogen fertiliser treatments that occur during the period of maximum productivity, i.e. during the summer period. Within the constraints of this study, mean leaf chlorophyll content values were shown to successfully predict shoot height and plot yield of *Miscanthus* crops. This could also be used as an indicator of the suitability of a site for crop growth. However, further study is required over several years to determine if this relationship continues over the lifetime of the crop.

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Chapter 7

The Effects of Organic and Inorganic Fertiliser Applications on Pot Trials and a Field Trial of *Miscanthus* x *giganteus*, *Arundo donax* and *Phalaris arundinacea*.

7.1 Introduction

All crops require water, light, carbon dioxide and nutrients for growth. Insufficient levels of any of these factors can lead to restricted growth, disease or plant death. The major nutrients required for plant survival are nitrogen, phosphorus, potassium, magnesium, sulphur and calcium, although several more minerals are required as trace elements. This study is concerned with nitrogen, phosphorus and potassium, since these are the nutrients most commonly applied in the form of fertilisers. They are referred to as the major or primary nutrients, because they are required in the largest quantities. Most crops show increased production in response to fertiliser applications, although if these exceed the crop's uptake then the remaining nutrients can contribute to environmental pollution.

Nitrogen is generally considered to be the most important fertiliser, as there is a strong relationship between nitrogen uptake, crop growth and yield (MAFF, 2000). It is the single most important nutrient concerned with protein and chlorophyll production, and is taken into plant cells as nitrate from soil solution (Yara Intl, 2008). Ammonium (NH_4) is also an important source of plant nitrogen, which is broken down into nitrate by soil organisms. Although nitrogen deficiency can result in reduced growth, excessive nitrogen has the same effect (MAFF, 2000). Phosphorus is also essential to plant growth, development and root development in particular, and is an important element in plant enzymes and DNA (Yara Intl, 2008). It is taken up by the plant as orthophosphates, as required during growth. Potassium is an essential element involved in the process of nitrate uptake from the soil, in addition to many other functions in plant growth and development. It is taken up by the plant as potassium (K^+) ions in association with nitrogen (Yara Intl, 2008).

Fertiliser application to all crops carries the risk of environmental pollution, and all excessive applications are, of course, financially wasteful. Nitrogen is soluble within both soil and plant matter and therefore very susceptible to losses by leaching and run-off. Application of manures with high ammonia content is an important contributory factor to nitrogen deposition, and thus contributes to acid rain (MAFF, 2000), although emissions can be reduced by application method and timing. Surface application of manures results in losses of around 40% of nitrogen through ammonia volatilisation, although emissions decrease with increased water content of the manure, which are thus incorporated into the soil more rapidly (MAFF, 2000).

Excessive build up of phosphorus in soil can cause water pollution through nutrient run off, and is an important contributory factor to freshwater eutrophication, which leads to algal blooms and disruption to aquatic ecosystems (MAFF, 2000). Recommendations therefore ensure that soil phosphorus levels do not exceed crop uptake (MAFF, 1998).

Once the crops' requirements are understood, fertiliser recommendations can be made to minimise pollution concerns, and a more productive and cost efficient crop can be grown. The Fertiliser Recommendations for Agricultural and Horticultural Crops (MAFF, 2000) is an indispensable guide for farmers to ensure that fertiliser applications to field crops are both cost effective and environmentally friendly. Arable, forage crops, grassland and vegetables are considered in terms of their nutrient requirements, and fertiliser recommendations are made, whilst accounting for soil fertility and method and timing of application.

Unfortunately the fertiliser recommendations for energy crops are less succinct. The *Miscanthus* Growers Handbook (Defra, 2007) recommends very low fertiliser application for *Miscanthus* crops in the UK, with fertiliser requirements during the first two years met by organic manures. There are no guidelines for fertiliser applications to *Arundo* or *Phalaris* crops within the UK.

Most studies of *Miscanthus*' response to fertiliser applications have used inorganic nitrogen. Results are contradictory, as studies have shown; no response to nitrogen fertiliser application (Christian *et al.*, 1999; Danalatos *et al.*, 2007; Himken *et al.*, 1997; Christian *et al.*, 2008); an optimal response to low nitrogen input of 40 kg/ha (Boehmel & Claupein, 2007); and a response to high application of 200kg/ha (Ercoli *et al.*, 1999). Similarly, *Arundo* studies have shown; no response to nitrogen input (Christou *et al.*, 2001); no response to fertiliser application over 100 kg/ha (El Bassam, 1998), or an increased response to application of 200 kg/ha (Angelini *et al.*, 2005). Studies of *Phalaris* have also shown no response to nitrogen application (Katterer *et al.*, 1998), an optimum nitrogen fertiliser application of 60 kg/ha (Christian *et al.*, 1999), and increased yield response to increased nitrogen input (Lewandowski & Schmidt, 2006).

The application of fertilisers to energy crops carries an additional concern as well as the risk of environmental pollution. As discussed in Chapter 4, excessive mineral levels within the harvested material can cause combustion problems or even deem the crop unsuitable for fuel use. It is therefore important that studies of energy crops also investigate the mineral levels that remain in the plant material at harvest.

In order to investigate several aspects of fertiliser effect, several experiments were set up and the results are presented in this chapter separately for ease of understanding. The four experiments are presented as follows:

Experiment 1: Pot trial 1: The effects of administering a standard amount of organic manure to *Miscanthus*, *Arundo* and *Phalaris* potted plants.

This study investigated the effects of applying a standard amount of cattle manure, chicken litter, pig manure, limed sewage cake and un-limed sewage cake to a pot trial of each crop. All these nutrient sources are readily available in the agricultural environment. The aims of the study were to determine if different manure applications had different effects on the crops in terms of growth and final yield. It also investigated whether different levels of nitrogen, phosphorus and potassium (N:P:K) in the manures had an effect on the N:P:K content of the plant matter at harvest.

Experiment 2: Pot trial 2: The effects of administering a standard amount of available nitrogen to *Miscanthus, Arundo* and *Phalaris* potted plants, in the form of organic manure.

This study investigated the effect of organic manure application to the three crops, where the amount of fertiliser applied was calculated by determination of the amount of nitrogen available to the plants. All pots received a standard amount of available nitrogen. By standardising the nitrogen content, it aimed to determine if other nutrients, in particular the phosphorus and potassium, had an effect on the growth or final yields of the crops. It also determined the effect of manure applications on the N:P:K content of the plant matter at harvest.

Experiment 3: Pot trial 3: The effects of administering a standard amount of inorganic nitrogen, inorganic phosphorus and inorganic potassium, to potted *Miscanthus, Arundo* and *Phalaris* plants.

This study investigated the effects of inorganic fertiliser application to potted plants, in terms of growth and final yield. It also determined if there was an effect on the N:P:K content of the plant matter at harvest.

Experiment 4: Field trial: The effects of administering a standard amount of available nitrogen to *Miscanthus, Arundo* and *Phalaris* crops, in the form of both organic manures and inorganic fertiliser.

This study investigated the application of a standard amount of available nitrogen to the three crops in a field situation and the effect on their growth or final yield. It also determined the effect fertiliser application had on the N:P:K content of the plant matter at harvest, and the effect on the N:P:K content of rhizome samples collected following harvest.

7.2 Methods

7.2.1 Fertiliser analysis

A sub-sample of cattle manure, chicken litter, pig manure, limed sewage cake and un-limed sewage cake, as applied to pot trial 1, were sent to Directlabs, Wolverhampton, for analysis of pH, percentage dry matter, total nitrogen, ammonium, phosphate and potash content.

7.2.2 Experiment 1: Pot trial 1

First year

45 *Miscanthus* and 45 *Arundo* rhizomes were planted in 10 litre pots containing multi-purpose compost in June 2005. A further 45 pots of compost were seeded with *P. arundinacea*. Five organic manures were sourced as follows; cattle manure, chicken litter, pig manure, limed sewage cake and un-limed sewage cake (Appendix 1). Sewage cake with lime added was readily available, and a smaller amount of cake without lime was also obtained. Five replicates of each fertiliser treatment were applied 5 weeks after planting in July 2005. All manures were weighed prior to application and a sufficient weight was applied to cover the surface soil, therefore pots receiving the same fertiliser treatment received approximately the same weight of manure, but weights and manure depths differed between treatments. The treatments were allocated randomly to a 5 pot by 9 pot arrangement, to include 5 replicate control pots per crop, and the remaining pots were used for the inorganic fertiliser trial (experiment 3). The control pots received no fertiliser application. Plants were watered daily with 1 litre water per pot.

Second year

All rhizomes were re-potted during spring 2006 into 30 litre pots containing multi-purpose compost. Organic manures were re-applied at a rate of 50 kg available nitrogen per hectare, to equate with the medium application rate of the field trial (see experiments 2 and 4 for details below). Plants were watered daily with 2 litres water per pot.

7.2.3 Experiment 2: Pot trial 2

Four organic fertiliser treatments were obtained for the second pot trial; cattle manure, chicken litter, pig manure, and limed sewage cake. It was not possible to obtain un-limed sewage cake for this trial. Application levels were determined in terms of the available nitrogen content of the treatment (see explanation below). These levels equated to low; 37.5 kg available nitrogen per hectare; and medium; 50 kg available nitrogen per hectare.

The *Miscanthus* and *Arundo* pot trials were planted in April 2006 using 10 litre pots filled with multi-purpose compost, at 1 rhizome per pot. *Phalaris* pots were seeded at a rate of 3 kg/acre. Five replicates of each fertiliser treatment were applied to the pots at both the low and medium application rates. The pots for each crop were arranged randomly within a block, to include five control pots, which received no fertiliser application. Soils were kept moist until germination and then watered daily with 1 litre water per pot during plant growth.

7.2.4 Calculation of available nitrogen of manures

In order to minimise environmental pollution, the Water Directive states that fertiliser applications should not contain in excess of 250 kg total nitrogen / ha (MAFF, 1998). However, as previously discussed, the total nitrogen content of manure is much higher than the amount of nitrogen present in a form suitable for plant uptake. Therefore for each of the treatment manures, the amount of nitrogen available for plant uptake (hereafter termed "available nitrogen") was standardised, in order to ensure that all pots in receipt of the low treatment level received 37.5 kg available nitrogen / ha and all pots in receipt of the medium treatment level received the equivalent of 50 kg available nitrogen / ha. The available nitrogen of each manure was calculated referring to both the MAFF guidelines (MAFF, 2000) and to the analysis of the manure samples from the previous pot trial. The MAFF guidelines refer to "typical nutrient content of manures after analysis of a large number of samples" (MAFF, 2000). However, analysis of the samples from the first pot trial treatments differed from these standard values. It was therefore decided that, for manures obtained from the same source as the first pot trial, the manure analysis results were consulted to calculate nitrogen content, and that for manures obtained from an alternative source to the first pot trial, the RB209 document (MAFF, 2000) was used for nitrogen content calculations (Table 7.1). The MAFF guidelines were consulted in all cases to determine the percentage of total nitrogen available for crop uptake for each manure type.

The large difference between the nitrogen content of the pig manure obtained for the first trial and the RB209 values can be explained by the fact that the original manure sample was collected from an area no longer in use by the animals, and therefore was not fresh. For all subsequent trials, pig manure was obtained from a different farm, and was obtained fresh (appendix 1).

All other differences between the first trial manure analysis and the standard values from RB209 were not deemed sufficiently different to justify the expenditure of having all manures re-analysed for the subsequent trials. Therefore the decisions as described above were adhered to for all fertiliser trials (second year of pot trial 1, pot trial 2 and field trial).

Treatment	Total N content (kg/tonne)		available N (%)	Available N (kg/tonne)	
	Manure analysis	RB209		Manure analysis	RB209
Cattle manure	5	6	20	1.0	1.2
Chicken (broiler litter)	33	30	35	11.5	10.5
Pig manure	4	7	20	0.8	1.4
Limed sewage cake	7	6	15	1.0	0.9
Un-limed sewage cake	7	7.5	15	1.0	1.1

Table 7.1: Total nitrogen and available nitrogen of each manure, as determined by manure analysis and as stated in RB209 (MAFF, 2000). The values used for calculations of quantities of fertiliser applications are highlighted.

7.2.5 Experiment 3: Pot trial 3

First year

The pots were planted and arranged as described in experiment 1. Inorganic fertilisers were applied at a rate of 60 kg/ha available phosphorus and 80 kg/ha available potassium, as advised by ADAS (A. Clarke, *pers. comm.*), combined with three application rates of available nitrogen (50 kg/ha, 100 kg/ha and 150 kg/ha).

Second year

Inorganic phosphorus and potassium were re-applied at a rate providing available nutrients of 60 kg/ha and 80 kg/ha respectively, with additional available nitrogen at three rates of 50 kg/ha, 100 kg/ha and 150 kg/ha.

Inorganic fertilisers were obtained from ADAS Pwllpeiran and were applied in the form of ammonium nitrate (34.5% N), triple superphosphate (46% P_2O_5) and muriate of potash (60% K_2O).

7.2.6 Experiment 4: Field trial

In spring 2006, the *Miscanthus*, *Arundo* and *Phalaris* crops at Llysdinam were each divided into 42 sub-plots measuring $6.25m^2$. Each sub-plot was separated by a minimum distance of 2.5m, and edge sub-plots were located a minimum of 2.5m from the crop edge.

Organic fertiliser treatments were determined from the most successful treatments observed within the first year of the first pot trial. Un-limed sewage cake was unavailable during 2006; so limed sewage cake was chosen for the field trial, in addition to chicken litter. Inorganic nitrogen was determined as the third treatment. Application rates were calculated as low (37.5 kg available nitrogen/ha), medium (50 kg available nitrogen/ha) and high (87.5 kg available nitrogen/ha).

Four replicates of each fertiliser at each application level were surface-applied during April 2006. Four sub-plots received no treatment. Treatments were distributed randomly within the sub-plots.

7.2.7 Measurements for all trials

7.2.7.1 Height

Height measurements were recorded from the soil to the top-most leaf ligule on each shoot per pot for each of the *Miscanthus* and *Arundo* pots for pot trials 1, 2 and 3. For the first pot trial, canopy heights were measured for *Phalaris* pots, determined as the height from the soil to the top of the canopy. For the second pot trial, height measurements were recorded for 5 *Phalaris* shoots per pot from the soil to the top-most leaf ligule, and the number of shoots per pot was recorded.

Measurements from the field trial differed from the pot trials, due to the increased number of shoots per plot. Ten random shoots per *Miscanthus* and *Arundo* sub-plot were measured for height from ground to the top-most leaf ligule. The number of shoots per plot was also recorded. Within the *Phalaris* sub-plots, three canopy heights were measured per plot.

Measurements were recorded monthly from summer until harvest. The sampling date which coincided with maximum plant height was used for analysis of growth effects. This occurred during November for all trials.

7.2.7.2 Yield estimates

All pot trials were harvested in December or January. The latest harvest date was identified after plant senescence as the plants showed the first signs of stem breakage and lodging. All above-ground plant matter from each pot was cut and weighed (fresh weight, FW). Plants were oven dried at 80°C to constant weight (dry weight, DW). Moisture content was calculated as the difference between fresh and dry weights. The field trial was harvested during the first week of March 2007. Fresh weights, dry weights and moisture contents were calculated as above.

7.2.8 Analysis of plant mineral content

For the first pot trial, one random shoot from each of the four replicate treatment pots receiving chicken litter, un-limed sewage, inorganic fertiliser and no treatment (control pots), were sent to Direct Laboratories, Wolverhampton for analysis of nitrogen, phosphorus and potassium content. No other plant matter analysis was carried out for financial reasons.

For the second pot trial and the field trial, three random shoots were taken from the harvested material of each pot or sub-plot. These were chopped finely and sent to Natural Resources Management Ltd, Berkshire, for analysis of nitrogen, phosphorus and potassium content. A section of a rhizome randomly sampled from each field trial sub-plot was removed at harvest, oven-dried and sent to the same laboratory for analysis of nitrogen, phosphorus and potassium.

7.2.9 Statistical analysis

For each trial, differences between the treatment groups were determined by one-way or two-way analysis of variance, and pair-wise comparisons were performed using Fishers' tests. Data were transformed as necessary to meet the assumptions of the tests. For all data not normally distributed after transformation, analysis was performed using Kruskal Wallis tests and pair-wise comparisons were made using Mann-Whitney tests. The Bonferroni correction was applied to multiple comparisons.

7.3 Results

7.3.1 Organic manure analysis

		Content (%)				
Treatment	pH	Dry matter	Total N	Ammonium	Phosphate	Potash
Cattle manure	7.7	18.9	0.49	0.006	0.32	0.33
Chicken litter	9.0	71.5	3.29	0.579	2.57	2.23
Pig manure	7.3	31.2	0.39	0.004	0.31	0.21
Limed sewage cake	7.6	26.6	0.7	0.07	1.48	0.05
Un-limed sewage cake	6.2	23.6	0.66	0.017	1.8	0.05

Table 7.2: Manure analysis of original samples as used in pot trial 1.

	Mean weight applied (g)				
	Total manure	Total nitrogen	Ammonium	Phosphate	Potash
Cattle manure	540	2.619	0.032	1.728	1.782
Chicken manure	510	16.774	2.953	13.107	11.373
Pig manure	700	2.695	0.028	2.170	1.470
Limed sewage	805	5.627	0.564	11.914	0.403
Un-limed sewage	325	2.135	0.055	5.850	0.163

Table 7.3: Quantity of manure applied to *Miscanthus* and *Arundo* pots in pot trial 1, and its total nitrogen, ammonium, phosphate and potash content.

	Mean weight applied (g)				
	Total manure	Total nitrogen	Ammonium	Phosphate	Potash
Cattle manure	220	1.067	0.013	0.704	0.726
Chicken manure	350	11.512	2.027	8.995	7.805
Pig manure	350	1.348	0.014	1.085	0.735
Limed sewage	370	2.586	0.259	5.476	0.185
Un-limed sewage	180	1.183	0.031	3.240	0.090

Table 7.4: Quantity of manure applied to *Phalaris* pots in pot trial 1, and its total nitrogen, ammonium, phosphate and potash content.

7.3.2 Experiment 1: Pot trial 1: The effects of administering a standard amount of organic manure to *Miscanthus*, *Arundo* and *Phalaris* potted plants.

7.3.2.1 First year manure application

For the first pot trial, sufficient manure to cover the surface of each pot was applied, and their respective weights are detailed in Tables 7.3 and 7.4. Less manure was applied to the *Phalaris* pots to prevent damage to the delicate early plant growth. Emerging *Miscanthus* and *Arundo* shoots were more robust and therefore it was possible to apply more manure. Following analysis of the manures it was possible to calculate the amount of total nitrogen, ammonium, phosphate and potash applied to each pot (Tables 7.3 and 7.4).

7.3.2.2 Pot trial 1: First year growth and yield effects

Miscanthus

There were no significant differences between the treatment groups in terms of mean shoot height. There were differences between the number of shoots per rhizome, with chicken litter and un-limed sewage producing significantly more shoots than the control pots ($P<0.05$, Figure 7.1a). A significant increase in the number of shoots per plant was also recorded in chicken litter when compared to both pig and cattle manure treatments ($P<0.05$).

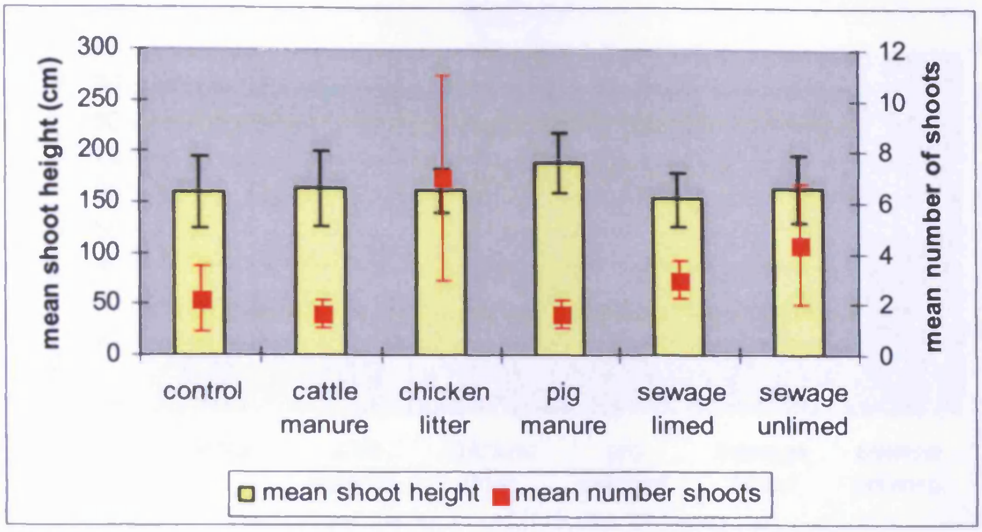
In the first year of pot trial 1, the pots receiving chicken litter and un-limed sewage produced yields that were significantly greater than the control pots ($P<0.05$, Figure 7.2a). There were no other differences between the treatments. The yield differences were therefore a result of differences in the number of shoots produced.

Arundo

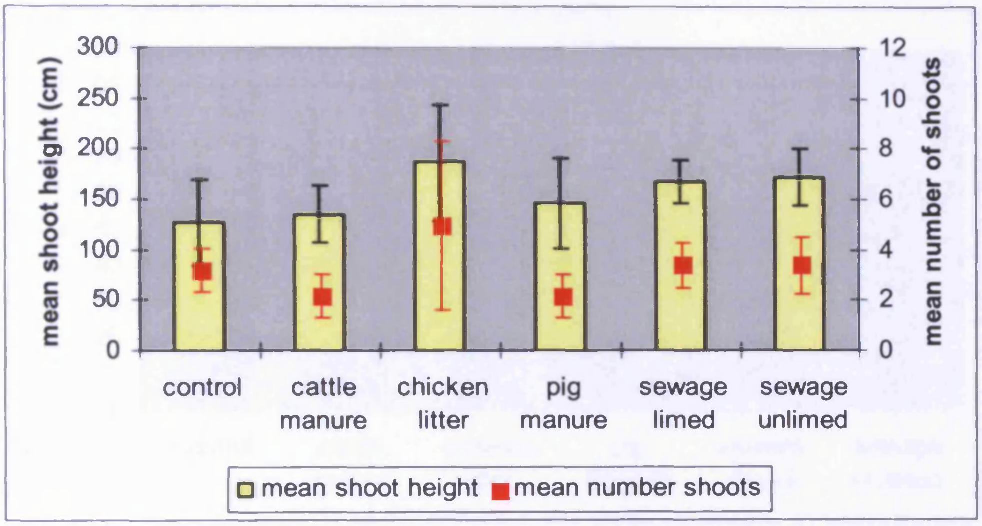
There were no significant differences between the mean number of shoots or mean shoot height (Figure 7.1b). However, the pots treated with chicken litter, limed sewage and un-limed sewage produced significantly greater mean yields than the control group (Figure 7.2b). There were no other significant differences between the treatments.

Phalaris

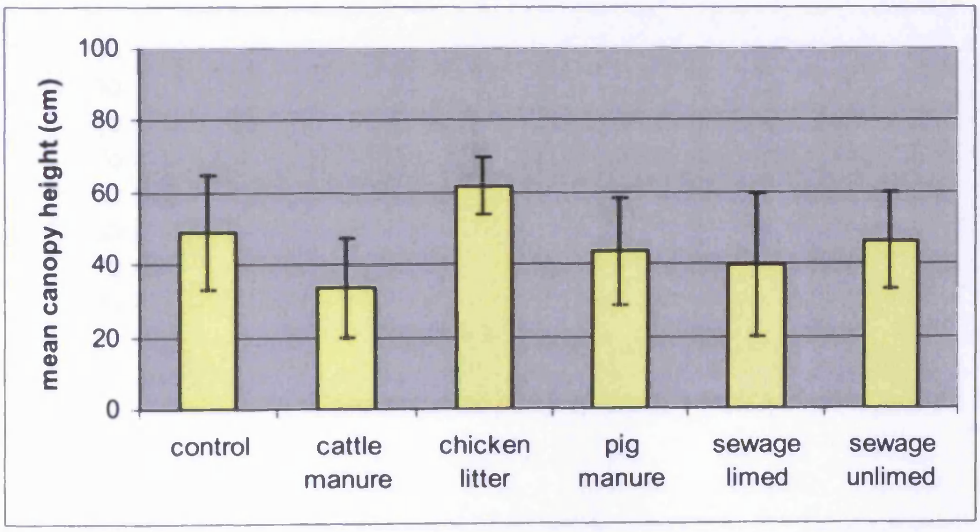
There were no significant differences between the canopy heights (Figure 7.1c) or between the dry weights of the *Phalaris* pots (Figure 7.2c).



(a) *Miscanthus*

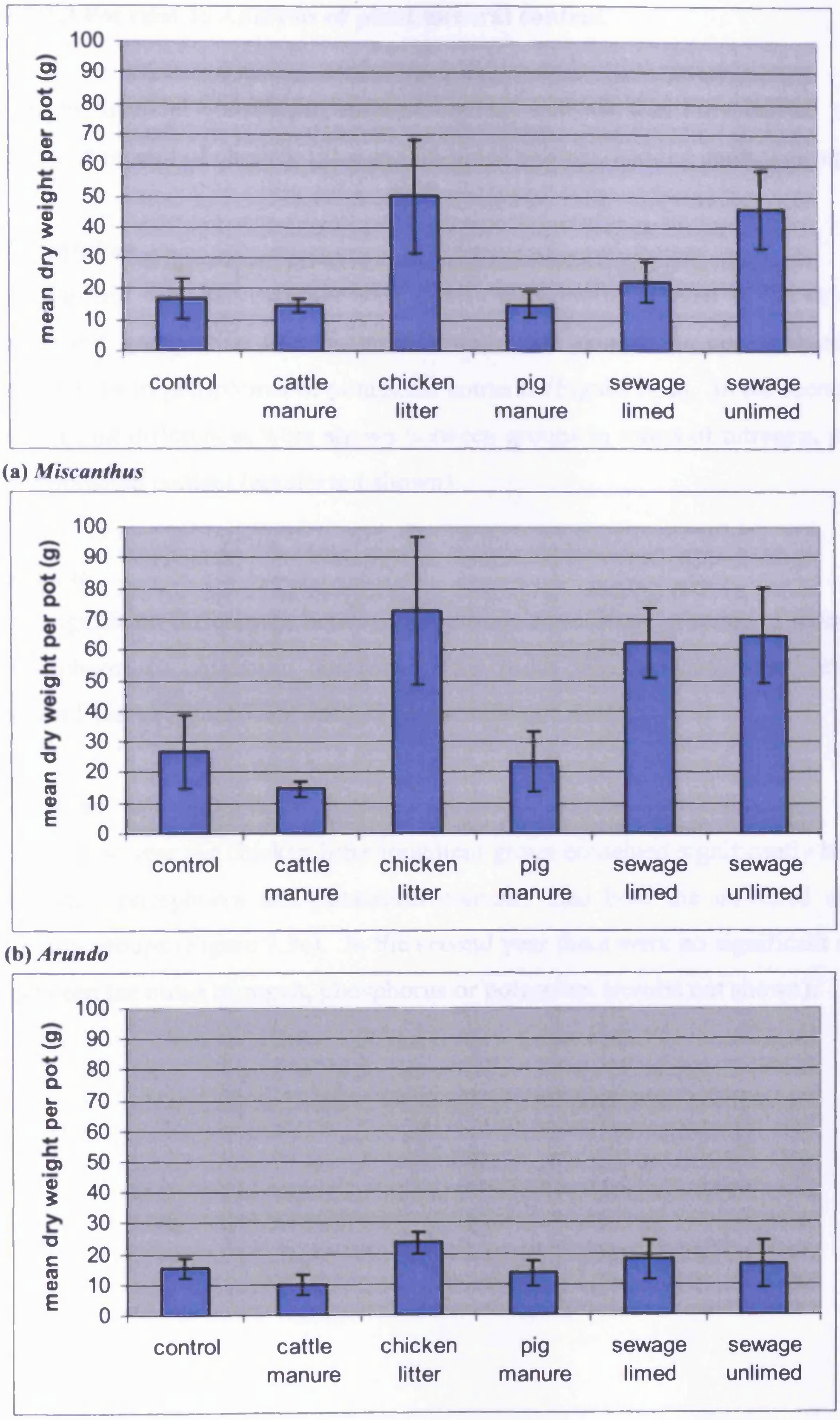


(b) *Arundo*



(c) *Phalaris*

Figure 7.1: Mean ±1 standard dev shoot height and number of shoots of (a) *Miscanthus*, (b) *Arundo* and (c) canopy height of *Phalaris* pots in the first pot trial. Note the change of scale for (c).



(a) *Miscanthus*

(b) *Arundo*

(c) *Phalaris*

Figure 7.2: Mean ±1 standard dev dry weight per pot for (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* pots in the first pot trial.

7.3.2.3 Pot trial 1: Analysis of plant mineral content

Due to financial constraints, mineral content analysis was only carried out on the plants in receipt of chicken litter and un-limed sewage, and the control group.

Miscanthus

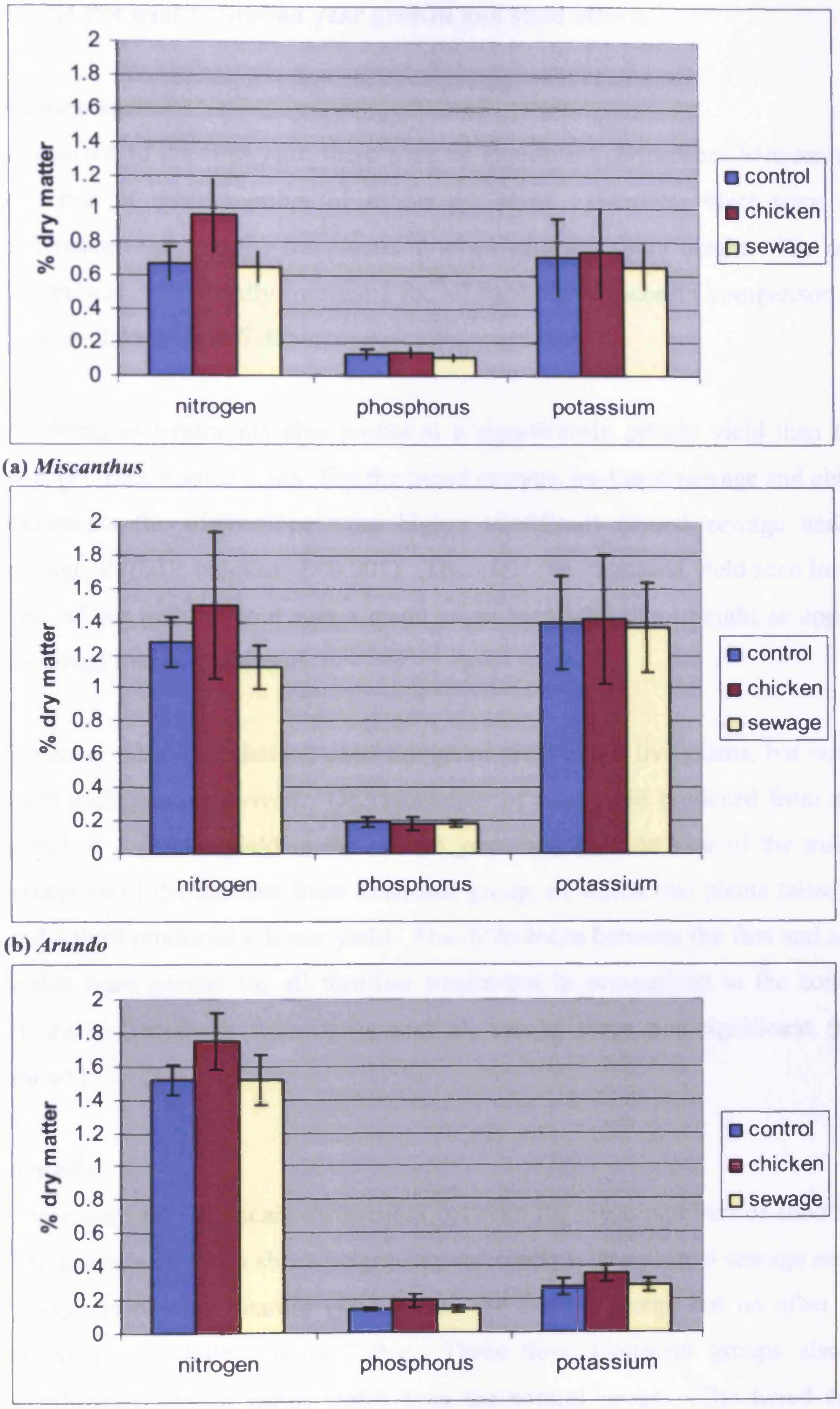
At the first year harvest there was significantly more nitrogen in the chicken litter treatment group than the un-limed sewage and control groups. There were no differences in phosphorus or potassium contents (Figure 7.3a). In the second year, no significant differences were shown between groups in terms of nitrogen, phosphorus or potassium content (results not shown).

Arundo

No significant differences between the groups were found in terms of mean nitrogen, phosphorus or potassium contents. This result was consistent for both first and second years (Figure 7.3b; second year results not shown).

Phalaris

In the first year the chicken litter treatment group contained significantly higher mean nitrogen, phosphorus and potassium contents than both the un-limed sewage and control groups (Figure 7.3c). In the second year there were no significant differences between the mean nitrogen, phosphorus or potassium (results not shown).



(a) *Miscanthus*

(b) *Arundo*

(c) *Phalaris*

Figure 7.3: Mean ±1 standard dev % nitrogen, phosphorus and potassium in above-ground dry matter of (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* at harvest, for the control group, chicken litter and un-limed sewage cake treatment groups in the first pot trial.

7.3.2.4 Pot trial 1: Second year growth and yield effects

Miscanthus

In contrast to the first year, there were no significant differences between the groups in terms of mean number of shoots per plant. However there were significant differences between the treatments in terms of mean shoot height. The mean shoot height was significantly increased for all treatment groups in comparison to control pots ($P<0.05$; Figure 7.4a).

All fertiliser treatments also produced a significantly greater yield than the control pots ($P<0.05$; Figure 7.5a). For the limed sewage, un-limed sewage and chicken litter treatments the differences were highly significant (limed sewage and un-limed sewage, $P<0.01$; chicken, $P<0.001$). Therefore the increased yield seen in the second year of the first pot trial was a result of an increased shoot height as opposed to an increased number of shoots.

However these calculations used the mean yield of all live plants, but not all plants from each group survived. On calculation of total yield produced from all pots, all achieved a greater yield in the second year than the first year of the trial, with the exception of the chicken litter treatment group, of which two plants failed to survive and a third produced a lower yield. The differences between the first and second year yields were greater for all fertiliser treatments in comparison to the control group. However, variances were large and all results were non-significant (results not shown).

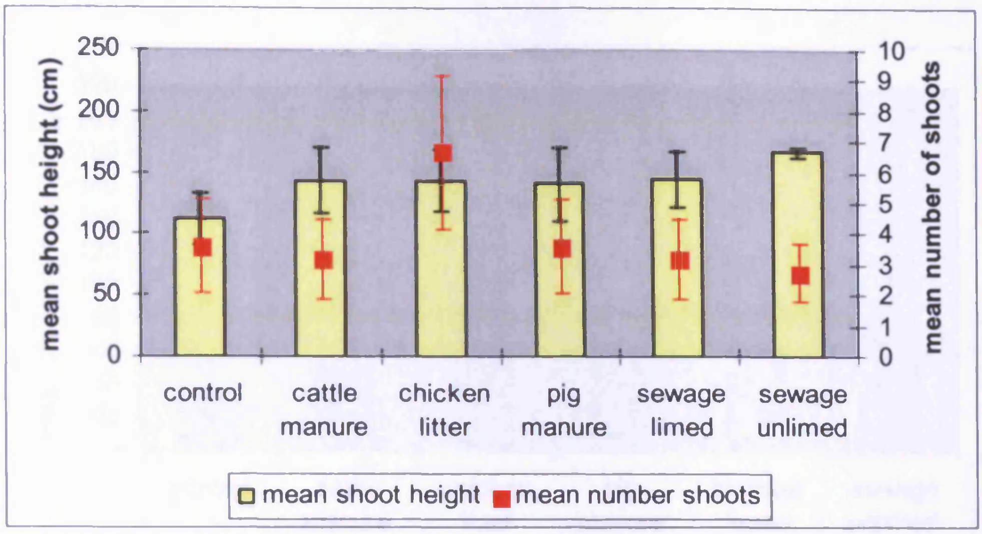
Arundo

There were no significant differences between the mean numbers of shoots per plant. The analysis of mean shoot height showed chicken litter, limed sewage and un-limed sewage to be significantly greater than the control group, but no other significant differences ($P<0.05$; Figure 7.4b). These three treatment groups also produced significantly greater mean yields than the control group. The limed sewage and chicken litter groups were also significantly greater than the cattle manure treatment group ($P<0.05$; Figure 7.5b).

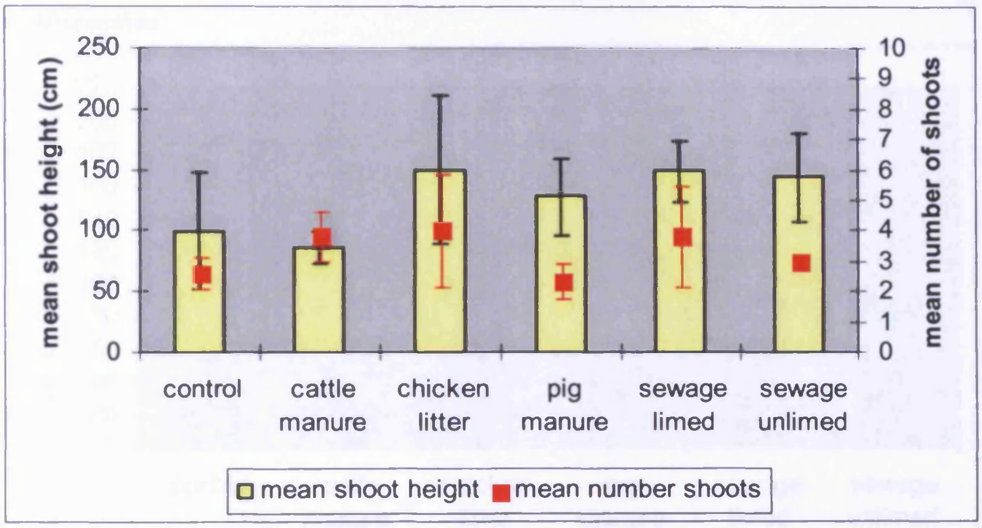
The mean dry weight from the first and second years showed an increased yield in all treatment groups, but not in the control group. However, differences between the first and second year means were not significant. *Arundo* survival was also affected in the second year of the trial. Only three of the five pots treated with pig manure survived to the second year. Four of the five pots treated with un-limed sewage survived. Within the chicken litter group, one of the plants failed to grow in the first year, but all four plants subsequently survived to the second year.

Phalaris

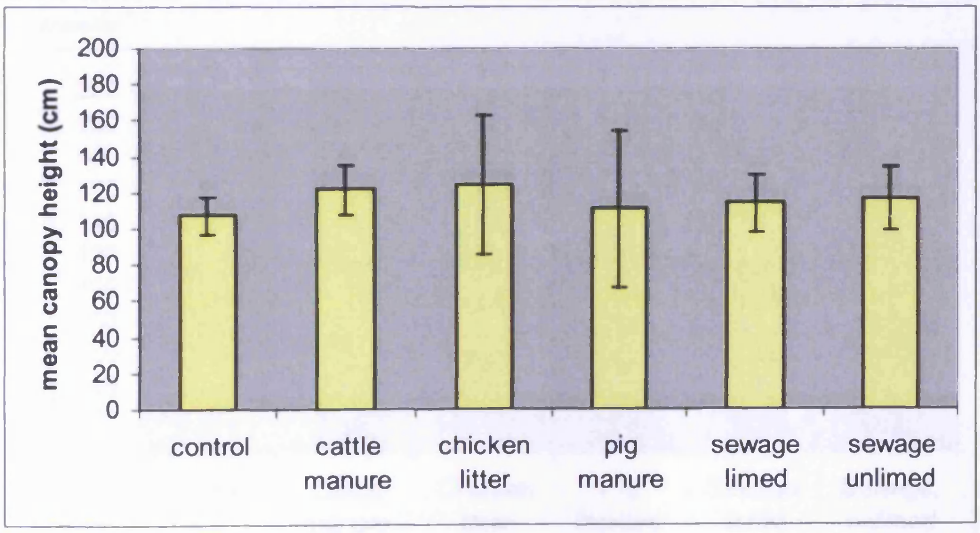
Canopy heights and yields of the treatment groups were analysed, but no significant differences were found (Figures 7.4c and 7.5c).



(a) *Miscanthus*

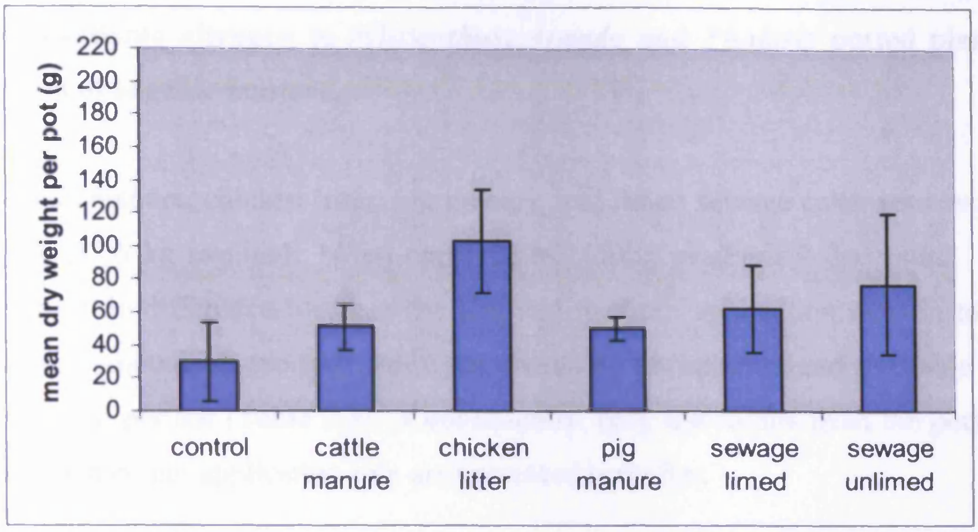


(b) *Arundo*

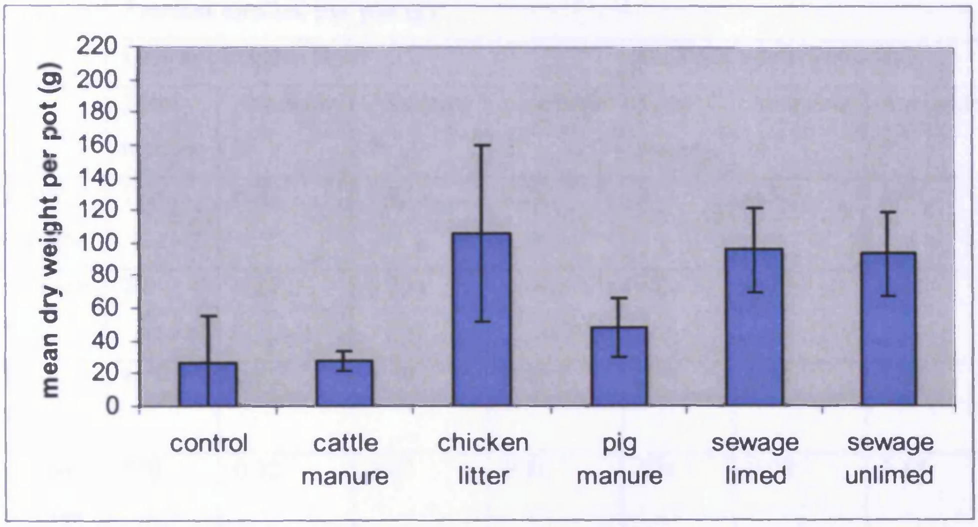


(c) *Phalaris*

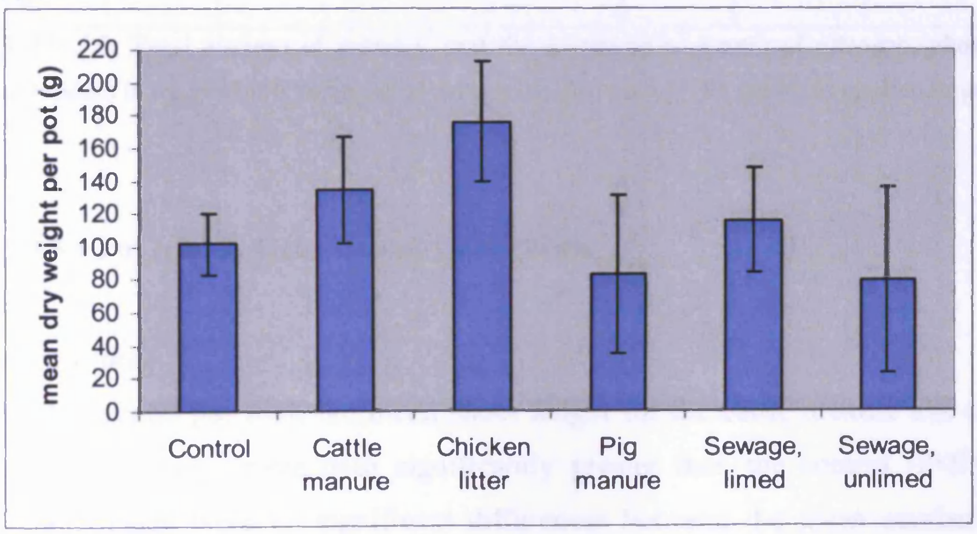
Figure 7.4: Mean ±1 standard dev shoot height and number of shoots of (a) *Miscanthus*, (b) *Arundo* and (c) canopy height of *Phalaris* pots in the second year of the first pot trial.



(a) *Miscanthus*



(b) *Arundo*



(c) *Phalaris*

Figure 7.5: Mean ±1 standard dev dry weight per pot for (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* pots in the second year of the first pot trial.

7.3.3 Experiment 2: Pot trial 2: The effects of administering a standard amount of available nitrogen to *Miscanthus*, *Arundo* and *Phalaris* potted plants, in the form of organic manure.

Cattle manure, chicken litter, pig manure, and limed sewage cake were applied at the low (37.5 kg available N/ha) and medium (50kg available N/ha) rates. There was very little difference between the low and medium application rates in terms of the actual amount of available nitrogen, available phosphorus and available potassium received per pot (Table 7.5). Consequently, only the results from the pots in receipt of the medium application rate are presented hereafter.

	Amount applied per pot (g)							
	Low application level				**Medium application level**			
	Total manure	Available N	Available P	Available K	Total manure	Available N	Available P	Available K
Cattle manure	193	0.23	0.81	2.78	257	0.31	1.08	3.70
Chicken litter	20	0.23	0.31	0.40	27	0.31	0.42	0.54
Pig manure	165	0.23	1.39	1.49	220	0.31	1.85	1.98
Limed sewage cake	220	0.22	1.63	0.11	294	0.29	2.18	0.15

Table 7.5: Total amount of manure, and the subsequent amount of nitrogen, phosphorus and potassium in an available form for plant uptake, for each of the manures applied to pot trial 2.

7.3.3.1 Pot trial 2: Growth and yield effects

Miscanthus

In the second pot trial, the mean shoot height for the cattle manure and pig manure treatment groups were both significantly greater than the control ($P<0.05$, Figure 7.6a). There were no significant differences between the mean number of shoots between treatments.

The increased shoot height of the cattle manure group only was reflected in its yield, which was significantly higher than the control group ($P<0.05$). There were no significant differences between the other groups (Figure 7.7a).

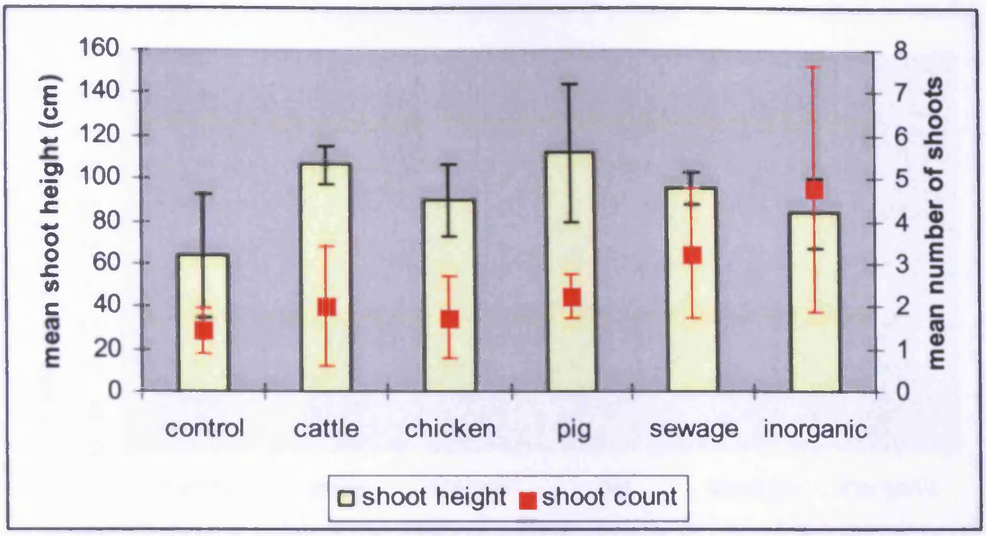
In general the yields of this trial were very poor, being roughly half the amount produced within the first year of the first pot trial. As fertiliser applications differed between the years, only the control pots can be directly compared. The mean dry weight obtained from the control pots from the first pot trial in the first year (16.8g / pot) was more than double that obtained from the second pot trial (6.9 g/pot).

Arundo donax

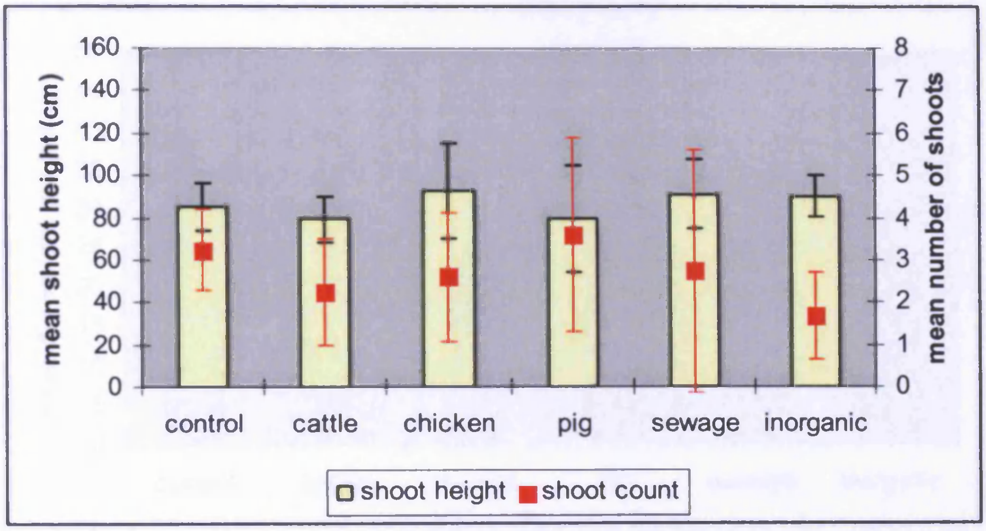
In the second pot trial, there were no significant differences between the treatment groups in terms of mean shoot height or mean number of shoots per plant (Figure 7.6b). The control group had the highest mean dry weight, but analysis showed there to be no significant differences between the groups' means (Figure 7.7b). However, the pooled data from all low application level treatments was significantly greater than the medium application level treatments in terms of mean shoot height and mean dry weight per pot ($P<0.05$; results not shown). In contrast to the *Miscanthus* trials, the control group mean yield (34.6 g/pot) was similar to the first year results from the first pot trial (26.9 g/pot).

Phalaris

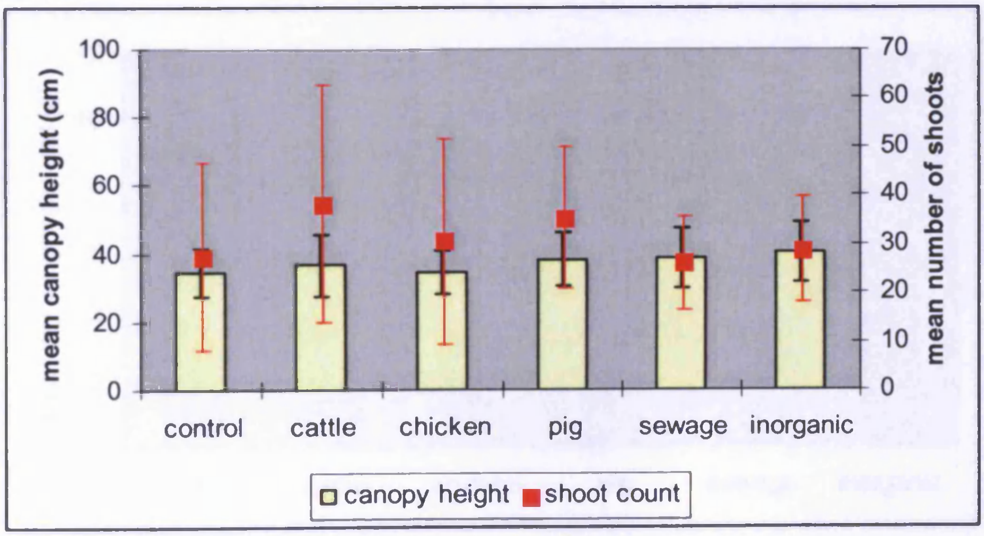
The second pot trial showed no significant differences between canopy heights, number of shoots or dry weights for the *Phalaris* treatment groups (Figures 7.6c and 7.7c).



(a) *Miscanthus*

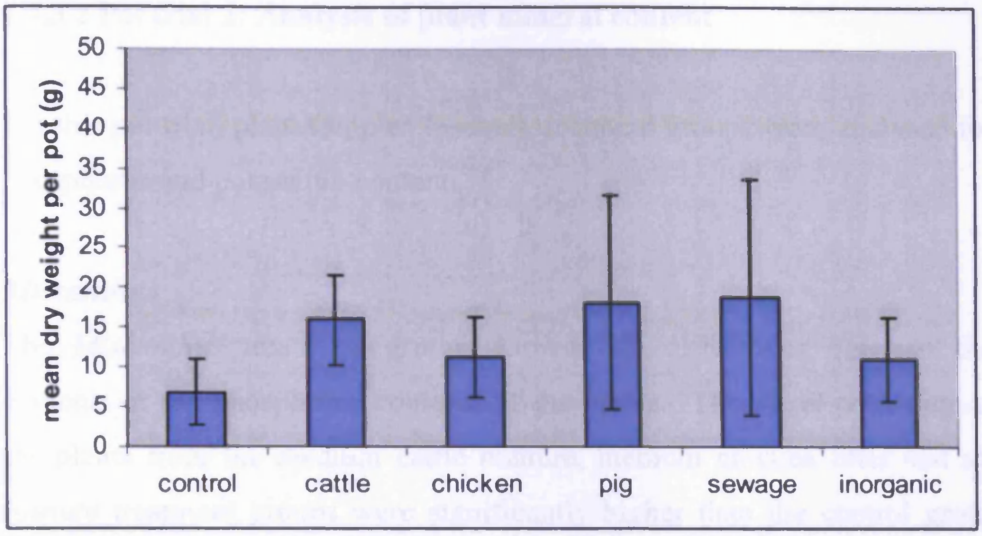


(b) *Arundo*

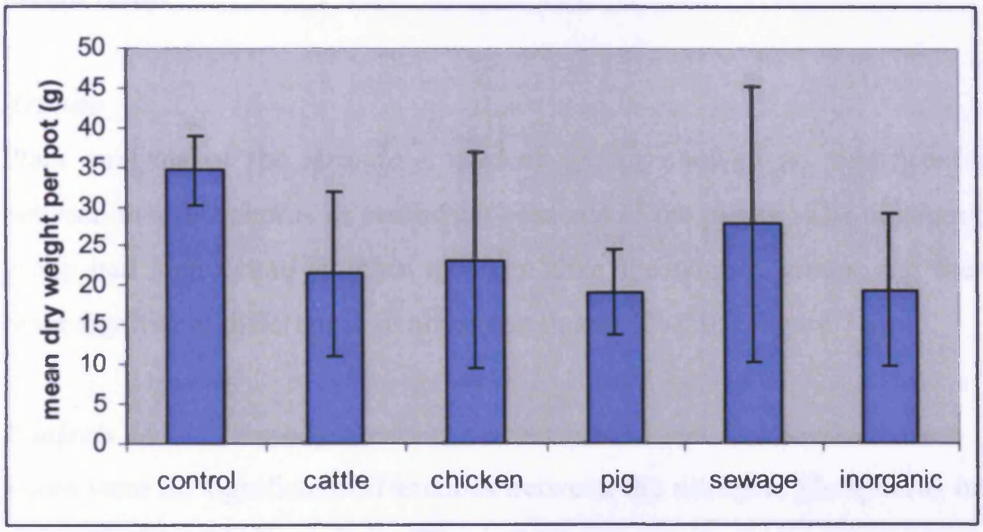


(c) *Phalaris*

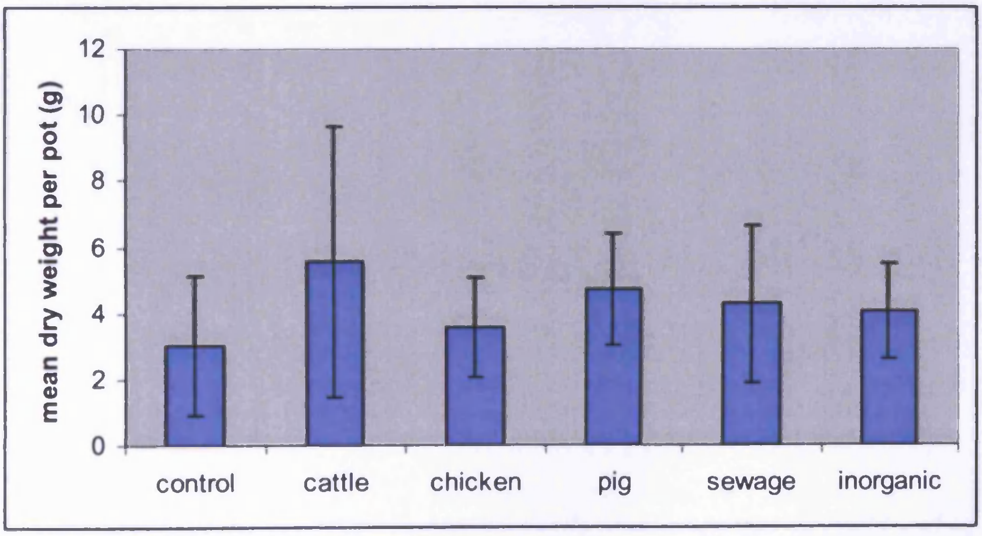
Figure 7.6: Mean ±1 standard dev shoot height and number of shoots of (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* pots in the second pot trial.



(a) *Miscanthus*



(b) *Arundo*



(c) *Phalaris*

Figure 7.7: Mean ±1 standard dev dry weight per pot for (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* pots in the second pot trial.

7.3.3.2 Pot trial 2: Analysis of plant mineral content

For this pot trial, plant samples from all treatment groups were analysed for nitrogen, phosphorus and potassium content.

Miscanthus

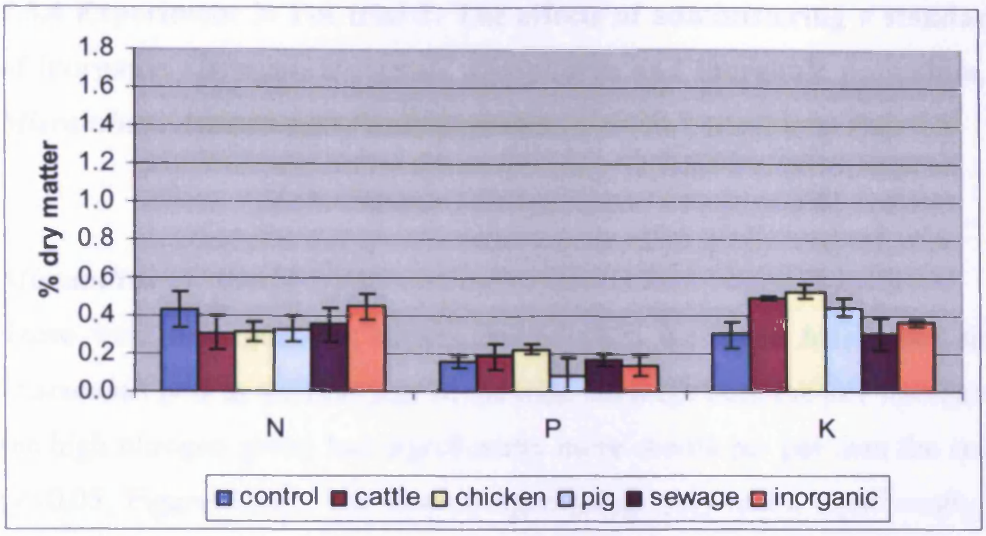
The *Miscanthus* treatment groups showed no differences between the nitrogen contents or the phosphorus contents of the plants. The mean potassium contents of the plants from the medium cattle manure, medium chicken litter and medium pig manure treatment groups were significantly higher than the control group ($P<0.05$, Figure 7.8a).

Arundo

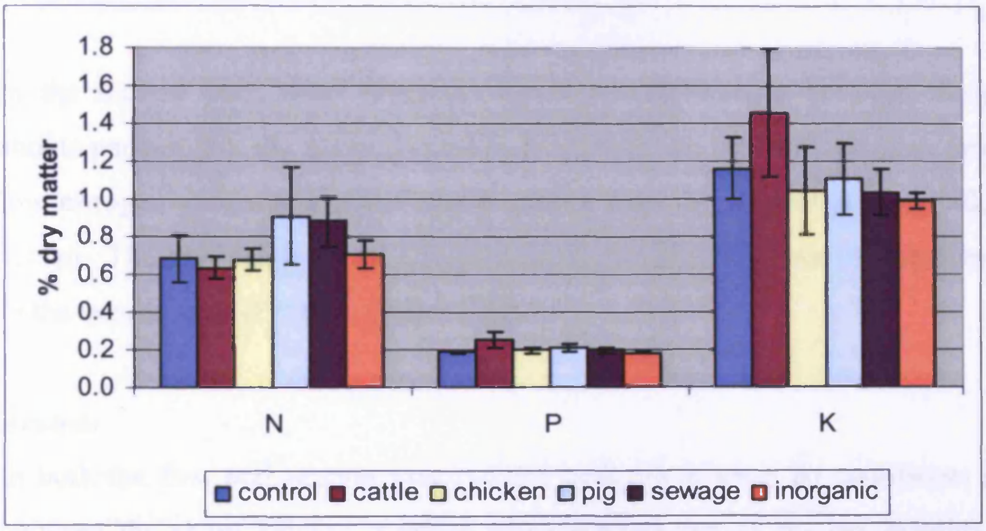
Plant analysis of the *Arundo* treatment groups showed no significant differences between the phosphorus or potassium contents of the plants. The medium pig manure group had significantly higher nitrogen than the control group, and there were no other significant differences in nitrogen content ($P<0.05$, Figure 7.8b).

Phalaris

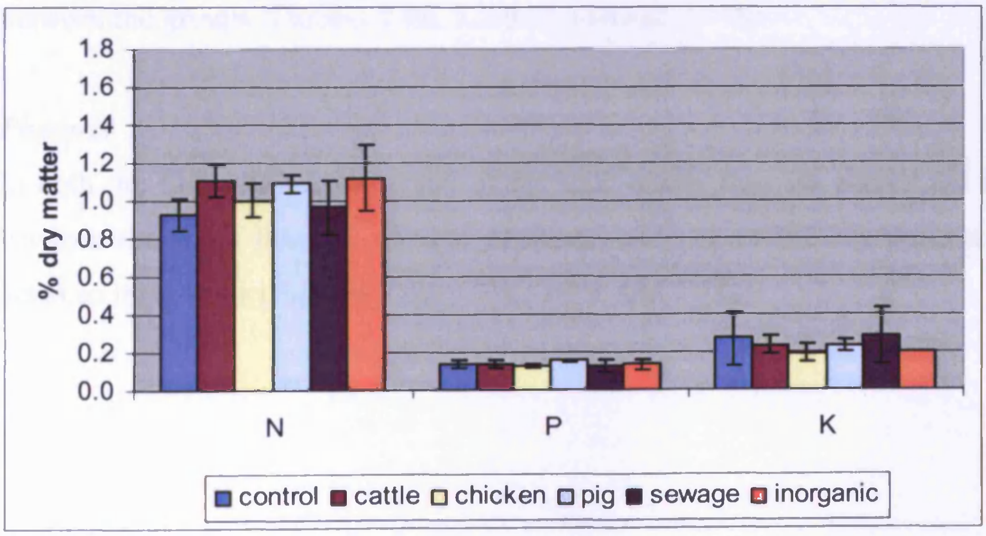
There were no significant differences between the nitrogen, phosphorus or potassium contents of the *Phalaris* plants (Figure 7.8c).



(a) *Miscanthus*



(b) *Arundo*



(c) *Phalaris*

Figure 7.8: Mean ±1 standard dev % nitrogen, phosphorus and potassium in above-ground dry matter of (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* at harvest, for the control group and all treatment groups in the second pot trial.

7.3.4 Experiment 3: Pot trial 3: The effects of administering a standard amount of inorganic nitrogen, inorganic phosphorus and inorganic potassium, to potted *Miscanthus*, *Arundo* and *Phalaris* plants.

Miscanthus

There were no significant differences between the mean height per shoot of the *Miscanthus* pots in the first year of the trial, although both the low nitrogen group and the high nitrogen group had significantly more shoots per pot than the control group ($P<0.05$; Figure 7.9a). The low nitrogen group only had a significantly higher dry weight than the control group ($P<0.05$; Figure 7.10a).

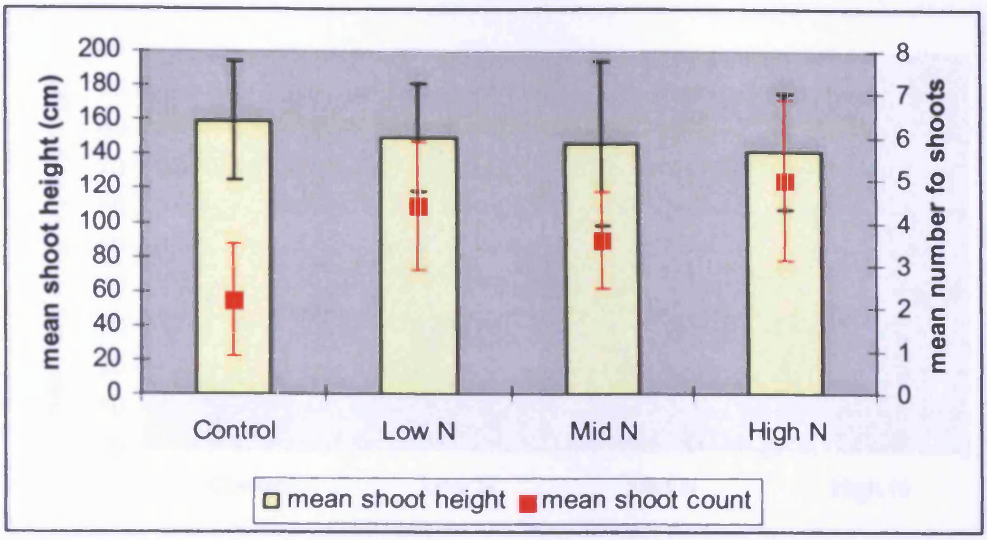
In the second year, there were no significant differences between the number of shoots per pot, but the mean shoot height of both the medium nitrogen group and the low nitrogen group was significantly greater than the control group ($P<0.05$; Figure 7.11a). The dry weight of all groups were significantly greater than the control group in the second year ($P<0.05$; Figure 7.12a)

Arundo

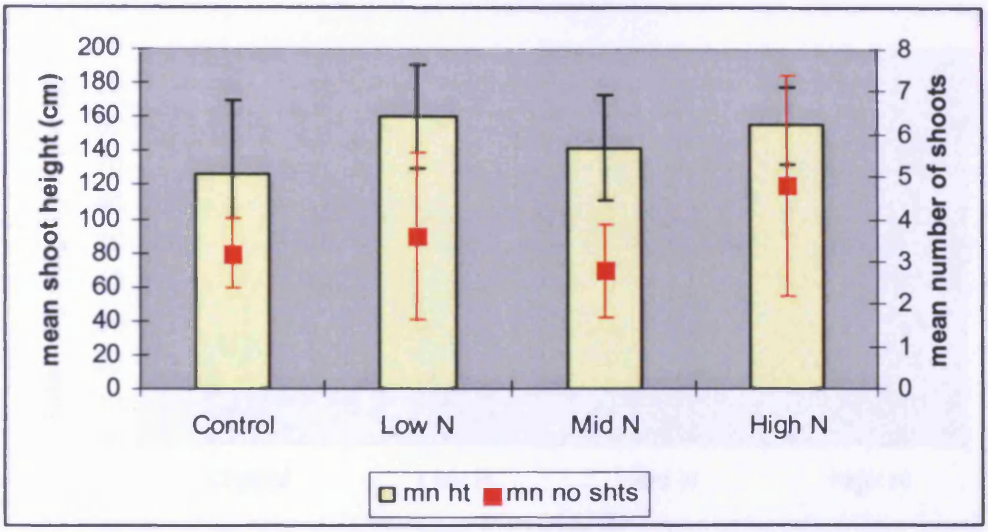
In both the first and second years of the trial, there were no significant differences between the shoot heights, number of shoots per pot, or the dry weights at harvest between the groups (Figures 7.9b, 7.10b, 7.11b and 7.12b).

Phalaris

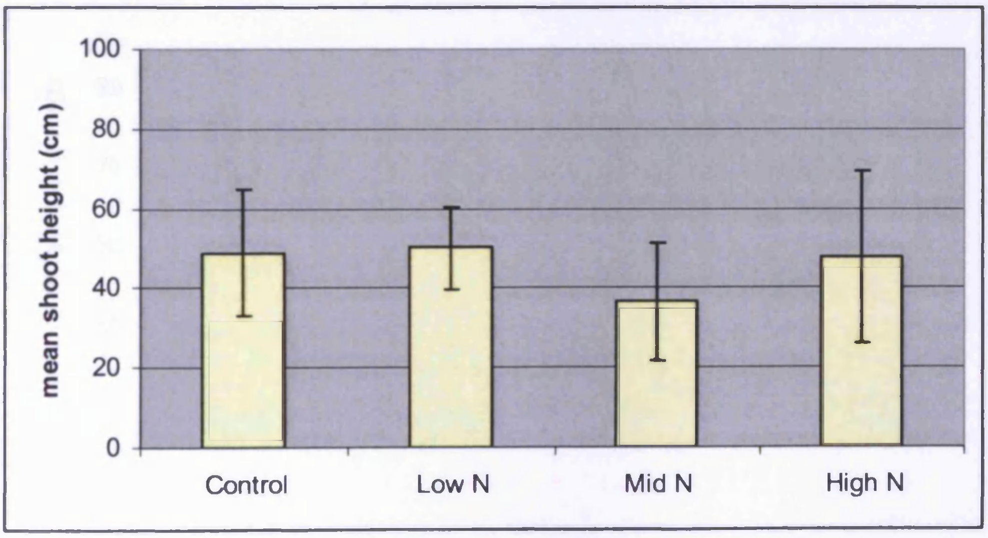
In both the first and second years of the trial, there were no significant differences between the shoot heights, number of shoots per pot, or the dry weights at harvest between the groups (Figures 7.9c, 7.10c, 7.11c and 7.12c).



(a) *Miscanthus*

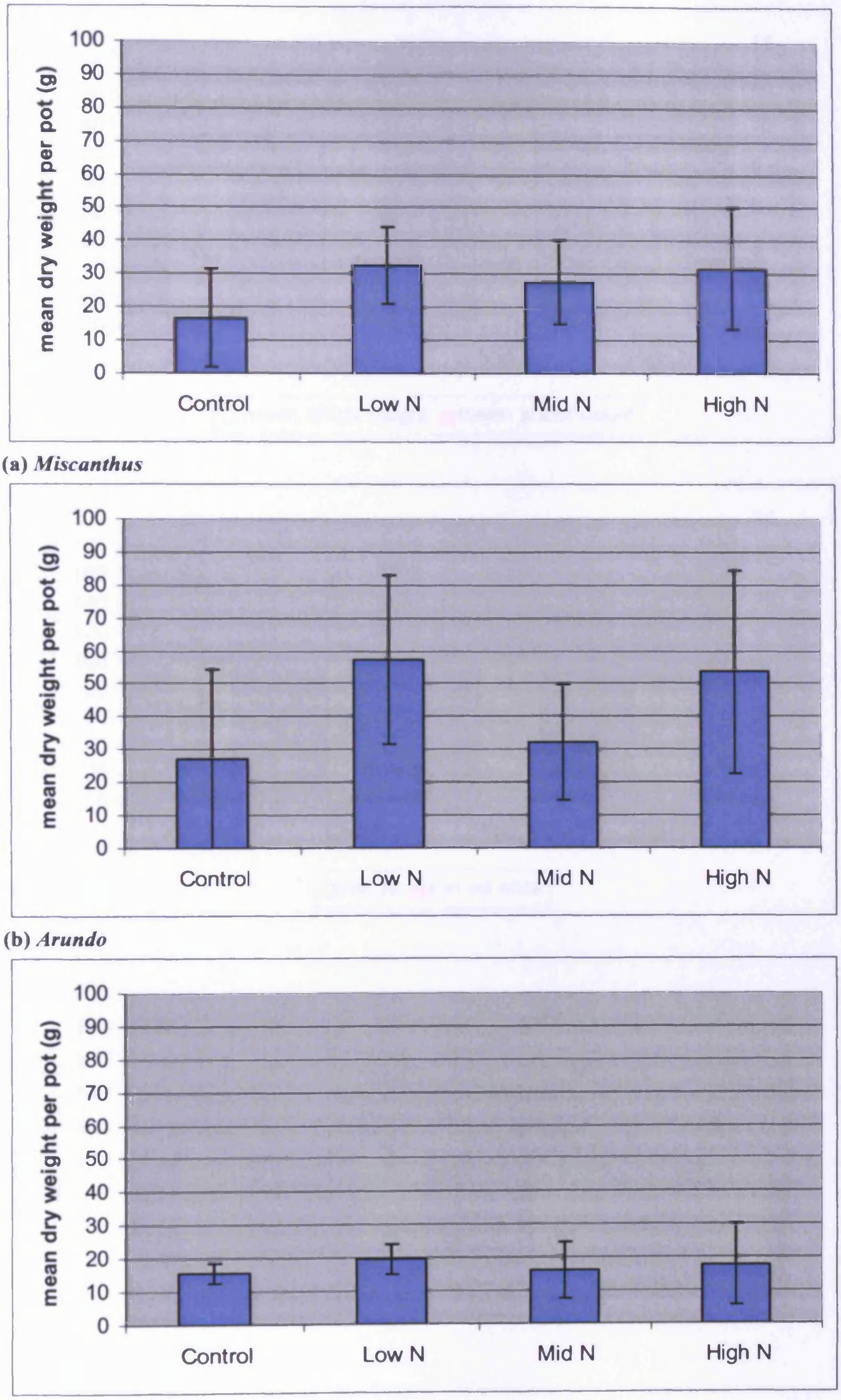


(b) *Arundo*



(c) *Phalaris*

Figure 7.9: Mean ±1 standard dev shoot height and number of shoots of (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* pots in the first year of pot trial 3.

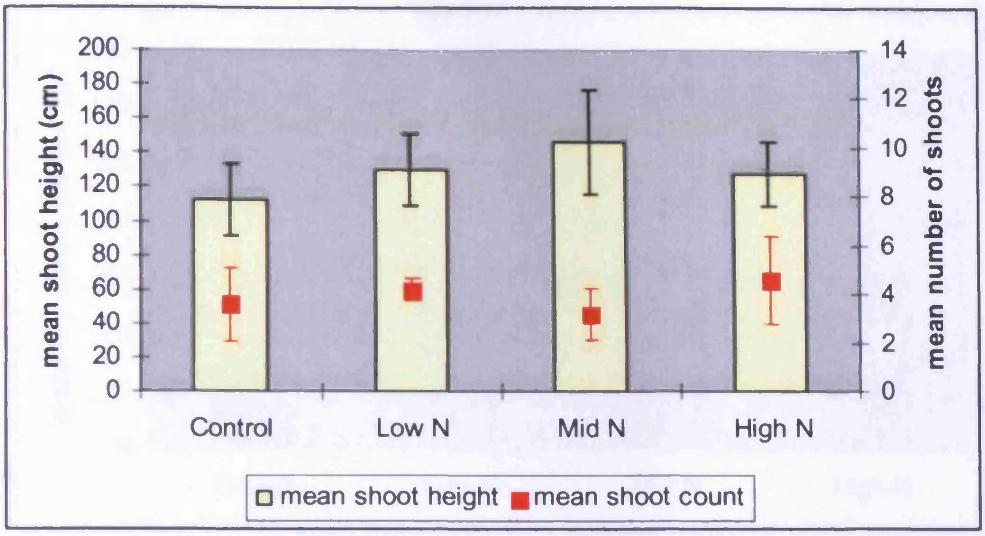


(a) *Miscanthus*

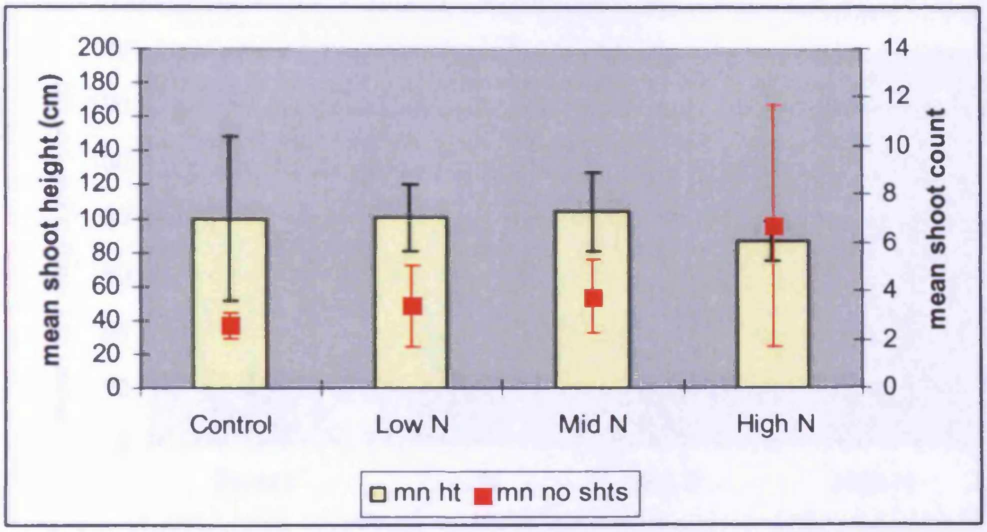
(b) *Arundo*

(c) *Phalaris*

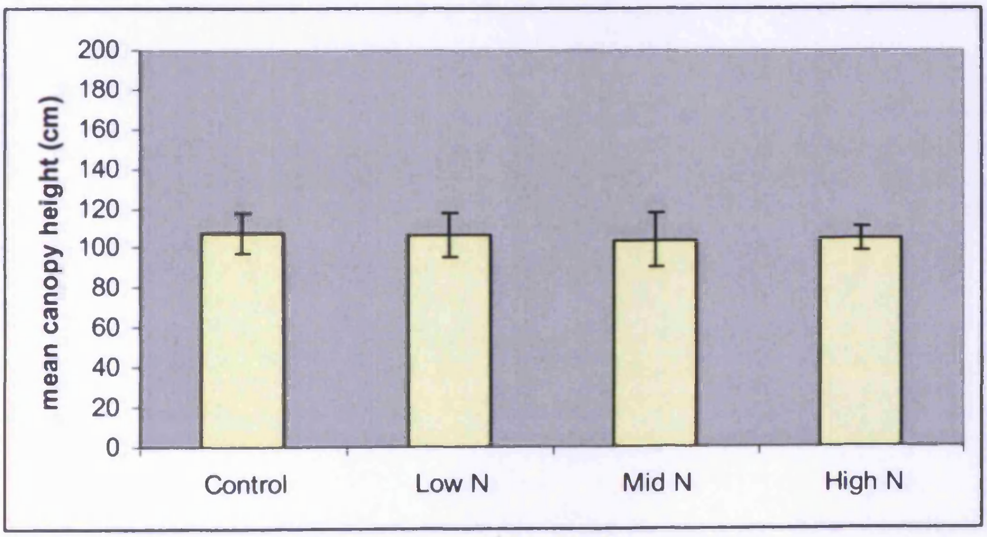
Figure 7.10: Mean ±1 standard dev dry weight per pot for (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* pots in the first year of pot trial 3.



(a) *Miscanthus*

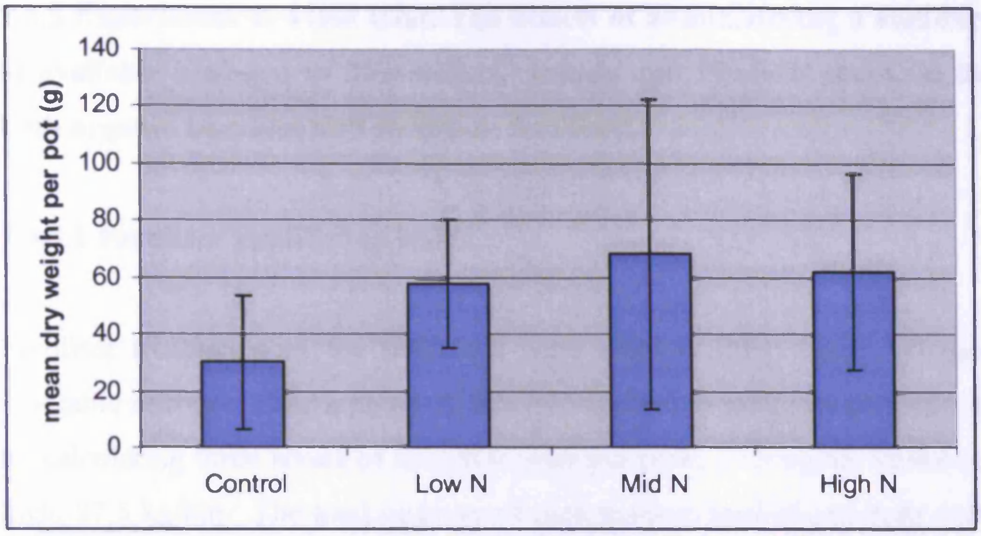


(b) *Arundo*

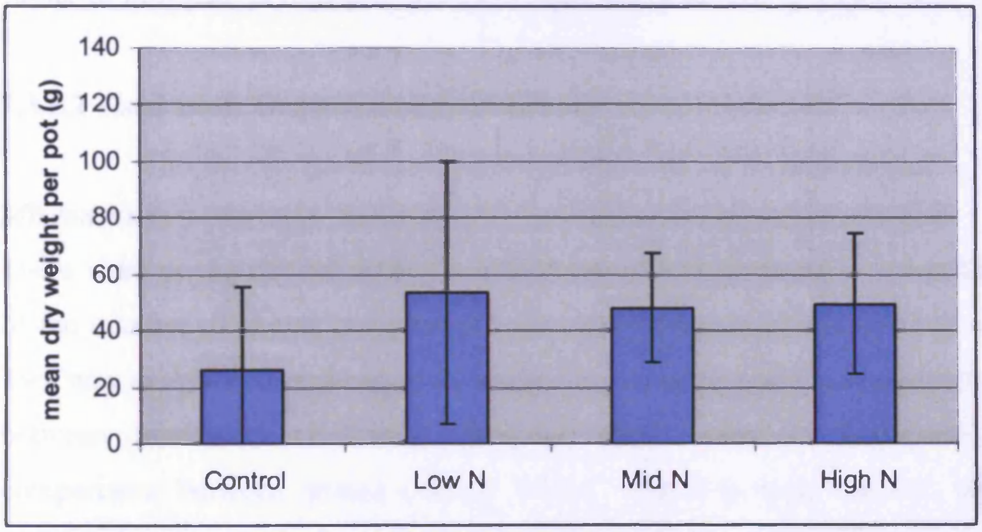


(c) *Phalaris*

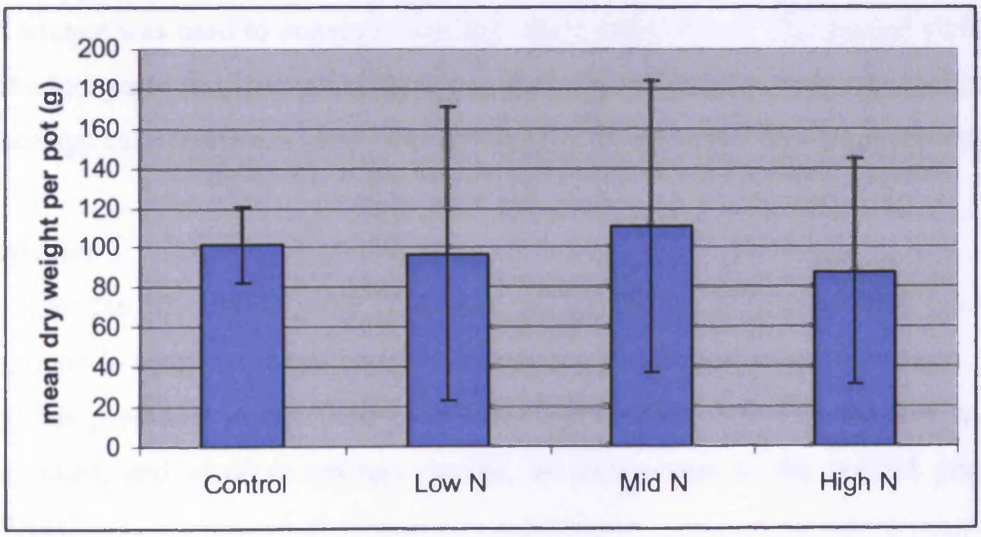
Figure 7.11: Mean ±1 standard dev shoot height and number of shoots of (a) *Miscanthus*, (b) *Arundo* and (c) canopy height of *Phalaris* pots in the second year of pot trial 3.



(a) *Miscanthus*



(b) *Arundo*



(c) *Phalaris*

Figure 7.12: Mean ±1 standard dev dry weight per pot for (a) *Miscanthus*, (b) *Arundo* and (c) *Phalaris* pots in the second year of pot trial 3.

7.3.5 Experiment 4: Field trial: The effects of administering a standard amount of available nitrogen to *Miscanthus*, *Arundo* and *Phalaris* crops, in the form of both organic manures and inorganic fertiliser.

7.3.5.1 Fertiliser application levels

Fertiliser treatments in the field trial were chicken litter, limed sewage cake and inorganic nitrogen. The amount of fertiliser applied to each sub-plot were determined by calculating three levels of available nitrogen (low, 37.5 kg/ha; medium, 50 kg/ha; high, 87.5 kg/ha). The total quantity of each manure applied and their corresponding values of nitrogen, phosphorus and potassium are described in Table 7.6.

7.3.5.2 Field trial: Growth and yield effects

Miscanthus

There were no significant differences between groups in terms of mean shoot height or the number of shoots per plant for the field trial data (Figures 7.13a and 7.13b). Two way analysis of variance of the yield data revealed a highly significant difference between treatments ($P=0.005$), although there were no significant pair-wise comparisons between groups (Figure 7.13c). The data from the low, medium and high application levels for each fertiliser type were pooled and one way analysis of variance was used to compare fertiliser types and control. The pooled yield data from the inorganic fertiliser plots were significantly higher than both the chicken litter and sewage cake treatment plots. No differences from control data were shown.

Arundo

As for the *Miscanthus* plots, there were no significant differences between the *Arundo* groups in terms of mean shoot height or the number of shoots per plant. The mean yields produced in the field plots were significantly lower in the low and medium chicken, and medium sewage groups, in comparison to the control group (Figure 7.14).

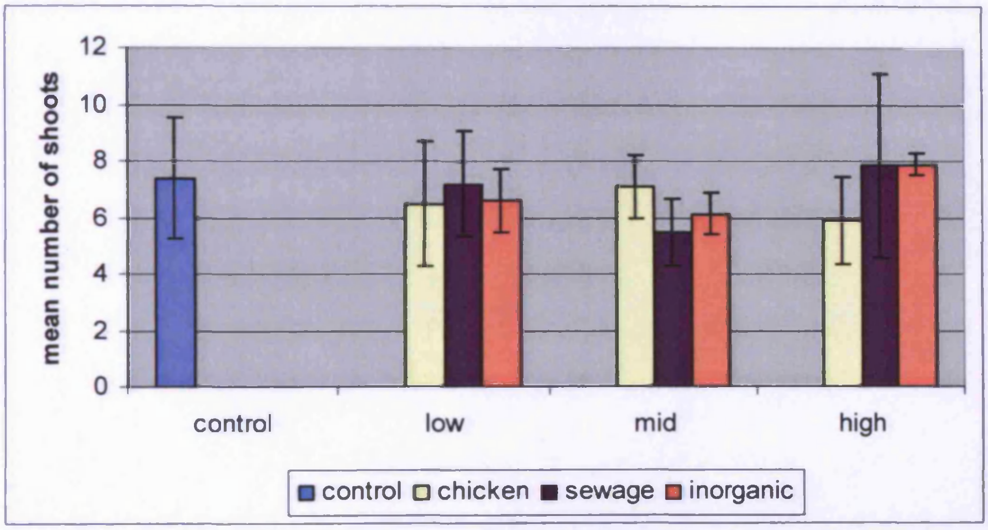
Phalaris

No significant differences were found between the canopy heights or yields for the *Phalaris* field plots (Figure 7.15).

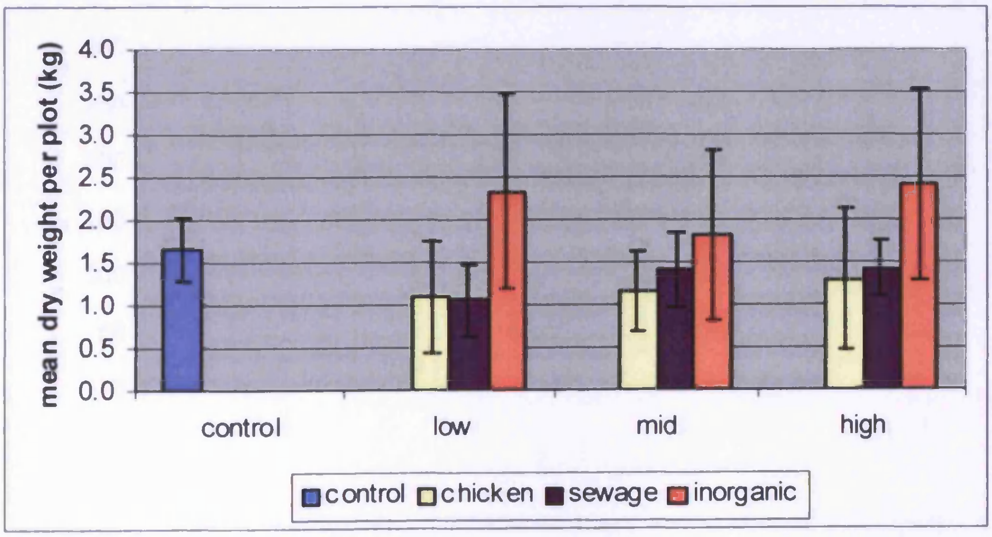
	Amount applied per pot (kg)											
Treatment	**Low application level**				**Medium application level**				**High application level**			
	Total manure	**N**	**P**	**K**	**Total manure**	**N**	**P**	**K**	**Total manure**	**N**	**P**	**K**
Chicken litter	2.0	0.02	0.03	0.04	2.7	0.03	0.04	0.05	4.8	0.06	0.07	0.10
Limed sewage cake	22.4	0.02	0.17	0.01	29.8	0.03	0.22	0.01	52.2	0.05	0.39	0.03

Table 7.6: The total amounts of manure applied to each sub-plot in the field trial, and their respective quantities of nitrogen, phosphorus and potassium applied.

(a)



(b)



(c)

Figure 7.13: Mean ±1 standard dev (a) shoot height, (b) number of shoots per plant, and (c) dry weight per plot at harvest, for *Miscanthus* sub-plots in the field trial.

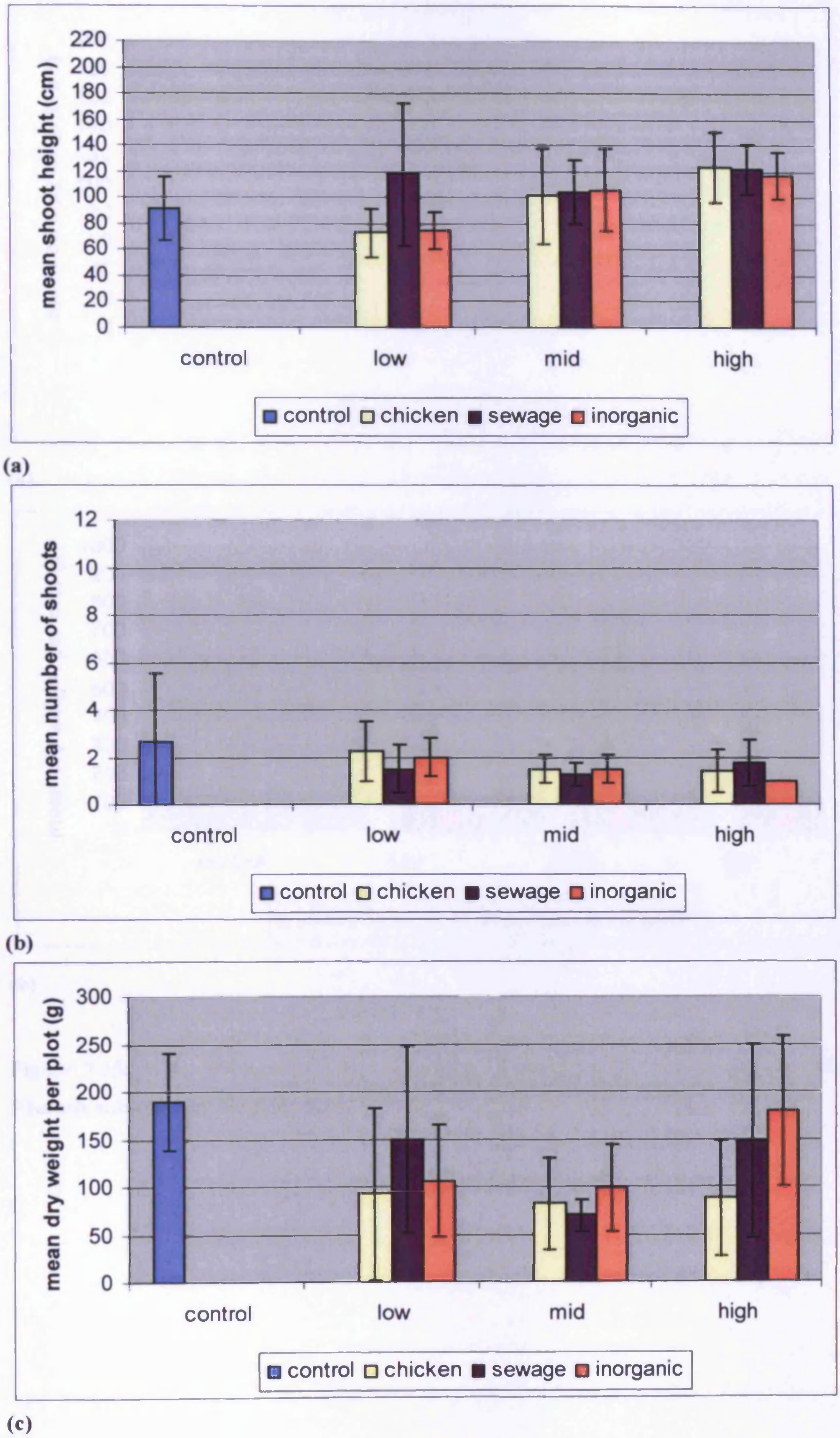
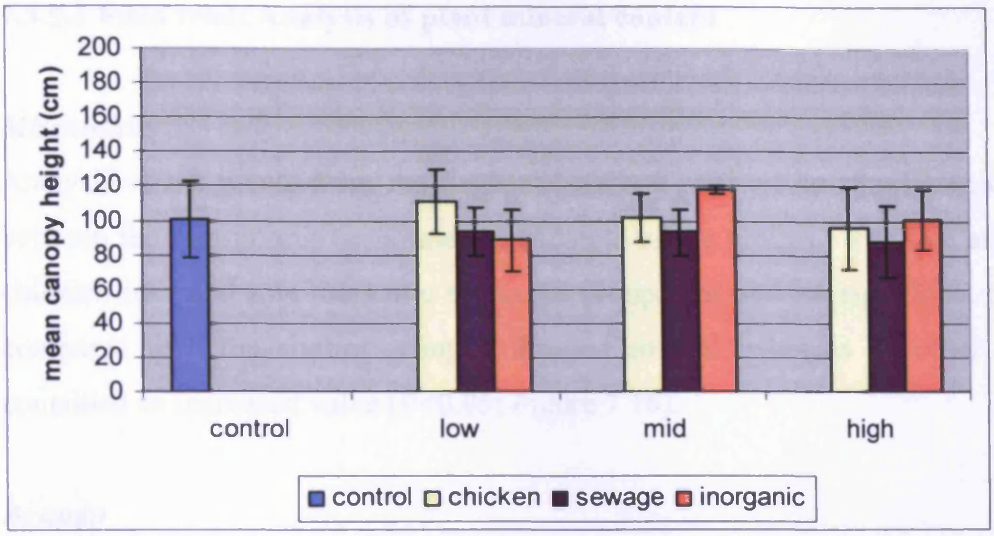
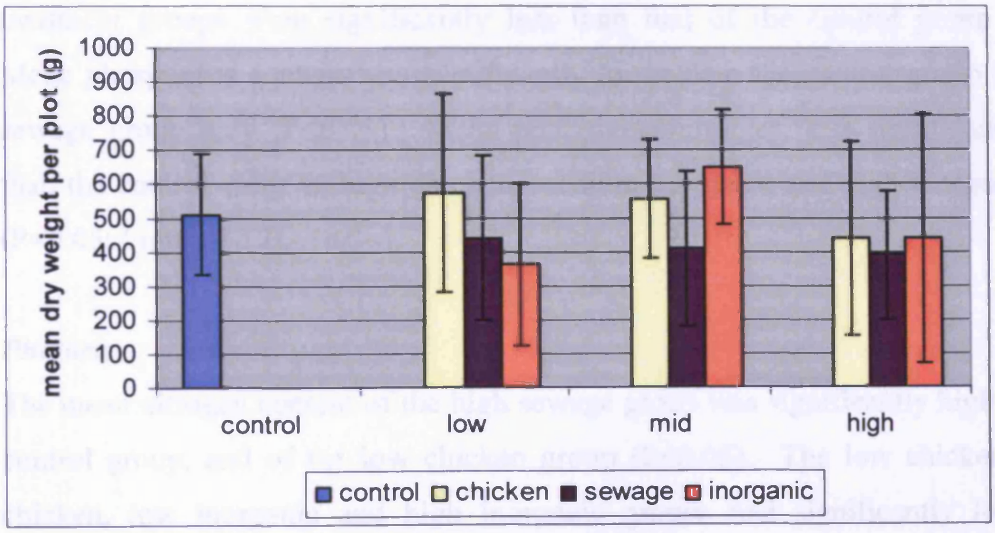


Figure 7.14: Mean ±1 standard dev (a) shoot height, (b) number of shoots per plant, and (c) dry weight per plot at harvest, for *Arundo* sub-plots in the field trial.



(a)



(b)

Figure 7.15: Mean ±1 standard dev (a) canopy height, (b) dry weight per plot at harvest, for *Phalaris* sub-plots in the field trial.

7.3.5.3 Field trial: Analysis of plant mineral content

Miscanthus

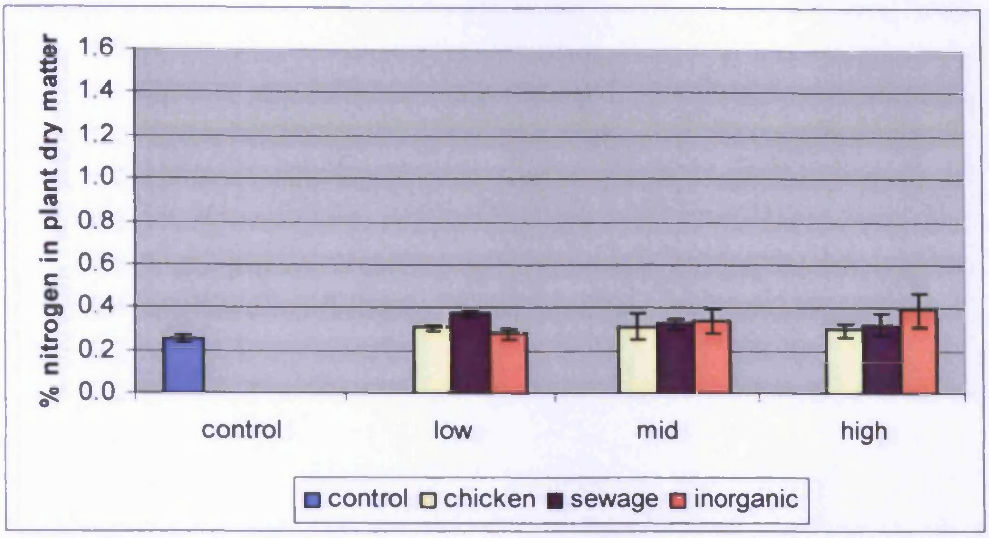
Analysis of the plants from the field trial harvest showed no significant differences between the phosphorus or potassium contents of the groups. The low and medium chicken litter and low inorganic treatment groups showed no significant differences compared with the control group's nitrogen content, whereas all other treatments contained an increased value ($P<0.05$; Figure 7.16).

Arundo

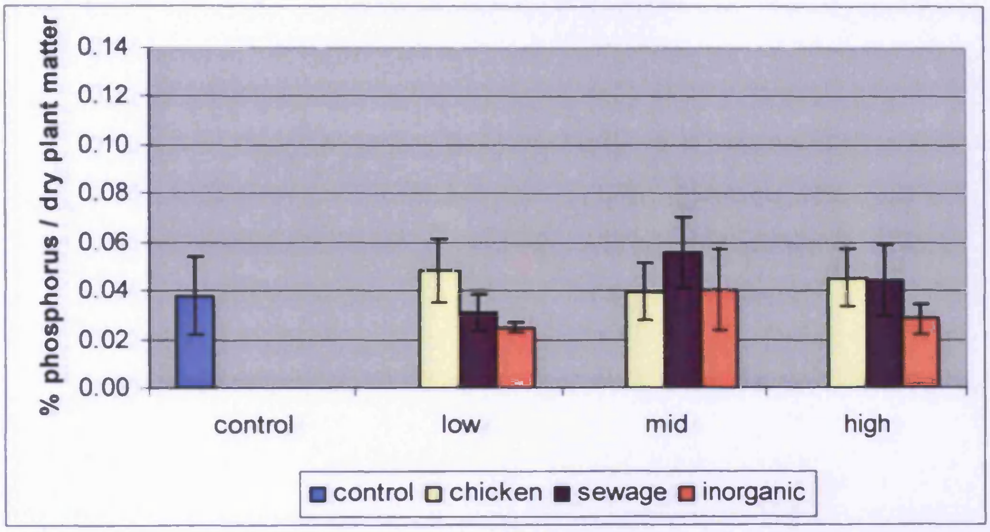
The mean nitrogen contents of the high chicken, low inorganic and high inorganic treatment groups were significantly less than that of the control group ($P<0.05$). Mean phosphorus content was significantly lower than the control group in the low sewage group only ($P<0.05$). Mean potassium, however, was significantly greater than the control value in high chicken, medium inorganic and high inorganic groups ($P<0.05$; Figure 7.17).

Phalaris

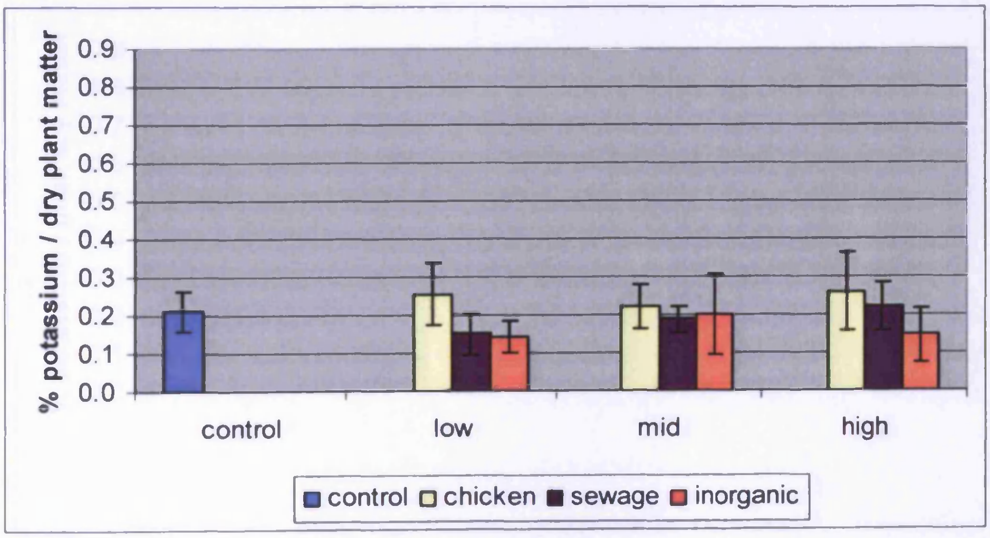
The mean nitrogen content of the high sewage group was significantly higher than the control group, and of the low chicken group ($P<0.05$). The low chicken, medium chicken, low inorganic and high inorganic groups had significantly lower mean potassium contents than the control group ($P<0.05$). There were no significant differences in phosphorus contents (Figure 7.18).



(a)

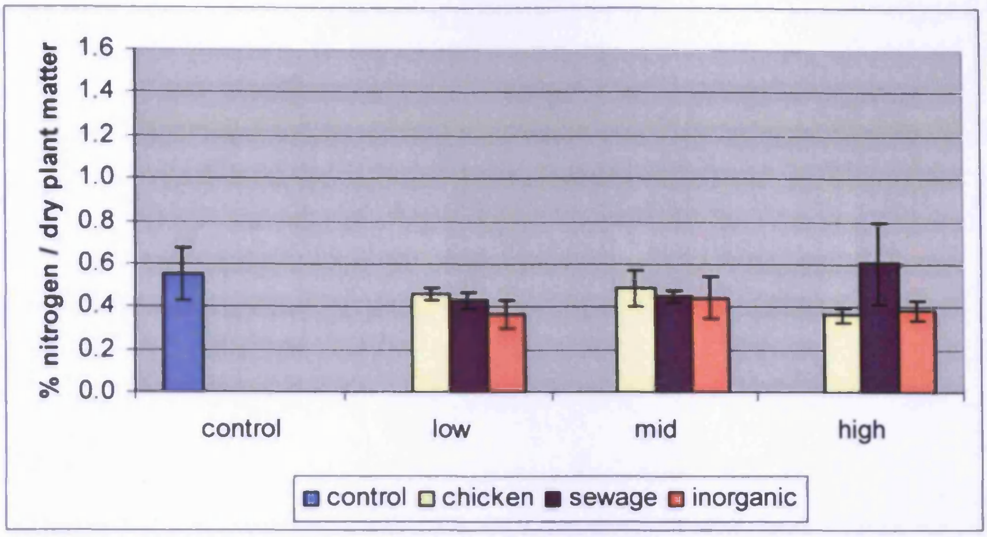


(b)

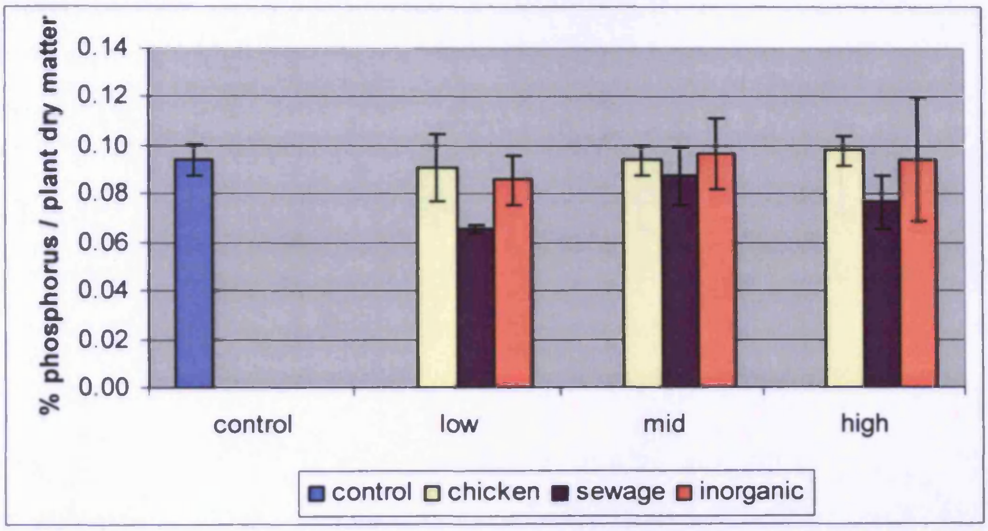


(c)

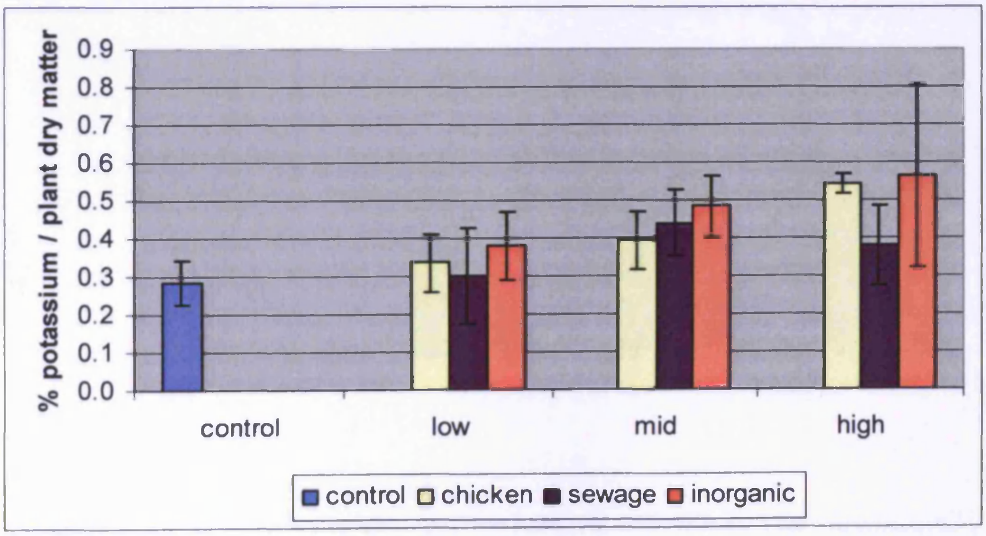
Figure 7.16: Mean ±1 standard dev percentage above-ground plant dry matter contents of (a) nitrogen, (b) phosphorus, and (c) potassium, for *Miscanthus* sub-plots in the field trial.



(a)

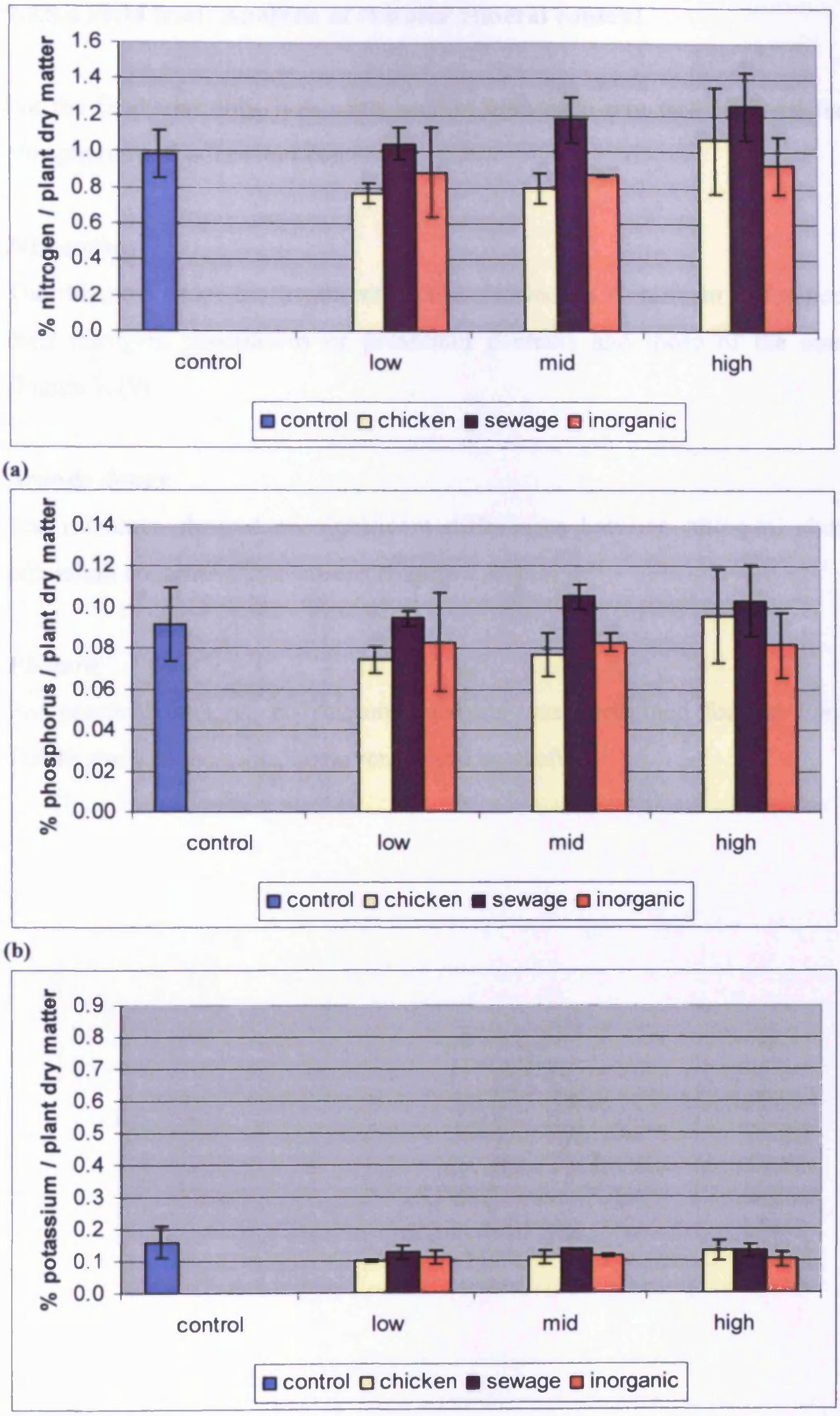


(b)



(c)

Figure 7.17: Mean ±1 standard dev percentage above-ground plant dry matter contents of (a) nitrogen, (b) phosphorus, and (c) potassium, for *Arundo* sub-plots in the field trial.



(c)

Figure 7.18: Mean ±1 standard dev percentage above-ground plant dry matter contents of (a) nitrogen, (b) phosphorus, and (c) potassium, for *Phalaris* sub-plots in the field trial.

7.3.5.4 Field trial: Analysis of rhizome mineral content

For the field trial only, a rhizome section from each plot was analysed for nitrogen, phosphorus and potassium content.

Miscanthus

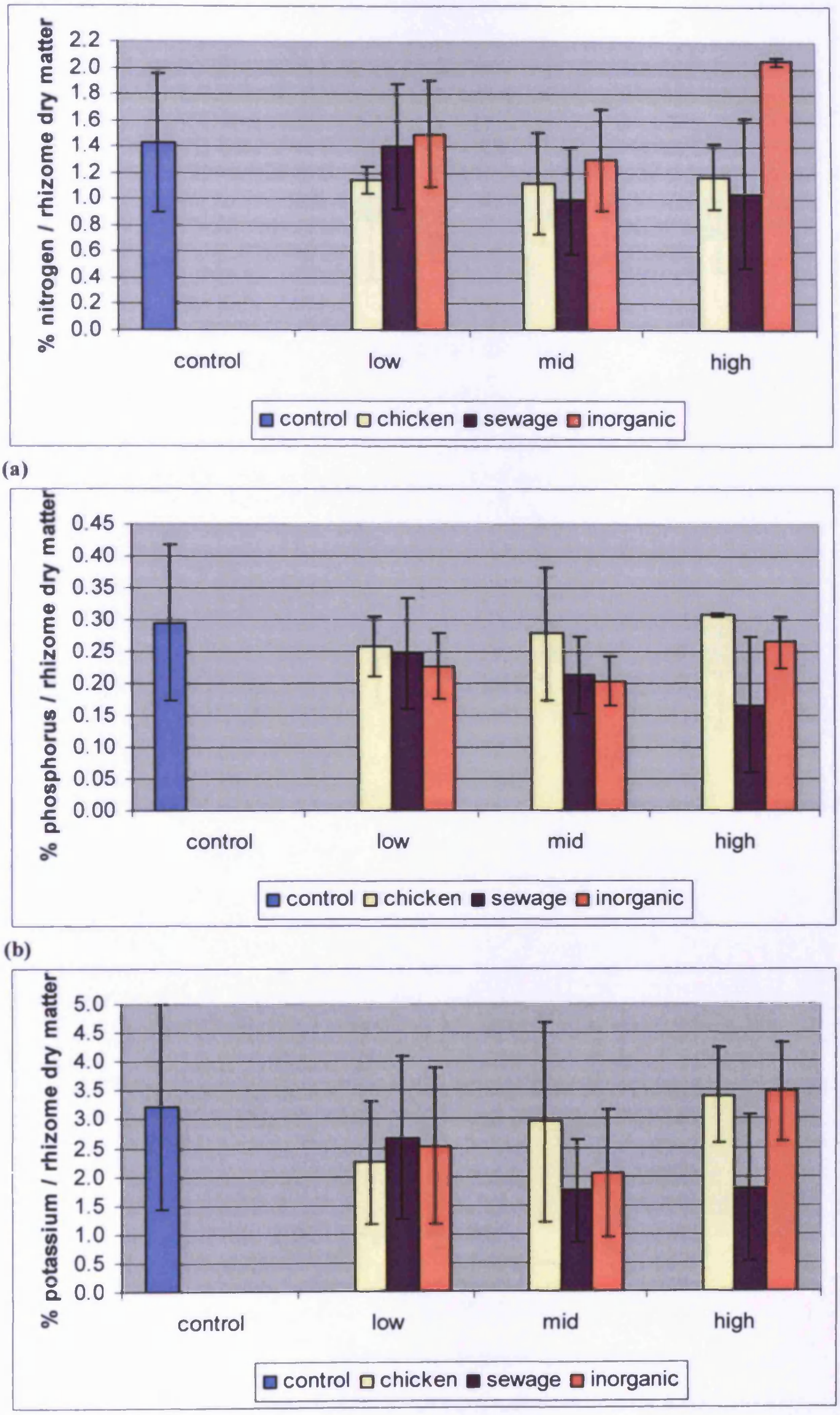
The rhizomes from the treatment groups showed no significant differences between their nitrogen, phosphorus or potassium contents and those of the control group (Figure 7.19).

Arundo donax

The rhizomes showed no significant differences between nitrogen, phosphorus or potassium contents of the groups (Figure 7.20).

Phalaris

For practical reasons, no rhizome analysis was performed for the *Phalaris* crop. Future study of the roots, however, would be useful.



(a)

(b)

(c)

Figure 7.19: Mean ±1 standard dev percentage rhizome dry matter contents of (a) nitrogen, (b) phosphorus, and (c) potassium, for *Miscanthus* sub-plots in the field trial.

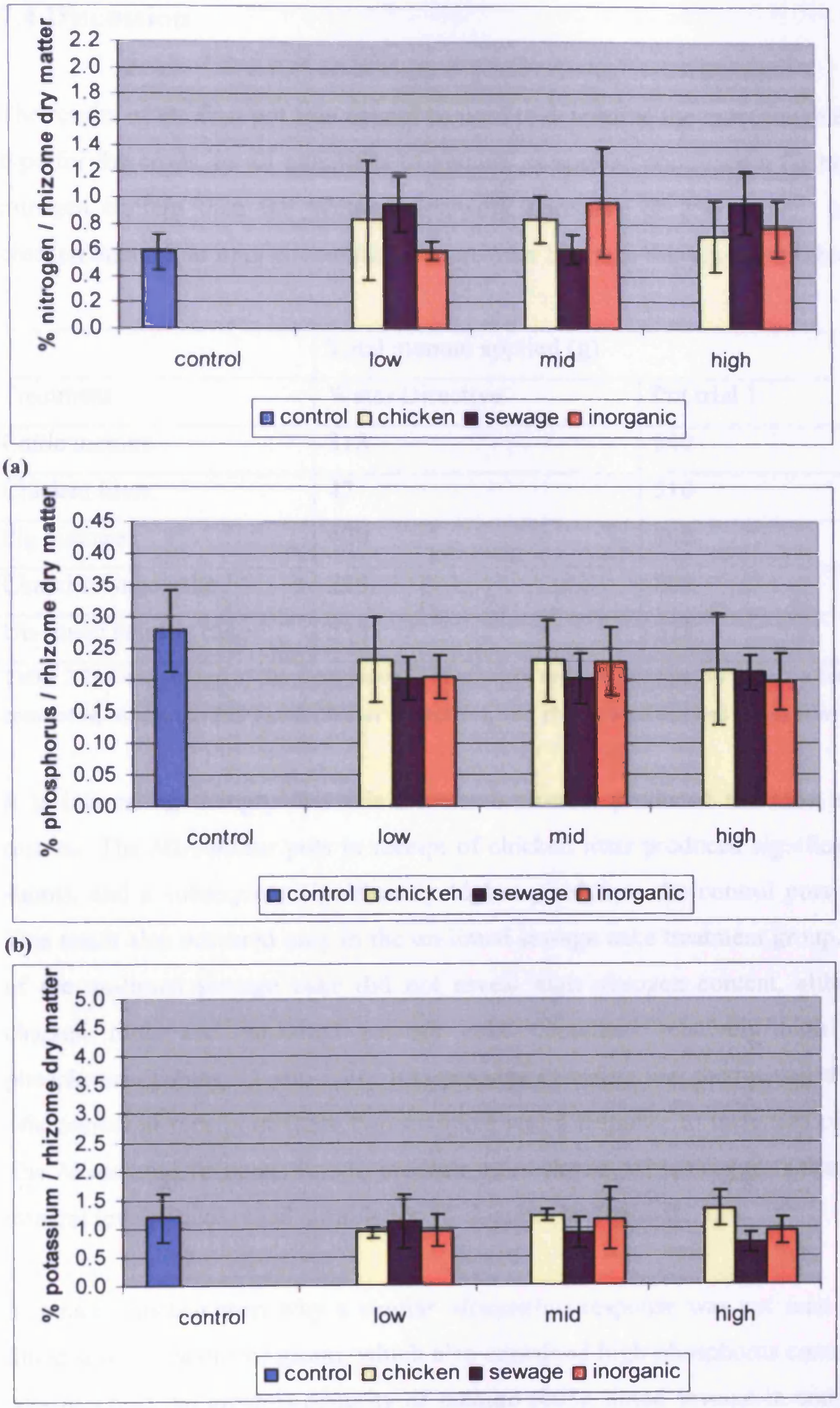


Figure 7.20: Mean ±1 standard dev percentage rhizome dry matter contents of (a) nitrogen, (b) phosphorus, and (c) potassium, for *Arundo* sub-plots in the field trial.

7.4 Discussion

The results of the first pot trial cannot be used to determine the most suitable fertiliser type for the crops, as all quantities of manure as applied contained a far higher total nitrogen content than the Water Framework Directive of 250 kg/ha. Indeed the chicken manure, as applied, contained more than 10 times this amount (Table 7.7).

	Total manure applied (g)	
Treatment	Water Directive	Pot trial 1
Cattle manure	318	540
Chicken litter	47	510
Pig manure	400	700
Limed sewage cake	220	805
Un-limed sewage cake	234	325

Table 7.7: Comparison of the total amount of manure required per pot to supply a total nitrogen content equivalent to 250 kg/ha (Water Directive), and the amount applied in pot trial 1.

It is interesting though, that this excessive amount produced the most significant results. The *Miscanthus* pots in receipt of chicken litter produced significantly more shoots, and a subsequent significantly higher yield than the control pots in year 1. This result also occurred only in the un-limed sewage cake treatment group. Analysis of the un-limed sewage cake did not reveal high nitrogen content, although both chicken litter and un-limed sewage cake contained relatively high levels of phosphorus (Tables 7.1 and 7.2). It is possible therefore that the increased growth of *Miscanthus* in receipt of these two manures was a response to increased phosphorus. The *Miscanthus* response was to produce more shoots, which suggests that increased mineral levels encouraged stimulation of rhizome bud growth.

It remains unclear then why a similar *Miscanthus* response was not seen within the limed sewage treatment group, which also contained high phosphorus content. These pots received the greatest quantity of manure (805g limed sewage in comparison to 325g un-limed sewage), so it is possible that this quantity of manure inhibited shoot growth in *Miscanthus*, but not in *Arundo*.

The *Miscanthus* plant matter from the chicken litter treatment group contained significantly more nitrogen at harvest, so some of the excess nitrogen was taken up and retained in the crop. This has implications for its use as a combustion crop, which are required to have low nitrogen content at harvest.

In the second year all manure treatment groups achieved a significantly greater height and dry weight than the control pots. This suggests that there was a delay in the plants' response to manure application, caused most probably by the slow release of organically bound nutrients. This was shown by Gutser *et al.* (2005), who recorded little effect of organic manures in the year of application, and by Lynn (1995) after sewage application to woodland ground flora. The chicken litter, limed sewage and un-limed sewage treatment groups showed a highly significant increased yield. As in the first year, this may have been an effect of increased phosphorus levels in the soil, although, as phosphorus is an essential mineral involved in root development, it is possible that these plants developed an improved root and rhizome structure. Although other studies have shown that the phosphorus requirement of *Miscanthus* can be met by soil reserves (Clifton-Brown, 2007) or very low fertiliser input (Christian *et al.*, 2008), this study provides evidence that high phosphorus input may produce an enhanced growth response.

It should also be noted that chicken litter contains greatly increased nitrogen levels, so the overall success of this group may have been a nitrogen response. Although many studies in the literature report no response of *Miscanthus* to nitrogen fertiliser (Christian *et al.*, 1999; Danalatos *et al.*, 2007; Himken *et al.*, 1997; Christian *et al.*, 2008) or report that a low nitrogen input is optimal (Boehmel & Claupein, 2007), Ercoli *et al.* (1999) showed that *Miscanthus* yields increased with increased nitrogen fertiliser applications of up to 200 kg/ha. It is possible therefore that the crop does indeed respond to excessive nitrogen input.

Although the chicken litter application increased *Miscanthus* growth in both the first and second years, it had a negative effect on plant survival (3 out of 5 plants survived), which suggests that excessively high mineral levels can cause plant death. Other studies of chicken litter applications have shown a potentially toxic build up of copper and zinc after successive applications (Cooper & Warman, 2000; Zhou *et al.*, 2005; Gascho & Hubbard, 2006), and a previous study of sewage sludge application to *Miscanthus* revealed accumulation of heavy metals in the roots and rhizome (Visser & Pignatelli, 2001).

Similarly to the *Miscanthus* results, the chicken litter, limed sewage and un-limed sewage groups produced a positive yield response in *Arundo*. All these groups produced a significantly greater dry weight at harvest than the control group in both years 1 and 2, and this was reflected in a significantly greater shoot height in year 2. As discussed with reference to *Miscanthus*, these three manures contain high phosphorus, but only chicken litter and limed sewage cake contain high levels of available nitrogen. It is therefore likely that both *Arundo* and *Miscanthus* respond to high levels of phosphorus application. There are few studies of *Arundo* in the scientific literature, although El Bassam (1998) suggests the crop has a phosphorus requirement in excess of 200 kg/ha. All manures in this trial exceeded this requirement. The response of *Arundo* therefore may continue with much higher phosphorus levels.

In *Arundo*, survival was affected in the pig manure and un-limed sewage cake treatment groups, with 3 out of 5 and 4 out of 5 plants surviving respectively. The worst survival therefore was shown by the pig manure treatment group, which cannot be explained by its nitrogen, phosphorus or potassium contents. Pig manure, however, contains the highest level of magnesium (1.2%; in comparison to 0.13% in un-limed sewage cake; 0.15% in limed sewage cake; 0.21% in cattle manure; and 0.73% in chicken litter). It is possible that magnesium levels, or other minor nutrients not analysed in this study, had an effect on *Arundo* survival.

The mulching effect of manure application in the first pot trial was not studied, although some organic mulch applications to pot trials of willow have produced increased yields (Heaton, 1999; Lowthe-Thomas, 2003). These have been attributed to increased soil moisture and decreased fluctuations in soil temperature. However the increased yields following chicken litter application in this study were not shown in willow, and it was shown that this manure did not have natural mulching properties (Heaton, 1999). Therefore, although it is likely that some of the organic manures applied in this study produced mulching effects, the increased growth of *Miscanthus* and *Arundo* in response to chicken litter was more attributable to nutrient content.

The *Phalaris* pots showed no response to fertiliser application in the first pot trial. *Phalaris* crops are generally poor during the establishment period (personal obs.) and therefore may be unsuitable for short pot trials. No significant differences between groups were also seen in the second pot trial. However no significant differences were shown in the field trial either, which suggests that the crops may not have a response to fertiliser application, as was found by Katterer *et al.* (1998). Further study is required to investigate any evidence of a delayed response to fertiliser application in subsequent years. However it is possible that higher levels of fertiliser application may produce a different response, as Lewandowski and Schmidt (2006) showed an increased response to increasing nitrogen input, and Partala *et al.* (2001) showed the uptake and translocation of ^{15}N-labelled nitrogen by *Phalaris* crops. Studies of wastewater application to *Phalaris* crops have shown uptake of nitrogen after treatment, with an increased yield response to increased nitrogen levels (Scheaffer *et al.*, 2008).

The mineral levels applied in the second pot trial and the field trial were much lower, as they were calculated using the MAFF guidelines. In contrast to the first pot trial, the chicken litter and sewage cake treatment groups applied to *Miscanthus* plants did not out-perform the control group in the second pot trial. Increased plant height was recorded in the cattle and pig manure treatment groups, and significantly increased yield was achieved by the cattle manure treatment group only.

As available nitrogen was regulated, the differences in growth were caused by other factors. There were no excessive quantities of minerals applied, so this trial is not comparable to the first pot trial. The sewage cake treatment contained the most phosphorus, although its effects were inconsistent in this trial, as there was high variation within the group. The mean dry weight per pot was highest in the sewage cake treatment group, but the results were not significant. The pig and cattle manure treatment groups received the most potassium; with cattle manure content nearly double that of pig manure. Thus the growth response was likely to have been a result of increased potassium in the manure. However, there was evidence of increased potassium uptake in the pig manure, cattle manure and chicken litter treatment groups, as these plants contained significantly more potassium at harvest. As potassium is an essential element involved in the plant uptake of nitrogen (Yara Intl, 2008), it is possible that, when nitrogen levels are fairly low, the importance of potassium increases to enable more efficient nitrogen uptake.

All *Miscanthus* treatment groups in this trial had a higher mean dry weight than the control group, although the results were not significant. This suggests that the crop responded to fertiliser treatment. The lowest values were recorded for the chicken litter group and the inorganic nitrogen group, which both received the least phosphorus. This re-enforces the concept that phosphorus is the most important mineral for enhanced *Miscanthus* growth.

The response of the *Arundo* plants to fertiliser application in the second pot trial showed large variation, and the variation within all treatment groups was larger than that shown in the control group. The mean dry weight per pot was higher in the control group than all treatment groups. This result was mirrored in the field trial, where the mean dry weight achieved by the control plots was greater than that achieved by all treatment plots. This suggests that fertiliser applications at these levels, in keeping with the Water Framework Directive, were insufficient to produce a growth response in *Arundo* plants, and may have inhibited crop growth in some way. The first pot trial showed that the crops do respond to higher application rates.

However, application of inorganic fertilisers to *Arundo* pots in year 1 produced an increased mean dry weight in the low and high application levels, although this result was not significant. In year 2, increased mean dry weights were achieved by all application levels of inorganic fertiliser, although this again was not significant. In the field trial, a greater mean dry weight was achieved by the high application level of inorganic nitrogen than its low and medium levels. This was slightly lower than that achieved by the control group, although higher than all other treatment groups. Despite all results being non-significant, this suggests that the nitrogen levels used in this trial were insufficient to meet *Arundo* requirements. Other studies have shown contrasting results, with a nitrogen requirement of 100 kg/ha shown by El Bassam

(1998), and no response to further applications; or an increased yield effect achieved with 200 kg N/ha (Angelini *et al.*, 2005).

However, the *Arundo* field trial was consistently unsuccessful due to very poor establishment of the crop and out-competition from *Phalaris* that was previously planted at the Llysdinam site. Therefore I do not think any more conclusions can be drawn from the field trial growth and yield results.

In the *Miscanthus* pots all inorganic fertiliser levels produced an increased mean dry weight in comparison to the control pots. This result was significant only in the low application level in the first year, although all application levels were significantly different from the control in the second year. There were no differences between the nitrogen application levels, so it is possible that, either a low nitrogen input of 50 kg/ha had a growth effect, or that the inorganic phosphorus and potassium application had a growth effect. Other studies have shown the annual nitrogen requirement of *Miscanthus* to be from 40 kg/ha (Boehmel & Claupein, 2007), to 50-70 kg /ha (Himken *et al.*, 1997), 69 kg/ha (Christian *et al.*, 2008), 93 kg/ha (Long & Beale, 2001) and 114 kg/ha (Lewandowski & Schmidt, 2006).

The poor performance of the second *Miscanthus* pot trial in comparison to the first pot trial suggests that the plants were under stress from other factors. The second pot trial was located within the field at Llysdinam whereas the first was located in a sheltered position at the edge of the car park, which may have offered protection from temperature fluctuations and wind exposure. It is possible that the exposed nature of the second trial caused poor crop success; as shown in other chapters the *Miscanthus* field crop at Llysdinam was very poor in comparison to other sites. The two *Arundo* pot trials did not show differences in crop success. This suggests that *Arundo* plants were less affected by the differences in site conditions. However as the two trials were planted in different years, it is also likely that there were differences in the quality of rhizomes planted between the years. The *Miscanthus* rhizomes from the two trials were sourced differently and may have been different in terms of rhizome size, nutrient content and number of buds present.

The *Miscanthus* field trial did not produce significant growth results for differences between treatment groups. Other studies likewise have shown no response of *Miscanthus* to fertiliser application in a field situation (Christian *et al.*, 1999; Danalatos *et al.*, 2007; Himken *et al.*, 1997; Christian *et al.*, 2008), and some suggest that rainfall is a more important factor affecting yield (Christian & Haase, 2001). In pot studies the irrigation is controlled by the experimenter and therefore may present a clearer image of the crop's response to fertiliser only, in the absence of other environmental factors. In a field situation the fertiliser effect can be over-ridden by more important environmental factors. It is also possible that the field trial did not show significant differences because the timing of the manure application was not suited to the crops' requirement for increased nutrient uptake. Christian *et al.* (1997) showed that only 14% of spring applied inorganic nitrogen was taken up by *Miscanthus* plants, which most probably obtained its shoot nitrogen from rhizome reserves. 40% of the applied fertiliser nitrogen was lost from the system, although this would probably occur less in a pot situation. Christian *et al.* (2008) suggest that fertiliser application may be more useful later in the growing season.

However, the mean dry weight produced from the plots receiving inorganic nitrogen was higher than that achieved by the chicken litter and sewage cake treatment groups. The inorganic treatment group also produced a higher mean dry weight than the control group, and this was most pronounced in the low and high application levels. As the mean dry weight produced by the chicken litter and sewage cake treatment groups were lower than that of the control, it appears that in a field situation the application of manures had an inhibitory effect on growth. All results were non-significant, so no conclusions can be drawn, but it appears that, in this trial, the application of inorganic nitrogen was most effective at increasing *Miscanthus* yield in the year of application. As other studies have shown a delayed plant response after application of organic manures (Lynn, 1995; Gutser *et al.*, 2005), further years' study is required to determine if the crops would show a second year response.

There was large variation in the field trial results, as can be expected when designing an experiment on an existing crop, which was already subject to variation in growth and survival from the previous two years' growth. Trials of plants grown from rhizomes are always limited by the quality of the rhizomes themselves, in terms of nutrient content and number of buds for shoot production. Therefore variation within treatment groups is inevitable.

Some significant results were shown with regard to increased nitrogen content of the *Miscanthus* shoot samples at harvest. No clear patterns existed between the low, medium and high sewage groups, the medium and high inorganic treatment groups, and the increased nitrogen content of these shoots. A fertiliser study over several years at Rothamsted also showed occasional and inconsistent differences in shoot nutrient contents (Christian *et al.*, 2008).

The plant analysis of the *Arundo* field trial, however, showed interesting results. The nitrogen content of the shoots from the low and high inorganic nitrogen treatment groups and the high chicken litter treatment group were significantly lower than the control group. Similarly the medium and high inorganic treatment groups and the high chicken litter treatment group contained significantly more potassium than the control group. This suggests an interaction between potassium and nitrogen content. It is known that potassium is essential to the uptake of nitrogen, so an increased level of the former mineral would be expected to reflect an increased level of the latter. This was not however reflected in this trial, although it is possible that extra nitrogen taken up during crop growth had been translocated to the rhizomes prior to harvest.

Although the results of the *Miscanthus* rhizome analysis were also non-significant, the high application of inorganic nitrogen appeared to coincide with a consistently high rhizome nitrogen content, which suggests that it was effectively taken up and stored by the plants. In contrast, the mean rhizome nitrogen content was lower in the chicken litter and sewage cake treatment groups than in the control group, which suggests that it was not stored when applied in this form, possibly because it was not immediately available to the crop. Himken *et al.* (1997) showed no effect of nitrogen fertiliser on the rhizome nitrogen content.

Overall the percentage nitrogen, phosphorus and potassium contents of the rhizomes were higher than that in the shoots. This suggests the presence of an efficient recycling system, where nutrients are taken up into the shoots when required and then stored in the rhizomes during senescence. This is supported by the large variation in the nutrient content of the rhizomes, which was greater than that shown within the shoots. This suggests that the rhizomes were storing and supplying nutrients as and when required by the shoots.

Further years' studies were not possible in this trial due to time constraints, and therefore it is impossible to conclude on the effectiveness of the fertilisers. Christian *et al.* (2006) showed that more inorganic nitrogen was taken up by *Miscanthus* in the year after application, and that this increased again in the third year. Clifton-Brown and Jones (2001) showed that overall *Miscanthus* yields increased with nitrogen fertiliser application over a 10 year period. It is likely however that studies of a low number of years may not report a fertiliser effect on *Miscanthus* yield because the crop can obtain its initial nutrient requirements from the soil reserves (Christian *et al.*, 2008; Clifton-Brown *et al.*, 2007) and then store sufficient quantities within its rhizome to exceed subsequent years' demands (Defra, 2007), but that eventually over time the nutrient off-take at harvest would lead to depletion of the soil and rhizome nutrient reserves that need to be replaced with fertiliser application, thus a yield response is seen in later years. As other studies have shown a delayed plant response after application of organic manures (Lynn, 1995; Gutser *et al.*, 2005), further years' study is required to determine if the crops would have shown a second year response.

7.5 Conclusions

Although chicken litter showed the greatest success during the first pot trial, its high nitrogen content means that only small quantities are permitted as a fertiliser application under Water Directive guidelines. Although it has a high percentage of its nitrogen available to crops, the decreased quantity of manure applied during this study meant that less phosphorus and potassium were applied, which appeared to negate its effect on crop success. However, this study standardised the amount of nitrogen applied to the crops for comparison purposes. Under Water Directive guidelines, application of chicken manure is permissible at the high application level (87.5kg available nitrogen/ha). As for sewage cake, a combination of low nitrogen availability and low potassium content render this treatment an unsuitable choice for fertiliser application to these field crops. Water Directive guidelines state that this manure should be applied at the low level (37.5kg available nitrogen/ha), whereas pig and cattle manures are permissible at the medium application level (50kg available nitrogen/ha). Pig, and especially cattle, manures were identified as most suitable for fertiliser application to *Miscanthus* crops in the second pot trial. Similarly cattle slurry was identified as the most suitable fertiliser application for willow (Heaton, 1999). Unfortunately in this study it was not possible to test these manures in a field situation, therefore further study is required to confirm these findings.

Both *Miscanthus* and *Arundo* crops were shown to increase growth in response to high phosphorus application. Further study is required to determine the optimum level of phosphorus application. In order to avoid the risks associated with high nitrogen and phosphorus applications from organic sources, it may be necessary to apply additional phosphorus as inorganic fertiliser. Financial analysis would be required to determine the cost effectiveness of this method.

The response shown in the first pot trial of both *Miscanthus* and *Arundo* to high nutrient application rates suggests these crops may have an important dual purpose application, as combustion and bio-filtration crops. They could be planted as buffers to intercept run-off from other crops or as a direct treatment to contaminated areas. Further study of these uses are required to ensure that the increased nutrient levels are used or translocated from plant material to the rhizomes prior to harvest, to avoid the combustion problems associated with increased plant mineral content. It is also necessary to further investigate the causes of plant death, as seen in the first pot trial, prior to exposing these crops to high contaminant levels.

This study showed no response of *Phalaris* to fertiliser applications. This may be because *Phalaris* does not take up and use additional nutrients beyond its immediate needs from fertilisers, or that the levels applied were insufficient to warrant a response, or that this crop produces a delayed response to fertiliser application. Further long-term study of this crop is therefore required.

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Chapter 8

8.1 General Discussion

As whole crop yields could not be recorded at all sites it was not possible to directly compare the success of each crop at each site. However, general comparisons can be made based on the yield estimates obtained from sub-sampling the crops, as discussed in Chapter 3. The most successful non-destructive sampling method for the *Miscanthus* crops studies were; the measurement of mean shoot height to calculate estimated shoot dry weight, and the measurement of mean total plant height to calculate estimated plant dry weight. The destructive sampling methods revealed that yield estimates based on plant dry weight were more accurate than those based on shoot weight. This was most probably because, at the sites in this study, it proved fairly easy to determine individual plants. At more dense or irregularly planted sites it can be difficult to distinguish individual plants, so yield estimates calculated from shoot measurements may be more effective.

All non-destructive sampling methods produced a highly significant relationship between on-site measurements and *Arundo* crop dry weight. The most consistent results were shown by measurement of mean shoot height during December and January, although the highest R^2 regression statistic was obtained from calculation of the shoot volume in November and December. It was therefore suggested that shoot width is important to yield estimates of *Arundo* crops, although this measurement was shown to have less impact during December and January. It is possible that shoot width did not alter during this period.

The highest yielding crop overall was the *Miscanthus* at Bluestone, followed by the *Miscanthus* and *Phalaris* at Llwynprenteg. The *Arundo* at Bluestone and Llwynprenteg were similar, and the sites at Llysdinam and Cae Clovers were clearly the least successful for all crops. Although Bluestone *Miscanthus* easily exceeded all other crops and sites, the rate of annual growth shown at this site was similar to that shown at Llwynprenteg (Figure 4.4). The increased Bluestone yield therefore was largely a result of a hugely successful first year of growth in 2003, which was prior to this study. This study highlighted many factors that may have created this first year success. The foremost factor, for which there are no data available, is probably the quality of the rhizomes planted in terms of size and number of buds, timing from rhizome harvest to planting and method and duration of storage (ADAS, 2006). The Bluestone *Miscanthus* plants produced more than double the mean number of shoots of *Miscanthus* plants at all other sites (Figure 4.2), which suggests that larger rhizome pieces with more viable buds were utilised.

Other studies have considered the soil clay content to be an important factor in *Miscanthus* establishment (El Bassam, 1998; Price *et al.*, 2004), when soil to rhizome contact is of utmost importance to rhizome survival (ADAS, 2006). The soil at Bluestone contained the least clay of all sites studied (Table 4.3), and the lowest yielding *Miscanthus* crop was grown at the site with the highest clay content (Llysdinam). However the overall survival of plants at Bluestone was reduced in comparison to other sites; therefore rhizome survival was not increased (Table 4.4). This may have been a result of patchiness within the crop caused by large fast growing plants shading out weaker growth. There were also considerable unavoidable flaws in the calculation of crop survival, as discussed in Chapter 4. Therefore the clay content of the soil may have been an important factor in the first year success of the Bluestone *Miscanthus*.

Increased first year yields of the *Miscanthus* at Bluestone, in comparison to Llwynprenteg, may have also been due to the site's climatic conditions. All *Miscanthus* crops were planted in April, irrespective of year of planting. In 2003, at Bluestone, and in 2004, at Llwynprenteg, the last air frost dates were in early April and therefore neither crop was affected by late frosts after planting. The temperatures during 2003 were not higher than in other years, and the cumulative maximum air temperature at Bluestone during the *Miscanthus* growing season, as defined in Chapter 4, was 3467.4°C, which was the lowest of all years (Table 4.9). The growing season ended with a relatively early air frost on 24 October 2003, resulting in a growing season of only 196 days, which was also the lowest of all years (Table 4.8). Rainfall during the *Miscanthus* growing season was sufficient (527.1mm), as determined by other studies (Clifton-Brown *et al.*, 2001; Long & Beale, 2001; Tuck *et al.*, 2006). As rainfall was shown to be sufficient at all sites in all years (Figure 4.30), and increased annual rainfall at Cae Clovers produced no yield response, the differences in *Miscanthus* success were suggested to be caused by other site factors.

During the year of *Miscanthus* establishment at Bluestone (2003) the site was subject to a shorter growing season and lower cumulative temperatures than the other years studied. However, the highest values were obtained from the 2004 climate data, which was the year of establishment for all other *Miscanthus* crops. Therefore only the *Miscanthus* at Bluestone would have been able to fully utilise the early start to the 2004 growing season and subsequent higher cumulative temperatures during the season, which would have produced a further increased growth response in year 2. Therefore the overall success of the Bluestone *Miscanthus* crop was most likely a combination of a successful establishment year, caused by large rhizomes planted in a low clay soil, followed by full utilisation of a prolonged second year growing season with increased cumulative temperatures during the season.

Of the site factors investigated in this study, soil clay content, growing season length and air temperatures during the growing season have been shown to be most important to *Miscanthus* growth. The nitrogen, phosphorus and potassium content of the soil and their agricultural grades were shown to have little effect on *Miscanthus* yield, as Llwynprenteg and Cae Clovers were the only sites to achieve premium grade for all three minerals. As has been discussed, Llwynprenteg *Miscanthus* was out-yielded by the Bluestone crop, whereas the Cae Clovers crop was very poor in comparison to Llwynprenteg. The latter two sites were very similar in terms of soil clay content. The growing season lengths were also very similar, as their proximity exposed the two sites to very similar frost dates. However, higher cumulative air temperatures were achieved at Llwynprenteg (Table 4.9), which suggests that temperatures during the growing season are crucial to *Miscanthus* crop success. However, temperatures experienced during the Llysdinam *Miscanthus* growing season (Table 4.9) were higher than those at Cae Clovers in all years, and higher than Llwynprenteg in years 3 (2006) and 4 (2007). Therefore the lack of crop success at Llysdinam was most likely a result of poor soil with high clay content (42%), although it is also likely that the field site at Llysdinam experienced lower temperatures than the sheltered location of the weather station. The soil association, as described by Rudeforth *et al.* (1984), is Cegin (cambic stagnogley soils) at

Llysdinam, whereas both Bluestone and Llwynprenteg are typical brown earths (Milford and Denbigh 1 association respectively), and Cae Clovers is a typical brown podzolic soil (Manod association). The Cegin association are seasonally waterlogged soils with slowly permeable subsoils. They occur mostly around Newtown and Llandrindod Wells in Powys, but also occupy some areas of west Dyfed, northern Anglesey and south of Aberystwyth (Rudeforth *et al.*, 1984). It is possible that this type of soil is unsuitable for *Miscanthus* growth, which would explain the lack of success experienced at Llysdinam in this study.

The second most productive crop in this study, as determined by whole crop harvest of all crops at the Llwynprenteg site in March 2007, was *Phalaris* (Table 3.13). However these results relate to third year *Miscanthus* and *Phalaris* crops and a second year *Arundo* crop, which was shown to produce a decreased yield in year 2. Few of the experiments in this study were carried out on *Phalaris* crops, as many experiments relied upon plant sub-sampling. In *Phalaris* crops it was not possible to determine individual plants, so sub-sampling was only possible by area. This proved to be very problematic, due to lodging of the crop during the growing season. Therefore it was decided to exclude this crop from many experiments in this study. Further research into this crop is clearly needed, and this study highlighted the possibility that it may be more suited to a two-cut cropping system, in an attempt to avoid the lodging problems. However, it was the only crop to achieve moisture content levels low enough for immediate combustion (Chapter 5); which would be considerably affected by earlier cutting. The mineral content of the harvested crop was shown to be below power station threshold levels for the dry material, but continued growth during the winter season resulted in new shoots being included in the harvested material. At Llwynprenteg this re-growth was sufficient to increase mineral content levels above the threshold, but not at Llysdinam (Chapter 5). The differences between the two sites were likely to be caused by decreased winter temperatures at Llysdinam, which resulted in less new shoot growth. Therefore this crop may be better suited to colder temperatures for energy crop purposes.

The *Arundo* crops at Bluestone and Llwynprenteg grew at a similar rate, although it is unlikely that they reached maturity during the three years of this study. European studies have reported very dense high yielding crops (El Bassam, 1998; Christou *et* *al.*, 2001; Angelini *et al.*, 2005), whereas in this study all plants carried less than 10 shoots, which were of varying height and width. The *Arundo* crop at Llysdinam was very poor, and was out-competed by the *Phalaris* crop formerly planted at this site, which had not been successfully eradicated. The lack of *Arundo* competitive advantage was most probably caused by the high clay content of the Llysdinam soil, and lack of soil preparation prior to planting, which would have prevented good soil to rhizome contact after planting (ADAS, 2006).

The agricultural grade of the soil was shown to have little effect on *Arundo* crop, as there was little difference between the crop yields at Llwynprenteg and Bluestone. This was most probably due to sufficient nutrient reserves being present in the rhizomes. However, decreased plant survival was shown at Llwynprenteg in comparison to Bluestone during the first winter (Table 4.4). The *Arundo* rhizomes were obtained from one source, stored together and planted by hand; therefore the survival data are more reliable than those produced for *Miscanthus*. This suggests that a proportion (30%) of the Llwynprenteg crop was lost during the first winter. This site experienced the first mild (-0.3°C) autumn air frost on 13 November, but was then subjected to 13 days of air frosts ranging from -2.1°C to -5°C from 16 November to 29 November, whereas Bluestone only experienced four days of air frosts of -1.6°C to -3°C from 20 November to 23 November. It is highly likely therefore that many above ground green shoots of the *Arundo* crop at Llwynprenteg were prematurely killed by the succession of severe frosts during November, which prevented sufficient nutrient translocation to, or development of, its rhizome and root structures.

Decreased yields in year 2 were produced from the *Arundo* crop at both Llwynprenteg and Bluestone, in comparison to years 1 and 3. During this year (2006) both sites experienced a summer period of higher temperatures and lower precipitation than the other years studied (Chapter 4). Therefore the decreased growth during this period reflected its sensitivity to warm, dry conditions, although this may have been a feature of immature plants that had not fully developed their root systems, and thus were unable to tap deeper water resources.

The growing season of *Arundo* crops was not determined in this study but all results indicated that it continued growth throughout the winter months. The chlorophyll meter study (Chapter 6) indicated that *Arundo* retained some green leaves and thereby retained its photosynthetic capability throughout the period. It also retained a constant level of nitrogen and phosphorus in both its leaves and cane, which were higher than the levels contained in *Miscanthus* plant matter. Potassium content fell slightly, which was most probably caused by leaching during the high winter rainfall experienced during this study, but levels remained higher than in *Miscanthus*. Correlation was significant between mean leaf chlorophyll content and nitrogen, phosphorus and potassium content of above-ground plant matter, and thus could be used to estimate mineral content of the crop.

Although the mean *Arundo* plant dry weight decreased from November to February in both years there were large variations between plants and no results were significant. Weight loss is indicative of leaf death and abscission, but the large variation in results suggested that different shoots were at different stages of maturity throughout the period. Indeed, personal observations were that plants were a constant mixture of tall, short, wide and thin shoots. The implications of these results for the use of *Arundo* as a combustion crop are inconclusive as the mineral threshold levels reported in the peer-reviewed literature (Jorgensen, 1997; Lewandowski & Kicherer, 1997; Kauter *et al.*, 2003; Yates & Riche, 2007; Monti *et al.*, 2008) are consistently lower than those contained in straw, which is successfully used for combustion (Christian *et al.*, 2006). Therefore *Arundo* may be suitable for combustion in purpose-built boiler systems or as a co-firing fuel.

However the moisture content of both *Arundo* and *Miscanthus* exceeded recommended levels in both years for all sites bar one. It would therefore be necessary to dry both of these crops after harvest prior to combustion. Other studies indicated that this could effectively be achieved by leaving the crops outdoors (El Bassam & Huisman, 2001; Nixon & Bullard, 2003; Luxton, pers. comm.), which may help to reduce their mineral content (Lewandowski *et al.*, 2003).

The results from this study indicated that *Miscanthus* leaves and cane contained sufficiently reduced levels of nitrogen, phosphorus and potassium from November to December onwards, but that the crop was most suitable for combustion after leaf abscission in December (Table 5.10). The nitrogen, phosphorus and potassium content of above ground plant matter was shown to be correlated with mean leaf chlorophyll content, which was suggested to be a useful method of determination of harvest date, which should ideally coincide with nutrient decline. Leaf chlorophyll was also shown to be correlated with *Miscanthus* shoot height and dry weight, which may be used as an indicator of site suitability for crop growth in future studies.

The fertiliser trials (Chapter 7) produced few clear results, which was partly caused by the delayed response of crops to organic manure application, as shown in other studies (Lynn, 1995; Gutser *et al.*, 2005). The unavoidable restriction of this study to one year for the second pot trial and field trial prevented observation of a second year response to the fertilisers. However this study indicated that both *Miscanthus* and *Arundo* have an increased growth response to high levels of phosphorus. This result was mirrored in Chapter 4, which showed a strong correlation between *Arundo* plant weight and soil phosphorus ($P=0.056$, $R^2=0.992$, $n=3$). At high application levels the increased response to organic phosphorus was shown in the first and second years' growth. At application levels determined in accordance with the Water Framework Directive (MAFF, 1998) the *Miscanthus* crop showed a response to increased levels of potassium. As potassium is an essential element involved in the plant uptake of nitrogen (Yara Intl, 2008), it is possible that, when nitrogen levels are fairly low, the importance of potassium increases to enable more efficient nitrogen uptake. The *Arundo* crop did not respond to the fertiliser levels applied under the Water Framework Directive (MAFF, 1998), which suggests that these levels were insufficient to produce a growth response.

The response shown in the first pot trial of both *Miscanthus* and *Arundo* to high nutrient application rates suggests these crops may have an important dual purpose application, as combustion and bio-filtration crops. They could be planted as buffers to intercept run-off from other crops or as a direct treatment to contaminated areas. Further study of these uses are required to ensure that the increased nutrient levels are used or translocated from plant material to the rhizomes prior to harvest, to avoid the combustion problems associated with increased plant mineral content. It is also necessary to further investigate the causes of plant death, as seen in the first pot trial, prior to exposing these crops to high contaminant levels.

Although chicken litter resulted in the greatest growth response during the first pot trial, its high nitrogen content means that only small quantities are permitted as a fertiliser application under Water Directive guidelines (MAFF, 1998). Although it has a high percentage of its nitrogen available to crops, the decreased quantity of manure applied meant that less phosphorus and potassium were applied, which appeared to negate its effect on crop success. However, this study standardised the amount of nitrogen applied to the crops for comparison purposes. Under Water Directive guidelines, application of chicken manure is permissible at the high application level (87.5kg available nitrogen/ha). As for sewage cake, a combination of low nitrogen availability and low potassium content proved this treatment to be an unsuitable choice for fertiliser application to these field crops. Water Directive guidelines state that this manure should be applied at the low level (37.5kg available nitrogen/ha), whereas pig and cattle manures are permissible at the medium application level (50kg available nitrogen/ha). Pig, and especially cattle, manures were identified as most suitable for fertiliser application to *Miscanthus* crops in the second pot trial. Similarly cattle slurry was identified as the most suitable fertiliser application for willow (Heaton, 1999). Unfortunately in this study it was not possible to test these manures in a field situation, but further study is required to confirm these findings.

This study has repeatedly highlighted the need for long-term studies of these energy crops in Wales. In Chapter 3 it was noted that shoot morphology may change as the crops age and that the yield estimate methods recommended may become less effective. Chapter 4 highlighted the effects of annual climatic differences on crop success, and therefore identified a need for long-term studies to "iron out" the annual differences. It also suggested that older crops may respond differently to climatic factors, due to increased root and rhizome development. In Chapter 5, the *Arundo* crops lost moisture during the first year's sampling period but failed to lose any moisture during the second year of sampling, which may have been a factor of age. Reference was made to other studies that reported differences in mineral composition of differently aged plants (Christian *et al.*, 2008; Monti *et al.*, 2008). The chlorophyll meter study (Chapter 6) was restricted to one year of study so it is unknown if age would effect the results. The fertiliser trial (Chapter 7) highlighted the need for study of the long term effects of manure applications, to understand the delayed crop acquirement of minerals and possible toxic mineral build-ups. It was suggested that studies of early crop growth do not report a fertiliser effect on crop yields because they can obtain their initial nutrient requirements from the soil or rhizome reserves (Christian *et al.*, 2008; Clifton-Brown *et al.*, 2007) and then store sufficient quantities within their rhizomes and roots to exceed subsequent years' demands (Defra, 2007). Eventually over time the nutrient off-take at harvest would lead to depletion of the soil and rhizome nutrient reserves that need to be replaced with fertiliser application, thus a yield response may be seen in later years.

8.2 Conclusions

This study identified soil type and texture to be of utmost importance to the success of both *Miscanthus* and *Arundo* crops. In particular low soil clay content was considered important in addition to good soil preparation prior to planting. These were deemed of particular importance to *Miscanthus* and *Arundo* crops because they are planted as rhizomes, which are irregularly shaped and therefore require non-clayey well-prepared soil to ensure good soil-rhizome contact. The Cegin soil association was shown to be unsuitable for *Miscanthus* and *Arundo* crops. Although not directly studied, rhizome quality including size and number of buds was also considered to be of importance.

Arundo was shown to be sensitive to harsh autumn frosts and to warm, dry conditions during the summer. *Miscanthus* was shown to respond positively to an increased growing season length and higher cumulative temperatures during the season. It was suggested that *Phalaris* may be more suited to lower winter temperatures for a one-cut system, but research of its use for a two-cut system is required. Soil type and texture was not shown to be important to *Phalaris* success, so this crop may be more suited to clay soils and those similar to the Cegin association.

Methods of monitoring *Miscanthus* and *Arundo* crops using non-destructive field measurements or chlorophyll meter readings were developed, and these were shown to provide good estimates of crop yield. Results obtained following application of organic fertilisers at recommended rates (MAFF, 2000) were inconclusive, partly due to the restricted time-length of the study, although indications were that cattle manure may be most suitable. Application of organic fertilisers at high levels in excess of MAFF recommendations showed an increased growth response of *Miscanthus* and *Arundo* plants to high phosphorus levels. However, plant survival was shown to be reduced by the highest manure application, and this was considered to be in response to a build-up of secondary minerals not studied. The results were considered to be of importance to biofiltration and bioremediation applications, but require further research.

Late harvest of both *Miscanthus* and *Arundo* crops was shown to be most suitable for their application as combustion crops, although both crops required further drying in order to meet a 25% moisture content threshold. The leaves of both crops contained higher nitrogen, phosphorus and potassium contents than the cane, and it was suggested that *Miscanthus* should be harvested after leaf abscission. *Miscanthus* plants fell to within the range of nitrogen and potassium threshold values, as reported in the peer-reviewed literature, during November – December, and total leaf abscission had occurred by February. *Arundo* crops did not lose their leaves, and therefore the harvested crop contained higher mineral levels than *Miscanthus*. *Arundo* plants showed a decline in mineral content of leaves and cane through the winter period but did not fall to within the nitrogen and potassium threshold range at both sites. Further research is required to determine if further decline in mineral content occurs in early spring, and if the use of desiccants is suitable to encourage leaf abscission.

Phalaris was the only crop to achieve moisture content levels low enough for immediate combustion, and its mineral content was shown to be below power station threshold levels for its dry material. However, continued growth during the winter season resulted in new shoots being included in the harvested material, and this was shown to be sufficient at the warmer site to increase mineral content levels above the threshold. Therefore this crop may be better suited to sites with colder temperatures, although further long-term study is required.

8.3 References

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Appendix 1

Sources of Organic Manure Samples.

Cattle manure:	Penllys Farm, Newbridge-on-Wye.
Chicken litter:	Five Turnings Farm, Knighton.
Pig manure (year 1):	Pigs Folly, Garth Mill, Garth.
Pig manure (year 2):	Woodhouse Farm, St. Harmon.
Sewage cake:	Kelda Water, Eign Sewage Works, Hereford.

Appendix 2

Site Photographs

Miscanthus at Llysdinam

Phalaris at Llysdinam

Arundo at Llysdinam

Phalaris, *Arundo* and *Miscanthus* (foreground to background) at Llwynprenteg

Miscanthus at Llwynprenteg

Phalaris at Llwynprenteg

Miscanthus at Bluestone 1

Arundo at Bluestone 2