Evaluation of *Miscanthus* Winter Hardiness and Yield Potential in Ontario

by

Ben Rosser

A Thesis

Presented to

The University of Guelph

In partial fulfilment of requirements

for the degree of

Master of Science

In

Plant Agriculture

Guelph, Ontario, Canada

ABSTRACT

EVALUATION OF *MISCANTHUS* WINTER HARDINESS AND YIELD POTENTIAL IN ONTARIO

Ben Rosser
University of Guelph, 2012

Advisor:
Dr. Bill Deen

Winter losses challenge the adoption of *Miscanthus* as a biomass crop in northern regions. This study was conducted to investigate the winter survival and yield potential of *Miscanthus* in Ontario. Twenty *Miscanthus* entries representing diploid *M. sacchariflorus* x *M. sinensis* hybrids, triploid *M. x giganteus* hybrids and diploid *M. sinensis* were established in 2008 at three locations in Ontario. First year winter survival ranged from 8-100% in Leamington, 0-100% in Elora and 0-89% in Kemptville. No difference in winter survival potential of the three species groups was observed in Leamington or Elora, but a diploid hybrid was significantly greater than all others in Kemptville. Establishment year culm height and basal circumference, and second year spring regrowth timing and biomass yield were associated with winter survival. Overall, winter severity increased from Leamington to Kemptville. The diploid hybrids were most winter hardy, followed by *M. x giganteus* types, followed by *M. sinensis*, though all had equivalent survival potential in Leamington and Elora.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Bill Deen, for his guidance throughout this project. Special thanks also to Dr. Stephen Bowley and Dr. Ashraf Tubeileh for their contributions through participating on my advisory committee For assistance in completing field work, I would like to thank Henk Wichers, Heather Engbers, the co-operators at the Leamington and Kemptville sites, and the countless number of seasonal employees who were involved. For financial support of this project, I would like to thank Mendel Biotechnology. For their support throughout this process, I would also like to thank my parents.

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1. INTRODUCTION

Strong global growth and development has increased demand for energy to refine, manufacture and transport products to support the lifestyles of an increasingly developing and globalized world. In recent decades, fossil fuels have become important sources of energy. However, with increasing demand, there has been developing concern over the sustainability of fossil fuels relating to their potential future sources and harmful byproducts of use, specifically large net carbon releases, which has spurred interest towards the use of alternative renewable energy sources. Potential alternatives are available including wind, solar, hydro, and biomass, all of which are touted to have greater environmental benefits relative to fossil fuels.

Biomass has had a long important use as an energy source for man, and includes that of wood which has been estimated to have been used for hundreds of thousands of years (Goren-Inbar et al, 2004). It is estimated that biomass currently supplies 10-14% of the world's energy (McKendry, 2002). Crops for energy use are also not new to society, including Ontario, citing the significant importance oats once had in crop rotations to fuel a horse-driven society (Hume, 2008).

Interest in energy production from crops has resurged in recent years, as evidenced by the recent growth in development of grain based ethanol and biodiesel industries spurred through the interest of replacing transportation fuel and fuel additives with cleaner burning renewable sources. In 2007, the Ontario government enacted a regulation requiring a minimum 5% ethanol blend for all gasoline sold in the province, with aims to increase the combustive efficiency of gasoline to reduce emissions of greenhouse gases and particulate matter (Ontario Ministry of the Environment, 2005). With similar aims, the Government of Canada similarly enacted regulation in 2010 requiring a minimum 5% blend of renewable fuels in gasoline, with a future amendment requiring 2% renewable content in diesel fuels and heating distillate oil provisioned for 2011 (Environment Canada, 2010). The enactment of these regulations demonstrates recognition of the importance of the development of renewable and environmentally respective sources of energy in Canada.

While grain derived biofuels may provide environmental benefits when compared to traditional fossil fuels, there are concerns about the sustainability of their adoption due to their limited improvements in net carbon release and net energy yields relative to fossil fuels and the limitations in total achievable grain production which will limit their

expansion and potential for offsetting fossil fuels (Hill et al, 2006). Grain based biofuels have also faced scrutiny through the development of a market which may compete for grains which would otherwise have been primarily grown for food purposes.

Given the limitations of grain-based bioenergy production, biomass has received attention as an alternative bioenergy feedstock due to its ability to overcome many of the challenges associated with grain-based bioenergy. In Ontario it has been proposed as an alternative energy source for heat and electricity generation. While non-agricultural biomass feedstocks such as sawdust and wood wastes are currently used in these roles, these sources suffer from supply limitations (Jannasch et al, 2001) and high processing and transportation costs from source regions in Ontario (Levin et al, 2011) which may hinder their expansion as a reliable and cost-effective feedstock beyond regions where they are produced. Thus, agricultural biomass has received attention as a viable alternative energy source.

In Ontario cropping systems, agricultural biomass sources can include crop residues as well as purpose-grown biomass crops. Crop residues may be widely produced in agricultural regions of Ontario, but harvestable quantities may be limited when considering sustainable removable rates. Kludze et al (2010) identified maintenance of organic matter as the key limiting factor in the sustainable harvest of crop residues in Ontario, noting that maintenance of organic matter is critical for long term productivity of agricultural land, and that residue removal was thus only sustainable if it left enough residue to at least maintain organic matter levels within a system.

Based on county level crop production estimates and organic matter formation and mineralization rates observed in Western Ontario, Kludze et al (2010) estimated that the province of Ontario could sustainably supply an average 1.1 million dry matter tonnes of corn, soybean and wheat residue combined without compromising long term soil productivity. Considering Ontario Power Generation alone has an estimated demand of 2 million tonnes of biomass for retrofitting a portion of their coal-fired power plant units in Ontario (Ontario Power Generation Inc., 2011), crop residues alone appear to be limited in their ability to sustainably supply biomass in large quantities in Ontario. Beyond limitations in crop residue quantity, concerns also exist with reliability of annual harvests, biomass quality, and the high costs of harvest and transport. Alternatively, Kludze et al (2010) identified high yielding dedicated biomass crops as a viable alternative option to reliably provide higher quantities of low cost agricultural biomass in Ontario.

Efficiencies through high, consistent yields and low production costs will be important aspects in the development of future biomass crops. Perennial grasses are an excellent fit for biomass systems through their perennial establishment, ability to recycle nutrients, and their potentially significant yields of high quality combustible biomass (Lewandowski et al, 2003). In terms of perennial grasses, C_4 photosynthetic types present an advantage to conventional C_3 types through their greater nutrient and water use efficiencies (Brown, 1978), suggesting the potential for higher biomass yields relative to C_3 types.

Two C_4 perennial grasses have been extensively analyzed for their potential as biomass crops; switchgrass (*Panicum virgatum L.*) primarily in North America, and species of the genus *Miscanthus* in Europe (Heaton et al, 2004). While research directly comparing the productivity of both species is limited, yield reports suggest *Miscanthus* will out-yield switchgrass, and therefore be a more attractive biomass crop (Heaton et al, 2004).. Recent trials in Illinois have confirmed this, with average established stands yielding 29.6 t ha^{-1} of *Miscanthus* compared to 10.4 t ha^{-1} for switchgrass over three sites throughout the state. Beyond yield, *Miscanthus* has demonstrated many key elements of efficiency: high nutrient use efficiency including some reports of no nitrogen response at sites (Christian et al, 2008); positive environmental benefits including less nitrogen leaching (Christian et al, 1998); greater potential for wildlife biodiversity (Smeets et al, 2009); and higher levels and improved quality of organic matter (Kahle et al, 2001).

Miscanthus is a C_4 perennial grass native to eastern Asia. Species currently studied for their biomass potential include *Miscanthus sinensis (M. sinensis)*, *Miscanthus sacchariflorus (M. sacchariflorus)*, and the hybrid *Miscanthus x giganteus (M. x giganteus)*. *M. sinensis* is a diploid species with a broad range along the Pacific coast of Asia. *M. sacchariflorus* exists as a tetraploid (or diploid) species with a range focused around east-central Asia. *M. x giganteus* is a sterile triploid, with significant evidence suggesting it is a natural allotriploid hybrid between diploid *M. sinensis* and tetraploid *M. sacchariflorus*. *M. x giganteus* has had significant interest as a biomass crop due to its significant productivity potential, and its sterile nature which may reduce the potential for invasiveness in non-native regions. Its sterile nature, however, makes stand establishment more costly as it must be vegetatively propagated which is more labour and capital intensive and slower relative to seed based biomass crops. Khanna et al (2008) demonstrated this in Illinois where they estimated rhizome/seed and planting equipment costs during the year of establishment to be \$416.10 ha^{-1} (\$407.78 USD ha^{-1},

where $1 CDN = $0.98 USD as of January 11, 2012 (Bank of Canada, 2012)) and $119.65 ha^{-1} ($117.26 USD ha^{-1}) for rhizome planted *Miscanthus* and seed planted switchgrass respectively. This places *Miscanthus* at a disadvantage to other potential biomass grasses which can be seeded via less intensive means, and reinforces the importance of good stand establishment. However, the significantly higher yield potential of *Miscanthus* may offset these higher propagation costs.

The potential for *Miscanthus* to yield well under cooler environments such as Ontario is supported by evidence that *Miscanthus* photosynthetic mechanisms have a high degree of cold tolerance. There is no evidence of decreased photosynthetic efficiency in cooler environments where *Miscanthus* has been shown to maintain a high biomass conversion coefficient unlike other C_4 plants (Beale and Long, 1995). Naidu et al (2003) investigated the impact of initial plant growth temperature on photosynthesis rates across a range of temperatures in *Miscanthus* and corn (*Zea mays* L.), two species which contain C_4 photosynthetic pathways. When photosynthesis was measured across a range of temperatures from 5°C to 38°C, it was observed that corn initially grown under cool conditions of 14°C days and 11°C nights had photosynthetic rates reduced by 80% compared to corn initially grown under warm conditions of 25°C days and 20°C nights. In contrast, no difference in photosynthesis rates were observed for *Miscanthus* grown under either cool or warm conditions, supporting the observations of Beale and Long (1995), and demonstrating that *Miscanthus* can maintenance photosynthetic efficiencies even in cooler environments.

One significant issue with the development of *Miscanthus* as a new crop is winter hardiness. For a biomass production system, winter hardiness would be manifested in measures of winter survival and also the ability to initiate regrowth in the spring and maintain productivity over the life of the stand. *Miscanthus* stand persistence is critically important given the relatively high costs of establishment. Winter losses would require replanting or stands could suffer from reduced yields and delayed evolution to the yield plateau of mature stands. This would further exacerbate the high costs associated with establishment, and could impact the feasibility of *Miscanthus* as a biomass source. In addition, this may increase weed pressure which may result in additional yield-loss or weed control costs . *Miscanthus* stands in Europe have been observed to persist and maintain productivity for twenty or more years. Amortization of establishment costs over this time period has a significant impact on establishment costs per tonne of production

for the stand. Thus, sufficient winter hardiness will be a key component which must be considered to fully realize *Miscanthus*' potential efficiencies.

While agronomic research including winter survival has been investigated in Europe, relevant work in North America is limited. In Ontario, no published research in regards to winter survival of Miscanthus has been observed. Research in Europe has demonstrated very significant differences in winter survival of genotypes across different environments (Clifton-Brown et al, 2001a). Lack of establishment due to winterkill has limited the expansion of cold sensitive genotypes to northern areas, demonstrating the importance of screening and proper genotype selection prior to stand establishment of field scale plantings. Further, little is known about the association between plant growth characteristics and winter hardiness, making the selection of potentially winter hardy genotypes and development of establishment practices which may promote winter survival difficult. This lack of knowledge may hamper the commercialization of *Miscanthus* in non-native regions where climates may challenge winter survival.

Predicting winter survival of *Miscanthus* in Ontario may be difficult, given the provinces' large size, there is significant variation in climate, ranging from a humid continental climate in the south to subarctic in the north (Carder, 1970). While latitude has a prevailing influence on mean temperatures, the presence of variation in topography and proximity to major water bodies creates regional variability. As a result of cool maritime influences from Hudson Bay, as well as cooling of air masses from the northern Great Lakes, Northern Ontario experiences a relatively cool growing season with lower heat unit accumulation relative to more inland areas of Canada with similar latitudes (Baldwin et al, 2000). As influenced by arctic air from the north, winters are cold and long, resulting in a relatively low number of frost free days ranging from 90-145 days per year based on current 30 year normals (OMAFRA, 2011). Southern Ontario is characterized by warm humid summers and cold winters. With its lower latitudes and moderating influences from the Great Lakes, growing seasons in southern Ontario are comparatively longer ranging from 125-190 frost free days (OMAFRA, 2011). In conjunction with summer influences from warm humid air from the Gulf of Mexico and Atlantic seaboard, there is greater heat unit accumulation than in the north, with highest levels associated with regions in close proximity to the southern Great Lakes and those with lower elevations (Baldwin et al, 2000). Increasing in annual accumulation from west to east, precipitation in northern Ontario is generally continental with higher levels during summer months relative to winter months, while precipitation distribution in southern

Ontario is generally uniform throughout the year as a result of lake and elevation effects (Baldwin et al, 2000). The variability of climate throughout Ontario and sensitivity of *Miscanthus* to winter conditions makes testing across the province crucial in order to identify best adapted genotypes.

The lack of experience and fundamental research with *Miscanthus* in Ontario and climatic differences between Ontario and existing research locations in Europe and Illinois made it difficult to withdraw valid conclusions for Ontario conditions from existing data . Therefore, the purpose of this study was to evaluate the winter hardiness characteristics of *Miscanthus* and to confirm the existence of variation in winter hardiness among *Miscanthus* genotypes under Southern Ontario conditions, and identify potential plant growth characteristics associated with winter survival.

2. LITERATURE REVIEW

2.1 *Miscanthus* background

Miscanthus is a genus of grasses native to Eastern Asia comprised of 11 to 12 species (Clifton-Brown et al, 2008). They are perennial rhizomatous grasses which utilize C_4 photosynthesis. This genus has commonly been used as an ornamental grass but has also generated considerable interest for use as a biomass crop during the past few decades. Species currently tested for biomass production include *M. sinensis*, *M. sacchariflorus* and *M. x giganteus*.

M. sinensis is native to eastern Asia, and cited to range from subtropical to subarctic environments (Numata, 1979). It is often classified as a tight clumping grass with little rhizome spreading. It is a fertile diploid species, and accessions in Europe have demonstrated significant genetic diversity within the species (Hodkinson et al, 2002a). In biomass tests in Europe it has displayed greater winter hardiness than other species, providing more consistent production in cooler climates, but is generally lower yielding than *M. x giganteus* species where both survive (Clifton-Brown et al, 2001a). While *M. sinensis* appears to be beneficial for adaptation due to its significant genetic variability, one caveat of this is that it is known to set viable seed, making it a potential invasive species in foreign regions (Atkinson, 2009).

In central Europe, third year dry matter yields of *M. sinensis* hybrids, defined as crosses between two *M. sinensis* genotypes, or a *M. sacchariflorus* and *M. sinensis* cross, ranged from 6.5-17.7 t/ha in England to 10.3-20.0 t/ha in Germany while third year dry matter yields of *M. sinensis* from collections in Denmark and Sweden originally sourced from Japan ranged in yield from 4.6-10.9 t/ha dry matter in England, and 9.1-12.8 t/ha dry matter in Germany (Clifton-Brown et al, 2001a). In northern Europe yields of *M. sinensis* hybrids and *M. sinensis* accessions were slightly higher, but could not be compared to *M. x giganteus* given the lack of survival.

M. sacchariflorus commonly exists as a diploid or tetraploid, and is native to warmer regions of east-central Asia (Deuter, 2000). Genotypes used in biomass testing have been characterized by relatively loose, spreading clumps with few, tall culms associated with long, thick rhizomes (Clifton-Brown et al, 2001). Biomass testing in Europe has been limited to one genotype, making conclusions about *M. sacchariflorus*' biomass potential difficult. For the genotype used, winter hardiness appears to be lower than *M. sinensis* with winter losses of 50% and 67% in Sweden and Denmark

respectively, much higher than all *M. sinensis* genotypes tested which ranged from 1-16% across the same locations (Clifton-Brown et al, 2001a). In regions where winter survival was high, third year yields for the *M. sacchariflorus* genotype tested were generally moderate, ranging from 11.1 t ha^{-1} in England to 12.6 t ha^{-1} in Germany; less than *M. x giganteus* but higher than *M. sinensis* for these same regions. Irrigated yields in Portugal however were very high and comparable to *M. x giganteus*, averaging 35 t ha^{-1} by third year. Similar to *M. sinensis*, *M. sacchariflorus* can pose some invasiveness risk as it has been observed to set fertile seeds even when grown outside of its native region, and has been characterized by having more vigorous rhizome growth relative to other *Miscanthus* species such as *M. x giganteus* which has more bunch-like growth habits (Meyer and Tchida, 1999). While results of the genotype currently published in literature do not appear to make *M. sacchariflorus* a strong candidate for biomass production, interest in this species still exists given its genetic contribution towards *M. x giganteus*.

M. x giganteus is commonly cited as a strong candidate biomass species, as its sterile nature limits its invasiveness risk when introduced in new regions. It was first introduced in Europe from Japan in the 1930's (Linde-Laursen, 1993). Although its exact mechanism of origin is unknown, ribosomal DNA sequencing supports the theory that it is a natural allotriploid hybrid of *M. sinensis* and *M. sacchariflorus* origin (Hodkinson et al, 2002b). It is believed to have originated from a natural overlapping region of both species in east-central Asia where it displays growth characteristics balanced between *M. sinensis* and *M. sacchariflorus*. Hodkinson et al (2002b) proposed two potential mechanisms of triploid hybrid formation. One consisted of a cross between a diploid of each species where the formation of an unreduced gamete to result in two genomes from one species. Another method was proposed by which a cross between a diploid from each species results in the formation of an allotetraploid which subsequently crosses with a standard diploid *M. sinensis*. Despite its yield potential, its adaptation is limited to warmer regions due to poor winter survival (Clifton-Brown et al, 2001a) and apparent low genetic diversity in European populations (Hodkinson et al, 2002a).

M. x giganteus has been a superior species in west central Europe due to its high productivity, with third year yields up to 18.7 and 29.1 t ha^{-1} in England and Germany respectively (Clifton-Brown, 2001a), higher than most *M. sinensis* hybrids in that region and considerably higher than natural *M. sinensis* entries which generally had below average location yields for all entries for each genotypes. In North America, published

Miscanthus data has generally been limited to *M. x giganteus* trials in Illinois where yields during the third to fifth years of establishment across three sites averaged 29.6t/ha of dry matter at full senescence (Heaton et al, 2008). Yields of a locally adapted switchgrass cultivar *Cave-in-Rock*, a native North American biomass crop averaged only 10.4 t/ha dry matter when grown across the same sites and years (Heaton et al, 2008).

2.2 Cost of establishment

Cost of establishment may have a major impact on the use of *Miscanthus* as a biomass crop (Jorgensen et al, 2000). The sterile nature of current sterile *M. x giganteus* clones makes planting difficult as vegetative propagation through rhizome separation or micropropagation is required. The propagation methods are much more intensive than seeded grasses causing establishment costs to be much higher for these systems.

Rhizome propagation requires digging and separation of rhizomes. Rhizomes are commonly planted immediately after separation although they may be stored for a short period or further separated for greenhouse plantlet propagation. Reflecting the degree of work, plant stock costs can be very high and have a large range in literature with estimates ranging from $0.05-0.58 (€0.04-0.45, where $1 CDN = €0.77 as of January 11, 2012 (Bank of Canada, 2012)) per plantable rhizome (Smeets et al, 2009), representing costs of $520-$5840 ha^{-1} (€400-4500 ha^{-1}) when planted at a standard density of 10,000 plants ha^{-1} . Previous literature estimates have suggested rhizome costs of $455-$100 ha^{-1} (€350-400 ha^{-1}) (Lewandowski et al, 2000), $975 ha^{-1} (€750 ha^{-1}) (Bullard, 2001). While economic analysis in North America is limited, one Illinois study has suggested rhizome seed-stock costs to be $340 ha^{-1} ($335 USD ha^{-1}) (Khanna et al, 2008). Additional establishment costs include planting, fertilization and chemical applications which can be highly variable depending on the methods used. Original rhizome planters consisted of modified potato planters which suffered from low capacities of 4-5 hectares per day while newer *Miscanthus*-specific planters may plant over 10 hectares per day (DEFRA, 2007). Even when using this newer technology, total establishment costs are still significant at $590 ha^{-1} ($580 USD ha^{-1}) in Illinois (Khanna et al, 2008).

Micropropagation is an alternative propagation method which utilizes tissue culturing and division of plantlet tillers to produce plant plugs. Although divisions can be rapid, micropropagated seed stock is much more expensive than rhizomes. Cost

estimates from literature for micropropagated plant stock range from \$3120-3900 ha^{-1} (€2400-3000 ha^{-1}) (Christian et al, 2004; Lewandowski et al, 2000) for a standard planting density of 10,000 plants ha^{-1}. Assuming planting, fertilizer and chemical costs are similar to rhizome establishment, current micropropagation techniques result in very high costs relative to rhizome planting. Given the observations of sub-optimal winter hardiness for some micropropagation methods (Christian and Haase, 2001; Clifton-Brown et al, 2007; Kim et al, 2010), this presents considerable financial risk.

Establishment of alternative biomass crops such as seeded grasses are shown to be much less expensive than *Miscanthus*. For switchgrass production in Illinois, Khanna et al (2008) have suggested costs of \$109 ha^{-1} (\$107 USD ha^{-1}) for seed, while total establishment costs including seed, chemical, fertilizer and planting to be approximately \$260 ha^{-1} (\$255 USD ha^{-1}). While actual establishment costs may vary from the literature, the relative difference of less than half of that suggested for *Miscanthus* in Illinois is an important consideration in the economics of the biomass systems. Although higher biomass yields of *Miscanthus* can offset these costs, this is only realized if *Miscanthus* survives and establishes as a productive stand. High establishment costs make replanting of *Miscanthus* highly undesirable, reinforcing the need for sufficient genotype screening for successful and productive stand establishment.

Overall it is evident that establishment costs of *Miscanthus* are very high relative to other biomass crops. While higher productivity may help amortize these costs, it is reliant on successful stand establishment. As a component of stand establishment, winter survival is critical to the economic viability of *Miscanthus* as a biomass crop.

2.3 Overwintering characteristics of species

Studies which have compared large numbers of genotypes among *Miscanthus* species for winter hardiness is limited. One of the most comprehensive tests is part of the European *Miscanthus* Improvement Project. In order to develop *Miscanthus* as a viable biomass crop, the European Union has funded the European *Miscanthus* Improvement project to test a large number of genotypes across a variety of European environments, with the goals of identifying the most adapted locations for specific genotypes/species to promote productive successful establishment, as well as to breed sterile, non-invasive genotypes for commercial development (Jorgensen and Muhs, 2001). In North America, research has been mostly limited to Illinois where the

University of Illinois has conducted some production research in regards to the use of *Miscanthus* as a potential biomass crop in the Midwestern United States.

M. x giganteus

Winter hardiness in higher latitudes has been a problem for the development of *M. x giganteus* as a biomass crop. Across four *M. x giganteus* entries tested in northern Europe, plant losses during the first winter ranged from 93-99% and 96-100% in Sweden and Denmark respectively, while losses in England, Germany and Portugal never surpassed 1%. Cold tolerance assessments of *M. x giganteus* rhizomes removed from fields in Germany under fully hardened state yielded a median lethal temperature (LT50) of -3.4^{o}C (Clifton-Brown and Lewandowski, 2000). Clifton-Brown and Lewandowski (2000) concluded that cold temperature tolerance must be a significant contributor towards winter hardiness, as the LT50s calculated from hardened rhizomes correlated with the actual soil temperatures and winter losses observed at other sites, particularly as they would have predicted high losses of *M. x giganteus* in Denmark and Sweden (Clifton-Brown et al, 2001a).

In North America, trials in Illinois have focused on a single *M. x giganteus* genotype which had been sourced from a local population originally transplanted from the Chicago Botanical Gardens (Heaton et al, 2008). Similar to central Europe, losses were negligible in central and southern Illinois but increased to 14% loss during the first winter in northern Illinois (Heaton et al, 2008). While incidence of winter losses was similar to central Europe, they were believed to have occurred with temperatures which were colder, leading the authors to suggest that the Illinois *M. x giganteus* clone may possess superior winter hardiness relative to the *M. x giganteus* tested in Europe (Heaton et al, 2008). This was supported with anecdotal evidence that established "Illinois" *M. x giganteus* has survived soil temperatures as low as -6^{o}C (Heaton et al, 2010), much lower than the LT50s measured for European genotypes. While low genetic diversity has been observed in European *M. x giganteus* populations (Greef et al, 1997; Hodkinson et al, 2002a), these results suggest that some variation in winter survival may be present, although no direct comparisons have been published between the "Illinois" *M. x giganteus* clone and any other *M. x giganteus*. No other evidence of winter survival issues in other published literature has been reported for central European conditions.

M. sinensis and M. sinensis hybrids

Biomass research from *Miscanthus* species other than *M. x giganteus* appears to generally be limited to Europe. Due to its high genetic diversity and parental contribution towards the highly productive *M. x giganteus* genotypes, research has also been conducted on true breeding *M. sinensis* as well as hybrid forms between *M. sinensis* and *M. sacchariflorus* accessions (Clifton-Brown et al, 2001a).

True breeding *M. sinensis* accessions demonstrated the potential for consistent winter survival in European tests. Clifton-Brown et al (2001a) demonstrated that in cooler climates *M. sinensis* genotypes originating from Japan have a greater winter hardiness capacity than other *M x giganteus*, *M. sacchariflorus*, and a number of *M sinensis* hybrids tested. Winter losses of five *M. sinensis* genotypes tested ranged from 1-15%, 5-16%, and 1-21% in Denmark, Sweden and England respectively, with no losses greater than 5% in either Germany or Portugal (Clifton-Brown et al, 2001a). It is important to note that while these accessions originated from collections in Japan, they had been sourced from collections in Denmark and Sweden, of which the Denmark collection had been pre-selected for winter hardiness and productivity in that region (Jorgensen, 1997). Regardless, the research demonstrated that winter hardiness capacity is present in *Miscanthus* species beyond the limited genetic resources of *M. x giganteus*.

Evaluation of select *M. sinensis* hybrids have demonstrated variability in winter survival (Clifton-Brown et al, 2001a). Plants designated as pure *M. sinensis* hybrids demonstrated variable winter hardiness at northern sites in Denmark and Sweden where winter survival was intermediate between the natural *M. sinensis* collections and that of *M. x giganteus*, ranging from 8-60% loss across both sites (Clifton-Brown et al, 2001a). A triploid *M. sinensis* hybrid had high winter survival similar to the pure *M. sinensis* collection, with highest losses in Sweden at 17% (Clifton-Brown et al, 2001a). A similar response was seen with two *M. sacchariflorus* x *M. sinensis* hybrids which over both northern sites did not suffer losses higher than 6% (Clifton-Brown et al, 2001a). Although losses were marginal, one diploid *M. sinensis* genotype actually had the greatest winter kill across all genotypes in Germany at 5.4% (Clifton-Brown and Lewandowski, 2002).

Clifton-Brown and Lewandowski (2000) confirmed the importance of rhizome cold tolerance in relation to winter hardiness when they demonstrated that the LT50 for the rhizomes from a full *M. sinensis* hybrid removed under field hardened conditions in Germany were -6.3^{o}C, significantly lower than the -3.4^{o}C observed for *M. x giganteus* sampled at the same time. The *M. sinensis* hybrids had better winter survival than *M. x*

giganteus at more northern locations with the authors noting how separation of genotypes reflected LT50s which were in line with recorded soil temperatures. No rhizome cold tolerance work on the true breeding *M. sinensis* collections appears to have been published.

Greater adaptation of *M. sinensis* to cooler environments has also been detected by differences in basal growth temperatures of leaf emergence following winter dormancy. Farrell et al (2006) identified base temperatures of 6.0-7.6^{o}C for leaf emergence of two true hybrid *M. sinensis* genotypes, lower relative to the base temperatures of 8.5^{o}C and 8.6^{o}C for the single *M. x giganteus* and *M. sacchariflorus* genotypes tested, respectively. Similarly, *M. sinensis* genotypes appeared to have greater shoot frost tolerance as evident by shoot LT50 values lower than those observed for *M. x giganteus* or *M. sacchariflorus* in the same study.

Differences in winter hardiness are evident between two of the currently most researched *Miscanthus* biomass candidates, *M. x giganteus* and *M. sinensis*. While *M. sinensis* appears to contain the potential for greater winter hardiness than *M. x giganteus*, it is evidently much more variable reflecting its large natural range in Eastern Asia. Across all genotypes, variability in winter survival is most evident at northern sites (Clifton-Brown et al, 2001a) making proper selection more critical than in southern areas when winter survival is not a significant production issue. Lack of reference genotype screening and relative adaptation make genotype recommendations for Ontario difficult.

2.4 Winter survival and propagation method

Given the sterile nature of the *M. x giganteus* species, vegetative propagation is required. Methods of vegetative propagation include rhizome division for direct replanting or production of greenhouse propagated plugs, as well as different micropropagation techniques through tissue culture. Propagation technique can have an important impact on winter survival with rhizome propagation often cited to be superior to micropropagation.

In Ireland in 1991, *M. x giganteus* was established under both rhizome and an unspecified micropropagation technique. Mortality rates of rhizome propagated and micropropagated plants by the fourth year were 14% and 47% respectively, with the majority of micropropagated losses apparently occurring during the first winter (Clifton-Brown et al, 2007). Sub-optimal winter survival of micropropagated *M. x giganteus* plantlets without significant establishment issues for rhizome propagates appeared to

occur in other studies in Ireland as well, with Christian and Haase (2001) citing winter mortality rates as high as 99% for winter 1993-1994. Similar observations have also been made in other parts of Western Europe as well (Christian and Haase, 2001; Clifton-Brown et al, 2001b).

While not directly compared with rhizome plantings, stands micropropagated via somatic embryogenesis in central Illinois demonstrated first year winter losses of 22% and 44% for a low density planting of *M. x giganteus* in 2006 and a high density planting in 2007, respectively (Kim et al, 2010). Although not compared within the same year, rhizome establishment of the "Illinois" *M. x giganteus* clone in 2002 did not suffer any losses during the first winter (Heaton et al, 2008).

Citing cases where micropropagated plants suffered high winter casualties and where rhizomes propagated plants did not, Lewandowski (1998) suggested that differences in root and shoot accumulation for plants from the various propagation methods may influence cold hardiness. This led Lewandowski (1998) to compare two micropropagation techniques, "in vitro tillering" consisting of regeneration via leaf node and apical meristem tissues, and "embryoid plant callus" consisting of regeneration via non-emerged inflorescences to rhizome propagation. While conclusions on cold hardiness were not made due to lack of sufficiently cold winter conditions, biomass accumulation varied with propagation method with embryoid culture having nearly twice the final above and below ground biomass yields than rhizome or in vitro tillering during the year of establishment in a field trial in Germany (Lewandowski, 1998).

In contrast to most micropropagation research, Plazek et al (2011) cited Podlesny (2005) who observed a higher frost tolerance of micropropagated plantlets via in vitro culture relative to rhizome propagated plantlets. In their experiment, Plazek et al (2011) also observed greater cold tolerance for *M. x giganteus* plants micropropagated in vitro via immature inflorescence relative to rhizome replants during the year of propagation. Unhardened micropropagated plants could still produce shoots after up to 14 days of exposure to $0^{o}C$, whereas unhardened rhizome propagated plantlets could not. After hardening at $12^{o}C$ for two weeks, and $5^{o}C$ for three weeks, in vitro plantlets could still produce shoots after 14 days at $-3^{o}C$, while rhizome plantlets could no longer produce shoots after only three days exposure to $0^{o}C$ (Plazek et al, 2011). Following one year of field establishment and exposure to winter, Plazek et al (2011) noticed no difference in shoot regrowth ability between propagation methods, suggesting that susceptibility to winter stresses is greatest during the year of establishment.

Current micropropagation methodologies appear to be insufficient to establishing stands even in areas of relatively low winter stress. The need for further research in regards to rhizome sizing, plant hardening and planting time is required to optimize winter survival of micropropagated plants has been addressed by Kim et al (2010). Basic principles of this research may be associated with improving winter survival of both micropropagated or rhizome propagated plants in more northern environments.

2.5 Winter hardiness in *Miscanthus*

Winter hardiness of perennial grasses can be influenced by a variety of physical, physiological and biotic factors (Larsen et al, 1994). In *Miscanthus*, differences in susceptibility to these stresses are evident by the variability in winter survival between genotypes and species across various sites in Europe (Clifton-Brown et al, 2001a). It has also been well documented that winter losses are most significant for *Miscanthus* during the first winter (Jorgensen and Schwarz, 2000; Christian and Haase, 2001, Heaton et al, 2008), suggesting survival during this period is most critical to stand viability.

The rhizome is the overwintering structure of *Miscanthus*, a role it plays through providing meristems for active new growth and through storing energy and nutrients for regrowth following dormancy (Moser and Jennings, 2007, Suzuki and Stuefer, 1999). This role has been supported in *M. x giganteus* through observation of seasonal fluxes in rhizome dry matter and nutrient concentrations coinciding with crop growth in the spring (Beale and Long, 1997), as well as in *M. sinensis* in natural grasslands in Japan where aerial plant parts have been observed to source nutrients from “below ground organs” from April until June (Stewart et al, 2009). Maintaining a minimum level of carbohydrate reserves has been suggested to be an important component of initiating new regrowth in perennial grasses (White, 1973). This concept appears to be firmly engrained in current *Miscanthus* research judging by the reiteration of the importance of early planting and proper agronomic practices to enhance above and below ground biomass accumulation to promote successful winter survival (Atkinson et al, 2009; Christian and Haase, 2001; Jorgensen and Schwarz, 2000; Lewandowski et al, 2000). The importance of sufficient reserves has also been reinforced by observations of *Miscanthus* which has failed to recover following frost damage to top growth during late spring frosts (Christian and Haase, 2001). Thus, it is expected that proper allocation of resources to rhizomes, and

sufficient reserve storage will play important roles in mediating winter stresses, and contribute towards successful stand establishment.

Physiological processes are expected to have an impact on winter preparation as well. Beyond anecdotal evidence, the relative importance of completing physiological processes such as sufficient biomass accumulation, reproductive maturity and natural senescence in regards to winter survival has not been well investigated, particularly in regions with harsher winter environments and limited experience with *Miscanthus*. While high winter survival is fundamental to the establishment of productive *Miscanthus* biomass stands, concurrently selecting for high yields is also critical to offset high establishment costs and to make *Miscanthus* a low-cost economically viable biomass crop. Little is known about the association of winter survival to potential yield contributing factors in current biomass accessions, and identification of correlations may provide insight into these relationships.

2.6 Relationship of winter survival with physiological processes

In terms of yield accumulation, early emergence is desirable to maximize light interception (Farrell et al, 2006), but can be concerning due to an increased risk of frost injury from which plants may not recover (Christian and Haase, 2001). As previously outlined, Farrell et al (2006) demonstrated lower basal growth temperatures for *M. sinensis* hybrids relative to *M. x giganteus* and *M. sacchariflorus* suggesting that they would be associated with earlier emergence in the spring. In northern European sites where an *M. x giganteus* genotype tested by Farrell et al (2006) with basal growth temperature of 8.5 ^{o}C suffered 93% loss during the first winter (Clifton-Brown et al, 2001a). In the meanwhile, a *M. sinensis* genotype with a lower basal growth temperature of 7.6^{o}C had winter losses as low as 1-17% across Denmark and Sweden, respectively. While only four genotypes were tested, and no strong correlation between winter survival and basal growth temperature was observed, this work suggests earlier emergence may not be associated with increased susceptibility to winter conditions. Leaves of *M. sinensis* demonstrated greater frost resistance than those of *M. x giganteus*, as demonstrated by lower shoot LT50s following exposure to frost (Farrell et al, 2006). In turf applications of the C_3 grass perennial ryegrass (*Lolium perenne* L.), a significantly negative correlation between ryegrass LT50s and turf height in the spring demonstrated that ryegrass varieties with greater cold tolerance had taller plants during spring

regrowth (Hulke et al, 2008). Based on the current evidence, research does not indicate any spring growth penalty to achieve improved winter hardiness.

Attaining maturity prior to a killing frost has been alluded to be important for the completion of processes such as remobilization of assimilates to rhizomes, an interruption of which may negatively impact winter survival (Clifton-Brown et al, 2001a, Christian et al, 2009). Flowering generally signifies the end of culm growth, and hence the majority of plant growth for genotypes where culms emerge in a uniform flush (Deuter, 2000). This is especially evident for biomass accessions in northern climates where flower timing appears to strongly dictate subsequent leaf senescence (Clifton-Brown et al, 2001a). As a result of cessation of growth, genotypes which flower early relative to the length of the growing season of a site have been shown to accumulate less biomass (Clifton-Brown et al, 2001a) which suggests early flowering is not a desirable trait for high yields. While late flowering may be conducive to increasing productivity, it may have a negative impact on winter hardiness as it represents a lesser degree of maturity prior to winter which may impair the completion of nutrient and carbohydrate remobilization to rhizomes (Clifton-Brown et al, 2001a).

This was supported by studies in Denmark where lower biomass nitrogen concentrations were observed for earlier maturing *M. sinensis* genotypes relative to later maturing genotypes of *M. sinensis*, and an *M. x giganteus* genotype which did not flower at all (Jorgensen, 1997). Jorgensen suggests nitrogen translocation to rhizomes was likely more efficient with earlier maturing genotypes while likely less efficient in *M. x giganteus* which still appeared to be “green and soft” into November (Jorgensen, 1997). While only a few genotypes were tested, the earlier maturing genotypes appeared to be associated with those which had better winter survival. Although work directly assessing the relationship between remobilization and flowering appears limited, Smith and Slater (2011) have addressed evidence from trials in England which indicate that flowering time coincides with nutrient remobilization timing within *Miscanthus*. Rhizome nutrient concentrations increase with flowering and senescence and is coupled with a decrease in biomass nutrient concentrations (Smith and Slater, 2011). Beale and Long (1997) have demonstrated that total above ground quantities of nitrogen, phosphorus and potassium in *M. x giganteus* peak in the months prior to peak biomass accumulation in the UK, coinciding with the seasonal minimum levels in rhizomes. Thereafter, observations of decreases in aerial biomass and nutrients with concurrent increases in

below ground biomass and nutrients strongly suggest a degree of remobilization occurs from aerial to below ground parts (Beale and Long, 1997).

While remobilization may not occur in other species, flowering has been observed to be an indicator of the downwards movement of carbohydrates towards underground portions of plants in other perennial species. Some perennial weeds in temperate regions rely on perennial root structures to overwinter, for which accumulation of carbohydrates and nitrogenous compounds are important for winter survival (Cyr et al, 1990). In several perennial weeds, basipetal translocation is also often associated with early reproductive stages (Orfanedes, 1993). Similarly, carbohydrate storage in the roots of perennial legumes reaches a maximum at or slightly after flowering, for which stored carbohydrates are important for winter survival and regrowth following defoliation (Moser and Jennings, 2007). Although results regarding the importance of remobilized nutrients and carbohydrates towards surviving structures for winter survival are not clear across all species, it is generally expected that completion of physiological stages which favour reserve accumulation will in effect favour winter survival.

Miscanthus studies in Europe have demonstrated that *M. sinensis* genotypes tend to flower earliest, while *M. x giganteus* tend to flower the latest, if at all, before killing frosts (Clifton-Brown and Lewandowski, 2002; Jorgensen, 1997). In more northern locations in Europe, early flowering was associated with earlier senescence (Clifton-Brown et al, 2001a). Early flowering and senescing genotypes were associated with higher winter survival in northern Europe while genotypes which failed to flower prior to frosts, such as *M. x giganteus*, failed to overwinter in more northerly locations. Early planting of *Miscanthus* has been one recommendation to ensure adequate growth and successful remobilization of “reserves” during the year of establishment of *Miscanthus* (Chistian and Haase, 2001). While current research is suggestive of the importance of completion of physiological maturity towards winter survival, there is no evidence to demonstrate that completion of physiological maturity is a requirement to achieve high winter survival.

Given the evidence that plant maturity may have an impact on winter hardiness, investigation is justified given the significant variation in flower timing in *Miscanthus* (Clifton-Brown et al, 2001a). Flowering control in *Miscanthus* is not wholly understood, and appears to be species dependent. *Miscanthus sinensis* biomass accessions are day length neutral (Jorgensen and Muh, 2001; Zub Brancout-Hulmel, 2010; Clifton-Brown et al, 2008). Flower emergence in *M. sinensis* has been well predicted by thermal-time

accumulation models (Clifton-Brown et al, 2008). This broad assumption however has been complicated by observations that ornamental *Miscanthus sinensis* accessions have only flowered under long day conditions (Cameron, 2004) and by observations of natural *M. sinensis* in Japan where flowering occurs earlier and with greater temporal variability in higher altitudes and latitudes (Stewart et al, 2009; Deuter, 2000). Stewart et al (2009) concluded that other genetic and environmental factors must also play a role in flower development in *M. sinensis*. Flowering in *M. x giganteus* is widely believed to be primarily influenced by photoperiod. This could be expected to be derived from its *M. sacchariflorus* parent which has demonstrated short-day photoperiod sensitivity (Deuter, 2000), although others suggest there may be variability in sensitivity to photoperiod response within this species (Clifton-Brown et al, 2008). There are also differences in the pattern of flowering between species. *Miscanthus sinensis* has longer flowering periods as related to continual emergence of new growing shoots throughout the growing season, while *M. sacchariflorus* flowering periods tend to progress uniformly reflective of its habit to have a significant proportion of total shoots emerge and progress within a relatively short time early in the season (Deuter, 2000). Given the parental influence of its progenitor species, length of flowering time in *M. x giganteus* is not clear. Stand age may also have an impact, as flowering has been reported to occur slightly earlier each year during the first three years of growth (Clifton-Brown and Lewandowski, 2002). Regardless of its influencing factors, variability in flower timing will lead to differences in maturity and stage of physiological processes of the plants as they are entering winter. Although anecdotal inferences have been suggested, the impacts of variability in plant maturity on winter survival does not appear to have been well investigated.

2.7 Relationship of winter survival with plant size and morphology

Differences in plant morphology are related to underlying physiological processes which may influence winter survival. Identification of morphologies which are associated with winter hardiness may assist in selection of *Miscanthus* genotypes which are winter hardy for adaptation and breeding purposes, assist in identifying potential underlying physiological mechanisms of winter hardiness, and aid in tailoring agronomic practices which may improve winter survival.

Relationships between plant morphology and winter survival do not appear to have been well investigated in *Miscanthus*. Clifton-Brown and Lewandowski (2000) have suggested that more cold tolerant genotypes tend to display a tighter ‘tussock’ growth

habit, reflective of a more compact rhizome structure relative to the spreading rhizomes of less winter hardy *M. x giganteus* genotypes, although this relationship has not been noted or investigated by others. While limited, correlations between morphology with potentially meaningful underlying physiological influences and winter hardiness have been observed in other perennial species. Research by Frankow-Lindberg (1999) demonstrated that exposure of non-adapted white clover (*Trifolium repens* L.) varieties to four winters in Sweden resulted in natural selection for plants with significantly reduced stolon internode lengths similar to those observed in the regional population. Reduced stolon extension rates were hypothesized to improve winter survival through influencing the underlying "carbon economy" of the plant, particularly through increasing the carbon source/sink ratio to maintain non-structural carbohydrate content which assist plants in tolerating winter stresses. In perennial ryegrass, Wood and Cohen (1983) demonstrated that sub-crown internode length was negatively correlated to winter survival observed in field conditions, an association suspected to be due to deeper crown establishment providing greater protection and insulation. They suggested sub-crown internode measurement could be a trait to help separate hardy and non-hardy perennial rye grass varieties.

There is significant variability in plant size within the *Miscanthus* genus in natural populations (Stewart et al, 2009; Yan et al, *In Press*) as well as between and within species and environments. There are ranges in height (Clifton-Brown et al, 2001a; Clifton-Brown and Lewandowski, 2002; Jezowski, 2008; Chen and Renvoize, 2005), shoot densities and number of tillers (Clifton-Brown et al, 2001a; Jezowski, 2008), culm diameters (Clifton-Brown and Lewandowski, 2002; Chen and Renvoize, 2005; Jorgensen, 1997) and plant circumferences (Jezowski, 2008). Variability in plant size appears to be greatest between the species groups, as well as within *M. sinensis* species and hybrids. In most instances, significant differences for measurements in *M. x giganteus* genotypes above are limited.

In *M. x giganteus*, plant morphology is also influenced by propagation method, with micropropagated plant characterized by a greater density of shorter culms relative to rhizome propagated plants (Clifton-Brown et al, 2007). Also, at low planting densities in Illinois, micropropagated plants have been observed to be significantly shorter in stem height and thicker in stem diameter relative to rhizome propagated plants (Kim et al, 2010), although the comparison was made across different years. Increasing plant

density for micropropagated plants significantly increased height beyond low density micropropagation or rhizome spacing (Kim et al, 2010).

While investigations of the relationship between winter survival and plant morphology is limited for *Miscanthus*, there is a relationship between plant size and winter survival. This was observed by Jorgensen and Schwarz (2000) who postulated that agronomic practices which are conducive to maximizing plant size during the year of establishment could be an important component of improving winter survival during the year of establishment. The importance of early planting during the year of establishment and other practices which enhance biomass accumulation for successful overwintering has been confirmed in other studies (Atkinson et al, 2009; Christian and Haase, 2001; Lewandowski, 2000). This relation was also supported by Atienza et al (2003) who suggested that identification of quantitative trait loci related to greater development and growth during the planting year could contribute towards enhancing winter survival through improving reserve accumulation and subsequent plant vigour. Indeed, above and below ground yields of *M. x giganteus*, *M. sacchariflorus* and *M. sinensis* during the year of establishment were observed to decrease as growing seasons shortened and, concurrently, winter losses increased across 5 different sites in Europe ranging from Portugal to Sweden (Clifton-Brown and Lewandowski, 2000).

Studies assessing the association of plant measurements and biomass accumulation during the year of establishment appear limited although Clifton-Brown and Lewandowski (2002) have observed strong associations between first year and third year measurements for both plant heights and yields, suggesting relative differences in measurements in the third year may be reflective of relative differences in the first. In the years following establishment, *Miscanthus* yields are observed to be positively correlated to the number of stems (Angelini et al, 2009; Jezowski, 2008), total plant diameter (Jezowski, 2008), and plant height (Jezowski, 2008). These observations suggest that an association between growth parameters during the year of establishment and yield may be made.

During the year of establishment of *M. x giganteus*, below ground biomass at the end of the harvest season is strongly correlated to aerial dry matter. This relationship has been observed by Christian et al (2009) who measured differences in establishment for rhizomes of varying ages and confirmed that above ground yield was linked to increased rhizome weight. This was also reflected in research analyzing differences in

biomass accumulation of two micropropagation methods relative to rhizome establishment for *M. x giganteus*, where above ground dry matter yield was positively correlated to below ground yield (Lewandowski, 2008). A similar trend was shown by Christian and Haase (2001) citing Jodl et al (1996) who demonstrated that rhizome mass development mirrored mass development of above-ground dry matter over two growing seasons, re-enforcing the importance of above-ground yield in the accumulation of below ground biomass.

Field experiments with two *M. x giganteus* genotypes across four sites in Europe failed to demonstrate significant differences between above ground and below ground yields, or winter survival during the year of establishment for the two genotypes making inferences about yield and winter survival difficult (Clifton-Brown and Lewandowski, 2000). In the same study however, above ground yields of *M. sinensis* were not proportional to below ground yields when observed across genotypes, and below ground yields did not appear to be associated with winter survival. This led the authors to conclude that rhizome size is not related to winter survival when observed across all genotypes across all species tested (Clifton-Brown and Lewandowski, 2000). This conclusion has been criticized by others noting that the evidence fails to exclude the possibility that a relationship between rhizome size and winter survival may exist within single genotypes (Jorgensen and Schwarz, 2000), suggesting further work in this area is necessary. These results may also be reflecting the lack of genetic diversity in European *M. x giganteus*, and the large genetic variation within the *M. sinensis* species as noted elsewhere (Greef et al, 1997; Hodkinson et al, 2002). Plazek et al (2011) provided further support for the importance of rhizome size where they anecdotally noted “considerably higher” cold tolerance for rhizome propagated plants regenerated with larger rhizomes versus those which were more finely divided.

Overall, relationships between morphological measurements and winter survival are not well known due to the lack of investigations in published research. Any potential relationships appear anecdotal. Confirmation of relationships could enhance genotype selection and establishment practices of *Miscanthus* in new regions where winter stresses may impede development, such as Ontario.

2.8 Hypothesis and objectives

Objective 1

Research in regards to genetic screening of *Miscanthus* biomass has generally been limited to Europe. The objective of this research was to screen multiple entries within several *Miscanthus* species groups (*M. x giganteus*, *M. sinensis*, and *M. sacchariflorus* x *M. sinensis* hybrids) for survival and productive stand establishment to determine if variability exists within or across these species groups in different locations in Ontario.

Hypothesis 1

Based on existing literature, it was hypothesized that *M. x giganteus* types would have poorest winter survival, especially at sites with harsher winters. *M. sinensis* types were expected to demonstrate superior survival and thus be the most productive at northern sites, whereas fertile hybrids would likely display survival intermediate between the other two groups.

Objective 2

Research from Illinois has suggested the potential for superior winter survival of a specific *M. x giganteus* clone originating from the Chicago Botanical Gardens relative to the *M. x giganteus* entries used in European research. The objective of this research was to evaluate differences in winter survival between the Illinois *M. x giganteus* clone, and *M. x giganteus* material sourced from Europe.

Hypothesis 2

Illinois researchers (Heaton et al, 2004) suggested that the Illinois *M. x giganteus* clone may be capable of surviving winter temperatures lower than those suggested for common European *M. x giganteus*, suggesting it may be more winter hardy. No direct comparisons between the Illinois *M. x giganteus* and other *M. x giganteus* accessions are known to have been published, thus it was hypothesized that the Illinois clone will show greater winter hardiness than other European types.

Objective 3

A key objective of this research was to correlate winter survival data with morphological measurements including culm length, compressed plant circumference, culm diameter, lodging rating, biomass yield, basal circumference and culm densities. Because the investigation of winter survival influences on yield is limited, establishment of correlations between these morphological measurements was expected to demonstrate how *Miscanthus* growth factors may be associated with winter hardiness.

Hypothesis 3

It was hypothesized that as morphological measurements that contribute to yield increase, winter survival would increase as well. Thus, culm length, compressed plant circumference, culm diameter, lodging rating, biomass yield, basal circumference and culm densities would all be positively correlated to winter survival.

Objective 4

To correlate winter survival with developmental measurements including winter survival assessment, spring 50 cm regrowth date, flower emergence scale, fall leaf senescence, percent flowering and post flowering tillers, and late season culm emergence assessment. Because investigation into physiological mechanisms of winter hardiness is limited, establishment of correlations may assist in identifying developmental traits that may have an association with improved winter hardiness to assist in genotype selection and future research efforts.

Hypothesis 4

It was hypothesized that winter survival would be positively correlated to measures of reproductive maturity such as inflorescence emergence, fall leaf senescence, and percent flowering and post flowering tillers. Spring 50cm regrowth was hypothesized to be positively correlated to winter survival. It was hypothesized that winter survival was negatively correlated to late season culm emergence.

3. METHODS

3.1 Trial locations and characteristics

Miscanthus trials were conducted at three Ontario sites: Leamington, Elora, and Kemptville, Ontario.

The Leamington trial was situated on a private field (42°08' N, 82°38' W) owned by Pyramid Farms. The site is characterized by a Brookston Clay Sand Spot Phase soil (Experimental Farm Service, 1946) belonging to the dark-grey gleisolic soil group. Weather information is based on 30 year climate normals (1971-2000) from the Environment Canada weather station located at Agri-Food Canada research station near Harrow, Ontario (Environment Canada, 2009). Snow fall is much lower relative to the other sites, and average minimum temperatures in Leamington are much warmer (Figure 3.1, Table 4.1) with only 126 days per year where minimum temperatures are lower than 0°C. Temperatures are the lowest in January where the monthly average daily minimum temperature is -7.9°C. In conjunction with warmer temperatures, Leamington also has a more moderate climate evidenced by the lesser temperature ranges between mean maximum and mean minimum monthly temperatures, as well as through the smaller transitional differences in minimum and maximum temperatures from month to month going into and out of winter (Table 4.1).

The Elora trial was situated on the Elora Research Station (43°38' N, 80°24' W) operated by the University of Guelph. The site is characterized by a London Loam soil (Canada Department of Agriculture, 1962) belonging to the grey-brown podzolic soil group. Weather information is based on 30 year climate normals (1971-2000) from the Environment Canada weather station located near Shand Dam in Fergus, Ontario (Environment Canada, 2009). Relative to other sites, winter conditions in Elora are cool, with significant snow fall (Figure 3.1, Table 4.1). Although snow fall is significant throughout winter months, a large decrease in snowfall during late winter may present a winter survival challenge given the fact that temperatures are still very cold. On average, Elora experiences 161 days where minimum temperatures are lower than 0°C, with the lowest average minimum monthly temperatures occurring in January at -11.7°C.

The Kemptville site is situated on the Winchester Research Station (45°03' N, 75°20' W) operated by the Kemptville Campus of the University of Guelph. The site is characterized by a North Gower Clay Loam soil belonging to the grey-brown podzolic soil group (Experimental Farm Service, 1951). Weather information is based on

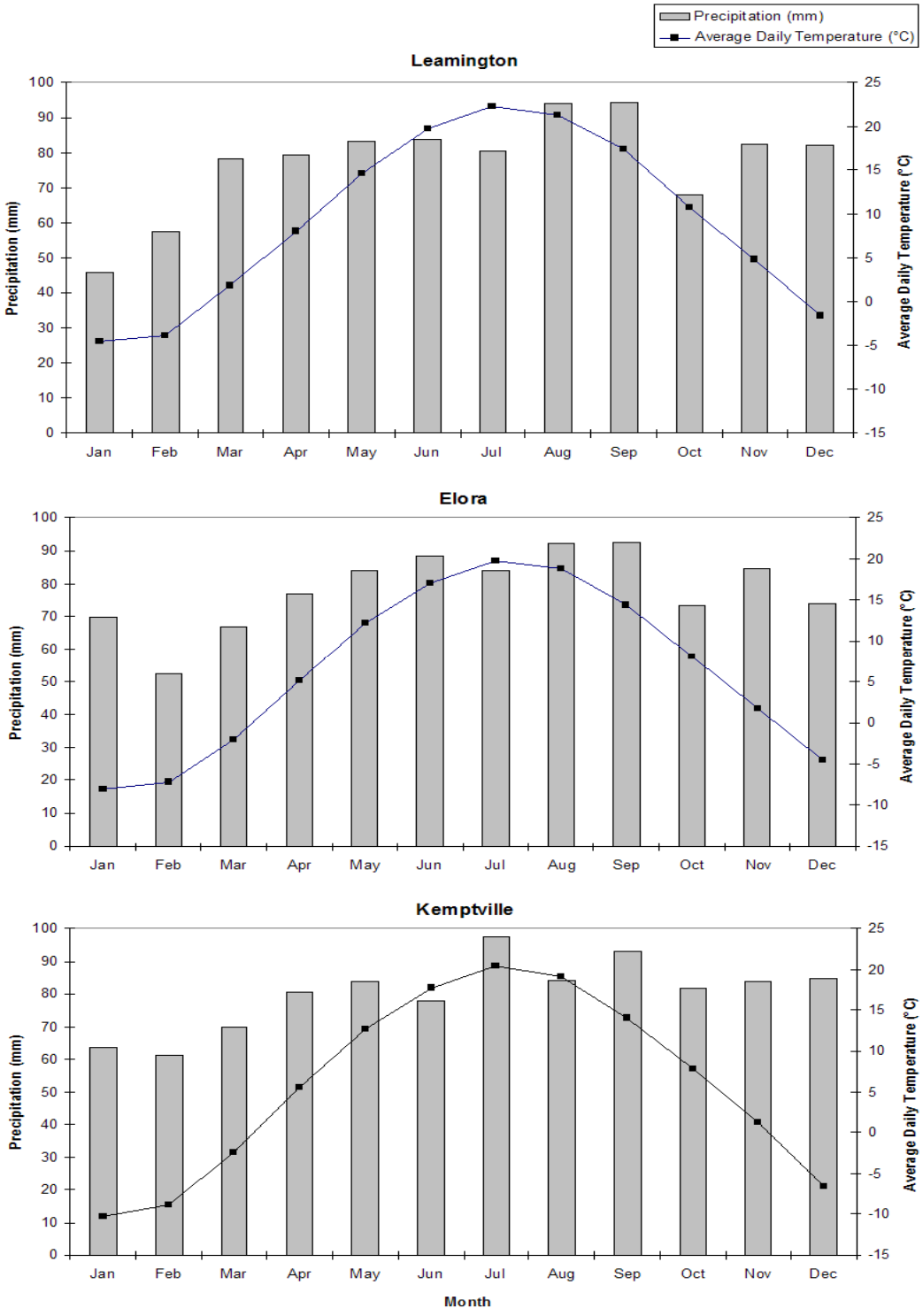

Figure 3.1 Average monthly weather normals (1971-2000) for Environment Canada weather stations nearest the test locations.

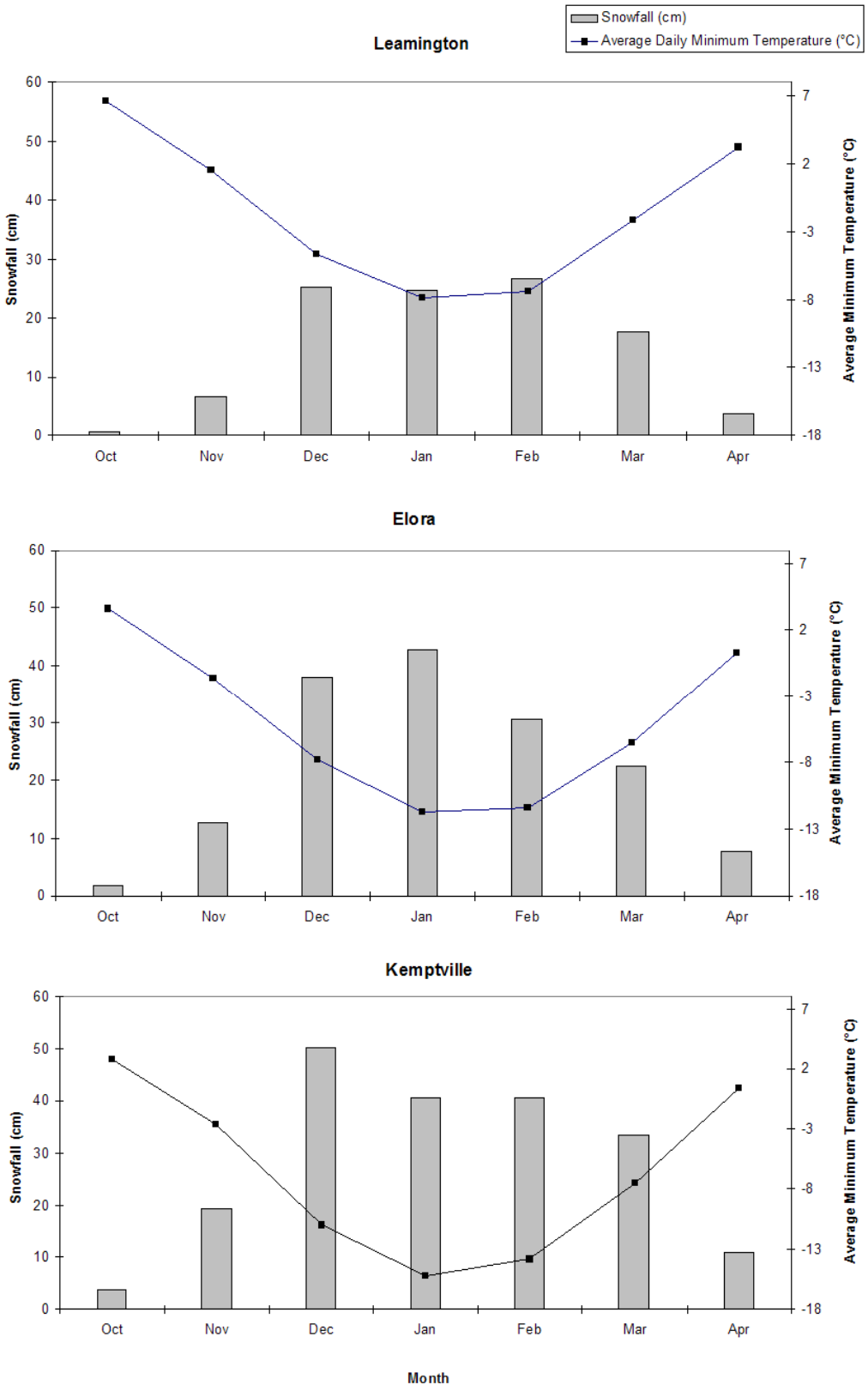

Figure 3.2 Average monthly weather normals (1971-2000) for Environment Canada weather stations nearest the test locations.

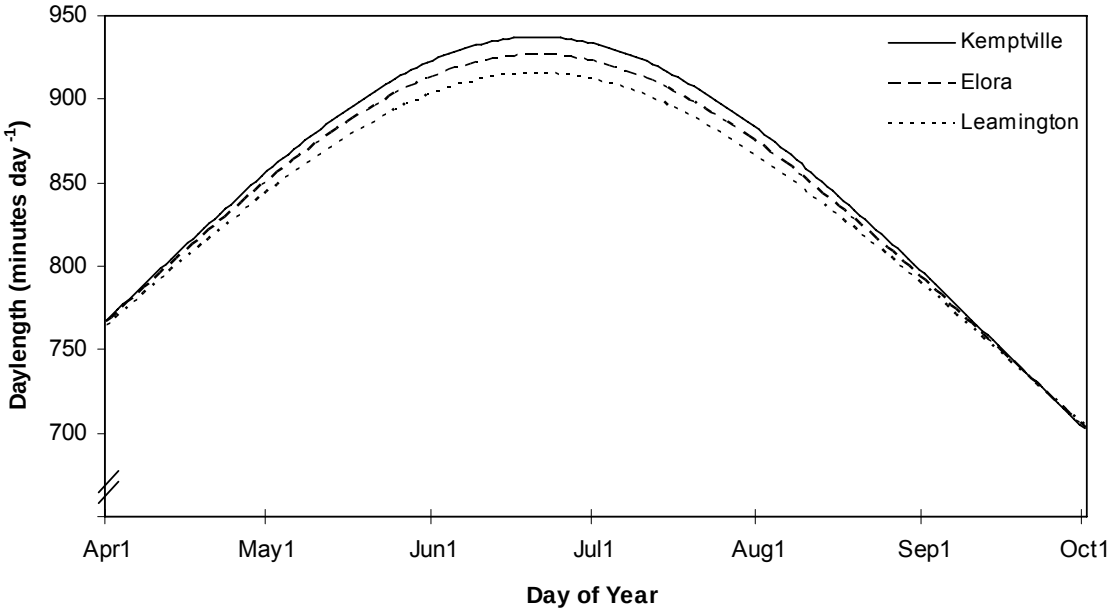

Figure 3.3 Theoretical day lengths during the growing season at the three *Miscanthus* trial locations in Ontario

30 year climate normals (1971-2000) from the Environment Canada weather station located in Kemptville, Ontario (Environment Canada, 2009). Relative to the other test sites, winter conditions in Kemptville are considered cold with generally consistent high snow accumulation (Figure 3.1, Table 4.1). Although late winter temperatures are still very cold, snowfall tends to still be consistently high. Similar to Elora, Kemptville experiences an average of 166 days where minimum temperatures are lower than 0°C. However, the lowest average minimum monthly temperatures in January are much colder at -15.2°C. Relative to the other sites, Kemptville also experiences much greater temperature variability with greater temperature ranges between mean maximum and mean minimum temperatures for each month, as well as through greater transitional differences in minimum and maximum temperatures from month to month going into and out of winter (Table 4.1).

The three locations were also characterized by differences in day lengths during the growing season (Figure 3.3), which may have an influence on plant development if daylight sensitivity exists within these entries. The day lengths presented are based on astronomical algorithms for the difference between sunrise and sunset during the year 2009 (NOAA, 2012), and do not take into account local influences such as differences in atmospheric conditions or shadows from nearby objects which may result in slight

variation. Given the differences in latitudes, the Kemptville location experienced the longest days in the summer while Leamington experienced the shortest. On the summer solstice (June 21) where day lengths are the longest with the greatest differences between locations, day lengths in Kemptville, Elora and Leamington were approximately 937, 926, and 915 minutes respectively.

3.2 Plant material

Twenty six *Miscanthus* entries representing three classification "groups" were evaluated. These included seven fertile *M. sacchariflorus x M. sinensis* hybrids hereafter referred to as "fertile hybrids", five sterile *M. x giganteus* types hereafter referred to as "*M. x giganteus* types", and 14 true breeding *M. sinensis* genotypes. Entries and descriptions are summarized in Table 3.1. *Miscanthus* vegetative propagules were sourced from Pyramid Farms (Leamington, Ontario).

3.3 Plot layout

Plots in trials were arranged in a randomized complete block design (Figure 3.4). Plot size was 3 m by 6.75 m and consisted of four rows of nine plants planted on a 75 cm grid pattern. Each location had four replications of all treatments arranged in four blocks. Within a block, there were 28-32 plots arranged in four tiers of eight plots. Empty alleys 2.5 m wide were maintained between tiers to facilitate harvesting and access to plots. Replications one and three in Elora and two and four in Leamington contained border plots between the plots for the two switchgrass varieties and the *Miscanthus* plots, while the remaining replications in Elora and Leamington and all of those in Kemptville did not have such border plots. The entire trial was surrounded by a minimum of two rows of *Miscanthus* plants to mitigate edge effects.

3.4 Plot establishment

Plots were established during the summer of 2008. Vegetative propagules were obtained in plug form and were planted by hand at the desired spacing by using a transportable grid template with marked 75 cm centers.

The Leamington location was prepared by disk and cultivator, with no fertilizer or herbicide used during the year of establishment. Initial planting occurred over several days from June 9th to 14th, with all plants being immediately watered in by hose.

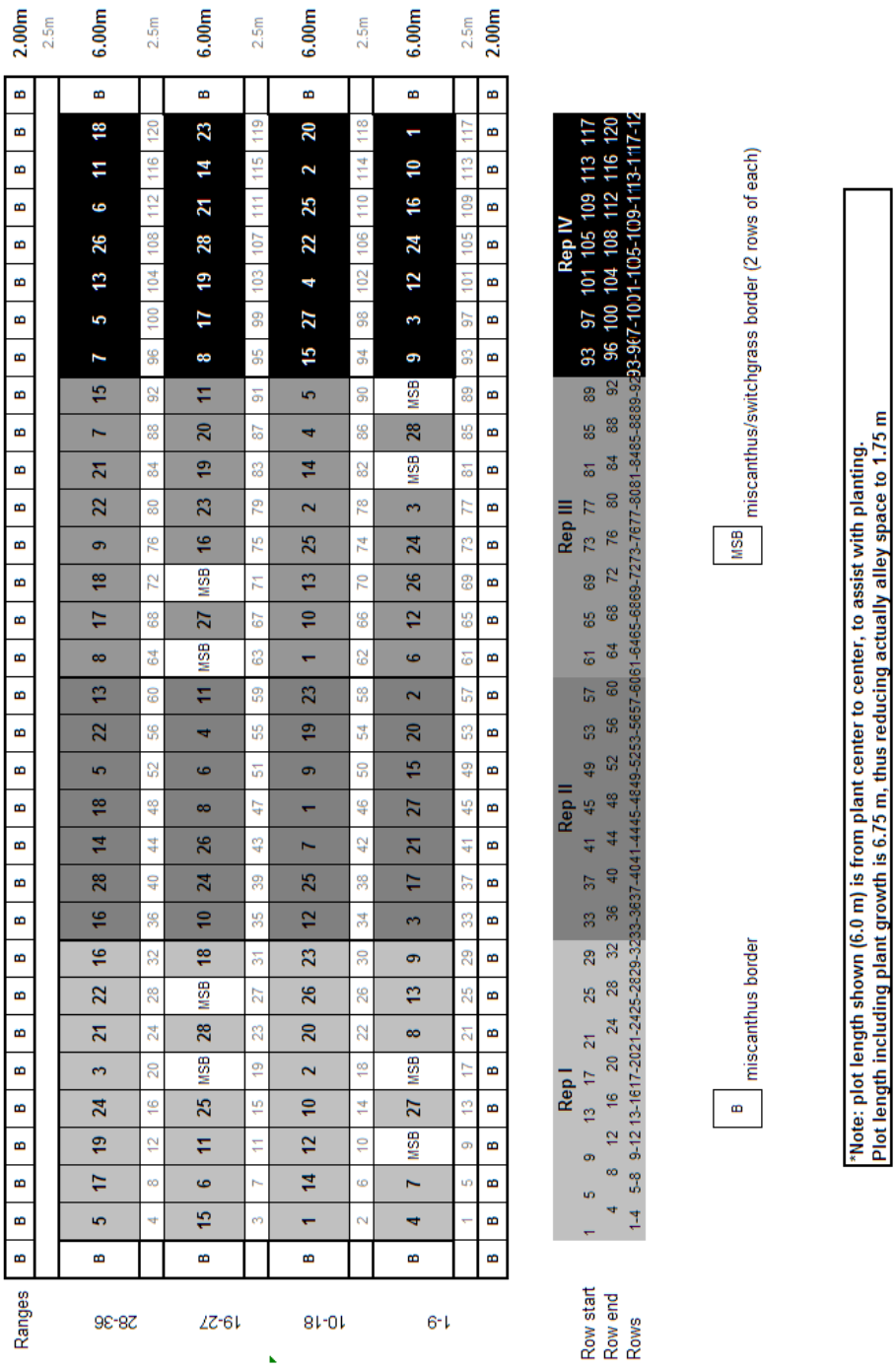

Figure 3.4 Example of test layout as conducted at the Elora, Ontario test location.

Table 3.1 Classification and description of the 26 *Miscanthus* entries evaluated across three locations in Ontario from 2008 to 2010.

Species Group	Entry #	Species Type	Ploidy	Description
M. sacchariflorus x M. sinensis hybrids	1	*M. sacchariflorus* x *M. sinensis*	2x	fertile diploid
	2	*M. sacchariflorus* x *M. sinensis*	2x	fertile diploid
	3	*M. sacchariflorus* x *M. sinensis*	2x	fertile diploid
	4	*M. sacchariflorus* x *M. sinensis*	2x	fertile diploid
	5	*M. sacchariflorus* x *M. sinensis*	2x	fertile diploid
	6	*M. sacchariflorus* x *M. sinensis*	2x	fertile diploid
	7	*M. sacchariflorus* x *M. sinensis*	2x	fertile diploid
M. x giganteus type	8	*M. sacchariflorus* x *M. sinensis*	3x	sterile triploid
	9	*M. sacchariflorus* x *M. sinensis*	3x	sterile triploid
	10	*M. x giganteus*	3x	sterile triploid
	11	*M. x giganteus*	3x	sterile triploid
	12	*M. x giganteus* ("Illinois" clone)	3x	sterile triploid
M. sinensis	13†	*M. sinensis*	2x	fertile diploid
	14†	*M. sinensis*	2x	fertile diploid
	15	*M. sinensis*	2x	fertile diploid
	16	*M. sinensis*	2x	fertile diploid
	17†	*M. sinensis*	2x	fertile diploid
	18	*M. sinensis*	2x	fertile diploid
	19	*M. sinensis*	2x	fertile diploid
	20†	*M. sinensis*	2x	fertile diploid
	21	*M. sinensis*	2x	fertile diploid
	22	*M. sinensis*	2x	fertile diploid
	23†	*M. sinensis*	2x	fertile diploid
	24	*M. sinensis*	2x	fertile diploid
	25†	*M. sinensis*	2x	fertile diploid
	26	*M. sinensis*	2x	fertile diploid

† Entries dropped due to insufficient propagules and/or poor establishment prior to the first winter 2008-2009.

Prior to plot establishment in Elora, glyphosate herbicide was applied to control established weeds, and the site was fertilized with a dry fertilizer blend of a 0-20-20 at a rate providing 60 kg ha^{-1} of both P_2O_5 and K_2O. The location was prepared by several cultivator passes one week prior to planting, and finished with a cultivator and crowfoot packer the day prior to planting. Most planting occurred from June 19th to June 20th, while *M. sinensis* entries 24 and 26 were not available to be planted until July 4th.

The Kemptville location was prepared by tillage, and fertilized with urea at a rate of 23 kg ha^{-1} of nitrogen on July 4th. The location was planted over July 7, 8, 9 and 11th, with irrigation provided during the same period. The herbicide 2,4-D was applied on August 14th to reduce competition from late emerging broadleaf weeds.

3.5 Plot maintenance during year of establishment

Plants which failed to survive the initial planting were replanted with extra plugs to ensure that all plots were complete prior to winter. In Leamington, replanting of missing, or dead plants was completed during the week of July 28, except for entry 26 which was only available in sufficient numbers to fill the first three reps. In Elora, plants which where missing due to insufficient number of plants for the original planting, or those which had not survived from the original planting were replanted over July 4, 7 and 8. From August 21-26, living plants were consolidated to fill any gaps from dead or unfilled plants from reps four to fill reps one, two and/or three as the number of living plants permitted. In Kemptville, no replanting of unfilled or dead plants occurred.

Following the establishment during the first summer, plant survival was recorded prior to winter to record the number of living plants, while those which were not living were assumed to have not survived from establishment, or never established due to insufficient number of plants. These pre-winter survival assessments were made on October 13 in Leamington, October 20 in Elora and October 28 and November 4, 6 and 7 in Kemptville. Live plant counts of entries across all sites during the establishment year are displayed in Table 3.2. Due to mortality and insufficient number of plants available for planting during the year of establishment, six *M. sinensis* entries, 13, 14, 17, 20, 23 and 25 were dropped from the study.

3.6 Plot maintenance during year following establishment

Weed control primarily consisted of hand hoeing plots throughout the growing season, performed as needed to prevent weed competition. In Elora, chemical application of Primextra II Magnum® (atrazine and metolachlor active ingredients) was also employed on May 11 to control early emerging weeds, applied at a rate of three L ha^{-1} of product. No chemical was applied at either the Leamington or Kemptville sites.
During the summer of the second year, winter survival measurements were made by assessing evidence of plant regrowth. In cases where plants of clonal *Miscanthus* entries 1-12 had died, replanted occurred with rhizomes.

In Leamington, missing clonal entries were filled from plants split from existing plant rhizomes within each plot on June 22. In Elora and Kemptville, within the week prior to the day of replanting, rhizomes were sourced from a propagation field operated by Pyramid Farms. *Miscanthus* plants were clipped of top-growth, dug from the field and rhizomes were washed and packed in crates using coco filings soaked by hose to

Table 3.2 Number of living plants present for each entry across each location as counted in October and November, 2008.

	Number of Plants†		
Entry	**Leamington**	**Elora**	**Kemptville**
1	139	144	117
2	138	144	123
3	138	132	115
4	140	144	73
5	129	143	130
6	140	129	113
7	132	142	119
8	139	143	102
9	141	142	140
10	144	142	89
11	137	143	116
12	137	142	128
13	124	108	0
14	77	72	0
15	126	137	4
16	83	53	72
17	78	70	0
18	82	54	12
19	103	73	3
20	117	36	0
21	78	55	18
22	62	74	47
23	89	55	0
24	78	118	34
25	78	111	0
26	43	116	18

† 144 plants (4 reps of 36) planted for each genotype at each location

conserve moisture. Crates were kept in cold storage (3°C) between digging and planting, and stored for no longer than one week. On planting day, rhizomes were hand separated into pieces consisting of a minimum of one viable rhizome internode and planted using hand shovels at approximately 50mm in depth. Initial replanting occurred in this fashion on June 8 and 16 in Elora and Kemptville respectively.

In Elora, rhizomes which again failed to re-grow following the initial replant were replaced with greenhouse propagated plug transplants originating from hand clipped rhizome pieces, or from fresh larger rhizomes dug from propagation fields for entries where plugs were not available. As a precaution, rhizomes were hand watered to the

point of soil saturation around the rhizome area, with all planting completed on July 16th No replanting occurred after the initial replanting at either Leamington or Kemptville, regardless of the success of the initial replant.

Following establishment year, *M. sinensis* propagules could no longer be sourced, and could thus not be replanted. In Leamington and Elora, surviving plants were consolidated into replicates 1, 2, 3 and 4, in that order, and occurred on June 9th in Leamington and June 22 in Elora. No consolidation occurred in Kemptville as no *M. sinensis* survived the first winter. In Elora and Kemptville, empty *M. sinensis* plant spaces were replaced with a unique 'European *M. x giganteus'* variety as a "filler" to mitigate any effects of missing plants, while spaces were left empty in Leamington.

3.7 Measurements

All measurements except for fall biomass yield were recorded on an individual plant basis and plot means computed

Fall stand counts - Plants were observed for being present and alive during the fall of the year of establishment in order to determine which plants where alive going into the first winter. Plants which were dead or had not been planted were recorded as not being present.

Winter survival – Plants were observed for several weeks in spring for new regrowth. Plants which failed to emerge with new growth by the end of May were considered to have not survived. Plants were recorded as either alive or non-alive, and survival computed as the percentage of living plants which were alive in the previous fall.

Spring 50 cm regrowth – Beginning in May, plants were rated for the Julian day they reached a height of 50 cm. Ratings were made biweekly until early August. Measurements were not completed in Kemptville for 2009.

Flower emergence – Plants were observed for full inflorescence emergence from flag leaf sheath, beginning the second week of June with ratings continuing biweekly until the end of October. The day of the year for the first observation where a plant had full inflorescence emergence was recorded.

Fall leaf senescence – Plants were observed for degree of natural leaf senescence of un-shaded upper canopy leaves as indicated by the transformation from green colouring. Ratings began during the second week of September, and consisted of rating the proportion of leaves which were fully green which was recorded on a percentage scale of 1 for 0-25% green, 3 for 26-50% green, 5 for 51-75% green and 7

for 76-100% green. Measurements were made until harvest, and reported as day of the year of the first observation of a plant reaching 50% or less green colouring.

Culm length – Height from the soil surface to the collar of the flag leaf of a representative culm for each plant was measured using a barcode ruler and scanner during the last week of October.

Compressed plant circumference – Culms were compressed at 1 m above the soil surface and aggregate tiller circumferences were measured using a bar-coded measurement strap. Measurements were made during the third week of October.

Culm diameter – The internode diameter of a representative mature culm was measured at 1 m from the soil surface using a digital caliper. Measurements were made during the third week of October.

Basal circumference – Circumference of all culms for each plant was measured at the soil surface using a sewing tape measure during the third week of October.

Percent flowering tillers – Plants were observed for the total percentage of tillers which had flowered during the growing season, as assessed by full inflorescence emergence from the flag leaf sheath. Measurements were made during the first week of November. Measurements were not made in Leamington in 2009.

Late season culm emergence – Following harvest, the number of culms less than 15 cm in height were counted for each plant.

Lodging rating – Plots were observed for lodged plants. Measurements were recorded as the percentage of plants within a plot which appeared to be lodged 45º or less from the horizontal during the last week of October.

Fall Biomass yield – Fall biomass yields were recorded at each location in 2009 and 2010. In Leamington, all 4 plots were first cut with a discbine leaving a 15cm stubble height, and subsequently harvested with a pull-type forage harvester blowing into a custom built Horstline® forage dump wagon equipped with load cells to determine harvest weights. Harvest in Leamington was completed on December 16, 2009 and December 9, 2010. In Elora, plots were direct harvested with a self-propelled forage harvester equipped with a 3 m Kemper® forage harvesting head collecting in the same Horst® forage dump wagon. All 4 rows were harvested leaving a 15 cm stubble height. Elora harvest was completed on November 12, 2009 and November 12, 2010. In Kemptville, the middle 2 rows of each plot were harvested, cut by hand with a hedge-trimmer at a stubble height of 15 cm. Cut plants were subsequently weighed in a plastic container on a portable weigh scale to determine harvest weights, with harvest

completed on November 19, 2009 and November 29, 2010. In Leamington and Elora, one representative plant from the middle of each plot was collected immediately prior to harvest to determine moisture content for dry mass yield conversions. Sample plants were weighed in field to determine final plot harvest yields, and were subsequently chipped into bags as subsample for calculating moisture. In Kemptville, subsamples were hand collected from the previously cut and weighed harvest plants. All subsamples were dried at 80°C until no changes in mass were detected.

Actual dates of measurements and assessments at the three locations are summarized in Table 3.3.

Table 3.3 Dates of *Miscanthus* measurements taken during the first and second years of establishment at three locations in Ontario.

	Leamington	Elora	Kemptville
	Measurements Made During Year of Establishment, 2008		
Culm length	Dec 1	Oct 17, 20, 22	Oct 28, Nov 4,6,7
Basal circumference	Nov 28	Oct 17, 20, 22	Oct 28, Nov 4,6,7
Fall stand count	Oct 13	Oct 20	Oct 28, Nov 4,6,7
	Measurements Made During Second Year of Establishment, 2009		
Winter survival	May 13	June 5	June 1
Spring 50cm regrowth	May 20, 26, June 3, 9, 16, 23	May 29, June 12, 25, Jul 9, 27, Aug 11	Not Completed
Flower emergence	Aug 4, 20, 25, Sept 11, 25, Oct 9	Aug 17, 31, Sept 17, Oct 1,14	Aug 24, Sep 7, 21, Oct 5, 19
Fall leaf senescence	Oct 9, 23, Nov 13	Sept 9, 21, Oct 14	Nov 4
Culm length	Oct 12	Oct 24	Oct 24
Compressed plant circumference	Dec 5	Oct 30	Oct 30
Culm diameter	Dec 3	Oct 21	Oct 21
Lodging rating	Oct 30	Oct 22	Oct 22
Percent flowering tillers	Not Completed	Oct 22	Oct 22
Biomass yield	Dec 16	Nov 12	Nov 12
Basal circumference	Dec 22	Dec 15	Dec 15
Fall culm regrowth			

3.8 Statistical analysis

Statistical analysis was conducted using SAS® 9.2 (SAS Institute Inc., Cary, NC) with all tests of significance being made at a type 1 error rate of 5%.

Analysis of variance for winter survival was conducted using the PROC MIXED function. Location, entry and location*entry interactions were used as fixed effects, while rep nested within location was used as a random variable. Residual analysis was completed by using the PROC PLOT function to plot residuals by predicted values to confirm the assumption of random distribution and homogeneity of error variance, and by plotting residuals by location and entry treatments and reps to visually assess for linearity and homogeneity of error variance. The assumption of normal distribution of errors was investigated by the Shapiro-Wilk statistic. Upon residual analysis of winter survival data, heterogeneity of errors for entry was observed. To account for this violation, an unstructured, between-subject heterogeneity model was fit through the use

of a REPEATED statement. No outliers were detected following Lund's test of studentized residuals.

Mean winter survival values for entries by locations were calculated as marginal means through the LSMEANS statement. Multiple pairwise comparisons between entries within locations were made using a Tukey's test.

Winter survival potential of species groups was investigated by a pairwise comparison of the winter survival of the two entries with the highest winter survival within each species groups within each location. Similarly, relative winter hardiness of European sourced *M. x giganteus* entries was tested by contrasts of both European entries to the "Illinois" entry.

Modeling of relationships between winter survival and plant measurements was made by viewing scatterplots of winter survival as a function of each measurement, where each data point represented the mean measurement of all plants within an individual plot and winter survival as the percentage of plants within the plot which had survived the first winter. Scatter plots were assessed for patterns at various scales, ranging from an aggregate scale including all entries across all locations, all entries within individual location scale, and by a species group within location scale. Where linear correlations were apparent, regression equations, coefficient of determination and significance were determined using PROC REG. Where non-linear relationships were observed, PROC NLIN was used to establish a non-linear regression. Non-linear regressions were fit with a linear-plateau or plateau-linear model by establishing a segmented linear equation (Bowley, 2008). For non-linear regressions, the slope, coefficient of determination and significance of correlation for the linear portion of the line were determined using PROC REG.

Variance analysis and comparisons of top yielding entries for each species group for 2009 and 2010 yields was conducted as outlined above for winter survival, while the Pearson product-momentum correlation coefficient for the linear correlation between 2010 yield data and 2009 yield data was completed using PROC CORR.

4. RESULTS AND DISCUSSION

4.1 Growing season conditions during the year of establishment

Weather recorded at the Environment Canada weather stations closest to the trial locations during the first year of establishment, as well as the 30 year climate normals (1971-2000) are all summarized in Table 4.1.

Leamington experienced above average mean maximum monthly temperatures from June through September, with minimum temperatures ranging from slightly above to a few degrees below seasonal. While precipitation was well above average during the initial planting month of June, precipitation was close to the 30 year normals for the months of July and September, but well below for the month of August at only 19% of the 30 year mean.

Elora experienced seasonal temperatures during the first three months of establishment from June to August, with maximum mean monthly temperatures within 0.5ºC of 30 year normals, and warmer than average to average mean monthly maximum temperatures during fall months of September to November. Monthly mean minimum temperatures ranged from slightly below to above seasonal. Precipitation was above average during the first three months of establishment ranging from 14-48% higher than 30 year averages, a trend which generally continued into the autumn months.

During the first months of establishment, Kemptville experienced mean, maximum and minimum temperatures ranging from seasonal average to slightly below seasonal average, a trend which continued into the autumn months with the exception of September which was slightly warmer than seasonal. Precipitation was well above average in the month prior to, and during establishment, being 105% and 44% higher than 30 year normals during the months of June and July respectively. August and September were below average at 70% and 46% of mean monthly precipitation respectively, with autumn months ranging from seasonal to below seasonal precipitation accumulation.

4.2 Winter conditions during the first winter following establishment

Winter conditions were relatively consistent across all three locations, with normal daily maximum temperatures during the month of December, but daily minimum temperatures which were several degrees below 30 year normals. In January, mean daily maximum and minimum temperatures were colder than normal, ranging from 2-3°C

below 30 year normals across all sites. During the months of February to April, mean monthly maximum and minimum temperatures were slightly higher than 30 year normals across most sites except for the minimum temperatures in Leamington for March and April. Precipitation during the winter months tended to be close to or higher than seasonal means, with all locations having at least 85% of normal winter precipitation during the months of December to March, except for Kemptville during January and February. While winter temperatures varied from their long term averages similarly within each site, the magnitudes were similar in such that the ranking of sites remained the same, with the least coldest temperatures experienced in Leamington, with intermediate temperatures experienced in Elora, and the coldest temperatures experienced in Kemptville. Similarly, differences between mean maximum and mean minimum temperatures were greater during the first winter months than experienced during the 30 year normals, suggesting greater temperature fluctuation, with the greatest spread consistent with the Kemptville location from September 2008 to May 2009.

4.3 Variability in winter survival during the year of establishment

Variability in winter survival was evident during the year of establishment in Ontario. Variance analysis of first year winter survival demonstrated highly significant ($P<0.0001$) sources of variation from locations, entries, and entry*location interactions (Table 4.2). As a result of the significant interactions, winter survival is discussed by entries within each location.

In general, winter survival was the greatest for most entries in Leamington where the winter was expected to be the least challenging, and decreased in Elora and Kemptville as winter severity increased (Table 4.3). Despite the differences in variability within species groups in Leamington, no significant differences in winter survival for any of the top surviving entries across the different groups was observed (Table 4.4), suggesting equivalent winter survival potential existed for each of the species groups based on the entries, and environmental conditions experienced at Leamington during the first winter.

In Elora, where winter conditions were expected to be moderately harsh in terms of the three test sites in this study, winter survival ranged from 0% for *M. sinensis* entries 16, 18, 19 and 26 to 100% for hybrid entry one (Table 4.3). Unlike Leamington, winter survival within the fertile hybrid group in Elora demonstrated a degree of variability with

Table 4.1 Mean monthly maximum and minimum temperatures and precipitation during the first 12 months of establishment of Miscanthus in Ontario in comparison to 30 year (1971-2000) normals at the closest Environment Canada weather stations with climate normals in Leamington, Elora and Kemptville, Ontario.

		June		July		August		September		October		November		December		January		February		March		April		May	
		2008	30-Yr	2008	30-Yr	2008	30-Yr	2008	30-Yr	2008	30-Yr	2008	30-Yr	2008	30-Yr	2009	30-Yr	2009	30-Yr	2009	30-Yr	2009	30-Yr	2009	30-Yr
Mean Daily Max Temp (°C)	Leamington	25.1	24.6	27.6	27.2	26.3	26.0	23.4	22.0	14.7	14.9	6.4	8.1	0.7	1.5	-4.5	-1.3	0.8	-0.3	6.6	5.8	13.5	12.7	19.8	19.8
	Elora	22.9	22.4	25.1	25.0	23.7	24.0	20.9	19.3	12.4	12.5	4.7	4.9	-1.4	-1.2	-6.0	-4.1	-1.0	-2.9	4.2	2.4	11.2	9.8	17.7	17.6
	Kemptville	24.1	23.6	25.3	26.4	24.3	24.9	21.2	19.6	13.1	12.7	5.8	5.2	-1.8	-2.1	-7.8	-5.3	-1.2	-3.7	4.6	2.5	12.8	10.8	17.4	18.6
Mean Daily Min Temp (°C)	Leamington	15.8	14.8	16.5	17.3	14.6	16.5	12.3	12.7	4.1	6.6	-0.3	1.5	-6.8	-4.6	-13.7	-7.9	-7.5	-7.4	-3.1	-2.1	2.9	3.2	8.8	9.5
	Elora	13.5	11.8	15.1	14.3	13.1	13.6	10.8	9.4	3.2	3.6	-2.0	-1.6	-9.0	-7.8	-15.3	-11.7	-10.6	-11.4	-5.3	-6.5	1.2	0.3	6.7	6.8
	Kemptville	13.9	11.9	14.5	14.4	12.6	13.2	9.2	8.5	1.6	2.8	-2.3	-2.7	-12.2	-11.0	-18.1	-15.2	-12.2	-13.9	-6.1	-7.5	0.5	0.4	5.8	6.8
Mean Monthly Precipitation (mm)	Leamington	134	84	82	81	18	94	94	94	31	68	97	83	102	82	43	46	75	58	120	78	117	79	14	83
	Elora	132	89	96	84	130	92	103	92	62	74	112	85	156	74	62	70	64	53	58	67	134	77	85	84
	Kemptville	160	78	141	98	59	84	43	93	87	82	52	84	77	85	43	64	49	61	101	70	150	81	81	84

Table 4.2 Variance analysis of establishment year winter survival of 20 *Miscanthus* entries at three locations in Ontario

Fixed Effects	Num df	Den df		F Value	Pr > F
Location	2	9		266.99	<0.0001
Entry	19	142		196.33	<0.0001
Location*Entry	38	142		53.52	<0.0001
Random Effects	**Group**	**Estimate**	**SE**	**Z Value**	**Pr > Z**
Rep(Location)		0.5	2.80	0.16	0.4349
UN(1,1)	entry 1	76.2	38.49	1.98	0.0239
UN(1,1)	entry 2	70.2	39.25	1.79	0.0369
UN(1,1)	entry 3	113.6	54.96	2.07	0.0194
UN(1,1)	entry 4	136.4	65.78	2.07	0.0191
UN(1,1)	entry 5	463.4	223.13	2.08	0.0189
UN(1,1)	entry 6	886.8	424.96	2.09	0.0185
UN(1,1)	entry 7	4.5	3.83	1.19	0.1180
UN(1,1)	entry 8	507.1	254.18	1.99	0.0230
UN(1,1)	entry 9	648.3	306.61	2.11	0.0172
UN(1,1)	entry 10	70.9	35.88	1.98	0.0240
UN(1,1)	entry 11	144.2	68.17	2.12	0.0172
UN(1,1)	entry 12	363.7	171.77	2.12	0.0171
UN(1,1)	entry 15	5.3	4.10	1.30	0.0968
UN(1,1)	entry 16	23.2	13.90	1.67	0.0473
UN(1,1)	entry 18	364.8	259.05	1.41	0.0795
UN(1,1)	entry 19	478.0	304.29	1.57	0.0581
UN(1,1)	entry 21	36.2	26.95	1.34	0.0896
UN(1,1)	entry 22	242.0	140.88	1.72	0.0429
UN(1,1)	entry 24	461.0	246.69	1.87	0.0308
UN(1,1)	entry 26	37.1	23.21	1.60	0.0551

values ranging from 56% for entry 6 to 100% for entry one. Variation in winter survival for the fertile hybrids appeared to be bimodal with the five top entries all well over 90% and not significantly different from one-another while the lowest three entries (six, eight and nine) had only partial winter survival, ranging 56-64%, none of which were significantly different than the lowest surviving entry six. These entries were all significantly lower than the 5 top surviving entries (Table 4.3). Similarly, variation also became evident within the *M. x giganteus* type group where winter survival ranged from 27% for entry 11 to 97% for entry 10, which were significantly different from one another (Table 4.5). The *M. sinensis* group displayed a wide variation in winter survival, ranging from 0% for entries 16, 18,

Table 4.3 Least squares mean winter survival (WS) and standard error (SE) of 20 *Miscanthus* entries after the year of establishment (2008) at three locations in Ontario. Means within the same letter groups (Sig) are not significant at a 0.05 probability within a location, as determined by Tukey's multiple means comparison.

Species Group	Entry	Leamington			Elora			Kemptville		
		WS (%)	SE	Sig	WS (%)	SE	Sig	WS (%)	SE	Sig
M. sacchariflorus x M. sinensis hybrids	**1**	99.1	4.41	A	100.0	4.43	A	37.1	4.41	BC
	2	99.4	4.17	A	99.3	4.17	A	49.1	4.15	B
	3	100.0	5.37	A	98.3	5.39	A	30.2	5.37	BCD
	4	98.3	5.79	A	97.9	5.79	A	12.6	5.80	CDE
	5	100.0	11.01	A	93.3	11.02	AB	55.0	11.01	ABC
	6	87.9	14.95	AB	56.1	14.95	ABCDEF	23.4	14.95	BCDE
	7	100.0	1.20	A	99.4	1.24	A	88.7	1.32	A
M. x giganteus type	**8**	99.9	11.17	A	61.7	11.17	ABCD	0.0	12.91	BCDE
	9	100.0	12.64	A	64.4	12.63	ABCDE	0.2	12.63	BCDE
	10	98.7	4.26	A	97.2	4.25	A	40.0	4.95	BC
	11	99.1	6.05	A	27.4	6.06	CDEF	0.0	6.05	DE
	12	99.0	9.55	A	70.3	9.54	ABC	2.8	9.54	CDE
M. sinensis	**15**	97.1	1.31	A	99.2	1.21	A	0.4	2.41	E
	16	93.3	2.58	A	0.1	2.96	F	0.0	2.94	E
	18	70.3	9.50	AB	0.2	13.43	DEFG	0.4	18.99	BCDE
	19	65.8	10.97	AB	0.2	12.66	DEFG	0.4	21.93	ABCDE
	21	94.0	2.86	A	52.5	4.01	BCG	0.4	5.68	DE
	22	71.4	7.95	AB	1.2	9.18	EF	0.0	11.23	BCDE
	24	30.3	10.69	BC	1.3	10.69	DEF	0.0	15.11	BCDE
	26	8.4	3.31	C	0.0	2.87	F	0.4	4.05	E

Table 4.4 Pairwise mean contrasts between the entries with the highest winter survival during the year of establishment for each species group in Leamington, Ontario. NS is Non-Significant at a probability of 0.05.

Group		Leamington						
		M.sa x *M.si*			M *x gig*		*M. sinensis*	
	Entry	3	5	7	8	9	15	21
M.sa x M.si	3		NS	NS	NS	NS	NS	NS
	5			NS	NS	NS	NS	NS
	7				NS	NS	NS	0.0498
M *x gig*	8					NS	NS	0.0309
	9						NS	0.0417
M. sinensis	15							0.0053
	21							

19 and 26 to 99% for entry 15. The majority of entries suffered nearly complete loss, including the low surviving entries 22 and 24 which were not significantly different than the entries with no winter survival. Only one entry, 21, demonstrated partial survival at 53% which was significantly different from the group of non/low surviving and high surviving entry 15 (Table 4.3).

Similar to Leamington, overlaps in winter hardiness across the different species groups was still evident based on the entries tested. When contrasting the top surviving entries for each species group, no significant differences in winter survival were observed, still supporting a high degree of winter survival potential for each of these species groups in conditions experienced in Elora.

Table 4.5 Pairwise mean contrasts between the entries with the highest winter survival during the year of establishment for each species group in Elora, Ontario. NS is Non-Significant at a probability of 0.05.

		Elora					
Group		*M.sa x M.si*		*M x gig*		*M. sinensis*	
	Entry	1	7	10	12	15	21
M.sa x M.si	1		NS	NS	0.0054	NS	<.0001
	7			NS	0.0030	NS	<.0001
M x gig	10				0.0110	NS	<.0001
	12					0.0031	NS
M. sinensis	15						<.0001
	21						

In Kemptville, where growing conditions were expected to be harsh in terms of these trials, winter survival ranged from 0% for several entries within all three species groups, to 89% for hybrid entry 7 (Table 4.3). Excluding those which did not survive in Elora, winter survival values were lowest for all entries in Kemptville. As in Elora, the fertile hybrid group demonstrated considerable variation with survival ranging from 13% for entry 4 to 89% for entry 7, which was significantly higher than all other hybrid entries within the location. While most of the remaining surviving entries were significantly different than zero, no others stood out as being significantly different than all others. Variability was also observed in the *M. x giganteus* type group where entry 10 displayed partial survival at 40%, which was significantly greater than the survival of the remaining entries which ranged from 0% to 3%. In the *M. sinensis* group, no entries survived the first winter.

Significant differences in winter survival were evident within both the hybrid and *M. x giganteus* species groups, and unlike the other locations, significant differences were observed between the top surviving entries for each species group. Survival of the hybrid entry 7 was significantly greater than all other entries in Kemptville, including the top surviving *M. x giganteus* entry 10, and all *M. sinensis* entries where no entries survived the first winter (Table 4.6). This demonstrated that for this selection of entries

and under conditions experienced in Kemptville, the greatest winter survival potential was within the hybrid species group.

Table 4.6 Pairwise mean contrasts between the two entries with the highest winter survival during the year of establishment for each species group in Kemptville, Ontario. NS is Non-Significant at a probability of 0.05.

		Kemptville					
Group		*M.sa x M.si*		*M x gig*		*M. sinensis*	
	Entry	7	5	10	12	18	19
M.sa x M.si	7		0.0028	<.0001	<.0001	<.0001	<.0001
	5			NS	0.0005	0.0140	0.0275
M x gig	10				0.0007	0.0452	NS
	12					NS	NS
M. sinensis	18						NS
	19						

Based on the entries and locations tested, variability in winter survival was apparent both within and between species groups during the year of establishment. The range of relative survival increased with winter severity as separation of entries was wider under increasingly harsh winter conditions. For *M. sinensis* in Kemptville, no entries survived the first winter. In the case of the entries tested, separation first appeared for *M. sinensis* within the Leamington location, followed by the *M. x giganteus* triploids in Elora, than the fertile diploids in Kemptville. Thus, relative winter survival was greatest for the fertile diploids, followed by the hybrid triploids, followed by *M. sinensis*. Excluding hybrid entry 6 which had relatively low survival of 56% in Elora, the hybrid entries appeared to maintain a consistently high degree of winter survival of 93% and greater except at the Kemptville location.

In the study by Clifton-Brown et al (2001a), variability in winter survival within location was very low among the four *M. x giganteus* accessions they used, as survival within sites was either very high or very low. This supported our results for Leamington, but not for Elora or Kemptville where conditions were harsher and significant differences between survival of entries occurred. It is possible that given the more limited geographic dispersion of the sites in Ontario relative to Europe, that this study was able to compare these entries across a more moderate gradient in terms of winter severity. Beyond this, genetic variability between existing *M. x giganteus* accessions in Europe has been reported to be very low, as suggested by analysis of surveys of material from botanical gardens and research stations in the UK (Hodkinson et al, 2002a) and

botanical and market gardens in central Europe (Greef et al, 1997). The four *M. x giganteus* accessions used by Clifton-Brown et al (2001a) represented two accessions selected from each of the only two distinct, yet very closely related *M. x giganteus* clusters identified from a collection of 31 accessions in the European survey by Greef et al (1997). Differences in genetic variability and lineage between the entries used in this study are unknown, and it is unknown how closely associated the entries of this study are relative to common *M. x giganteus* in Europe.

Variability for winter survival within the *M. sinensis* group has not been well characterized, though genetic variation within *M. sinensis* is reported as being high (Greef et al, 1997; Hodkinson et al, 2002a) Its native territory is often cited as being extensive, including regions with subarctic climates (Numata, 1979). Work by Clifton-Brown et al (2001a) demonstrated variability in winter survival within *M. sinensis*, with one of the five genotypes having a consistently high loss relative to the other *M. sinensis* collection genotypes in England, Denmark and Sweden. This variability in survival was reflected within *M. sinensis* in Elora where a few entries appeared to display exceptional survival relative to the others, although the relative differences were much greater in Elora. The natural *M. sinensis* selections used by Clifton-Brown et al (2001) had been selected in Japan and had undergone some prescreening for winter hardiness (Jorgensen, 1997) which may have reduced the extent of variability, while the extent and aim of selection behind the entries used in this trial are unknown. In the same trials, two full diploid *M. sinensis* hybrids acquired from Deuter in Germany demonstrated poorer winter survival relative to the genotypes collection from Japan, with losses for one of the German genotypes as high as 60% and 50% in Denmark and Sweden respectively, supporting variability for winter survival within the species. Beyond winter survival, within species variability for morphological measurements has been observed for natural selections of *M. sinensis* in China (Yan et al, 2011). Screening of multiple fertile hybrids has not been well published. Clifton-Brown et al (2001a) demonstrated high winter survival of a diploid and aneuploid *M. sacchariflorus* x *M. sinensis* hybrids, with no winter losses greater than six percent for both genotypes across all sites. While it is difficult to make conclusions on the limited work, both parent species, *M. sacchariflorus* and *M. sinensis* are noted to possess genetic variability (Greef et al, 1997; Hodkinson et al, 2002a), which may support the observation of variability suggested by reduced winter hardiness for certain fertile hybrid entries such as entry six in Leamington and Elora.

Variability within species groups is an important finding. Selecting entries solely by species group is insufficient to guarantee winter survival. This was especially evident as increasing winter severity results in the separation of entries within groups. Thus, when selecting entries to use in new regions where winter survival may be an issue, such as Ontario, proper entry selection appears to be critical for ensuring winter survival and subsequent stand establishment. Entry screening will be an important consideration to identify suitably adapted entries.

When the winter survival potential of species groups was compared using the top surviving entries of species groups within a location, no significant differences in winter survival were observed between species groups at Leamington or Elora, only at Kemptville. Even though winter pressure was evident for some *M. sinensis* entries in Leamington and a variety of entries across all species groups in Elora, no significant differences were observed for top surviving entries at either site. In Elora, entry 15 had significantly greater winter survival than all other *M. sinensis* entries within that location, and appeared to account for the lack of significant differences with the fertile hybrid and *M. x giganteus* type groups, reflecting the degree of variability within that group for this selection of entries. The data suggests that, based on the entries used, all species groups had equivalent winter survival potential at these two sites. As winter conditions became harsher at the Kemptville site, hybrid entry 7 had significantly greater winter survival than the top surviving entries of *M. x giganteus* or *M. sinensis*, suggesting that the best source of winter hardiness within this selection of entries was in the hybrid species group, and that species selection for winter survival for these entries only becomes relevant for winter severity beyond that experienced in Elora in 2008-2009.

These findings are consistent with the trends in within group variability where the hybrid entries had consistently numerically higher winter survival relative to most *M. x giganteus* and *M. sinensis* entries in Elora, and most *M. sinensis* entries in Leamington. In general, the hybrids had greater winter hardiness than the *M. x giganteus* species group which had greater winter hardiness than the *M. sinensis* species group.

While studies comparing winter survival of a number of *Miscanthus* entries across a gradient of winter environments are limited, the findings of the present study do not directly conform with those previous reports. A study across five locations in Europe by Clifton-Brown et al (2001a) revealed that *M. x giganteus* entries were more susceptible to winterkill relative to *M. sinensis* and *M. sinensis* hybrid groups when grown in regions with challenging winter conditions. The authors observed very low

winter survival rates ranging from 0-7% for four *M. x giganteus* entries at sites in Denmark and Sweden, whereas the collection of *M. sinensis* genotypes and *M. sacchariflorus* x *M. sinensis* hybrids had winter survival as high as 95% and 98% respectively in Sweden, and 99% for both groups in Denmark. Work completed by Clifton-Brown and Lewandowski (2000) supported the observations by Clifton-Brown et al (2001a) when they found lower LT50 values for two full *M. sinensis* hybrids relative to two *M. x giganteus* entries which had been used in the study by Clifton-Brown et al (2001a). Both hybrids had lower LT50 values, including one at -6.3°C, relative to the -3.4°C LT50 which was observed for the two *M. x giganteus* entries. These hybrids had been progeny of plants selected from "cool temperate Asia" (Clifton-Brown and Lewandowski, 2000). Pre-selection for winter hardiness of parents of the *M. sinensis* entries in the European trials (Jorgensen, 1997) may have accounted for the observation of greater winter hardiness for the *M. sinensis* entries relative to the *M. sinensis* hybrids and *M. x giganteus* at sites with greater winter severity in Europe. It is unknown if the lack of prescreening of this sort had influenced selections for the *M. sinensis* entries used in Ontario. Given the recurring observations of high genetic variability and an extensive natural range for the *M. sinensis* species (Yan et al, 2011), it is possible that the *M. sinensis* entries used in Ontario were not as well adapted to cooler regions as those used in the study by Clifton-Brown et al (2001a), and thus did not reflect the winter survival potential of the species..

The greater survival of the fertile hybrid entries was consistent with Clifton-Brown et al (2001a) where a single diploid *M. sacchariflorus* x *M. sinensis* hybrid maintained winter survival of 99% and 98% in Denmark and Sweden respectively, higher than all *M. x giganteus* accessions, and similar to the cold selected *M. sinensis* collection. As mentioned, intermediate survival of the *M. x giganteus* types between the hybrid and *M. sinensis* groups in Ontario was not consistent with Clifton-Brown et al (2001a) where it was among the poorest winter survivors relative to the entries in other species groups. Within certain environments, such as Elora, winter survival within the *M. x giganteus* types was highly variable ranging from very low (27% for entry 11) to very high (97% for entry 10). While no variability was evident within the four true *M. x giganteus* accessions in Clifton-Brown et al, there was one *M. sacchariflorus* x *M. sinensis* hybrid labeled as an "aneuploid" which maintained winter survival of 94% in both Denmark and Sweden. If this was a triploid hybrid, then it would support the variability observed within *M. x giganteus* types in Ontario.

Even though the hybrid species had the greatest winter survival potential (entry seven in Kemptville), within species variability still makes entry selection important, as selection based on species group alone would not have necessarily ensured adequate survival for a commercially successful establishment.

Selection of entries with high winter survival and low variability is ideal to reduce the risks associated with stand loss. Stability analysis of mean winter survival of entries across all environments demonstrated a strong association of winter survival with variation in winter survival as measured by the coefficient of variation (Figure 4.1). As mean winter survival across all sites increased, coefficient of variation across sites decreased in a fairly linear fashion, suggesting a strong negative relationship. Entry seven, with highest winter survival of 96% demonstrated the lowest coefficients of variation of 7%, while entry 26 with the lowest mean winter survival of 2.8% demonstrated the highest coefficient of variation of 173%. Nearly all entries in the lower than average winter survival and higher than average variability quadrant were *M. sinensis*, while nearly all entries in the higher winter survival and lower variability quadrant were fertile hybrids or sterile triploid hybrids. Overall these results suggest that with these entries for these environments, selection for high winter survival during the year of establishment concurrently selects for low winter survival variability.

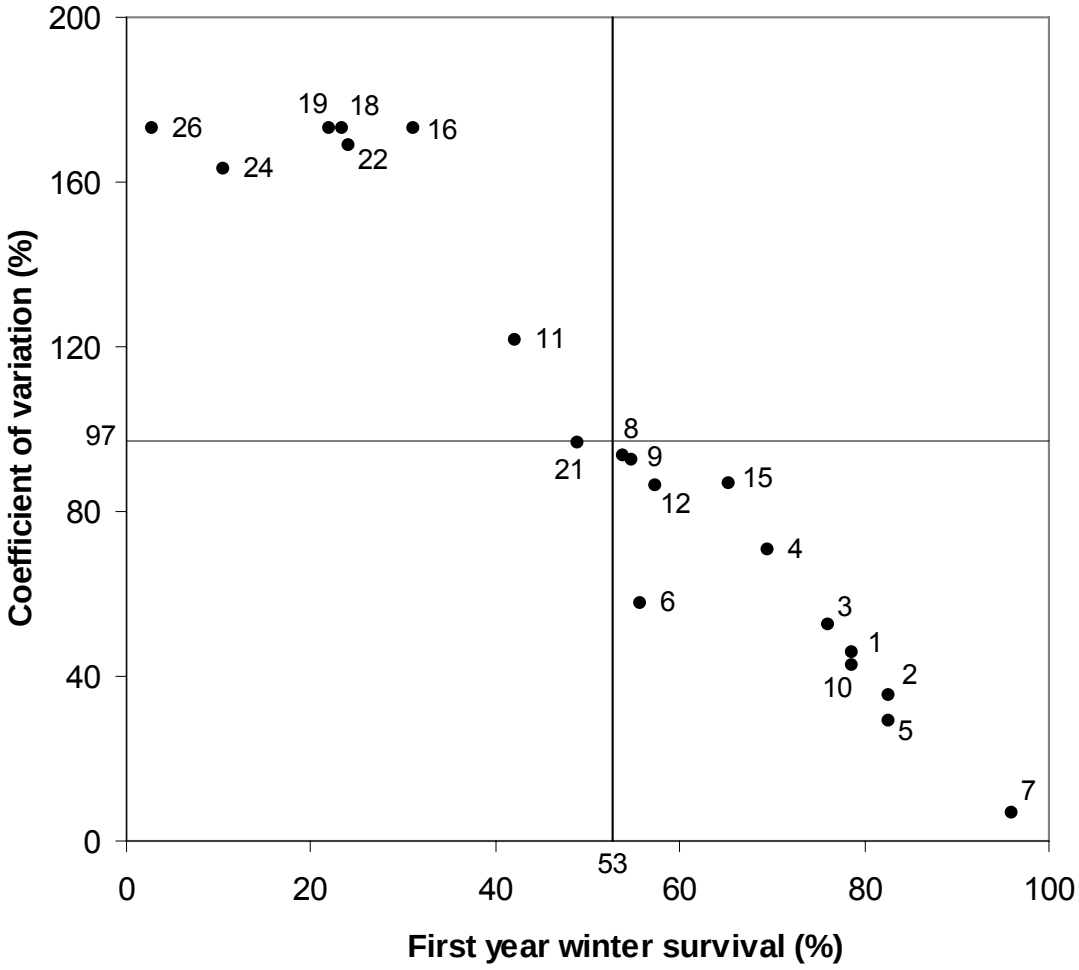

Figure 4.1 Stability analysis of first year (2008/2009) winter survival of 20 *Miscanthus* entries averaged across three locations in Ontario.

Plant condition and vigour during the year of establishment may have influenced winter survival. The winter survival results observed in this study may be biased if variability in vigour during the initial growing season was evident across entries. To investigate this, plant survival during the 2008 establishment period was compared to winter survival during the establishment year. In Leamington and Elora, establishment survival was the percentage of plants surviving from the initial planting time until immediately prior to the establishment growing season mortality replant, with the expectation that higher mortalities reflect lower plant vigour. In Kemptville, where no replanting occurred during the establishment year, establishment survival was the percentage of plants which survived from planting until prior to the first winter. Figure 4.2

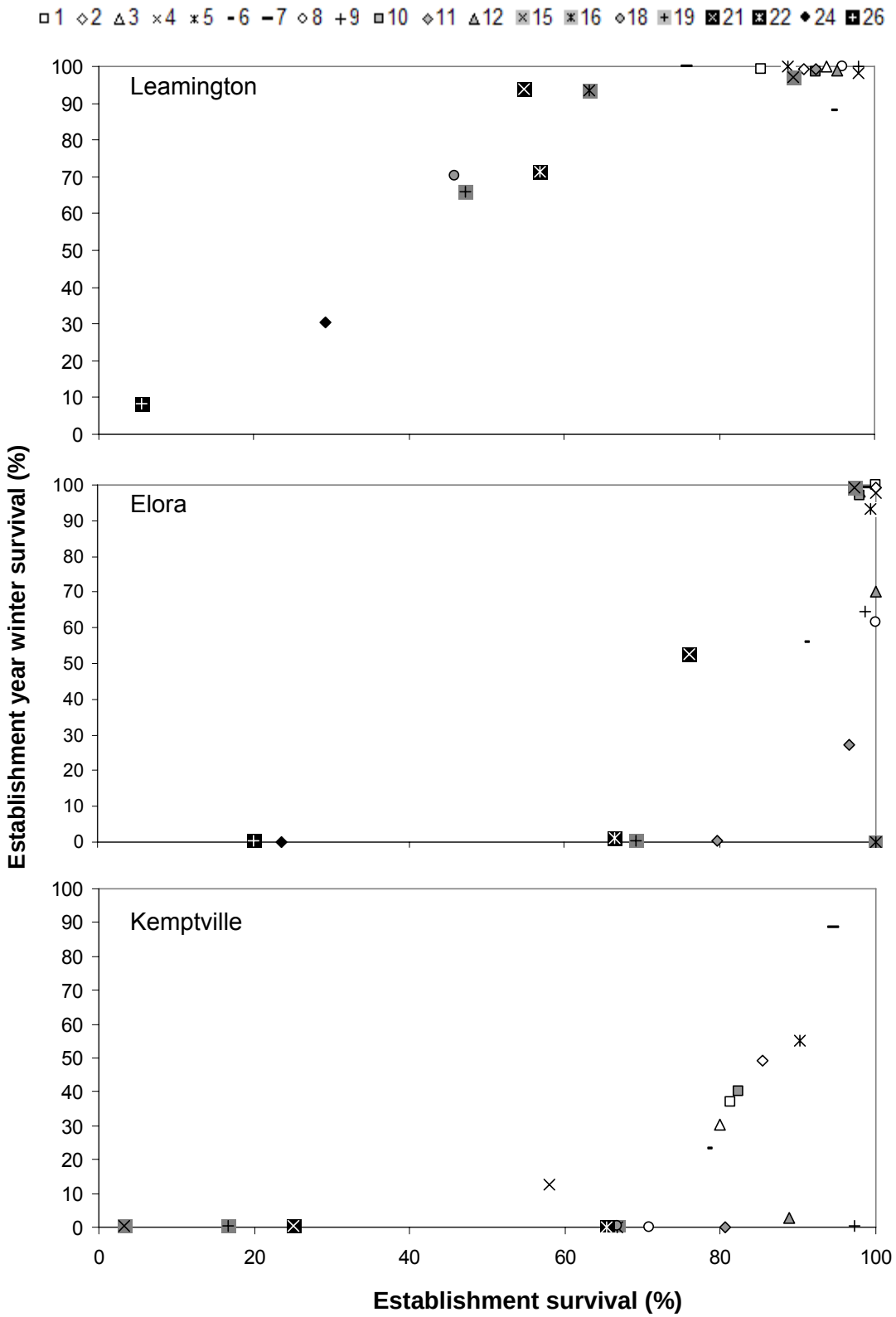

Figure 4.2 Establishment year winter survival as a function of establishment survival during the first growing season for 20 *Miscanthus* entries at three sites in Ontario, 2008. (data presented in appendix 4.1)

presents establishment survival as a function of winter survival, where each data point represents a mean for each entry within each location.

While the majority of entries in Leamington had both very high establishment and winter survival, several *M. sinensis* with poorer winter survival demonstrated a negative relationship where decreasing levels of survival during establishment were associated with decreasing levels of winter survival. In Elora, lower levels of survival during the establishment year appeared to be associated with lower winter survival values, although high survival during the establishment period did not necessarily guarantee good winter survival. This was evident for entries with establishment survival of over 90% where winter survival ranged from 0-100%. Similar results were observed in Kemptville where lower establishment survival was associated with lower winter survival, but high establishment survival did not always guarantee high winter survival.

4.4 Variation in winter survival of European *Miscanthus x giganteus* accessions versus North American tested "Illinois" *Miscanthus x giganteus*

Winter survival for the Illinois entry ranged from 99% in Leamington to 70% in Elora to 3% in Kemptville, suggesting it may not be well adapted to all growing regions within Ontario. When grown in Illinois, winter survival was 100% in southern and central Illinois, but dropped to 86% in Northern Illinois (Heaton et al, 2008), suggesting that winter conditions in Ontario are more challenging than those experienced in Illinois.

Differences in first year winter survival rates within the *M. x giganteus* species group were more evident under certain environments within Ontario. In Leamington, winter survival was nearly 100% for all *M. x giganteus* entries (Table 4.3), and thus no significant differences in winter survival were observed between the Illinois and non Illinois *M. x giganteus* entries. In Elora, variability in winter survival among *M. x giganteus* entries was evident. Of the entries present, the "Illinois" entry had moderate winter hardiness; it was significantly lower than the entry 10 which had nearly full survival of 97%, and was not significantly different than entries eight and nine at 62% and 64% survival respectively, but was significantly greater than entry 11 at 27%. Winter survival rates of all *M. x giganteus* entries dropped greatly in Kemptville. Entry 10 was significantly higher than the Illinois entry which was not significantly different from the remaining *M. x giganteus* entries which all failed to survive the first winter in Kemptville.

Heaton et al (2008) observed that the Illinois entry persisted under conditions which they expected to be colder than those predicted by Clifton-Brown and

Lewandowski (2000) for adequate survival of *M. x giganteus* entries from Europe, leading them to suggest that there was greater cold tolerance for the Illinois entry. This was disconcerting towards the introduction of entries from the potentially highly productive and sterile *M. x giganteus* species in Ontario, as genetic diversity within common accessions of this species groups in Europe is often reported as being low (Greef et al, 1997; Hodkinson et al, 2002a) which suggested improvements for winter hardiness beyond those observed in the Illinois entry may be difficult, and winter conditions within Ontario were expected to be harsher than those experienced in northern Illinois where the Illinois entry began to suffer stand establishment issues due to winterkill. While the background of the entries used in this trial is not known, these results suggest that variability is present either through natural *M. x giganteus* or can be created through breeding.

These results demonstrate that even within the selection of entries used, entries with equal or greater winter hardiness than the *M. x giganteus* entry “Illinois” exist, and suggests that the potential exists to adapt *M. x giganteus* entries to regions with winter severity greater than that experienced in northern Illinois, including parts of Ontario.

While this work is important to confirm the adaptive potential of *M. x giganteus* entries for certain environments in Ontario, it is difficult to relate the significance of these findings to existing reports of winter survival of non-Illinois *M. x giganteus* entries, given the differences between test locations and lack of reference entries between sites. Clifton-Brown and Lewandowski (2000) identified LT50s of hardened rhizomes as a potential predictor of *M. x giganteus* winter survival when they were compared with minimum soil temperatures across five test sites in Europe as conducted by Clifton-Brown et al (2001a). However, no soil temperatures were recorded during the year of establishment to draw any relative connection.

Table 4.7 Pairwise mean differences in winter survival between *M. x giganteus* entries 9, 8, 10 and 11 relative to "Illinois" clone following the year of establishment at three locations in Ontario.

	Entry	Estimate	Std. Error	DF	Pr > t
Leamington					
Illinois vs. Nagara	(12 vs. 10)	0.3	10.43	133	0.9781
Illinois vs. M1 Select	(12 vs. 11)	- 0.1	11.29	133	0.9926
Illinois vs. Genotype 8	(12 vs. 8)	- 1.0	14.68	133	0.9481
Illinois vs. Genotype 9	(12 vs. 9)	- 1.2	15.83	133	0.9383
Elora					
Illinois vs. Nagara	(12 vs. 10)	- 26.8	10.41	133	0.0110
Illinois vs. M1 Select	(12 vs. 11)	43.0	11.30	133	0.0002
Illinois vs. Genotype 8	(12 vs. 8)	8.6	14.68	133	0.5569
Illinois vs. Genotype 9	(12 vs. 9)	5.9	15.81	133	0.7087
Kemptville					
Illinois vs. Nagara	(12 vs. 10)	- 37.3	10.74	133	0.0007
Illinois vs. M1 Select	(12 vs. 11)	2.9	11.27	133	0.7994
Illinois vs. Genotype 8	(12 vs. 8)	3.1	16.03	133	0.8469
Illinois vs. Genotype 9	(12 vs. 9)	2.6	15.82	133	0.8703

4.5 Correlations of morphological traits with winter survival

Culm height appeared to vary with location with mean culm lengths decreasing from Leamington to Kemptville, likely reflecting differences in growing seasons. Variability was observed within locations as well, with differences between entries. Variability in plant height has been well established for *Miscanthus* plants in native regions (Stewart et al, 2009; Yan et al, *In Press*) as well as in biomass related research (Clifton-Brown et al, 2001a; Clifton-Brown and Lewandowski, 2002; Jezowski, 2008; Chen and Renvoize, 2005). Given the variability, relationships between culm length and winter survival varied depending on location as well. At the Leamington location where winter survival was very high for most entries, correlations between culm length and winter survival were only significant ($p<0.0001$) when the lower surviving *M. sinensis* entries (15-26) were considered (Figure 4.2; *M. sinensis* specific correlation not shown). This was interesting as the range of heights where the linear relationship was significant for *M. sinensis* entries corresponded with the same range of heights for the other species groups where no response in winter survival was observed due to very high survival. At Elora and Kemptville, culm length in the year of establishment was observed to be positively correlated to winter survival (Figure 4.2) with linear and linear-plateau relationships respectively. In Elora, the plateau portion of the curve appeared consistent with fertile hybrid and *M. x giganteus* type entries which where often characterized by greater heights and winter survival, while the sloping portion primarily consisted of the range of *M. sinensis* entries with some lower surviving fertile hybrids as well. Unlike

Leamington, winter survival response appeared consistent to culm height across all three species groups.

Direct associations between plant height and winter survival appear limited in the literature, although comparisons in above ground and below ground development have been noted, which may have implications for winter hardiness. During the year of establishment, *M. x giganteus* below ground biomass at the end of the harvest season appears to be strongly correlated to aerial dry matter (Christian et al., 2009), while Christian and Haase (2001) cited Jodl et al (1996) who demonstrated that rhizome mass development mirrored mass development of above-ground dry matter over two growing seasons.

Similar to culm height, mean basal circumferences appeared to generally decrease from Leamington to Kemptville, while variability within locations was evident by the ranges in circumferences across the various entries. Relationships with winter survival did not appear consistent across the locations (Figure 4.3). At the Leamington location winter survival appeared to improve with increasing basal circumference up until approximately 59 cm, above which point winter survival reached a plateau just below 100%. The linear portion was a weak relationship (R^2=0.41) with apparent wide ranges in winter survival for given basal circumferences, and consisted of primarily shorter and poorer surviving *M. sinensis* entries.

At the Elora location, basal circumference generally increased with winter survival. While a significant (P>0.0005) linear relationship was fit, the relationship was weak (R^2=0.27) as a broad range of winter survival values were often evident for any given circumference. Entries with high survival often had larger basal circumferences, but the larger basal circumferences did not appear to guarantee good winter survival, as observed for entries 11, 12 and five. The surviving *M. sinensis* entries (15, 21, 22 and 24) tended to have basal circumferences lower than those observed for the other species groups, and appeared most supportive of a positive linear relationship.

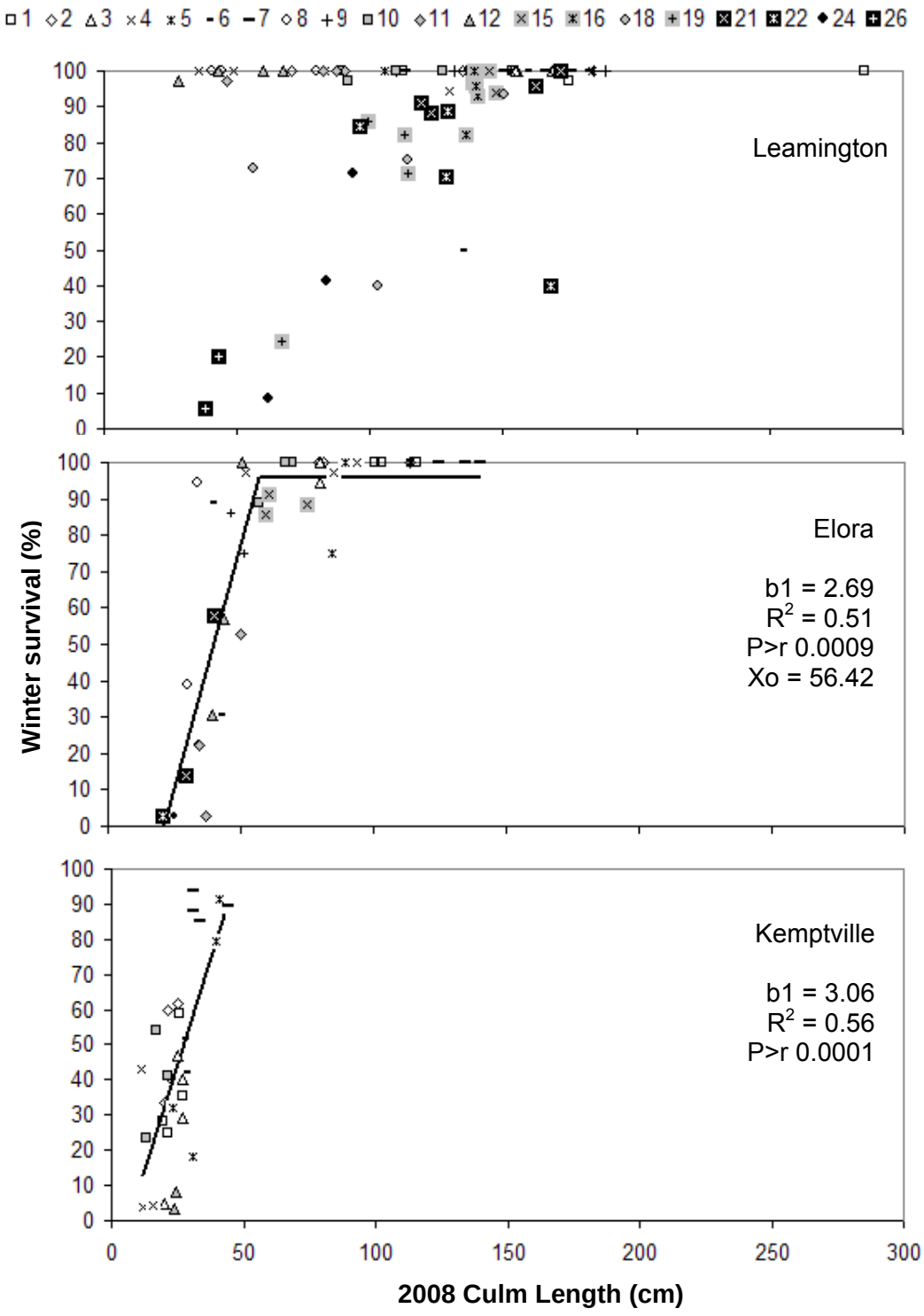

Figure 4.3 Plot means of winter survival of 20 *Miscanthus* entries following year of establishment correlated to plot means of fall 2008 culm lengths at three locations in Ontario, where entries are designated by the symbols in the legend above, models fit by the black solid lines, and where b1, R^2, and Xo represent slope and coefficient of determination of the sloping line and the junction point of the sloping and non-sloping lines respectively. (data presented in appendices 4.2 - 4.4)

At the Kemptville location no relationship was observed, as large relatively equivalent ranges in winter survival were observed across all basal circumferences. Previous studies have observed that more cold tolerant *Miscanthus* entries tend to display a tighter 'tussock' growth habit (Clifton-Brown and Lewandowski, 2000), which would not support the results displayed here. Studies with other species have drawn connections between reduced internode lengths and winter survival. Wood and Cohen (1983) observed greater winter hardiness in perennial ryegrass with reduced sub-crown internode length which they proposed to be associated with greater crown protection, and Frankow-Lindberg (1999) observed increased winter tolerance of white clover with reduced internode lengths of stolons, suggesting it promoted accumulation of non structural carbohydrates. Basal circumferences as measured during the year of establishment in this study would likely have been primarily reflecting the number of emerged culms. Variation for shoot densities and number of tillers (Clifton-Brown et al, 2001a; Jezowski, 2008) has been observed in other studies. It is possible that the number of emerged culms could have overridden any influences from rhizome morphology, especially considering the limited opportunity for development during the first 4 months of growth. As a result, it is possible that this measurement could be more reflective of plant vigour or biomass accumulation.

Relationships with other morphological measurements were limited. No relationship was observed with culm diameter. Some limited relationships were observed with second year culm height and basal circumference, but may have been remnants of first year measurements. Compressed circumference did demonstrate a degree of positive relationship for the fertile hybrids at Elora and Kemptville where measurements in the lower range were associated with much poorer winter survival (not shown). Given the thin culms which were consistent across all fertile hybrids, this likely reflected fewer second year culms per plant for plots with poor winter survival during the year of establishment. It is difficult to interpret these second year measurements in terms of winter survival potential as they are not "predictive" of winter survival, and variability in the different measures that is evident may simply be a product of differences in susceptibility to winter stresses which may influence plant vigour during the second growing season.

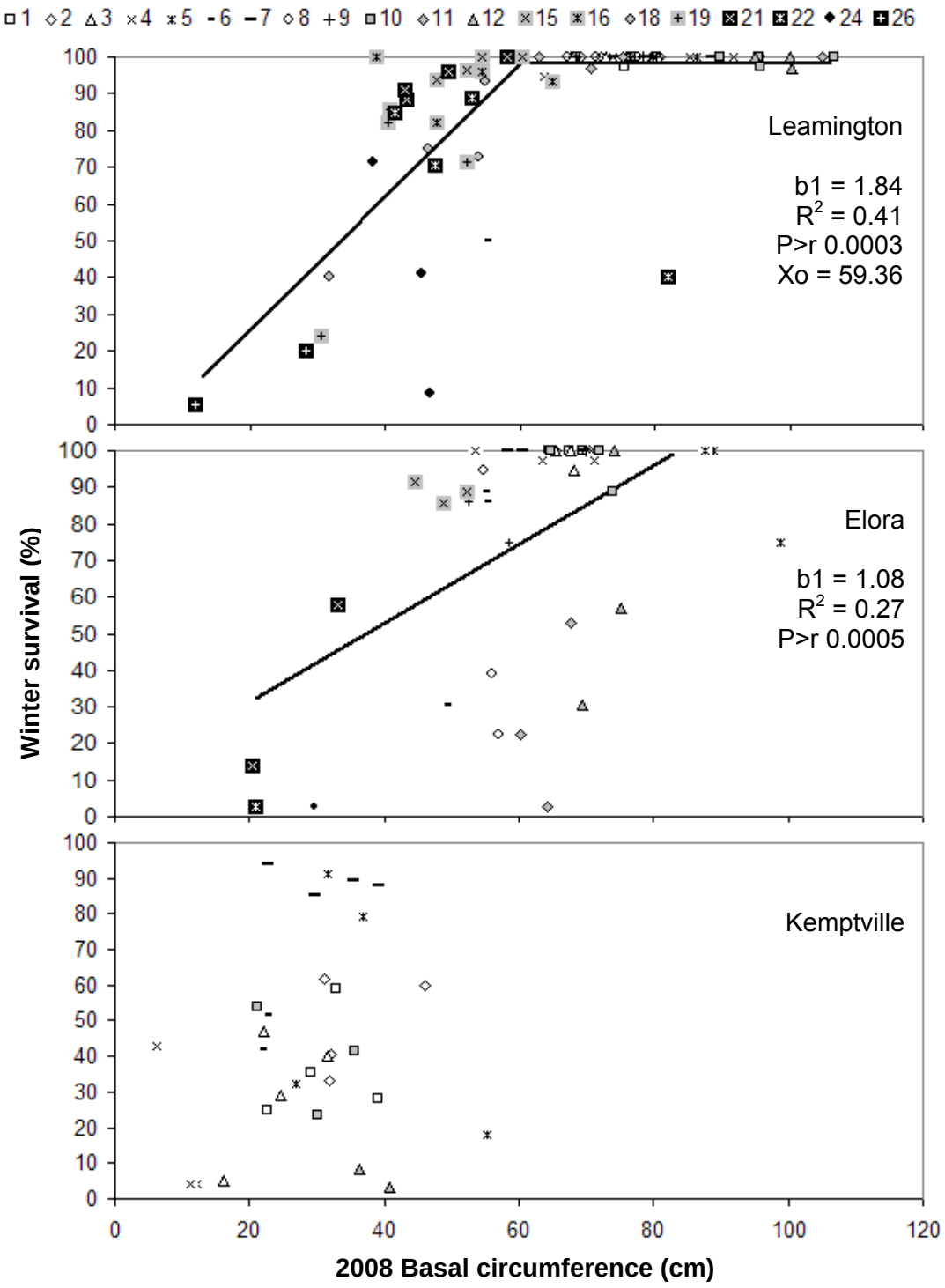

Figure 4.4 Plot means of winter survival of 20 *Miscanthus* entries following year of establishment correlated to plot means of fall 2008 basal circumferences at three locations in Ontario, where entries are designated by the symbols in the legend above, models fit are designated by the black solid lines, and where b1, R^2, and Xo represent slope and coefficient of determination of the sloping line and the junction point of the sloping and non-sloping lines respectively. (data presented in appendices 4.5 - 4.7)

Overall, the positive relationship between morphological measurement size and winter survival is in agreement with existing literature recommendations which promote the use of early planting and other agronomic practices that maximize plant size during the year of establishment to facilitate winter survival (Jorgensen and Schwarz, 2000; Atkinson et al, 2009; Christian and Haase, 2001; Lewandowski et al, 2000). This also supports Atienza et al (2003) who identified enhanced vigour and growth during the establishment year as a breeding objective towards improving winter survival through the premise of enhanced reserve accumulation and subsequent plant vigour.

In spite of the associations made between plant size and winter hardiness, no measure of above or below ground yields was made during the year of establishment to verify the relationship between these morphological features and rhizome development. However, the identification of positive associations between plant size and winter survival in this study demonstrates the importance of plant development during the year of establishment under Ontario conditions.

4.6 Correlations of developmental traits with winter survival

Flowering, senescence and late season culm emergence were not measured until the year after establishment and were used to characterize the relative maturity and dormancy across the selection of *Miscanthus* entries in this study.

Flowering during the second year of establishment was variable in terms of both the occurrence and timing of flowering across all entries. Flower timing was similar for all species groups in Leamington, with the earliest entries commencing in the third week of August. No clear relationship with winter survival was evident, however, given the very high winter survival.

The lack of relationship between flowering and winter survival was most evident in Elora where survival was variable. Mean flowering date was earliest for the fertile hybrids, occurring during the last week of August. Entries with very good winter survival ranged from fertile hybrids which demonstrated early full flowering to the *M. x giganteus* type entry 10 which had high survival but never flowered in any of the first three growing seasons. The surviving *M. sinensis* entries in Elora tended to flower later than the fertile hybrids, with a few plots with very low survival demonstrating very late flowering relative to fertile hybrids or higher surviving *M. sinensis* (not shown).

In Kemptville, flowering was only observed for the fertile hybrids. The majority of average flowering dates occurred during the third week of August where there was wide variation in winter survival for given flowering dates. Survival appeared to decrease with dates for flowering during subsequent weeks. Given that measurements were made the year following establishment, it is difficult to ascertain whether these late flowering dates were associated with, or influenced by potential winter stresses given the poorer winter survival at this site.

While a degree of flowering was observed in the year of establishment, no records were made at any sites. Based on some high surviving specific entries in Elora which did not flower during the second or third growing seasons, and presumably not during the year of establishment, flowering does not appear to be critical for winter survival across these entries within the environment. The Kemptville data support this through the observation that good winter survival is not necessarily associated with early flowering. This was evident by the observation of the non-flowering *M. x giganteus* entry 10 which had winter similar to or greater than a number of flowering fertile hybrid entries.

The late flowering of *M. sinensis* is in contrast with experiences in Europe where it has been observed to flower early, while the lack of flowering for *M. x giganteus* at Elora and Kemptville was in line with observations where it flowered the latest if at all before killing frosts (Clifton-Brown and Lewandowski, 2002; Jorgensen, 1997). Early flowering entries in previous studies were generally associated with higher winter survival in northern Europe while entries which failed to flower prior to frosts, such as *M. x giganteus*, failed to overwinter in more northerly locations (Clifton-Brown et al, 2001a).

No clear relationship was observed between winter survival and percent flowering culms at either Elora or Kemptville (not shown). No measurements were recorded in Leamington. The fertile hybrids and *M. x giganteus* types appeared to have very uniform culm development where the initial culm “flush” developed in unison and accounted for the majority of total culms by the end of the season. Given this, these entry ratings generally reflected flowering observations with either a very low or very high proportion of flowering culms. In Elora, the *M. sinensis* entries had greater variability as culms within single plants ranged from various levels of vegetative development to full inflorescence emergence by the end of the growing season. While not measured, it is possible this could reflect a longer period of active culm emergence throughout the growing season. Associations between the variability of culm maturity

within *M. sinensis* plants and the poorer winter survival of this species could be a topic for future investigation.

No relationship between winter survival and fall leaf senescence during the second year was observed at Leamington or Elora. In Elora, some fertile hybrids appeared to demonstrate advanced senescence relative to other entries, but entries such as *M. x giganteus* type 10 displayed very little leaf senescence while demonstrating good winter survival, suggesting senescence is not critical for winter survival for these entries under these environments. No senescence measurements were made in Kemptville during the second year of growth.

The relationship between maturity and winter survival often appears to be based on the role plant maturity is expected to have towards ensuring adequate rhizome reserve replenishment. If rhizome replenishment is strongly associated with winter survival, the results from this study do not support the association between winter survival and reproductive maturity timing, suggesting panicle emergence may not be a good indicator of sufficient minimum reserve accumulation in these environments.

Research from established *M. x giganteus* stands in Europe has demonstrated that rhizome dry matter and nutrient levels reach seasonal minimums in the May (Christian and Haase, 2001) to July (Beale and Long, 1997) timeframe, after which levels begin to recover due to accumulation of dry matter and nutrients. While no flowering dates were discussed, these periods are at least a month or more prior to flowering for even the earliest flowering entries used in Ontario, suggesting rhizome accumulation may be well under way by the time flowering does occur.

This would also support the lack of apparent requirement for senescence. The lack of relationship with winter survival demonstrated here may suggest that any remobilization at time of senescence is inconsequential for winter survival in these environments for these entries. This was supported by Beale and Long (1997) who observed decreases in above ground and increases in below ground nutrient concentrations starting mid to late summer in the UK, with the greatest nutrient concentration decreases occurring in green tissues.

Asynchronous remobilization and maturity has been observed in another perennial rhizomatous grass *Phragmites australis* where seasonal rhizome dry matter minimums and subsequent initiation of rhizome translocation occurs a few months prior to panicle emergence (Karunaratne et al, 2004). It does not appear known whether timing of reproductive maturity influences the timing or rate of rhizome replenishment, that is

whether the earlier flowering entries such as the fertile hybrids would reach dry matter minimums sooner than the non-flowering *M. x giganteus* types. If flower timing and rhizome dry matter minimums are connected, it may be possible that adequate reserve accumulation for winter survival in these environments had already occurred prior to inflorescence emergence. These results are promising as they suggest that selecting for winter survival does not require selecting for flowering genotypes. Non-flowering genotypes may utilize a longer growing season which may deliver greater yield potential versus earlier flowering types which have been suggested to yield less (Clifton-Brown et al, 2001a).

Late season culm emergence was observed in a number of entries as the presence of young, green culms less than 15 cm in height following harvest. Although actual emergence dates of late culm flushes were not observed, their presence was expected to reduce winter survival as they suggested a lack of dormancy and use of plant reserves which may come at the expense of winter hardiness. Despite expectations, no strong correlation between late season culm emergence and winter survival in the following year of establishment was observed (data not shown).

Day of 50 cm regrowth in the spring was recorded only at the Leamington and Elora locations. At the Leamington location, where winter survival was high, there was no relationship between winter survival and regrowth (Figure 4.4) which occurred as early as the 140th day of the year. At the Elora location the relationship between winter survival and regrowth demonstrated a plateau-linear relationship (Figure 4.4). *Miscanthus* plots that achieved regrowth prior to the 161st day of the year had nearly full winter survival, while those which achieved regrowth later began to demonstrate a negative relationship. Based on this observation, it is expected that these regrowth dates would also be fairly representative of relative emergence dates, as plant growth rates did not appear to vary greatly across the entries used in this trial.

Evidence of the positive relationship between winter survival and early regrowth is in contrast to the work of Farrell et al (2006) on *M. sinensis* hybrids, *M. x giganteus* and *M. sacchariflorus* where basal growth temperatures were not well correlated with winter survival. Similar results however have been observed in perennial ryegrass where Hulke et al (2008) observed that varieties with greater cold tolerance had greater growth and upright stature in the spring.

The correlation observed between 50 cm regrowth date and winter survival may be usefuel to the development of high yielding, cold tolerant *Miscanthus* entries. Early

emergence has the potential to extend the growing season of *Miscanthus*, and would be expected to aid biomass accumulation. More research is required to test the relationship over the non-responsive portion of the curve; testing of these entries under harsher winter conditions would be needed to confirm if the negative correlation holds for these entries as well.

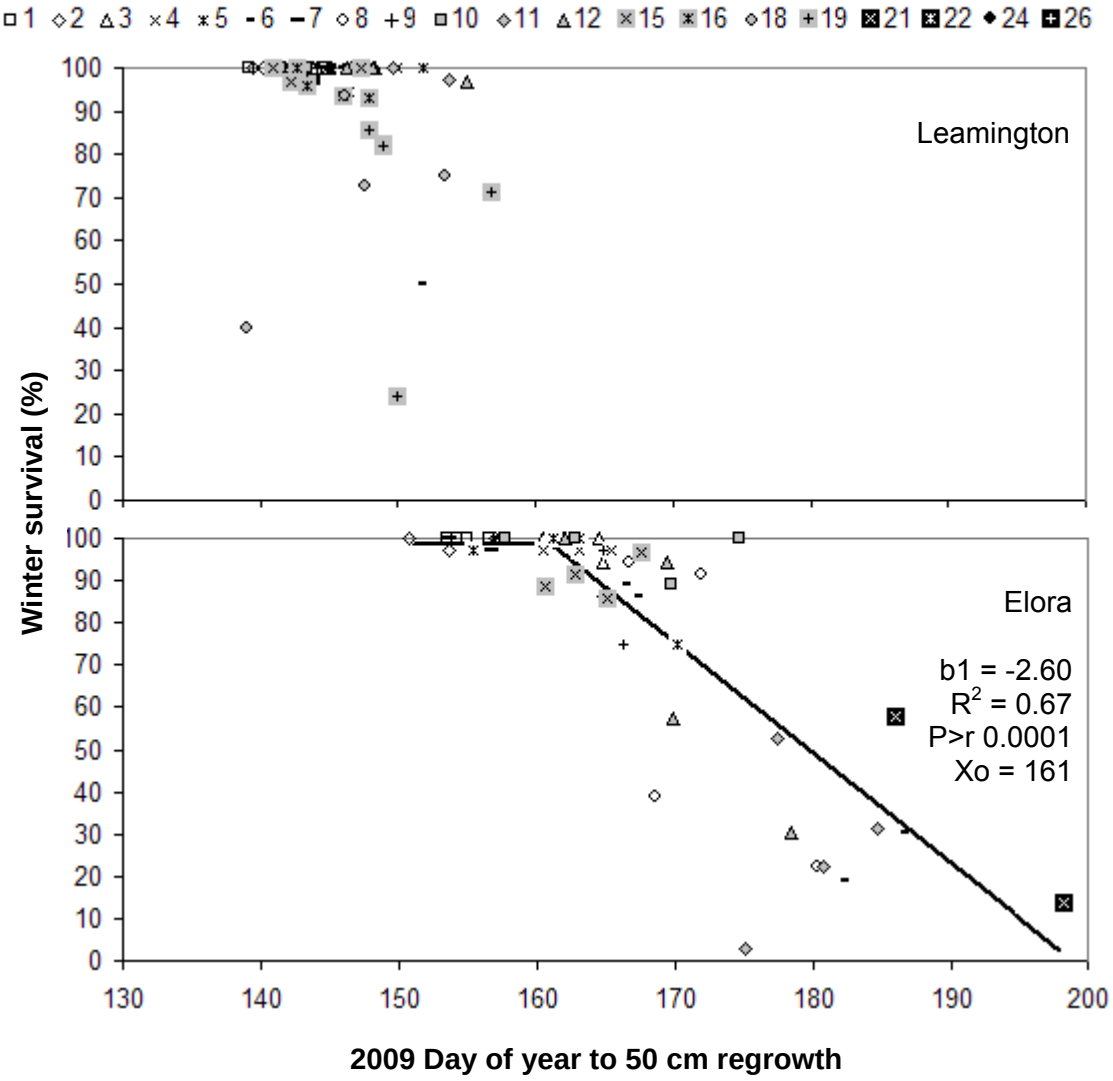

Figure 4.5 Plot means of winter survival of 20 *Miscanthus* entries following year of establishment correlated to mean day of year to 50 cm regrowth at two locations in Ontario, where entries are designated by the symbols in the legend above, models fit are designated by the black solid lines, and where b1, R^2, and Xo represent slope and coefficient of determination of the sloping line and the junction point of the sloping and non-sloping lines respectively. (data presented in appendices 4.8 - 4.10)

Despite the advantages for biomass accumulation, early regrowth of *Miscanthus* could also lead to greater risk of frost injury. Average date (1976-2005) of last frost (0°C) at the Elora and Leamington locations are May 11th-17th and April 25th, respectively (OMAFRA, 2011), while a 10% frost risk is still apparent fourteen days after. At the Elora location, 50 cm regrowth was achieved as early as day 150 (May 30th) for entry 2, with a degree of emergence having already taken place for most entry 2 plants within Elora by the first winter survival check on May 6th, making this entry susceptible to frosts during the early regrowth period. The ability of these early emerging entries to survive late season or repeated frosts following emergence needs to be assessed.

No clear relationship between winter survival and lodging was observed. Based on the suggestions that full plant maturity may help ensure adequate remobilization of nutrients for replenishment of rhizomes, it was hypothesized that in-season lodging may hamper these processes and compromise winter survival. It appeared that most lodging occurred during the growing season when plants became heavy with top-growth, and when assessed at harvest time, plants which were still lodged did not appear to suffer constrictive stem damage. In 2010, plots with a high incidence of lodging often had portions of plants where culms had lodged while the remainder stayed erect. The non-lodged culms appeared to continue with flowering and senescence while the lodged culms appeared to remain green and did not flower or senesce. Lodging was not measured during the year of establishment, although given the smaller stature of plants did not appear to be an issue.

Overall, the lack of relationship between maturity and dormancy indicators with winter survival in the year of establishment seems to contradict observations in previous studies. However, it is possible that winter survival in the year of establishment is predominantly influenced by other factors such as the degree of growth and biomass accumulation, as indicated in the previous section and by other studies (Atkinson et al, 2009; Christian and Haase, 2001; Lewandowski et al, 2000).

4.7 *Miscanthus* entry effects on yield

4.7.1 Yields during first full growing season, 2009

Variance analysis of fall 2009 yields demonstrated significant sources of variation from locations, entries, and location*entry interactions (Table 4.8). Yields of entries was displayed as the actual harvested mean yields for each location*entry combination (Table 4.9), with no adjustments made for winterkill.

Table 4.8 Variance analysis of *Miscanthus* yields for 20 entries across three locations during autumn 2009.

Fixed Effects	**Num *df***	**Den *df***	**F Value**	**Pr > F**
Location	2	9	76.20	<0.001
Entry	19	138	23.09	<0.001
Location*Entry	38	138	6.67	<0.001
Random Effects	**Estimate**	**SE**	**Z Value**	**Pr > Z**
Rep(Location)	0.20	0.219	0.90	0.1850
Residual	4.96	0.593	8.35	<0.001

Table 4.9 Mean dry biomass yields for 20 *Miscanthus* entries across three locations in Ontario during the autumn of 2009, the second year of establishment.

	LS Means					
	Leamington		**Elora**		**Kemptville**	
Entry	**Yield (t ha^{-1})**	**SE**	**Yield (t ha^{-1})**	**SE**	**Yield (t ha^{-1})**	**SE**
1	7.60	1.135	13.96	1.135	0.82	1.135
2	8.22	1.135	15.58	1.135	1.89	1.135
3	9.40	1.135	10.00	1.135	0.55	1.135
4	9.98	1.135	12.88	1.135	0.06	1.309
5	9.08	1.135	11.95	1.135	2.59	1.309
6	7.36	1.135	5.28	1.135	1.31	1.135
7	6.58	1.135	16.09	1.135	5.36	1.135
8	9.52	1.135	6.46	1.135	0.01	1.309
9	12.78	1.135	7.49	1.135	0.00	1.135
10	15.03	1.135	18.27	1.135	1.96	1.309
11	8.81	1.135	1.84	1.135	0.00	1.135
12	8.30	1.135	7.66	1.135	0.02	1.135
15	4.27	1.135	4.64	1.135	0.00	2.260
16	2.01	1.308	0.00	1.308	0.00	1.309
18	2.38	1.135	0.00	1.599	0.00	2.258
19	2.29	1.135	0.00	1.308	0.00	2.258
21	2.98	1.135	0.29	1.599	0.00	2.260
22	1.44	1.135	0.00	1.308	0.00	1.600
24	1.41	1.135	0.00	1.135	0.00	1.600
26	0.06	1.599	0.00	1.135	0.00	1.600

When comparing species group yields across the three locations, there were some inconsistencies in terms of where maximum yields were recorded. Numerically, yields of the *M. sacchariflorus* x *M. sinensis* hybrids were generally highest in Elora, where the highest yields across all locations were recorded for six of the seven entries. Yields of *M. x giganteus* and *M. sinensis* tended to be highest in Leamington, where maximum yields were identified for four of the five *M. x giganteus* entries and seven of the eight *M. sinensis* entries.

Yield potential of species groups was compared by comparing the top two yielding entries across each species group within each location. In Leamington, entry 10 which was the highest yielding entry across the whole site had a yield that was significantly higher than the highest yielding entries in both the *M. sacchariflorus* x *M. sinensis* hybrids and the *M. sinensis* species groups (Table 4.10). The next highest yielding entry in the *M. x giganteus* group did not have significantly higher yields than the highest in the *M. sacchariflorus* x *M. sinensis* hybrid group, but did relative to the *M. sinensis* group.

Table 4.10 Pairwise mean contrasts between the two top-yielding entries from each species group within the Leamington location for second-year stand yields in autumn 2009. NS is Non-Significant at a probability of 0.05.

		Leamington					
Group		*M.sa x M.si*		*M. x gig*		*M. sinensis*	
	Entry	4	3	10	9	15	21
M.sa x M.si	4		NS	0.0017	NS	0.0004	<0.0001
	3			0.0005	0.0337	0.0014	<0.0001
M. x gig	10				NS	<0.0001	<0.0001
	9					<0.0001	<0.0001
M. sinensis	15						NS
	21						

In Elora, entry 10 was also the highest yielding of all entries during 2009, although it was not significantly different than the highest yielding *M. sacchariflorus* x *M. sinensis* hybrids seven and two (Table 4.11). Other than the second-highest yielding *M. x giganteus* entry 12, all the top yielding entries in each group were significantly higher yielding than the top yielding entry in the *M. sinensis* group, which was the only entry to experience any appreciable yield accumulation.

Table 4.11 Pairwise mean contrasts between the two top-yielding entries from each species group within the Elora location for second-year stand yields in autumn 2009. NS is Non-Significant at a probability of 0.05.

Group	Entry	Elora					
		M.sa x M.si		*M. x gig*		*M. sinensis*	
		7	2	10	12	15	21
M.sa x M.si	7		NS	NS	<0.0001	<0.0001	<0.0001
	2			NS	<0.0001	<0.0001	<0.0001
M. x gig	10				<0.0001	<0.0001	<0.0001
	12					NS	0.0002
M. sinensis	15						0.0260
	21						

In Kemptville, entry seven of the *M. sacchariflorus* x *M. sinensis* hybrid species group was the highest yielding across all entries. While it was not significantly different from the next highest yielding hybrid entry five, it was significantly higher yielding than the top *M. x giganteus* entry, entry 10, which was the only *M. x giganteus* entry to accumulate any appreciable yield (Table 4.12). As a result of no winter survival, no yield accumulation occurred for any of the *M. sinensis* entries.

Table 4.12 Pairwise mean contrasts between the two top-yielding entries from each species group within the Kemptville location for second-year stand yields in autumn 2009. NS is Non-Significant at a probability of 0.05.

Group	Entry	Kemptville					
		M.sa x M.si		*M. x gig*		*M. sinensis*	
		7	5	10	12	15	16
M.sa x M.si	7		NS	0.0478	0.0009	0.0333	0.0019
	5			NS	NS	NS	NS
M. x gig	10				NS	NS	NS
	12					NS	NS
M. sinensis	15						NS
	16						

4.7.2 Yields during second full growing season, 2010

Similar to 2009, variance analysis of fall 2010 yields demonstrated significant sources of variations from locations, entries and entry*location interactions (Table 4.13), with yield by entries within locations displayed in Table 4.14.

Table 4.13 Variance analysis of *Miscanthus* yields for 20 entries across three locations during autumn 2010.

Fixed Effects	Num *df*	Den *df*	F Value	Pr > F
Location	2	9	86.98	<0.001
Entry	19	142	27.72	<0.001
Location*Entry	38	142	6.00	<0.001
Random Effects	**Estimate**	**SE**	**Z Value**	**Pr > Z**
Rep(Location)	0	.	.	.
Residual	10.80	1.243	8.69	<0.001

Table 4.14 Mean dry biomass yields for 20 *Miscanthus* entries across three locations in Ontario during the autumn of 2010, the second year of establishment.

	LS Means					
	Leamington		Elora		Kemptville	
Entry	Yield (t ha^{-1})	SE	Yield (t ha^{-1})	SE	Yield (t ha^{-1})	SE
1	12.66	1.643	18.86	1.643	5.16	1.643
2	12.64	1.643	22.69	1.643	11.05	1.643
3	12.45	1.643	15.99	1.643	5.01	1.643
4	15.58	1.643	16.37	1.643	0.79	1.643
5	11.23	1.643	16.45	1.643	11.32	1.897
6	13.09	1.643	14.90	1.643	7.91	1.643
7	11.11	1.643	21.84	1.643	15.28	1.643
8	15.47	1.643	18.51	1.643	0.37	1.643
9	19.74	1.643	15.24	1.643	0.54	1.643
10	18.38	1.643	24.43	1.643	8.95	1.643
11	17.41	1.643	6.52	1.643	0.06	1.643
12	14.66	1.643	16.06	1.643	0.11	1.643
15	7.85	1.643	11.04	1.643	0.00	3.286
16	6.14	1.643	0.00	1.897	0.00	1.897
18	5.46	1.643	0.00	2.323	0.00	3.286
19	4.11	1.643	0.00	1.897	0.00	3.286
21	5.25	1.643	1.59	2.323	0.00	3.286
22	3.23	1.643	0.00	1.897	0.00	2.323
24	1.02	1.643	0.00	1.643	0.00	2.323
26	0.56	2.323	0.00	1.643	0.00	2.323

The highest recorded yields occurred in Elora where the top three yielding entries within a location were observed for *M. x giganteus* entry 10 at 24.4t/ha, and *M. sacchariflorus* x *M. sinensis* hybrid entries two and seven at 22.7 and 21.8t/ha respectively. For the hybrid entries, yields were consistently numerically highest in Elora for all entries, while Leamington yields were numerically higher than those in Kemptville for all but two entries. For *M. x giganteus* entries, highest yields occurred in Elora entries eight, ten and 16, while the remaining entries nine and 11 were highest in Leamington. *M. x giganteus* yields in Kemptville were much lower than the other sites. Leamington had the greatest *M. sinensis* yields across all sites for all entries except for entry 15 which yielded the highest in Elora.

Yield potential of species within locations was assessed by comparing the top two surviving entries as described for the 2009 yield data. In Leamington, no significant difference in yields were observed between the top yielding hybrid entry four and the top two yielding *M. x giganteus* entries nine and 10 (Table 4.15), while the second highest yielding hybrid entry six was significantly lower than the *M. x giganteus*. All were significantly higher than the two top yielding *M. sinensis* genotypes 15 and 16.

Table 4.15 Pairwise mean contrasts between the two top-yielding entries from each species group within the Leamington location for second-year stand yields in autumn 2010. NS is Non-Significant at a probability of 0.05.

		Leamington					
Group		*M.sa x M.si*		*M. x gig*		*M. sinensis*	
	Entry	4	6	9	10	15	16
M.sa x M.si	4		NS	NS	NS	0.0011	<0.0001
	6			0.0048	0.0242	0.0257	0.0033
M. x gig	9				NS	<0.0001	<0.0001
	10					<0.0001	<0.0001
M. sinensis	15						NS
	16						

In Elora, no significant differences in yield were observed between the top two yielding hybrid entries two and seven and the top two yielding *M. x giganteus* entries 10, while all of the above were significantly higher yielding than the top yielding *M. sinensis* entries 15 and 21, of which entry 15, similar to 2009, was the only *M. sinensis* entry in Elora to accumulate any appreciable yield (Table 4.16).

Table 4.16 Pairwise mean contrasts between the two top-yielding entries from each species group within the Elora location for second-year stand yields in autumn 2010. NS is Non-Significant at a probability of 0.05.

Group		Elora					
		M.sa x M.si		*M. x gig*		*M. sinensis*	
	Entry	2	7	10	8	15	21
M.sa x M.si	2		NS	NS	NS	<0.0001	<0.0001
	7			NS	NS	<0.0001	<0.0001
M. x gig	10				0.0120	<0.0001	<0.0001
	8					0.0016	<0.0001
M. sinensis	15						0.0011
	21						

In Kemptville, the highest yielding hybrid entry seven was significantly higher yielding than all other entries within that location (Table 4.17). The highest yielding *M. x giganteus* entry 10 was again the only entry within that species to accumulate any appreciable yield in 2010. While significantly lower yielding than top hybrid entry seven, there was no significant difference in yield between the second highest yielding hybrid entry five, while it was significantly higher yielding than all other *M. x giganteus* and *M. sinensis* entries.

Table 4.17 Pairwise mean contrasts between the two top-yielding entries from each species group within the Kemptville location for second-year stand yields in autumn 2010. NS is Non-Significant at a probability of 0.05.

Group		Kemptville					
		M.sa x M.si		*M. x gig*		*M. sinensis*	
	Entry	7	5	10	9	26	19
M.sa x M.si	7		NS	0.0072	<0.0001	<0.0001	<0.0001
	5			NS	<0.0001	0.0002	0.0034
M. x gig	10				0.0004	0.0020	0.0161
	9					NS	NS
M. sinensis	26						NS
	19						

There appeared to be a strong positive association between first year and second year yields during the first two full years of establishment as evident by the correlation coefficient of 0.88 ($P<0.0001$) (Figure 4.5). While yields tended to increase from 2009 to 2010, yield increases appeared relatively proportional to first year yields, suggesting that yield limitations that occurred during the first full year of establishment persist to the second full year of establishment. Given the relationship between yield and winter survival, winter survival during the year of establishment appears critical to the establishment of a productive, economically viable *Miscanthus* stand.

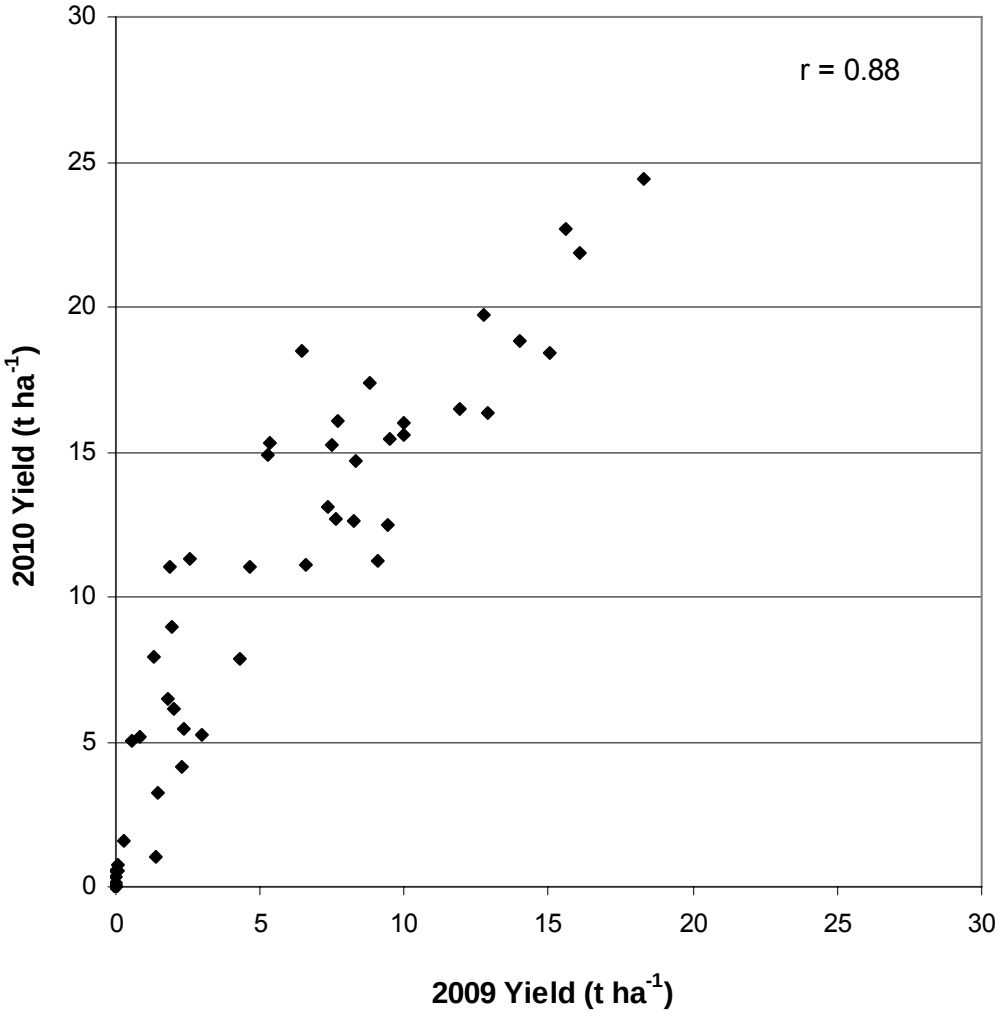

Figure 4.6 Scatter plot of mean location yields for 20 entries across three locations for 2010 harvest relative to 2009 in Ontario.

4.7.3 Importance of winter survival for yield

High levels of winter survival during the establishment year are essential for yield potential in the subsequent year. At all three locations poor winter survival was consistently associated with reduced yields (Figure 4.6). Within those entries that achieved winter survival levels approaching 100%, yield varied considerably. At the Elora and Leamington locations yield varied between 9.4-19.6 and 6.1-18.3 oven-dried t ha^{-1}, respectively, for entries with greater than 95% winter survival. At both the Elora and Leamington locations, the *M. x giganteus* entry 10 tended to have the highest yield. This observation is consistent with previous studies that have demonstrated that *M. x giganteus* has superior productivity than most *M. sinensis* hybrids in that region and considerably higher than natural *M. sinensis* entries which generally had below average location yields for all entries for each genotype (Clifton-Brown, 2001a). It is interesting to note however that in Elora in the second year, the yield potential between top-yielding *M. x giganteus* and top yielding fertile hybrids was non-significant, and numerically similar.

While there appears to be high variability in yield for entries with very high winter survival, likely due to factors beyond winter survival, the relations suggest that winter survival becomes strongly associated with yield at levels just below 100% winter survival at both Leamington and Elora where a significantly positive linear association becomes evident. In Kemptville where winter survival did not surpass 94%, only a significant positive linear correlation was observed.

These results indicate that winter survival is critical to maximize productivity, as rarely did plots having less than 90% winter survival have high yields. This negative correlation would be expected given the stand loss that would occur, and suggests that *Miscanthus* is not able to compensate for the loss during the establishment years under the practices used in this study. Replanting would be required to overcome reductions in productivity as a result of stand loss, which would result in additional costs

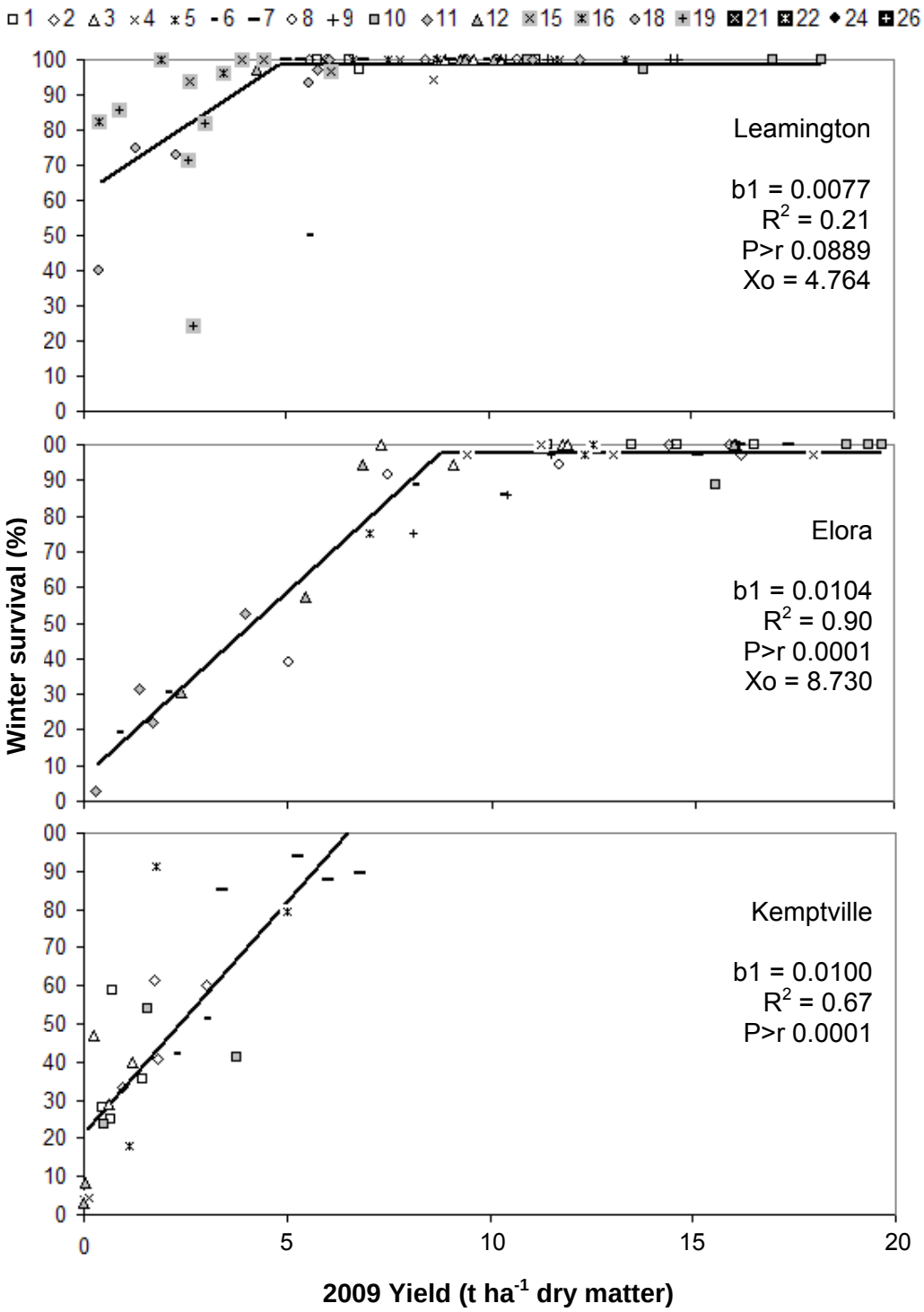

Figure 4.7 Plot means of winter survival of 20 *Miscanthus* entries following year of establishment correlated to plot means of oven dried biomass yields at three locations in Ontario, where entries are designated by the symbols in the legend above, models fit are designated by the black solid lines, and where b1, R^2, and Xo represent slope and coefficient of determination of the sloping line and the junction point of the sloping and non-sloping lines respectively. (data presented in appendices 4.11 - 4.12)

5. CONCLUSIONS

5.1 Research summary

Research regarding the viability of *Miscanthus* as a biomass crop in Ontario is limited, however research conducted in Europe has identified winter survival during the year of establishment as a key limitation towards the successful stand establishment, and adoption of *Miscanthus* as a biomass crop in northerly regions of Europe.

Miscanthus trials were conducted at Leamington, Elora, and Kemptville Ontario, and were arranged across a gradient of winter severity, and represented differences in soil type and growing conditions. Twenty six *Miscanthus* entries representing three classification "groups" were evaluated, and included seven fertile *M. sacchariflorus x M. sinensis* hybrids, five sterile *M. x giganteus* types and 14 full *M. sinensis* genotypes. Trials were arranged in a randomized complete block design, and were established by plug form in June and July of 2008. Six *M. sinensis* entries were dropped from the experiment as a result of failing to establish at all locations during the year of establishment.

5.2 Variability in winter survival

In efforts to identify the importance of species selection, and winter survival potential of *Miscanthus* under Ontario conditions, winter survival during the year of establishment was recorded. Based on European research, it was hypothesized that the poorest and greatest winter hardiness would be demonstrated by the *M. x giganteus* and *M. sinensis* groups, respectively. The winter during the year of establishment experienced colder than average temperatures during several winter months at all sites, suggesting conditions could have been harsher than what would be experienced for a thirty year average.

For the *Miscanthus* entries and locations in this study, winter survival was dependent on location, with most entries having the greatest survival at Leamington, of which many were 90% or greater, and all having the least survival at Kemptville, where most were less than 50%.

In general, the greatest and most consistent winter hardiness was within the fertile hybrid group, while the least was within the *M. sinensis* group. Despite this, no significant differences in winter survival potential of the best surviving entry in each species was evident except for in Kemptville where entry seven of the fertile hybrids had

significantly greater winter hardiness than the most winter hardy *M. x giganteus* type. No *M. sinensis* entries survived the first winter in Kemptville. Plant vigour of entries during establishment appeared to have an impact on winter hardiness, as high mortalities during the initial establishment season appeared to be associated with higher winter mortality.

Variation in winter survival within species was observed, as separation of entries widened with increased winter severity. For the entries tested, within species separation first appeared for *M. sinensis* at the Leamington location, followed by the sterile *M. x giganteus* triploids in Elora, than the fertile diploid hybrids in Kemptville. Thus, relative winter survival was greatest for the fertile hybrids, followed by the sterile triploid *M. x giganteus* types, followed by *M. sinensis*.

In Ontario conditions, winter survival is a concern during the year of establishment. In regards to winter survival of the entries used in this study, species selection did not appear to be a significant concern until the harshest winter conditions at Kemptville, though genotype selection within species was an important consideration, especially as winter severity increases. These results demonstrate the potential for adaptation of *Miscanthus* under Ontario conditions, but reinforce the need for proper genotype screening and selection. Given the differences in characteristics (productivity, plant composition, sterility) between species groups, flexibility in species selection does not appear to be a limiting concern for these entries until winter severity conditions greater than those experienced in Elora.

5.3 Winter hardiness potential of the "Illinois" *M. x giganteus*

Significant variation within the *M. x giganteus* species group was observed when winter pressure was greater than that at Leamington, where survival was over 98% for all entries, and no significant differences were observed. In Elora, the "Illinois" clone (entry 12) displayed winter survival significantly less than entry 10, no significant difference between entries eight and nine, and survival significantly greater than entry 12. In Kemptville, winter survival was 0% for all *M. x giganteus* entries except for entry 10 which was significantly highest at 40%. These results indicate that the "Illinois" clone does not have superior winter survival, and that greater winter survival potential exists within the *M. x giganteus* group. These results are promising for the adaptation of *M. x giganteus* in regions which experience winter severity greater than that of Illinois.

5.4 Relation between winter survival and morphological measurements

Timely planting and vigorous growth is often noted as a requirement for successful stand establishment and winter survival for *Miscanthus*. One objective of this research was to identify relationships between morphological measurements and winter survival during the year of establishment in attempts to provide insight towards manageable factors which may contribute to winter survival.

Measurements made in fall 2008, prior to the first winter, were most associated with winter survival. Culm length was associated with winter survival for genotypes under winter pressure, as it was positively associated with winter survival for *M. sinensis* in Leamington and Elora. No relationship was observed across the other species groups in Leamington, while in Elora the heights of other species groups revealed a linear-plateau relationship. Basal circumference was positively associated with winter survival in both Leamington and Elora, while no strong relationship was observed in Kemptville.

Relationships between second year measurements and survival did not appear as clear or intuitive in terms of attempting to describe winter hardiness.

These results demonstrate that increased plant size going into the first winter, as represented by height and basal circumference, may indicate high potential for winter survival. Management practices which promote strong growth may play important roles as entries are grown in increasingly marginal winter environments relative to their winter hardiness.

5.5 Relation between winter survival and developmental measurements

Associations between developmental characteristics and winter survival are often alluded, particularly the importance of maturity and senescence towards winter survival. Limited associations between developmental factors and winter survival were observed. The 50 cm regrowth days recorded following the first winter demonstrated a plateau-linear relationship in Elora where the winter survival was negatively correlated with regrowth beyond a certain date, but no relationships were observed in Leamington. No strong relationships were evident between the other measurements including flowering date, flowered tiller ratings or senescence at any of the other sites. This was supported by the survival of certain entries which failed to flower and remained green until hard fall frosts. These results indicate that winter survival does not come at the expense of yield potential, as early spring regrowth and season long vegetative growth did not appear to be associated with reduced winter survival.

5.6 Limitations of the research

Overarching limitations of the conclusions of this research are the limited number of unique *Miscanthus* entries, which given the variability within species such as *M. sinensis* may only provide limited insight of their genetic potential, and given the lack of background of the selections used in this trial, may not well represent the *Miscanthus* population as a whole. Conducting research on a greater number of genotypes from a dispersed, representative sampling of the natural population could improve inferences.

First year winter survival, often identified as critical for the persistence of the stand, is only reflective of the conditions experienced during the 2008-2009 winter. Based on the temperatures experienced, this winter appeared to be slightly harsher than usual across all locations. Differences in winter severity could change the conclusions made here, particularly in reference to the winter survival potential of entries and species at the various sites. Conducting stand establishment and winter survival assessments for several consecutive years could capture the variability between winters, and create more confident screening data, particularly in terms of making recommendations towards commercial plantations. Given the variability observed between these locations, more extensive testing across locations intermediate to these could also increase accuracy of planting recommendations for future Ontario producers.

In conjunction with winter survival potential, agronomic methods used may not have been ideal, differences in plant quality and health, storage and transportation, planting timing and weed control may have influenced these results. In determining true winter survival potential, further research into agronomic practices which enhance winter survival may increase the potential for some entries and species, particularly if agronomic*entry interactions exist.

The simple correlations used in this study should be interpreted with caution. Identification of associations between winter survival and growth factors may be circumstantial between the locations, entries and practices used. As observed in other species, simple correlations may not always identify true underlying relationships, or confer any meaningful contributions towards winter survival (Frankow-Lindberg, 1999).

Developmental measurements were not made until the year following the first winter. Based on the conclusions made, it is assumed that developmental characteristics of the second year are correlated to those of the establishment year. Moreover, while many developmental associations were not observed across entries within these

locations, these results fail to demonstrate what impact stage of development may have on winter hardiness within these entries.

5.7 Implications of findings

These findings indicate that *Miscanthus* entries exist which are capable of surviving the winter during the first year of establishment under southern Ontario conditions, and successfully produce biomass during subsequent years, thus demonstrating *Miscanthus*' potential as a biomass crop in Ontario.

Identification of associations between winter survival and morphological and developmental measurements have provided some tools which may aid in screening for potential genotypes for environments where winter hardiness is important, such as Ontario. Identification of vigorous growing genotypes (strong establishment year growth, early spring growth) in these environments may assist in making genotype selection recommendations, and contribute towards future breeding efforts.

Identification of associations between winter survival and morphological and developmental characteristics may provide insight for future research towards investigating plant mechanisms which contribute towards winter hardiness. The presence of relationships with potentially manageable factors (ie. fall growth) also demonstrates the need for further agronomic research to identify optimum establishment practices which may enhance winter survival potential.

In general, this research demonstrated that the fertile diploid hybrids were the most winter hardy, followed by the *M. x giganteus* types, followed by the *M. sinensis* group. Despite this generality, equal winter hardiness potential existed within all species groups at both the Leamington and Elora environments. These findings are encouraging, and suggest winter hardiness sufficient for some regions of Ontario can be selected for within these species groups. This is promising for future breeding efforts which may improve varieties through combining winter hardiness with more desirable biomass production characteristics. Widespread screening across more *Miscanthus* genotypes and locations throughout Ontario would further strengthen this research, and increase the accuracy for more local recommendations of existing material.

Overall, these findings may contribute towards further research which may assist in reducing the risks associated with adaptation of *Miscanthus* as a viable, commercial biomass crop in Ontario.

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7. APPENDICES

Appendix 4.1 Establishment survival and winter survival for 20 *Miscanthus* entries across three locations during the year of establishment in Ontario.

Entry	Location					
	Leamington		Elora		Kemptville	
	Establish Survival (%)	Winter Survival (%)	Establish Survival (%)	Winter Survival (%)	Establish Survival (%)	Winter Survival (%)
1	85	99	100	100	81	37
2	91	99	100	99	85	49
3	94	100	98	98	80	30
4	98	98	100	98	58	13
5	89	100	99	93	90	55
6	94	88	91	56	78	23
7	76	100	99	99	94	89
8	96	100	100	62	71	0
9	98	100	99	64	97	0
10	92	99	98	97	82	40
11	92	99	97	27	81	0
12	95	99	100	70	89	3
15	90	97	97	99	3	0
16	63	93	100	0	67	0
18	46	70	80	0	67	0
19	47	66	69	0	17	0
21	55	94	76	53	25	0
22	57	71	66	1	65	0
24	29	30	†		24	0
26	6	8	†		20	0

† denotes entry was not received during initial planting

Appendix 4.2 Plot means for first year winter survival (WS) and fall 2008 culm length as measured at Leamington, Ontario.

Entry	Rep 1		Rep 2		Rep 3		Rep 4	
	WS (%)	Length (cm)	WS (%)	Length (cm)	WS (%)	Length (cm)	WS (%)	Length (cm)
1	100	285	100	153	100	113	97	174
2	100	110	100	70	100	135	100	171
3	100	168	100	155	100	145	100	155
4	94	130	100	83	100	49	100	35
5	100	153	100	184	100	105	100	172
6	100	181	100	135	100	158	50	134
7	100	166	100	150	100	175	100	113
8	100	80	100	169			100	44
9	100	188	100	184	100	132	100	136
10	100	110	100	128	100	90	97	91
11	100	90	100	88	100	82	97	46
12	97	28	100	67	100	43	100	60
15	94	147	100	144	100	140	97	139
16	93	141	96	140	100	139	82	136
18	93	150	73	56	75	114	40	103
19	82	113	86	99	71	114	24	67
21	88	123	91	119	96	162	100	171
22	89	129	85	96	71	128	40	168
24	71	94	41	83			8	62
26	20	43			6	38		

Appendix 4.3 Plot means for first year winter survival (WS) and fall 2008 culm length as measured at Elora, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	Length (cm)	WS (%)	Length (cm)	WS (%)	Length (cm)	WS (%)	Length (cm)
1	100	116	100	103	100	100		
2	100	113			100	81		
3	94	80	100	80	100	88		
4	100	94	97	85	97	52		
5	100	114	75	85			100	89
6	86	60	31	42	89	39		
7	100	135	100	124	100	140		
8	94	34	39	30	22	34		
9			75	52				
10	100	69	89	57	100	67		
11	22	35			3	37	53	50
12	100	51	31	39	57	44		
15	86	59	91	61			89	75
16								
18								
19								
21	14	29	58	40				
22	3	21						
24					3	25		
26								

Appendix 4.4 Plot means for first year winter survival (WS) and fall 2008 culm length as measured at Kemptville, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	Length (cm)	WS (%)	Length (cm)	WS (%)	Length (cm)	WS (%)	Length (cm)
1	28	19	25	21	35	27	59	26
2	60	22	62	25	41	22	33	20
3	40	27	5	20	29	27	47	25
4	4	12			4	16	43	11
5	18	31	32	23	79	40	91	41
6					42	28	52	27
7	88	31	85	33	94	31	89	44
8								
9								
10	24	13	54	17	41	21		
11								
12			3	24			8	25
15								
16								
18								
19								
21								
22								
24								
26								

Appendix 4.5 Plot means for first year winter survival (WS) and fall 2008 basal circumference (B Circ.) as measured at Leamington, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)
1	100	80	100	77	100	68	97	76
2	100	69	100	72	100	76	100	81
3	100	81	100	80	100	73	100	68
4	94	64	100	74	100	92	100	85
5	100	80	100	96	100	86	100	86
6	100	78	100	96	100	73	50	55
7	100	74	100	89	100	79	100	74
8	100	78	100	76			100	67
9	100	76	100	68	100	78	100	80
10	100	107	100	90	100	95	97	96
11	100	75	100	63	100	105	97	71
12	97	100	100	100	100	95	100	100
15	94	48	100	60	100	54	97	52
16	93	65	96	55	100	39	82	48
18	93	55	73	54	75	47	40	32
19	82	41	86	41	71	52	24	31
21	88	43	91	43	96	50	100	58
22	89	53	85	42	71	47	40	82
24	71	38	41	46			8	47
26	20	28			6	12		

Appendix 4.6 Plot means for first year winter survival (WS) and fall 2008 basal circumference (B Circ.) as measured at Elora, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)
1	100	65	100	70	100	68		
2	100	66			100	70		
3	94	68	100	68	100	66		
4	100	53	97	63	97	71		
5	100	89	75	99			100	88
6	86	55	31	49	89	55		
7	100	58	100	61	100	69		
8	94	55	39	56	22	57		
9			75	59				
10	100	65	89	74	100	72		
11	22	60			3	64	53	68
12	100	74	31	70	57	75		
15	86	49	91	44			89	52
16								
18								
19								
21	14	20	58	33				
22	3	21						
24					3	30		
26								

Appendix 4.7 Plot means for first year winter survival (WS) and fall 2008 basal circumference (B Circ.) as measured at Kemptville, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)	WS (%)	B Circ. (cm)
1	28	39	25	23	35	29	59	33
2	60	46	62	31	41	32	33	32
3	40	32	5	16	29	25	47	22
4	4	12			4	11	43	6
5	18	55	32	27	79	37	91	32
6					42	22	52	22
7	88	39	85	30	94	23	89	35
8								
9								
10	24	30	54	21	41	36		
11								
12			3	41			8	36
15								
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21								
22								
24								
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Appendix 4.8 Plot means for first year winter survival (WS) and spring 2009 50cm regrowth date (50cm Reg.) as measured at Leamington, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	50cm Reg. (DOY)	WS (%)	50cm Reg. (DOY)	WS (%)	50cm Reg. (DOY)	WS (%)	50cm Reg. (DOY)
1	100	141	100	139	100	145	97	144
2	100	142	100	140	100	142	100	140
3	100	145	100	144	100	148	100	144
4	94	146	100	147	100	147	100	150
5	100	146	100	143	100	152	100	145
6	100	146	100	147	100	145	50	152
7	100	142	100	142	100	141	100	145
8	100	141	100	143	100	143	100	145
9	100	143	100	144	100	144	100	142
10	100	143	100	142	100	143	97	144
11	100	147	100	148	100	150	97	154
12	97	155	100	146	100	148	100	148
15								
16								
18								
19								
21								
22								
24								
26								

Appendix 4.9 Plot means for first year winter survival (WS) and spring 2009 50cm regrowth date (50cm Reg.) as measured at Elora, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	50cm Reg. (DOY)	WS (%)	50cm Reg. (DOY)	WS (%)	50cm Reg. (DOY)	WS (%)	50cm Reg. (DOY)
1	100	154	100	153	100	155	100	157
2	100	151	100	157	100	157	97	154
3	94	165	100	162	100	160	100	165
4	100	161	97	165	97	163	97	160
5	100	161	75	170	100	163	97	155
6	86	167	31	186	89	166	19	182
7	100	157	100	154	100	157	97	157
8	94	167	39	169	22	180	91	172
9	75	166	86	165			97	165
10	100	158	89	170	100	175	100	163
11	22	181	3	175	53	177	31	185
12	100	162	31	178	57	170	94	169
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Appendix 4.10 Plot means for first year winter survival (WS) and fall 2009 yield as measured at Leamington, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})
1	100	11.191	100	5.784	100	6.590	97	6.819
2	100	5.559	100	8.459	100	9.400	100	9.461
3	100	9.650	100	10.304	100	8.712	100	8.938
4	94	8.649	100	11.654	100	11.786	100	7.834
5	100	7.545	100	13.397	100	6.636	100	8.721
6	100	10.202	100	3.831	100	9.886	50	5.525
7	100	5.355	100	6.888	100	9.079	100	5.006
8	100	10.238	100	11.091	100	6.022	100	10.729
9	100	14.663	100	10.432	100	11.491	100	14.521
10	100	17.037	100	18.261	100	10.991	97	13.847
11	100	12.279	100	6.051	100	11.106	97	5.789
12	97	4.273	100	10.165	100	9.298	100	9.453
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Appendix 4.11 Plot means for first year winter survival (WS) and fall 2009 yield as measured at Elora, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})
1	100	16.434	100	13.456	100	14.573	100	11.385
2	100	15.816	100	14.341	100	16.016	97	16.129
3	94	9.074	100	11.752	100	11.873	100	7.315
4	100	11.212	97	9.420	97	17.901	97	12.999
5	100	15.988	75	7.015	100	12.489	97	12.290
6	86	10.217	31	2.000	89	8.078	19	0.819
7	100	16.066	100	17.262	100	16.007	97	15.041
8	94	11.650	39	5.028	22	1.672	91	7.475
9	75	8.076	86	10.392			97	11.475
10	100	19.270	89	15.491	100	19.589	100	18.728
11	22	1.706	3	0.289	53	3.975	31	1.368
12	100	15.971	31	2.393	57	5.439	94	6.849
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22								
24								
26								

Appendix 4.12 Plot means for first year winter survival (WS) and fall 2009 yield as measured at Kemptville, Ontario.

	Rep							
	1		2		3		4	
Entry	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})	WS (%)	Yield (t ha^{-1})
1	28	0.451	25	0.681	35	1.443	59	0.718
2	60	3.016	62	1.736	41	1.834	33	0.960
3	40	1.217	5	0.093	29	0.641	47	0.228
4	4	0.007			4	0.126	43	0.042
5	18	1.115	32	0.002	79	4.997	91	1.790
6					42	2.254	52	3.001
7	88	5.998	85	3.390	94	5.244	89	6.811
8								
9								
10	24	0.513	54	1.565	41	3.764		
11								
12			3	0.016			8	0.062
15								
16								
18								
19								
21								
22								
24								
26								