

Genetic and Environmental Factors Affecting Switchgrass Performance and Quality in Quebec

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TABLE OF CONTENTS

TABLE OF CONTENTS	ii
LIST OF TABLES	iv
LIST OF FIGURES	v
ABSTRACT	vii
RÉSUMÉ	viii
ACKNOWLEDGMENTS	ix
LIST OF ABBREVIATIONS	x
CONTRIBUTION OF AUTHORS	xi
CHAPTER 1	1
GENERAL INTRODUCTION	1
1.1 OBJECTIVES	3
1.2 HYPOTHESES	3
CHAPTER 2	4
REVIEW OF LITERATURE	4
2.1 BIOLOGY OF SWITCHGRASS	4
2.2 BIOLOGY OF BIG BLUESTEM	5
2.3 SWITCHGRASS IN CANADA	6
2.4 FACTORS AFFECTING SWITCHGRASS YIELD	7
2.4.1 Cultivar choice	8
2.4.2 Establishment factors	9
2.5 Factors affecting switchgrass quality	10
CONNECTING TEXT FOR CHAPTER 3	14
CHAPTER 3	15
PERFORMANCE OF SWITCHGRASS AND BIG BLUESTEM SELECTIONS IN SOUTHERN QUEBEC	15
3.1 ABSTRACT	16
3.2 INTRODUCTION	16
3.2 MATERIAL AND METHODS	18
3.2.1 Plant materials	18
3.2.2 Field management and data collection	19
3.2.3 Laboratory analyses	21
3.2.7 Statistical analyses	21
3.3 RESULTS AND DISCUSSION	22
3.3.1 Biomass yield	22
3.3.2 Tiller density	23
3.3.5 Tiller morphology/composition	26
3.3.3 Height	27
3.3.4 Maturity	28
3.3.6 Effects of harvest date on biomass yield and quality variables	28

3.5 CONCLUSION	31
CONNECTING TEXT FOR CHAPTER 4	45
CHAPTER 4	46
RENOVATION STRATEGIES FOR POORLY ESTABLISHED SWITCHGRASS FIELDS	46
4.1 ABSTRACT	47
4.2 INTRODUCTION	47
4.3 MATERIAL AND METHODS	48
4.3.1 Field management	48
4.3.2 Statistical analysis	49
4.4 RESULTS AND DISCUSSION	50
4.5 CONCLUSION	51
CONNECTING TEXT FOR CHAPTER 5	57
CHAPTER 5	58
CORRELATIONS BETWEEN SOIL CHARACTERISTICS AND SPRING- HARVESTED SWITCHGRASS BIOMASS COMPOSITION	58
5.1 ABSTRACT	59
5.2 INTRODUCTION	59
5.3 MATERIALS AND METHODS	61
5.3.1 Site details	61
5.3.2 Elemental analyses	62
5.3.3 Statistical analyses	62
5.4 RESULTS AND DISCUSSION	63
5.5 CONCLUSION	66
CHAPTER 6	73
FINAL CONCLUSION AND SUMMARY	73
CHAPTER 7	75
RECOMMENDATIONS FOR FUTURE RESEARCH	75
LITERATURE CITED	76
APPENDICES	86

LIST OF TABLES

Table 2.1: Characteristic traits of biomass conversion feedstocks.....	13
Table 3.1: Origins of the commercial switchgrass and big bluestem varieties evaluated in the present study and the selections derived from them through a local selection programme in Southern Quebec.....	32
Table 3.2: Yield of 11 selections of switchgrass and 3 selections of big bluestem seeded in 2010 and harvested in fall 2011 and 2012 at two sites in Southern Quebec (in Mg ha ⁻¹).	33
Table 3.3: Moisture content (%) at fall harvest of 11 selections of switchgrass and 3 selections of big bluestem at two sites in Southern Quebec.	34
Table 3.4: Fraction of reproductive tillers, average dry matter mass per reproductive tiller, and average dry matter mass per tiller overall of 11 selections of switchgrass in 2011 and 2012 grown at 2 sites in Southern Quebec and harvested in October.	38
Table 3.5: Fall 2011 and spring 2012 values for yield, moisture content, cell wall components, ash and higher heating value (HHV) of three selections of switchgrass and three selections of big bluestem in Ste-Anne-de-Bellevue, Quebec.	43
Table 3.6: Fall 2011 and spring 2012 values for yield, moisture content, cell wall components, ash and higher heating value (HHV) of three selections of switchgrass and three selections of big bluestem in Cookshire-Eaton, Quebec.....	44
Table 4.1: ANOVA test results for the effects of seeding, nitrogen and herbicide on the yield of switchgrass plots in Cookshire-Eaton, QC.	53
Table 5.1: Mean, maximum, minimum and standard deviation of soil characteristics at 0-15 cm in switchgrass fields at 58 Quebec and Ontario sites. All values are in mg kg ⁻¹ unless otherwise noted.	68
Table 5.2: Mean, maximum, minimum and standard deviation of soil characteristics at 15-30 cm in switchgrass fields at 58 Quebec and Ontario sites. All values are in mg kg ⁻¹ unless otherwise noted.	69
Table 5.3: Mean, maximum, minimum and standard deviation of spring-harvested Cave-in-Rock switchgrass samples from 58 sites in Quebec and Ontario. All values are in mg kg ⁻¹ unless otherwise noted.	70
Table 5.4: Rotated principal components (PCs) and Communality estimates (CE) from soil data across 58 environments in switchgrass fields in Southern Quebec and Ontario. 71	
Table 5.5: Coefficients and multiple coefficient of determination for stepwise regression of switchgrass biomass dependent variables on rotated PCs of corresponding soil variables obtained through PCA.	72
Table A5.6: Sampling locations for switchgrass biomass and soil samples collected in 2011 in Quebec and Ontario. Mowed column indicates fields that were mowed and left in windrows over winter.	103
Table A5.7: Sampling locations for switchgrass biomass and soil samples collected in 2012 in Quebec and Ontario. Mowed column indicates fields that were mowed and left in windrows over winter.	104

LIST OF FIGURES

Figure 3.1: Number of tillers m ⁻² of 11 selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	35
Figure 3.2: Number of tillers m ⁻² of Sunburst lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	36
Figure 3.3: Number of tillers m ⁻² of 11 selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	37
Figure 3.4: Height of 11 selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	39
Figure 3.5: Height of 11 selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	40
Figure 3.6: Mean stage count of 11 selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	41
Figure 3.7: Mean stage count of 11 selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	42
Figure 4.1: Average dry matter yield of switchgrass plots in Cookshire-Eaton, QC subject to various renovation treatments in the spring of the post-seeding year (O, no treatment, S, no-till seeding, N, nitrogen fertilizer). Results are for the renovation year and illustrate the re-seeding by nitrogen fertilizer interaction.	54
Figure 4.2: Average dry matter yield of switchgrass plots in Cookshire-Eaton, QC subject to various renovation treatments in the spring of the post-seeding year (O, no treatment, S, no-till seeding, H, herbicide, N, nitrogen fertilizer). Results are for the post-renovation year.	55
Figure 4.3: Experimental plot after seeding with a Brillion no-till disc seeder. Note the compacted soil, incomplete closing of the furrows and bisected plant on the right.	56
Figure A3.8: Number of tillers m ⁻² of Summer lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	86
Figure A3.9: Number of tillers m ⁻² of Cave-in-Rock lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	87
Figure A3.10: Number of tillers m ⁻² of Sunburst lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	88
Figure A3.11: Number of tillers m ⁻² of Summer lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	89

Figure A3.12: Number of tillers m ⁻² of Cave-in-rock lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	90
Figure A3.13: Height of Summer lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	91
Figure A3.14: Height of Sunburst lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	92
Figure A3.15: Height of Cave-in-rock selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	93
Figure A3.16: Height of Sunburst lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	94
Figure A3.17: Height of Summer lineage of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	95
Figure A3.18: Height of Cave-in-rock lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	96
Figure A3.19: Phenology of Summer lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	97
Figure A3.20: Phenology of Sunburst selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	98
Figure A3.21: Phenology of Cave-in-rock selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	99
Figure A3.22: Mean stage count of Sunburst lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	100
Figure A3.23: Mean stage count of Summer lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	101
Figure A3.24: Mean stage count of Cave-in-rock lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.	102

ABSTRACT

Switchgrass (*Panicum virgatum* L) is a native North American perennial grass species with promising applications as a bioenergy feedstock. Three studies were conducted to identify genetic and environmental factors affecting switchgrass performance and quality. A first study was conducted to evaluate the performance of new selections made in Southern Quebec, Canada. Eleven selections of switchgrass from 4 base populations and 3 selections of big bluestem (*Andropogon gerardii* Vitman), another promising perennial grass, were evaluated at two sites in Southern Quebec during the first 2 production years. Considerable variation among selections was found for all traits examined, however, differences varied by year and site. Biomass dry matter yield across all switchgrass selections and environments averaged 7.2 Mg ha⁻¹ for a fall harvest. Biomass was also harvested in the spring for some selections to determine changes in yield, moisture content, fibre, ash, and energy content caused by harvest date. The spring harvest resulted in lower yield and moisture content, and higher cellulose content. A second study was conducted to evaluate various methods to renovate poorly established switchgrass fields. Reseeding with a no-till seeder initially negatively impacted yields, however, the combination of no-till reseeding with herbicide and N fertiliser applications increased yields in the post-renovation year compared to the use of only applying N and reseeding. Finally, a third study examined the relationships between soil characteristics and biomass quality in spring-harvested switchgrass fields. There was a significant relationship between soil parameters and biomass Si ($R^2 = 0.74$) and ash ($R^2 = 0.45$) content. Weaker relationships were seen between soil parameters and biomass Ca and Mg content, while other important elements including K showed no relationship to any of the measured soil variables.

RÉSUMÉ

Le panic érigé (*Panicum virgatum* L) est une graminée indigène d'Amérique du Nord qui est une prometteuse source de biomasse. Trois études ont été menées pour évaluer les aspects génétiques et environnementaux affectant la performance et la qualité du panic érigé. Une première étude a évalué la performance de nouvelles sélections faites dans le sud du Québec, Canada. Onze sélections de panic érigé originant de 4 populations originales et 3 sélections de barbon de Gérard (*Andropogon gerardii* Vitman), une autre graminée prometteuse, ont été évaluées pendant les 2 premières années de production. Une variation substantielle a été observée entre les sélections pour toutes les variables étudiées, cependant ces différences entre sélections variaient selon les années et sites. Le rendement de biomasse sèche moyen pour toutes les sélections et environnements était 7.2 Mg ha^{-1} pour une récolte d'automne. La biomasse a aussi été récoltée au printemps pour certaines sélections afin d'évaluer les changements en rendement, humidité, contenu en cendres, énergie, et fibres causées par différentes dates de récolte. Une récolte au printemps a réduit le rendement en biomasse et le niveau d'humidité, et a augmenté les teneurs en cellulose. Une deuxième étude a été faite pour évaluer plusieurs méthodes pour rénover des champs de panic érigé mal établies. Un re-semis direct a initialement réduit les rendements, mais la combinaison de ce traitement avec un herbicide et une application d'azote a augmenté les rendements en biomasse l'année suivant la rénovation comparé aux parcelles traitées uniquement avec de l'azote et réensemencée. Finalement, une troisième étude a évalué les relations entre les caractéristiques du sol et la qualité de biomasse du panic érigé récolté au printemps. Il y avait une relation significative entre les paramètres du sol étudiés et le niveau de Si dans la biomasse ($R^2 = 0.74$) et le contenu en cendres ($R^2 = 0.45$). Des relations plus faibles ont été observées entre les caractéristiques du sol et les teneurs en Ca et Mg de la biomasse, mais aucune relation a été observée pour d'autres éléments importants, incluant le K.

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LIST OF ABBREVIATIONS

ADF – Acid Detergent Fiber

ADL – Acid Detergent Lignin

DM – Dry Matter

DOE – Department of Energy

Ha – Hectare

HHV – Higher Heating Value

IRDA - Institut de Recherche et de Développement en Agroenvironnement

Kg – Kilogram

MAPAQ – Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec

MSC – Mean Stage Count

NDF – Neutral Detergent Fiber

ODT – Oven Dry Tonnes

PLS – Pure Live Seed

REAP-Canada – Resource Efficient Agricultural Production Canada

SRF – Short Rotation Forestry

CONTRIBUTION OF AUTHORS

This thesis has been written in the form of manuscripts. This format has been approved by the Faculty of Graduate Studies at McGill University as described in the “Guidelines for Thesis Preparation and Submission”. This research was designed by the candidate in cooperation with Dr. Philippe Seguin, thesis supervisor and co-author of all manuscripts. The candidate conducted all field studies and data collection, compiled and analyzed the results and wrote the manuscripts and thesis under the supervision of Dr. Philippe Seguin. Roger Samson provided all germplasm used in the experiments and provided technical expertise as well as supervising re-seeding and field logistics. Laboratory analyses were conducted by the candidate under the supervision of Dr. Arif Mustafa. Huguette Martel assisted with field supervision, technical support and consultation. The present thesis is composed of 5 chapters. The first and second chapters are the General Introduction and Review of Literature, respectively. Chapters 3, 4 and 5 represent the field and laboratory experiments and were written in the form of manuscripts to be submitted for future publication.

All three manuscripts were co-authored by the candidate, Dr. Philippe Seguin, Department of Plant Science, McGill University, Dr. Arif Mustafa, Department of Animal Science, McGill University, Roger Samson, REAP-Canada and Huguette Martel, MAPAQ, Direction régionale de l’Estrie. Erik Delaquis, the primary author, performed all experiments, data analysis and manuscript writing. Dr. Philippe Seguin provided the funds and assistance for the research, including supervisory guidance and reviewing of the manuscript. Dr. Arif Mustafa provided technical assistance and guidance in all laboratory analyses undertaken by the candidate. Roger Samson provided all germplasm used in the experiments and provided technical expertise as well as supervising re-seeding and field logistics. Huguette Martel assisted with field experiments and technical support. All authors were involved in the editing of the manuscripts.

CHAPTER 1

GENERAL INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a perennial grass native to North America. A dominant component of the tallgrass prairie ecosystem, switchgrass has long been known as a useful crop for forage, conservation plantings, and as an inexpensive alternative source of straw (Panciera and Jung, 1984; Schmer *et al.*, 2005; Liebig *et al.*, 2008; Brejda *et al.*, 1994; Hopkins *et al.*, 1995a). Recently the development of biomass energy systems has renewed interest in the crop as a feedstock for various conversion platforms, notably direct combustion either alone, as part of a mixture or co-fired with coal (Sanderson *et al.*, 1996; McLaughlin and Kszos, 2005; Keshwani and Cheng, 2009). The results of research programmes over several decades in Canada indicate that this species is an ideal candidate for biomass production due to its deep-rooting perennial nature, ability to produce high yields on marginal land, tolerance to drought and cold winters, and simple management with the use of conventional hay equipment (Weaver 1968; McLaughlin and Kszos 2005; Vogel *et al.* 2011).

Despite this interest for switchgrass very limited selection or genetic improvement has been done for this species, with the few most advanced selections available only being the results of a few selection cycles from fragmented base populations (Casler and Smart 2013). In particular there is a lack of adapted selections for Eastern Canada with all the currently available switchgrass selections originating from the USA. The shorter growing season and colder winters of Southern Quebec put particular pressures on populations and there is thus a need to select material specifically adapted to local environmental conditions.

Switchgrass establishment has proven to be one of the most difficult aspects of the production of this crop, as it is a slowly developing species that only reach its full developmental and yield potential in the second year after seeding (McLaughlin and Kszos 2005). Producers have identified the development of renovation strategies in the event of poor establishment as a research priority (H. Martel, personal communication). Strategies for renovating perennial switchgrass and other forage fields include the application of

herbicides, reseeding the affected areas with a no-till drill and the application of N fertilizer (Panciera and Jung, 1984; USDA, 1986; Seguin, 1998). Although renovation techniques have been studied thoroughly for other forage crops, little research has been done on renovation strategies for switchgrass stands.

Biomass energy crops capture solar energy and transform it into chemical energy through photosynthesis. This stored energy in the form of plant tissues can later be harvested and converted to more useful forms of energy such as heat, electricity or liquid fuels. Switchgrass is typically used for combustion, however the presence of trace elements such as K, Na, Cl, and Si are detrimental to the combustion process, causing the formation of deposits and excess ash as well as corroding and damaging boilers, leading to increased operating costs (Jenkins *et al.* 1998; Miles *et al.* 1996; Johansson *et al.* 2003). The concentrations of these elements can be reduced in switchgrass by leaving the crop to overwinter in the field, allowing precipitation to leach minerals from the tillers while the loss of leaves and panicles reduces yield but lowers Si levels (Adler *et al.* 2006; Vermerris 2008; Kim *et al.* 2011). Greenhouse experiments have also demonstrated that Si levels in the growing medium influence the level of uptake in the plant (Nabity *et al.* 2012), but little work has been done studying the relationships between soil characteristics and biomass quality in field settings. There is a need for research examining the various management and environmental factors affecting switchgrass quality for use as a biomass feedstock.

1.1 OBJECTIVES

1.2.1

a. To determine the performance of new switchgrass selections in Southern Quebec.

b. To determine the impacts of harvest date on switchgrass yield and quality.

1.2.3 To determine the most effective strategies for renovating poorly established switchgrass stands.

1.2.4 To determine relationships between soil parameters and spring-harvested switchgrass quality.

1.2 HYPOTHESES

1.1.1 Locally made switchgrass selections display improved agronomic characteristics compared to their parent populations.

1.1.2 Renovation strategies that include no-till reseeding, herbicide application, and N application will increase biomass yield of poorly established switchgrass fields.

1.1.3 There is a relationship between soil characteristics and biomass quality in spring-harvested switchgrass.

CHAPTER 2

REVIEW OF LITERATURE

2.1 BIOLOGY OF SWITCHGRASS

Switchgrass (*Panicum virgatum* L.) is a perennial C₄ warm-season grass native to North America. Its distribution ranges throughout the North American continent East of the Rocky mountain range, and from 55°N latitude to Central Mexico (Vogel *et al.* 2011). Switchgrass is a major constituent of the North American tallgrass prairie, a formerly extensive ecosystem which has been reduced to less than 1% of its historical range due largely to conversion to farmland (Vogel *et al.* 2011).

Across this large range switchgrass has evolved into two separate and largely distinct ecotypes: a lowland form seen in humid Southern habitats and an upland form found in Northern and drier environments (Wulfschleger *et al.* 2010; Sanderson *et al.* 1996). These ecotypes differ not only in habitat but in ploidy level; all lowland types are tetraploid ($2n = 4x = 36$), while upland types may be tetraploid or octaploid ($2n = 8x = 72$) (Vogel *et al.* 2011).

Switchgrass plants may produce over 300 tillers plant⁻¹ (Smart, Moser, and Vogel 2004) and from 600 to >1300 tillers m⁻² in fully established swards (Boe and Casler 2005), with tillers reaching up to 2 m in height (Martinez-reyna and Vogel 2008). The results of a fifty-year prairie plant study in the American Midwest found switchgrass to be the deepest-rooted of all prairie grasses examined, reaching a rooting depth of >3m (Weaver 1968).

Existing selections of switchgrass demonstrate tolerance to extreme heat and cold, drought and flooding (Vogel *et al.* 2011). Seedlings are also highly acid-tolerant, having been observed growing on soils with pH as low as 3.7 (Stucky *et al.* 1980). Switchgrass has repeatedly demonstrated the ability to produce appreciable yields on sites with otherwise marginal agricultural applications (McLaughlin and Kszos 2005).

Due to these characteristics, switchgrass has drawn attention as a promising potential crop for conservation planting (Panciera and Jung 1984; Liebig, Schmer, and Vogel 2008; Schmer *et al.* 2005), summer forage production (Panciera and Jung 1984;

Brejda *et al.* 1994; Hopkins *et al.* 1995a), and more recently for commercial biomass production (Sanderson *et al.* 1996; McLaughlin and Kszos 2005; Keshwani and Cheng 2009). Biomass crops are agricultural species which efficiently capture and store photosynthetic energy, producing large amounts of energy-rich plant materials which can be harvested, stored, and later transformed into commercially useful forms of energy.

Research conducted across a wide latitudinal range of the American Great Plains has demonstrated significant soil organic carbon increases in switchgrass fields under cultivation for bioenergy at both the 0-30cm and 0-120cm depths (Liebig, Schmer, and Vogel 2008). These increases are thought to be caused by a combination of processes including root mass deposition, rhizodeposition of photosynthetically fixed C and decreased erosion losses (Liebig *et al.* 2005). Soil organic carbon stored in the top 5 cm of soil alone by warm season grasses has been calculated to reach up to 3 Mg C ha⁻¹ yr⁻¹, and to result in greater soil and environmental benefits when planted on marginal land (Blanco-Canqui 2010).

The natural senescence of plant tillers at the end of the growing season is accompanied by the translocation of non-structural carbohydrates and N-rich materials to the plant's underground storage tissues; for this reason the delaying of the harvest season until as late as possible in the fall is advisable for maximizing long-term yields in a one-cut system (Parrish and Fike 2005). These large underground carbohydrate reserves allow switchgrass plants to recover well from winter injury or heaving, making the species well adapted to cooler zones where corn and soybean production is marginal (Kludze, Deen, and Dutta 2011).

2.2 BIOLOGY OF BIG BLUESTEM

Like switchgrass, big bluestem (*Andropogon gerardii* Vitman) is a warm-season grass native to North America. Along with indiangrass (*Sorghastrum nutans*), these three species constitute the majority of the tallgrass prairie ecosystem (Vermerris 2008). Although less researched than switchgrass, studies in Canada have identified big bluestem as the highest-yielding warm-season grass other than switchgrass (Jefferson *et al.* 2002). Big bluestem is a deep-rooted grass which may attain 3 m in height and a rooting depth of 2.7 m (Vermerris 2008). Although it can be multiplied through crown division or by

rhizomes, the principal method of propagation is by seed. The presence of hairy awns on relatively small seeds has slowed adoption by making seeding difficult with conventional equipment, however, there has been success with the use of mechanical debearding (Tober *et al.* 2008).

Big bluestem has been named as a candidate species for use in forming polycultures with switchgrass and other native grasses in mixed prairie systems (Samson *et al.* 2005). Mixed prairie plantations have been shown to provide many benefits including increased indigenous avian and arthropod diversity, abundance and species richness (Robertson *et al.* 2010; Gardiner *et al.* 2010). Polycultures of the two species have also been reported to be extremely well suited to certain types of energy production applications and to have very low N requirements (Mulkey, Owens, and Lee 2007). In some settings such polycultures may even outyield switchgrass monocultures (Zamora *et al.* 2013). Breeding and selection programmes based in the United States have produced several improved varieties since the crop's genetic potential was first recognized in the 1950s, with releases including Bison, Bonilla, Prairieview, Sunnyview, Pawnee, Champ, Kaw and Niagara (Newell and Peters 1957; Vermerris 2008; Jung *et al.* 1990; Madakadze *et al.* 1992). Advantages of big bluestem cultivation include enrichment of local biodiversity as well as higher N use efficiency and *in vitro* fermentability than other warm season-grasses, the latter being of particular interest in ethanol production systems (Vermerris 2008).

2.3 SWITCHGRASS IN CANADA

Switchgrass is currently in production on approximately 1500 ha in Quebec, with a rate of increase in acreage of around 30% annually (Huguette Martel, private communication). This increase, without the benefit of government subsidy programs, implies considerable producers' interest. Despite this significant expansion there remains a lack of germplasm adapted specifically to Eastern Canada's distinct growing conditions. Quebec's colder winter temperatures, fewer summer growing degree days and diminished photoperiod all contribute to the necessity for locally adapted selections. In Quebec, switchgrass has found market opportunities as livestock bedding, a component of livestock feed rations, primary material for biocomposite applications and as a substrate

for mushroom production. It has also been suggested as an option for high value but ecologically sensitive agricultural lands bordering on waterways or other highly erosion-prone areas (Kludze *et al.* 2013). However, perhaps the greatest potential application lies as a feedstock for bioenergy production.

Due to its geography and climate, Canada has a long history of studying potential bioenergy crops. Serious efforts began in the 1980s, with the University of Toronto studying hybrid willow, and a forest group based at the Ministry of Natural Resources in Brockville, Ontario working on hybrid poplar. Research interest in Canada surrounding short-rotation forestry (SRF) was informed by similar work in moist temperate regions of Europe such as Sweden and Belgium. Canadian SRF researchers linked with the US Department of Energy (DOE) in Oak Ridge, Tennessee. In 1985 the DOE began research on identifying herbaceous crops with promising characteristics as bioenergy candidate species, testing 34 candidate species on 31 sites across a large range of soil types over the next 2 decades. A major review of this work found switchgrass to be the most promising of the species evaluated due to its consistently high ranking by all institutions involved (Wright 2007). Reasons given by the participants for their preference included the crop's high potential for yield, deep rooting patterns, ability to propagate by seed, use of existing inexpensive hay equipment to harvest, and high carbon sequestration potential (Vogel *et al.* 2011; Wright 2007).

The DOE then funded a decade of research which highlighted the possibilities for yield increases with the selection of improved genetics adapted to local environments. Taken together, the DOE's research programmes showed that switchgrass could be a competitive new biomass crop in Eastern North America. This led to the DOE listing switchgrass as the 'model bioenergy species' for the next round of research beginning in 1992 (Parrish and Fike 2005). Since this period, a significant increase in interest in Quebec and Ontario has stimulated the development of a parallel Canadian programme.

2.4 FACTORS AFFECTING SWITCHGRASS YIELD

The yield potential of switchgrass grown for biomass production varies widely according to environmental and genetic factors, notably showing a strong inverse correlation with latitude of germplasm origin (Casler *et al.* 2004; Wullschleger *et al.*

2010). Under a single-cut system for biomass production, yield response varies due to many factors, notably including cultivar (Fike *et al.* 2006; Lemus *et al.* 2002; Jefferson and McCaughey 2012), site (Fike *et al.* 2006; Hopkins *et al.* 1995a), rainfall (Muir *et al.* 2000), seeding date (West and Kincer 2011) and N fertilization (Vogel *et al.* 2011; Muir *et al.* 2000).

2.4.1 Cultivar choice

The experiences of the DOE's body of research indicated that the identification of appropriate locally adapted varieties resulted in yield increases of up to 50%, and in Northern latitudes the varieties Cave-in-Rock, Trailblazer and Sunburst were found to be the highest yielding (McLaughlin and Kszos 2005). The currently grown commercial varieties in Eastern Canada were all selected for performance by breeding programmes in more Southerly climates. It must be noted that in this largely undomesticated crop, commercial cultivars are typically only 3-4 cycles away from their wild base populations (Casler and Smart 2013). The current cultivar of choice for Eastern Canada is Cave-in-Rock, which makes up approximately 90% of the area presently under cultivation despite being a selection made in Southern Illinois at 37°N.

A strong inverse correlation between latitude of origin and yield potential has been identified (Casler *et al.* 2004; Boe 2007), however, it has been recommended that moving germplasm more than 5°N (about 500km) or >1 hardiness zone from its origin will result in unsatisfactory stands due to winter injury (Casler *et al.* 2004; Casler *et al.* 2007). For this reason, cultivar selection in Canada is typically centered around finding a high yielding variety which is not susceptible to winter injury at that particular latitude. A recent study conducted in Saskatchewan and Manitoba confirmed this, concluding that only American cultivars with the most Northerly latitudes of origin were appropriate at the study sites (Jefferson and McCaughey 2012). The authors of the same study also noted the failure of the popular Cave-in-Rock variety to persist in the study environment due to winter injury.

Studies of tiller dynamics in switchgrass swards have shown a high propensity for excess tillering early in the season, resulting in extensive tiller mortality later in the growing period. It has been demonstrated that the major cause of tiller mortality in

switchgrass is understory shading, with larger tillers shading out the smaller ones (Ong, Marshall, and Saoar 1978). Although there is significant vascular connection between tillers, radiocarbon studies indicate that the large reproductive stems translocate little resources to the shaded vegetative tillers beneath (Ong, Marshall, and Saoar 1978). The overproduction of tillers early in the season therefore represents a significant loss of plant energy during the early growing period, as the small vegetative tillers die and wilt onto the ground throughout the growing period. These diminutive tillers are not harvestable at the end of the season, having largely fallen or retted onto the soil surface, and therefore represent a significant waste of energy by the plant.

Previous research has indicated that selections at the seedling stage can be an effective method for modifying yield-related traits such as tiller number in both switchgrass and big bluestem populations (Smart, Moser, and Vogel 2004) and producing populations with increased yield per tiller (Smart and Moser 1999). An evaluation of genetic variability and relationships with various phenotypic traits have also confirmed that adequate variability exists within individual cultivars of switchgrass to justify selection and breeding work within populations (Das, Fuentes, and Taliaferro 2004). Taken together, these findings suggest a large opportunity for selection programmes to develop adapted selections for Eastern Canada's unique environments.

2.4.2 Establishment factors

A major hurdle identified in producer acceptance of switchgrass as a commercial crop is its slow and sometimes difficult establishment (Aiken and Springer 1995; Parrish and Fike 2005). The main causes of poor establishment are poor seed quality, inappropriate seedbed preparation and planting procedures, weed competition due to lack of proper weed control methods, and lack of rainfall throughout the establishment period (Mitchell and Vogel 2012; Smart and Moser 1999; Curran *et al.* 2011). With the exception of rainfall, all of these problems can be addressed by modifying management techniques.

Like many perennial crops, switchgrass does not attain full yield potential in the first year. The year of seeding is generally considered to be an establishment year, with the subsequent year representing the first production year. Appropriately managed

switchgrass plantations reach 33-66% of their maximum potential in the first production year (McLaughlin and Kszos 2005), while the second production year may often be considered as indicative of long-term yield potential. It has been suggested that there may also be some variability in length of establishment period between cultivars (Parrish and Fike 2005).

Previous research has identified the stand frequency threshold for successful establishment of a switchgrass field for biomass production to be about 40%, while stand frequencies above this threshold were shown not to limit subsequent biomass yields in the long term (Schmer *et al.* 2005). This is likely associated with the ability of switchgrass to tiller, self-seed or spread by rhizomes to fill gaps. A 10-year research programme at eight sites in the upper Southeast US detected no decrease in yield over the long term after the stand reached maturity (Fike *et al.* 2006).

Seed size and planting depth have been shown to have an effect on emergence rates (Mitchell and Vogel 2012), while seed size alone has been shown to have little effect on long-term growth of seedlings given appropriate planting conditions (Smart and Moser 1999). Seeding rates of over 100-200 PLS m⁻² have also been shown to have little effect on population establishment or density (Vogel 1987).

2.5 Factors affecting switchgrass quality

Plants convert solar energy into stored chemical energy in the form of tissues; principally these are cellulose, hemicellulose and lignin. Along with these tissues, each crop also contains varying amounts of cell solubles as well as minerals and silicates taken up from the soil during growth. Herbaceous species are generally recognized to contain higher amounts of cellulose and hemicellulose (Saidur *et al.* 2011) as well as macronutrients, silica and ash (Jenkins *et al.* 1998), while woody biomass is typically highly lignified and contains low levels of ash. In herbaceous species cell walls comprise 40-80% of the biomass, the percentage varying with species and maturity (Vogel and Jung 2001). The cell walls of grasses differ from those of dicots, containing cellulose, hemicellulose, lignin, and acids including p-coumaric, phenolic and ferulic acids (Vogel 2008; Sarath *et al.* 2011).

Biomass feedstocks are converted into useful forms of energy through a variety of mechanisms, ranging from straightforward combustion to more advanced second-generation platforms. Among the latter, promising technologies in development today are gasification, biogas, cellulosic ethanol and pyrolysis systems. There is currently a very high degree of interest and investment in the production of liquid biofuels as an additive to conventional oil-based petrochemicals. Densified herbaceous feedstocks can also be co-fired directly in coal-fired power generation plants, while some modern heat and power cogeneration plants are using undensified straw in Denmark and England.

Along with each of these conversion technologies comes a different set of optimal biomass characteristics for maximizing efficient energy production. For this reason, the various feedstocks available may be more or less conducive to use depending on each conversion method (see Table 2.1). The selection of the optimal feedstock for each application can therefore be based on certain characteristics, one of which is related to the concentration and composition of inorganic compounds in each feedstock.

Two types of inorganic compounds are typically found in biomass. The first are inherent minerals absorbed by the plant and dispersed throughout the tissues during growth; these are sometimes referred to as being atomically dispersed. The second type are contaminants often arising from harvest and processing. Examples of this are dirt or clay pressed onto bales of straw or on the surface of logs. In woody biomass this type of inorganic contaminant can make up a large percentage of the ash.

The ash component of herbaceous feedstocks including switchgrass is made up largely of silicon, but also includes oxides of elements including K, Na, S, Cl, P, Ca, Mg, and Fe (Saidur *et al.* 2011). Aside from forming ash, these species are also involved in undesirable complex chemical reactions during the combustion process which lead to damaging fouling and slagging of boilers (Jenkins *et al.* 1998; Miles *et al.* 1996; Saidur *et al.* 2011). Warm season grasses such as switchgrass tend to have lower ash contents than other herbaceous biomass crops due to the fact that they are C₄ species which utilize water more efficiently than their C₃ counterparts (Jones *et al.* 2011). Because silicon is mainly taken up by plants in the form of silicic acid dissolved in water this decrease in water consumption means that C₄ grasses generally accumulate lower levels of silicon.

A recently developed management practice that has been shown to have significant effects on biomass quality is harvest date. In Canada, switchgrass is typically harvested once per year, while in more Southerly areas with longer growing seasons or when forage production is the key consideration multiple-cut systems are sometimes employed (Brejda *et al.* 1994; Parrish and Fike 2005). In a typical biomass production one-cut system the crop may be harvested in fall or left in the field to overwinter until spring. In the latter case the crop may either simply be left standing or mowed and overwintered in windrows. Canada, representing the Northern limit of production, faces the added challenge of trying to balance fall harvest dates to allow the plants to go completely dormant while still completing the harvest before conditions become too wet or snow falls (Samson 2007). Many switchgrass producers also engage in the production of other cash crops such as corn or soy, making the ability to harvest in the spring during a less labour-intensive period attractive.

Overwintering principally reduces the mineral fraction through leaching with exposure to seasonal precipitation, thereby increasing biofuel quality (Adler *et al.* 2006; Vogel *et al.* 2011). However, significant losses in harvestable yield may occur with this strategy in years with heavy snowfall, and during the winter exposed plants are prone to breakage. Reductions of up to 40% in biomass yield have been reported in Pennsylvania, due largely to the loss of panicles, leaves and stem components such as cell solubles through leaching (Adler *et al.* 2006). The heavy snow load in environments such as Quebec can also flatten the material, making it more difficult for machinery to pick it up. The effects of a delayed harvest system on switchgrass yield and quality in Quebec require further investigation to properly evaluate the potential applications of this strategy.

Table 2.1: Characteristic traits of biomass conversion feedstocks

Process	Key desirable traits	Key undesirable traits
Combustion	High energy content, nonspecific for cellulose, hemicelluloses and lignin	High alkali content, ash, high moisture content
Gasification	High energy content, nonspecific for cellulose, hemicellulose and lignin	Tars, high alkali content, ash
Biogas	High digestibility, high cellulose, fat, starch and sugar content	High N, highly fibrous, high lignin
Cellulosic ethanol	High cellulose content	High lignin
Pyrolysis	High cellulose and hemicellulose content	High moisture content

CONNECTING TEXT FOR CHAPTER 3

In this study we examined the performance of several new local selections of switchgrass and big bluestem and their parent cultivars. We also examined the differences in quality in a select few of those varieties harvested in fall and spring.

The following chapter was written as a manuscript which will be submitted for future publication. The manuscript was co-authored by the candidate, Dr. Philippe Seguin, Department of Plant Science, McGill University, Dr. Arif Mustafa, Department of Animal Science, McGill University, Roger Samson, REAP-Canada and Huguette Martel, MAPAQ, Direction régionale de l'Estrie.

. The candidate carried out the experiments and was the primary author of the manuscript. Dr. P. Seguin provided funds and assistance for this research, including supervisory guidance and reviewing of the manuscript. Laboratory analyses were conducted in the laboratory and under the supervision of Dr. Arif Mustafa. Mr. Roger Samson provided all germplasm and selections used in the experiment as well as technical assistance in field management and data collection. Huguette Martel provided field and technical support. All authors contributed to editing the manuscript.

CHAPTER 3

PERFORMANCE OF SWITCHGRASS AND BIG BLUESTEM SELECTIONS IN SOUTHERN QUEBEC

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3.1 ABSTRACT

Switchgrass (*Panicum virgatum* L.) and big bluestem (*Andropogon gerardii* V.) are warm-season grasses with promising characteristics for bioenergy production across North America and Europe. Despite serious interest in recent years, little work has been done on developing regionally adapted selections for Eastern Canada. New selections made from four promising switchgrass cultivars and one big bluestem cultivar in Southern Quebec were evaluated for their performance and agronomic characteristics including tiller density, height and rate of maturity. Significant variation was detected among selections for all measured characteristics, with differences varying by site and year. The responses of various advanced selections suggested that in some cases the selection process modified certain characteristics of the switchgrass cultivars. The effects of harvest date were also evaluated through the comparison of fall and spring harvested materials. Spring harvesting drastically reduced yield and slightly increased cellulose content, but did not affect energy, lignin or hemicellulose contents.

3.2 INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a perennial C₄ grass native to North America which has historically been used as a summer forage, for conservation plantings and as a source of straw for livestock bedding (Panciera and Jung 1984; Liebig, Schmer, and Vogel 2008; Schmer *et al.* 2005). Recently interest has shifted towards biomass energy applications, with emerging markets and government-sponsored environmental targets backed by research indicating significant production potential in Eastern Canada (Kludze *et al.* 2013). Interest for switchgrass has led to an increasing number of breeding and selection programmes and the release of several new commercial selections in the 1980s (Trailblazer, Forestburg, Dacotah, KY1625), 1990s (Sunburst, Shawnee) and 2000s (High Tide, Carthage, BoMaster, Performer, Cimarron, Colony) (Jefferson and McCaughey 2012; Vogel *et al.* 2011; Casler 2012). Despite a rapid yearly increase in acreage dedicated to the production of this crop in Eastern Canada, these selections all represent the efforts of plant material improvement centers located in the Northern United

States, with little selection being done *in situ* in Eastern Canada's production environment.

Big bluestem (*Andropogon gerardii* Vitman), another native warm-season grass species, is also a major constituent of the tallgrass prairie ecosystem which has been demonstrated to be the highest-yielding warm-season grass other than switchgrass in the Eastern North American context (Jefferson *et al.* 2002). While producing high biomass yields, big bluestem also has higher N use efficiency and greater *in vitro* fermentability than other warm season-grasses, making it especially interesting for ethanol production systems (Vermerris 2008). Big bluestem has been used for forming polycultures with switchgrass and other native grasses in mixed prairie systems (Samson *et al.* 2005), which have demonstrated many benefits including increased indigenous avian and arthropod diversity and species richness (Robertson *et al.* 2010; Gardiner *et al.* 2010), and in some settings may outyield switchgrass monocultures (Zamora *et al.* 2013).

Switchgrass production in Quebec currently covers about 1500 ha, with the acreage increasing by approximately 30% per year (Huguette Martel, private communication). The vast majority of this acreage is the variety Cave-in-Rock, a selection made from a field in Cave-in-Rock, Illinois in 1958. Previous experiences indicate that appropriate regional selections can significantly increase yield potential in a given environment (McLaughlin and Kszos 2005), but despite this there are currently no commercial varieties available which have been specifically developed for the Eastern Canadian environment, nor to our knowledge has any selection work been conducted locally for big bluestem.

Previous studies with switchgrass and big bluestem have confirmed the effectiveness of seedling-stage selection for developing selections with modified tiller number (Smart, Moser, and Vogel 2004) and increased yield per tiller (Smart and Moser 1999). It has also been demonstrated that selection and breeding programmes have the capacity to alter fiber composition in switchgrass (Sarath *et al.* 2011), an important characteristic for energy crops. Fiber content, including lignin and cellulose concentrations, have been demonstrated to be important parameters for multiple conversion technologies, and thus important breeding targets for biomass crops (Vermerris 2008).

A selection programme located in Southern Quebec using bulk selection at the seedling stage followed by selection at maturity in spaced-plant nurseries has been underway since 1991 by REAP-Canada (Ste-Anne-de-Bellevue, QC, Canada). Through several cycles of selection new populations of switchgrass and big bluestem have been produced (Table 3.1).

The development of a spring harvest system for switchgrass is another potential avenue for improving biomass quality for energy applications. In biomass crops the leaching of minerals from plant tissues throughout the winter has been shown to result in lower mineral content (Adler *et al.* 2006; Burvall 1997), while the loss of leaves may decrease the concentration of major ash components such as silicates (Clarke, Eng, and Preto 2011). Silicon is the primary ash component in herbaceous biomass, and elevated levels are problematic for commercial combustion processes (Jenkins *et al.* 1998). Spring harvesting also carries the added advantages of reducing labor demands during peak harvest time for commercial cash crops and drastically reducing moisture content at harvest to 12-14 %, which eliminates the need for drying (Samson 2007) and is beneficial for baling and storage (Adler *et al.* 2006). Adler *et al.* (2006) examined the impacts of a spring harvest on the fiber properties of switchgrass in Pennsylvania, but only for the Cave-in-Rock variety.

The goal of the present project was to evaluate the agronomic characteristics of several commercial varieties of switchgrass and big bluestem along with advanced selections made from the original populations to determine the effects of a local selection process on their performance in Southern Quebec. Several of these selections were also subjected to both a spring and fall harvest regime to determine the effects of this management strategy on yield, fiber and energy characteristics of harvested biomass.

3.2 MATERIAL AND METHODS

3.2.1 Plant materials

Selection lines and parent germplasm were supplied by REAP-Canada's selection programme, based in Southern Quebec (Sainte-Anne-de-Bellevue, QC, Canada). All cultivars and selections evaluated in the present study were upland varieties. A total of 11 switchgrass and 3 big bluestem selections were evaluated. These included 3 switchgrass

parent germplasm and their selections (Table 3.1). Sandlover, a selection from NU-942 from the University of Oklahoma, was also evaluated bringing the total switchgrass selections evaluated to 11. In addition, 2 selections of big bluestem were also evaluated alongside their parent germplasm prairie view. The big bluestem selections made from prairie view Indiana big bluestem (Burgdorf, 2009). The selections prairie view early and prairie view II were selected from an 800 spaced-plant nursery of prairie view Indiana big bluestem originally seeded in 2008.

The Bluejacket I and Bluejacket II (2010) selections were made from sunburst switchgrass using recurrent restricted phenotypic selection (Burton, 1974). The population of Bluejacket II was selected for reduced tillering at the seedling stage (Smart *et al.* 2004). The Bluejacket early selection was derived from 50 plants which exhibited early spring growth in the year after establishment in a 1 ha seed field of Bluejacket switchgrass in Valleyfield, Quebec. The plants were dug up in the spring of 2008 and seed collected in the fall of 2009.

Cave-in-rock II was derived from a seed collection in the fall of 2006 from 30 superior plants in an 8 year old switchgrass field in Valleyfield, Quebec. The population was subsequently selected for reduced tillering at the seedling stage (Smart *et al.*, 2004). Seed derived from superior plants from a 200 space plant nursery were collected in fall 2009. Cave in Rock early was derived from seed collected in fall 2009 from 30 early maturing switchgrass plants in a 10 year old field of Cave-in-Rock switchgrass in Valleyfield Quebec.

The Tecumseh I and Tecumseh II selections were made from Summer switchgrass using recurrent restricted phenotypic selection (Burton 1974). The population of Tecumseh II was selected for reduced tillering at the seedling stage (Smart *et al.*, 2004). Seed derived from superior plants from a 200 spaced-plant nursery were collected in fall 2009.

Sandlover was derived from a 1000 spaced-plant nursery using recurrent restricted phenotypic selection (Burton 1974). The original source population was Northern Upland NU-942 obtained from Charles Taliaferro Charles at the University of Oklahoma in 2006.

3.2.2 Field management and data collection

Plots were arranged in a randomized complete block design with four replications at two sites in Southern Quebec; Sainte-Anne-de-Bellevue (45°25'32.45"N, 73°55'48.63"W, 37 m elevation) and Cookshire-Eaton (45°20'57.49"N, 71°47'08.40"W, 243 m elevation). Plot size was 4 x 5 m in Sainte-Anne-de-Bellevue, and 2.4 x 5 m in Cookshire-Eaton. The soil at the Sainte-Anne-de-Bellevue site was a free-draining St. Bernard sandy clay loam, while the Cookshire-Eaton site was a class 3/4 imperfectly-drained Magog stony loam. All selections of both species were seeded in spring 2010 using a Fabro no-till seeder (Fabro Enterprises, Swift Current, SK, Canada) with an 18 cm row spacing. The seeding rate used for all selections was 300 pure live seeds (PLS) m⁻². Nitrogen was applied at both sites as urea at a rate of 50 kg N ha⁻¹ in May of 2011 and 2012; no other fertilization was done and weeds were manually controlled as needed throughout experimentation.

Plant height was determined in each plot bi-weekly from May to September in 2011 and 2012. The height of ten plants was measured in each of three subplots selected at random within each plot, with the height recorded as the tallest overall part of the plant. Tiller numbers were also determined bi-weekly in 2011 and 2012 in three 50 x 50 cm quadrats placed randomly in each plot. Tiller counts were continued until tiller die down at the beginning of the transition to dormancy at the end of each growing season. Phenology stages were determined based on sampling 10 plants at random from each plot. Phenology was scored based on the mean stage count (MSC) as described by Moore *et al.* (1991). Big bluestem selections were not included in tiller counts and were measured for height once at the end of the growing season.

All plots were harvested in the fall using a flail-type forage harvester (Fabro Enterprises, Swift Current, SK, Canada) with a cutting surface of 0.6 x 5 m and a cutting height of 10 cm. The harvest dates for 2011 and 2012 were November 1 and October 23 in Ste-Anne-de-Bellevue and October 24 and October 17 in Cookshire-Eaton, respectively. In addition, the selections from Cave-in-Rock switchgrass and Prairieview big bluestem were also harvested in the spring to determine yield differences related to harvest time. The 2012 spring harvest dates were April 19th and April 30th in Ste-Anne-de-Bellevue and Cookshire-Eaton, respectively. All harvested biomass was weighed and subsamples of approximately 500 g were collected from each plot to determine biomass

yield on a dry matter basis. These sub-samples were also used to determine the moisture, ash, fiber, and energy content of the harvested biomass for selections that were harvested both in the fall and the spring. At the Cookshire-Eaton site difficult conditions and smaller plot width meant that only a hand-harvest was possible in the spring.

3.2.3 Laboratory analyses

Subsamples taken from each plot were dried for 48 hours at 60°C and then reweighed to determine moisture content (AOAC 1990, method No. 934.01). Dry samples were then ground with a Model 4 Thomas-Wiley Laboratory Mill forage grinder (Thomas Scientific, Swedesboro, NJ, USA.) to pass through a 1 mm screen. The ash fraction was determined using a Thermolyne (Dubuque, Iowa) muffle furnace (AOAC 1990, method No. 924.05). Ground samples were analyzed for neutral (NDF) and acid (ADF) detergent fiber using an Ankom Fiber Analyzer (Ankom Technology Corporation, Macedon, NY, USA) by incubating the samples in neutral (Van Soest *et al.* 1991) and acid detergent solutions (AOAC 1990, method no. 9738.18), respectively. Acid digestible lignin (ADL) was determined by washing ADF residues with 20 N H₂SO₄ (AOAC 1990, method No. 9738.18). From the results of these analyses cellulose, hemicellulose and lignin contents were calculated (Hemicellulose = NDF – ADF, Cellulose = ADF – ADL, Lignin = ADL – mineral ash). Energy content was determined using a Parr adiabatic bomb calorimeter (Parr Oxygen Bomb Model 1341EB, Calorimeter Thermometer Model 6772, Parr Instrument Company, Moline, IL) to calculate higher heating value (HHV).

3.2.7 Statistical analyses

The experiment design used at both sites was a randomized complete block design with four replications. All data were analyzed using the GLM procedure of the SAS statistical software program (SAS Institute, 2003). Unless otherwise noted, tests were considered significant at the 5% probability level. Only significant results are discussed in the text unless otherwise noted.

3.3 RESULTS AND DISCUSSION

3.3.1 Biomass yield

Switchgrass biomass yield across selections, years, and sites averaged 7.2 Mg ha⁻¹ with individual site-year averages ranging between 3.8 and 9.9 Mg ha⁻¹ (Table 3.2). Large differences were observed between sites, yields averaging 4.9 and 9.5 Mg ha⁻¹ in Cookshire-Eaton and Ste-Anne-de-Bellevue, respectively. Yields of all selections were greater in Ste-Anne-de-Bellevue than in Cookshire-Eaton, although differences in the ranking of selections varied at the two sites. The overall much lower yields observed in Cookshire-Eaton may be due to several environmental factors. The site is at a higher elevation and had a heavier, imperfectly drained soil and more intense weed pressure compared to Ste-Anne-de-Bellevue. Yields were higher in 2012 than 2011 for all selections at both sites except Bluejacket 2, for which yield declined from 9.7 to 9.1 Mg ha⁻¹ at Ste-Anne-de-Bellevue. Switchgrass usually reaches 33-66% of its yield potential in the first production year, while the second production year is generally indicative of long-term yield potential (McLaughlin and Kszos 2005). Variation in switchgrass yield across sites, cultivars, and fertilizer use has previously been reported (Lemus *et al.* 2002; Fike *et al.* 2006; West and Kincer 2011; Hopkins *et al.* 1995a), with a recent meta-analysis reporting a mean yield of 8.7 ± 4.2 Mg ha⁻¹ over 39 trials conducted across the United States (Wullschlegel *et al.* 2010). Jannasch *et al.* (2001) reported an average yield of 9.2 Mg ha⁻¹ for switchgrass in Southern Quebec, while average yields of 7.0 and 9.0 Mg ha⁻¹ were reported in Minnesota (Zamora *et al.* 2013) and Iowa (Lemus *et al.* 2002), respectively. Big bluestem biomass yield averaged 5.5 Mg ha⁻¹ across all selections, sites, and years but were even more variable than switchgrass, with values ranging between 1.9 and 8.2 Mg ha⁻¹ in individual site-years. As was the case with switchgrass, selections produced higher yields in 2012 and at the Ste-Anne-de-Bellevue site. Previous experiences have suggested that big bluestem may not reach maximum yields until 3-4 years after seeding (Vermerris 2008), a result supported by the large increases in yield and plant height observed between years in the present study.

Variation in yield was also observed between selections, with significant differences generally occurring between selections of a different lineage and not often between selections in the same lineage (Table 3.2). However, overall trends were

observed for higher yields in some of the advanced selections when compared to their parent cultivars. The Cave-in-rock 2 selection had the highest average yield at both sites in 2011, while in 2012 it significantly outyielded all other selections in Cookshire-Eaton but ranked second to Summer in Ste-Anne-de-Bellevue. This could be related to the unusually dry summer conditions across Eastern North America in 2012, which may have given the earlier-maturing Summer cultivar an advantage. Cave-in-Rock selections generally performed at least as well or better than the other selections tested, confirming the results of other studies which have found Cave-in-Rock to be well adapted and high yielding in Southern Quebec (Madakadze, Coulman, Peterson, *et al.* 1998).

All selections in the Sunburst lineage performed very poorly in Cookshire-Eaton, and in both 2011 and 2012 significantly higher stem rust (*Puccinia* spp.) incidence was noted on those selections relative to all other selections in the study (data not shown). Some disease pressure was also noted on the Bluejacket selections in Ste-Anne-de-Bellevue, which may explain the decreased yield of Bluejacket 2 in 2012. Stem rust infections are the most common disease reported in switchgrass, and may cause significant damage including stunting, early flowering, and reduced yield (Lemus *et al.* 2002). The prevalence of rust infections on all Cave-in-Rock selections was extremely low, similarly to results from previous research (Redfearn *et al.* 1997). Gustafson *et al.* (2003) evaluated the genetic variation in resistance to rust disease in switchgrass populations including Summer, Sunburst and Cave-in-rock. Sunburst had the highest susceptibility to rust, while Cave-in-Rock, having evolved under heavy rust pressure in humid Southern Illinois, was the least susceptible. The Summer cultivar was originally selected for rust resistance (Casler 2012), and none of the selections in the Summer lineage were noted to have been affected by disease in the present study. Disease pressure may thus explain the yield depression observed in all the Sunburst lineage selections in the present study, particularly in 2012 and at the humid Cookshire-Eaton site.

3.3.2 Tiller density

Significant differences in tiller number were observed among selections at all measurement points in both years at Ste-Anne-de-Bellevue (Fig. 3.1). At the first

measurement period (early June in 2011, late May in 2012), the mean tiller density per square meter across all entries was 906 in 2011 and increased to 1033 in 2012, with Sunburst producing the most tillers m^{-2} both years (1307 in 2011, 1375 in 2012) and the Tecumseh I selection producing the least (580 in 2011, 733 in 2012). Due to the large variability between plots, the significant differences observed were often between selection lineages and not between selections within a lineage. By the end of August, tiller density in Ste-Anne-de-Bellevue decreased by an average of 22 and 30% in 2011 and 2012, respectively. The magnitude of the decrease varied considerably depending on the selection lineage. The average decrease in tiller density throughout the season across both years was 38% for Sunburst selections, 19% for Summer selections, 26% for Cave-in-Rock selections, while no decrease was observed for Sandlover. All subsequent selections of the Sunburst lineage displayed reduced tillering early in the season (Fig 3.2). The variable degree of tiller loss across selections ultimately had an equalizing effect on tiller density between all selections. The average tiller density at the first measurement period was 969 tillers m^{-2} with a standard deviation of 183; by the last measurement period this had decreased to an average of 690 tillers m^{-2} with a standard deviation of 61.

In Cookshire-Eaton stands were considerably thinner and more variable, but significant differences were still observed between selections at all measurement periods (Fig. 3.3). At both sites, differences between selections were much larger at the beginning of the season, with only stands with the highest initial density self-thinning throughout the season. However, due to the thinner stands at this site tiller mortality was greatly reduced. In 2011, the low initial tiller density in Cookshire-Eaton resulted in no observed decrease over the course of the season, however in 2012 all stands displayed higher initial tiller density, and correspondingly average tiller mortality increased to 14% over the season. Once stands had more fully established at the Cookshire-Eaton site in 2012, trends in average tiller decrease also began to align more closely with those seen at Ste-Anne-de-Bellevue with an average decrease of 25% across all Sunburst lineage selections, 21% across Cave-in-Rock lineage selections, and 11% across Summer selections, while Sandlover did not decrease. Additional figures displaying tiller density can be found in the appendix (A3.8 – A3.9).

High tiller mortality as the season progresses is common in grasses, with crops such as ryegrass losing 60% of their tillers prematurely (Ong, Marshall, and Saoar 1978). The large majority of these are small vegetative tillers which senesce in the sward after being shaded out by the larger reproductive tillers. In perennial grasses such as switchgrass high vegetative tiller production occurs in the spring with subsequent mortality throughout the season (Mitchell *et al.* 1998). Previous studies have also reported differences in tiller density among populations (Boe and Beck 2008; Das, Fuentes, and Taliaferro 2004).

The results of the present study support previous findings that indicate there is likely an optimal tiller density for efficient capture of solar radiation which swards target through increased vegetative tiller production or self-thinning. This optimum is known as equilibrium density (Zarroug, Nelson, and Sleper 1984). Madakadze *et al.* (1998) found that populations of switchgrass had differing leaf area indices associated with various heights of their canopy. The authors also found that leaf angle and leaf area index varied by cultivar, indicating that different populations have different canopy structures. The variability in tiller mortality among selections in the present study may indicate that tiller plasticity also plays a large role in this variability. Boe and Casler (2005) reported a large degree of plasticity in tiller morphology within populations. In the present study this plasticity seemed to allow tiller density across many selections to converge towards equilibrium density. The Sunburst population had the highest initial density and displayed >40% tiller mortality over the season (Fig. 3.1, Fig. 3.3), while conversely Sandlover, the selection with the lowest initial tiller density, on average did not display any tiller mortality.

A trend was also noted for differences in decrease in the most advanced selections when compared to their parent cultivars, appearing to be due to lower initial tiller density in the advanced selections at the beginning of the season. Redfearn *et al.* (1997) examined tiller density in switchgrass plots in Iowa and Nebraska managed for forage and found G x E interactions to be present for both yield and tiller density, with Cave-in-Rock producing the lowest tiller number but highest forage yield. The authors noted that plant density differed with location even between selections from the same base population. In the present study at Ste-Anne-de-Bellevue average tiller mortality over both years was

10% lower in Bluejacket 2 than in Sunburst, and 13% lower in Cave-in-Rock 2 than in the Cave-in-Rock base population. However, there was an average increase in tiller loss in the Summer lineage, with mortality on average 16% higher in Tecumseh 2 than in Summer. This was likely due to Tecumseh 2 establishing more quickly and having a higher initial density than Summer in 2011. In 2012, the Summer population was more fully established and there was no significant difference between the two. These trends suggest some shifts in progress among the selection lineages in the study.

3.3.5 Tiller morphology/composition

The percentage of reproductive tillers, average weight per reproductive tiller and average weight per tiller can be seen in Table 3.4. The reproductive fraction increased from 2011 to 2012 in Cookshire-Eaton, while the opposite was observed at Ste-Anne-de-Bellevue. This is likely a response to the low and high initial densities over the same period in Cookshire-Eaton and Ste-Anne-de-Bellevue, respectively. In general, the Sunburst lineage produced the lowest percentage of reproductive tillers while the Summer lineage produced the highest. This same result was noted between the Sunburst and Summer base populations by Boe (2007) in South Dakota, who also found significant variation between cultivar and location. Reproductive tillers have been found to be six times heavier than vegetative tillers (Boe and Casler 2005), and they persist throughout the season while some vegetative tillers senesce. Biomass yield in swards has been shown to be associated with fraction of reproductive tillers (Boe and Casler 2005) and increased mass tiller⁻¹ (Boe 2007). At both sites, Cave-in-Rock 2 reproductive tillers were among the heaviest of all selections. The Sunburst lineage consistently ranked among the lowest selections in terms of average overall tiller mass, while Cave-in-Rock 2 again ranked consistently among the highest selections. Given the fact that the Cave-in-Rock 2 selection did not have the highest tiller density or fraction of reproductive tillers, this suggests that mass reproductive tiller⁻¹ had an important effect on yield.

Madakadze *et al.* (1992) reported an average increase in tiller density (22%), height and diameter between the first and second production years. Although the authors did not note if exclusively reproductive tillers were measured, overall plant height invariably reflects the height of the tallest reproductive tiller as vegetative tillers are much

shorter. The present study confirms average increases in yield, density, height, and average mass of reproductive tillers, but not necessarily an increase in mass of tillers overall. This data suggests that the yield increases are likely due to an increase in the average weight of reproductive tillers and an average increase in the number of the smaller vegetative tillers.

3.3.3 Height

Significant differences were observed between the heights of selections at all periods observed throughout all site-years (Fig. 3.4, Fig. 3.5), however, trends varied by site. The trends observed were generally supported by previous studies which have indicated that later maturing varieties produce taller tillers due to their slow rates of leaf appearance (Madakadze *et al.* 1992). In all 4 site-years, Cave-in-Rock 2 was significantly taller than the other selections in its lineage at both sites in 2012, reaching an average of 201 cm in Ste-Anne-de-Bellevue. Over all site-years this represented an average increase of 12 cm or 7% over the parent cultivar. Despite a difficult initial establishment year, the Cave-in-Rock selections were the three tallest recorded in 2012 at Cookshire-Eaton. The dry summer of 2012 allowed the Tecumseh and Tecumseh 2 selections to perform very well in Ste-Anne-de-Bellevue, attaining the second and third highest average heights, however, there was no significant increase in height over the parent cultivar, Summer.

As with tiller density, the height of the Sunburst lineage selections were depressed at the Cookshire-Eaton site (Fig. 3.5). The Bluejacket and Bluejacket 2 selections performed well in 2011, but only Bluejacket 2 maintained height potential in 2012, with Sunburst and Bluejacket becoming the two worst-performing selections.

No meaningful height differences were observed among big bluestem selections, however there was a significant increase in height in the second year at both sites. Average height increased from 144 to 192 cm (33%) at the Cookshire-Eaton site and from 194 to 216 cm (11%) at Ste-Anne-de-Bellevue in 2011 and 2012 respectively. Additional figures displaying height data for all selection lineages can be found in the appendix (A3.13 – A3.18).

3.3.4 Maturity

There were significant differences observed among maturity stages at both sites for all measurement periods (Fig. 3.6, Fig. 3.7). The differences observed were almost exclusively between selections in different selection lineages and not within a lineage. The Sunburst selections displayed the earliest maturity, followed by the Summer selections, with the Cave-in-Rock and Sandlover selections displaying the latest maturity. These findings are supported by recent literature linking latitude of origin with maturity (Casler *et al.* 2004; Boe 2007). The latitudes of origin of the selections in this study rank in the same order as the maturity observed (Table 3.1). The moisture content of the selections at harvest also mirrored this pattern, with significant differences observed between selections related to maturity at harvest (Table 3.3). No significant moisture differences were observed between selections in a single lineage. Additional figures displaying mean stage count by selection lineage can be found in the appendix (A3.19 – A3.24).

Previous studies using artificial lighting have demonstrated that switchgrass maturity can be delayed in germplasm of Northern origin with control of day length, suggesting that dormancy in this species is a true photoperiodic response (Parrish and Fike 2005). Similar results have been found for big bluestem (Vermerris 2008). This reinforces the concept that maturity date is a trait strongly linked to latitude of origin and will likely prove to be very difficult for breeders to modify from the base population. For example, the Summer cultivar was originally selected for early maturity, while Sunburst was selected for large seed size and mass (Casler 2012). Despite this, the maturity ratings observed in the present study indicate that, as with the ‘early’ selections, the maturity of these selections largely remained dictated by their respective latitudes of origin.

3.3.6 Effects of harvest date on biomass yield and quality variables

In Ste-Anne-de-Bellevue, significant differences were observed between fall and spring harvest dates for yield, moisture content, and cellulose content (Table 3.5). No differences in lignin content were observed with the exception of the prairie view population. Yield differences were significant between species at both harvest dates, with switchgrass selections outyielding the big bluestem. Spring harvest resulted in an average

41% yield reduction compared to a fall harvest. At Cookshire-Eaton, an average yield loss of 14% was observed which was only significant in the highest-yielding selection, Cave-in-Rock 2, which had an average loss of 56% (Table 3.6). The low yield loss values at that site are likely a result of the variable establishment and thin stands in 2011. Reductions of up to 40% in biomass yield have also been reported in Pennsylvania, where this yield loss was attributed to the loss of panicles and leaves, the leaching of stem components such as minerals and cell solubles (Adler *et al.* 2006), and the translocation of mobile nutrients and N-rich compounds into underground perennial structures, a process which may continue into December (Parrish and Fike 2005). These processes have also been shown to decrease the ash fraction in the spring (Adler *et al.* 2006; Burvall 1997), an effect that was only noted in this study at Cookshire-Eaton and not at Ste-Anne-de-Bellevue. The lack of change in ash content at Ste-Anne-de-Bellevue may have been due to soil contamination. The colour of the ash in all spring samples collected at that site was a deep brick orange, while the samples collected in Cookshire-Eaton were the typical white. This may have been due to the exposed nature of the plots in relation to dust from nearby roads and spring soil tillage, or to increased soil contact due to the particularly heavy snowfall.

The decrease in moisture content in the spring biomass compared to the fall averaged 74%, falling from 45 to 11% moisture content by dry weight (Table 3.5). The very high fall values for Cave-in-Rock are a result of its late maturity and the harvesting process; material was harvested directly and not left to dry in windrows as is common producer practice (Samson 2007). The calorific value for combustion of herbaceous biomass declines linearly with rising moisture content, making this an important measure for certain applications (Prochnow *et al.* 2009). However, drying usually also takes place in covered storage, with fall-baled material at 16-17% declining to 12-14% by the following spring (Samson 2007).

The cell walls of grasses contain cellulose, hemicellulose, lignin, and acids including p-coumaric, phenolic and ferulic acids (Vogel 2008; Sarath *et al.* 2011). The increase in cellulose content is likely related to increased stem fraction and the reduction of mineral and cell soluble contents in overwintered materials. Big bluestem selections tended to have slightly higher cellulose contents than switchgrass, again likely due to

increased stem fraction. Jefferson and McCaughey (2012) found differences in cellulose content between spring-harvested cultivars with Cave-in-Rock having a significantly lower cellulose content than either Summer or Sunburst, which had no difference between them. In the present study, the original populations of Cave-in-Rock switchgrass and Prairieview big bluestem both had higher cellulose concentrations than the advanced selections, although these trends were only significant in the spring. These trends were not seen at the Cookshire-Eaton site, likely due to the different overall tiller composition and plant heights described earlier. Previous studies have reported very significant differences between stem and leaves in concentration of ash fraction elements including K, Ca (Lemus, Parrish, and Wolf 2009) and Si (Lanning and Eleuterius 1989; Samson *et al.* 2008), allowing even small differences in leaf and stem fractions to affect analyses for ash or energy.

There were no significant differences in HHV observed between selections or between harvest dates, with Ste-Anne-de-Bellevue and Cookshire-Eaton averaging 18.9 and 19.3 kJ g⁻¹ respectively over both harvest times. A previous analysis of four varieties of fall-harvested switchgrass determined an average energy content of 18.8 ±0.2 kJ g⁻¹ with no significant differences between varieties (Hu *et al.* 2010), while Madakadze *et al.* (1998b) reported a lower energy yield of 17.4 kJ g⁻¹ for Cave-in-Rock harvested in the fall in Southern Quebec.

Turn *et al.* (1997) have indicated that the leaching process increases HHV in herbaceous biomass through removal of inorganic constituents. However, in the present study no effect was observed for harvest time on HHV. Experiences with spring harvesting reed canarygrass (*Phalaris arundinaceae* L.) in Sweden demonstrated significant reductions in Cl, K, S, alkali, ash and several other important quality factors, but curiously also a decrease in HHV (Burvall 1997). The authors also noted large variation in HHV and ash content related to soil type, with clay soils having much lower values than humus rich or sandy soils. The elemental composition of spring-harvested switchgrass, relationship to soil factors and implications for energy applications are discussed in depth later in this document (See chapter 5).

In a variety trial with 8 varieties Sladden *et al.* (1991) found no difference in cellulose or ash content, and only slight differences in hemicellulose, with the variety

Summer ranking at the low end of the spectrum. The authors also found Cave-in-Rock to be significantly lower than several other cultivars for both ADF and NDF. The authors concluded that little difference was present in fiber composition between switchgrass cultivars when harvested at the same maturity and that differences observed were likely related to maturity at harvest.

3.5 CONCLUSION

Significant differences were observed among selections for yield, tiller density, height, maturity, reproductive tiller fraction and tiller mass. These differences varied between sites. Sunburst germplasm-derived selections proved to be particularly ill-suited to the more humid environment and heavier, imperfectly-drained soil at Cookshire-Eaton. The differences in maturity and moisture content observed appear to correspond to the germplasm latitude of origin, and earlier maturity typically related to lower moisture content at harvest. Big bluestem was slower to establish than switchgrass, requiring a longer establishment period to attain yield potential.

Delayed spring harvest resulted in a large decrease in moisture content and a slight increase in cellulose content, however yield losses approached 40%. Unlike in previous studies the ash content was not affected by harvest date, nor was the HHV. These findings should be taken into account when considering spring harvesting as a management practice for Eastern Canada.

The selections tested in the present experiment were the result of several cycles of selection and do not represent commercial varieties, but steps towards their development. In some cases the effects of this selection process have modified plant traits within a given lineage. The trend for increased yield in the Cave-in-Rock 2 selections suggests that the continuation of a local selection programme could further increase yields. With considerable increases in switchgrass acreage in recent years, the introduction of adapted cultivars to Eastern Canada could prove very beneficial. The results of this study indicate that local selection programmes have the potential to modify characteristics including height and tiller density to produce adapted varieties of switchgrass for Southern Quebec.

Table 3.1: Origins of the commercial switchgrass and big bluestem varieties evaluated in the present study and the selections derived from them through a local selection programme in Southern Quebec.

Variety/Selection	Year of Release	Site of Origin	Latitude of origin (°N)	References
Switchgrass				
Cave-in-Rock Cave-in-Rock 2 Cave-in-Rock Early	1958	Cave-in-rock, Illinois	37	(Jefferson and McCaughey 2012)
Summer Tecumseh Tecumseh 2	1953	Nebraska City, Nebraska	41	(Jefferson and McCaughey 2012; Elbersen 2001)
Sunburst Bluejacket Bluejacket 2 Bluejacket Early	1998	Union County, South Dakota	43	(Jefferson and McCaughey 2012; Elbersen <i>et al.</i> 2000)
Sandlover	2009	Northern Nebraska	36-40	(Vermerris 2008; Hopkins <i>et al.</i> 1995b)
Big bluestem				
Prairieview Prairieview 2 Prairieview Early	1994	20 sites across Indiana	38-41	(Burgdorf, 2009)

Table 3.2: Yield of 11 selections of switchgrass and 3 selections of big bluestem seeded in 2010 and harvested in fall 2011 and 2012 at two sites in Southern Quebec (in Mg ha⁻¹).

Entry	Ste-Anne-de-Bellevue		Cookshire-Eaton	
	2011	2012	2011	2012
Switchgrass				
Sunburst	7.7	9.1	3.6	5.6
Bluejacket 1	9.0	9.1	4.2	4.8
Bluejacket 2	9.7	9.1	5.0	5.3
Bluejacket early	8.1	9.1	3.6	4.7
Summer	9.2	11.2	4.9	6.8
Tecumseh 1	8.2	10.4	2.1	4.2
Tecumseh 2	9.3	10.3	4.6	6.7
Cave-in-Rock	10.0	10.3	3.5	7.3
Cave-in-Rock 2	10.2	11.1	5.9	9.0
Cave-in-Rock early	8.8	9.9	2.5	6.4
Sandlover	9.3	9.7	1.6	5.3
Switchgrass mean	9.0	9.9	3.8	6.0
Big Bluestem				
Prairie view	6.0	7.5	2.1	5.1
Prairie view 2	5.6	8.8	1.9	5.6
Prairie view early	6.6	8.4	1.6	6.1
Big bluestem mean	6.1	8.2	1.9	5.6
Overall mean	8.4	9.6	3.4	5.9
P value	<.0001	0.0046	<.0001	0.0004
LSD (5%)	1.1	1.7	1.4	1.6

Table 3.3: Moisture content (%) at fall harvest of 11 selections of switchgrass and 3 selections of big bluestem at two sites in Southern Quebec.

Entry	Ste-Anne-de-Bellevue		Cookshire-Eaton	
	2011	2012	2011	2012
Switchgrass				
Sunburst	36.8	34.1	33.8	27.6
Bluejacket 1	36.1	33.8	31.6	31.8
Bluejacket 2	35.5	35.4	32.7	30.1
Bluejacket early	38.1	35.2	30.1	28.0
Summer	39.7	31.9	26.1	24.4
Tecumseh 1	37.7	35.8	37.5	29.3
Tecumseh 2	40.3	34.3	31.4	20.2
Cave-in-Rock	46.1	41.0	43.6	29.8
Cave-in-Rock 2	45.8	44.5	44.9	39.6
Cave-in-Rock early	44.1	41.8	35.5	31.6
Sandlover	49.1	44.5	38.7	34.8
Switchgrass mean	40.8	37.5	35.1	29.7
Big Bluestem				
Prairie view	43.7	39.6	44.3	38.9
Prairie view 2	46.8	41.0	35.6	30.9
Prairie view early	43.8	39.0	44.0	33.7
Big bluestem mean	44.8	39.9	41.3	34.5
Overall mean	41.7	38.0	36.4	30.8
P value	<.0001	<.0001	0.0002	0.0023
LSD (5%)	3.3	3.2	7.9	7.6

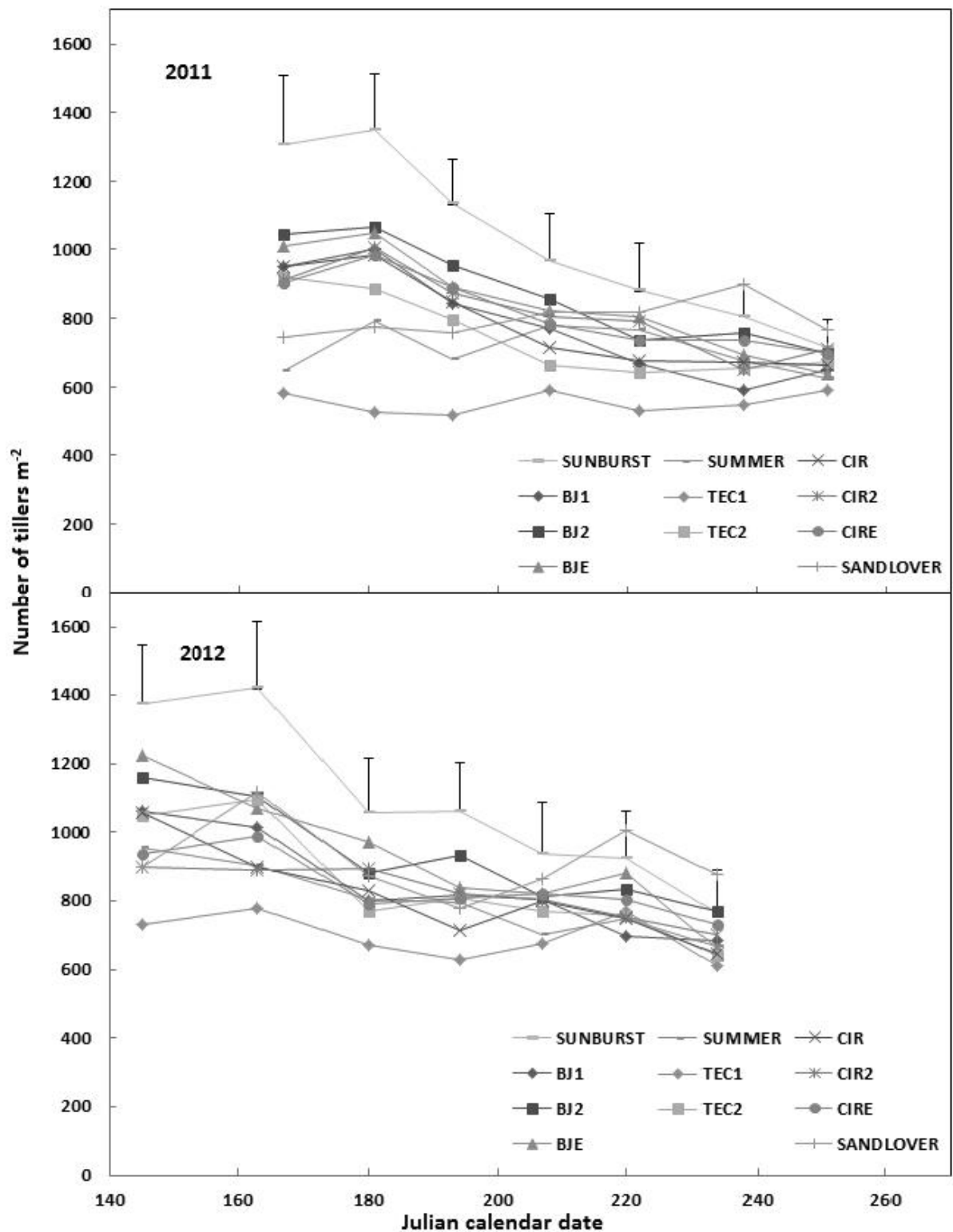


Figure 3.1: Number of tillers m⁻² of 11 selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

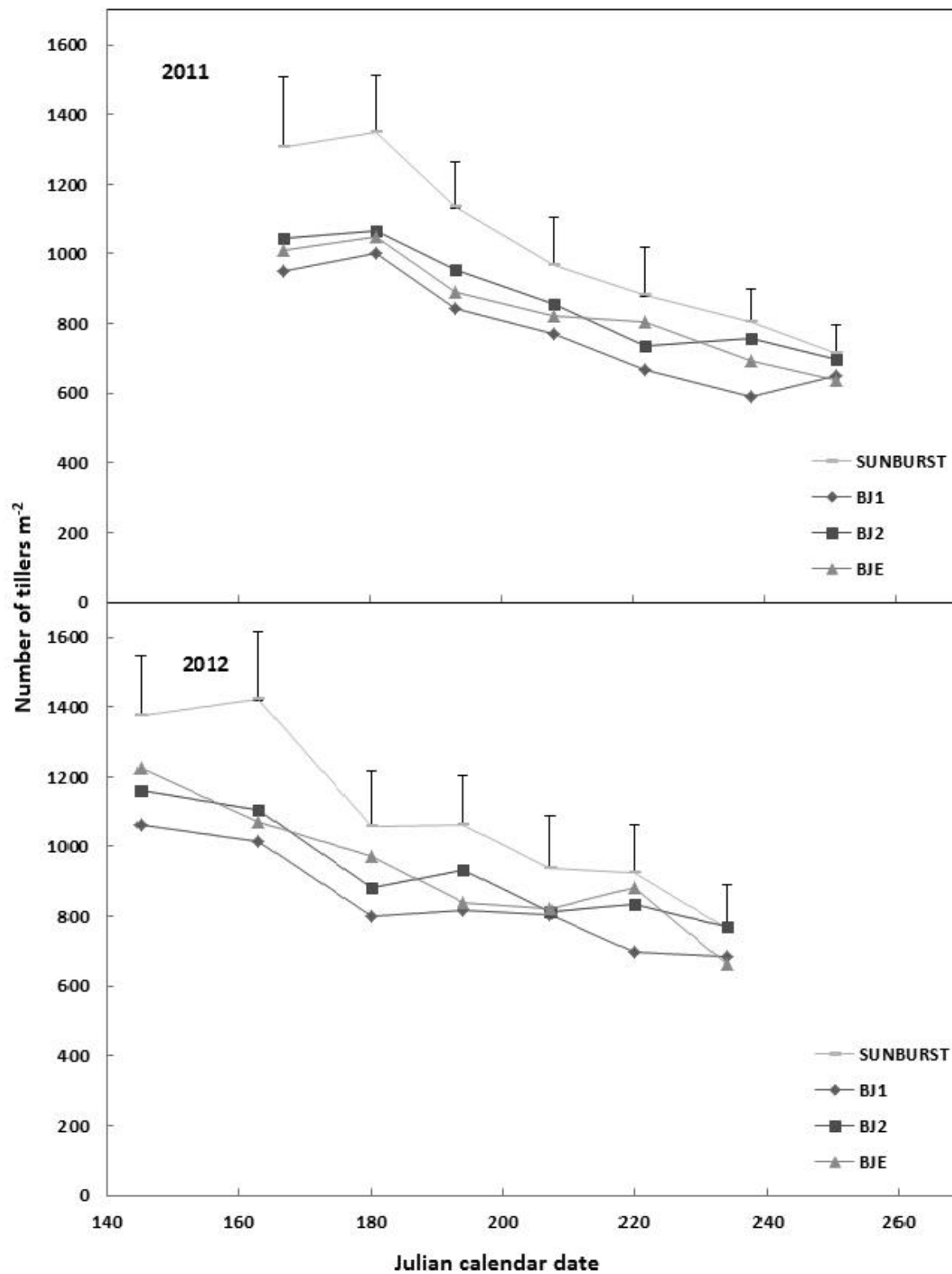


Figure 3.2: Number of tillers m^{-2} of Sunburst lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

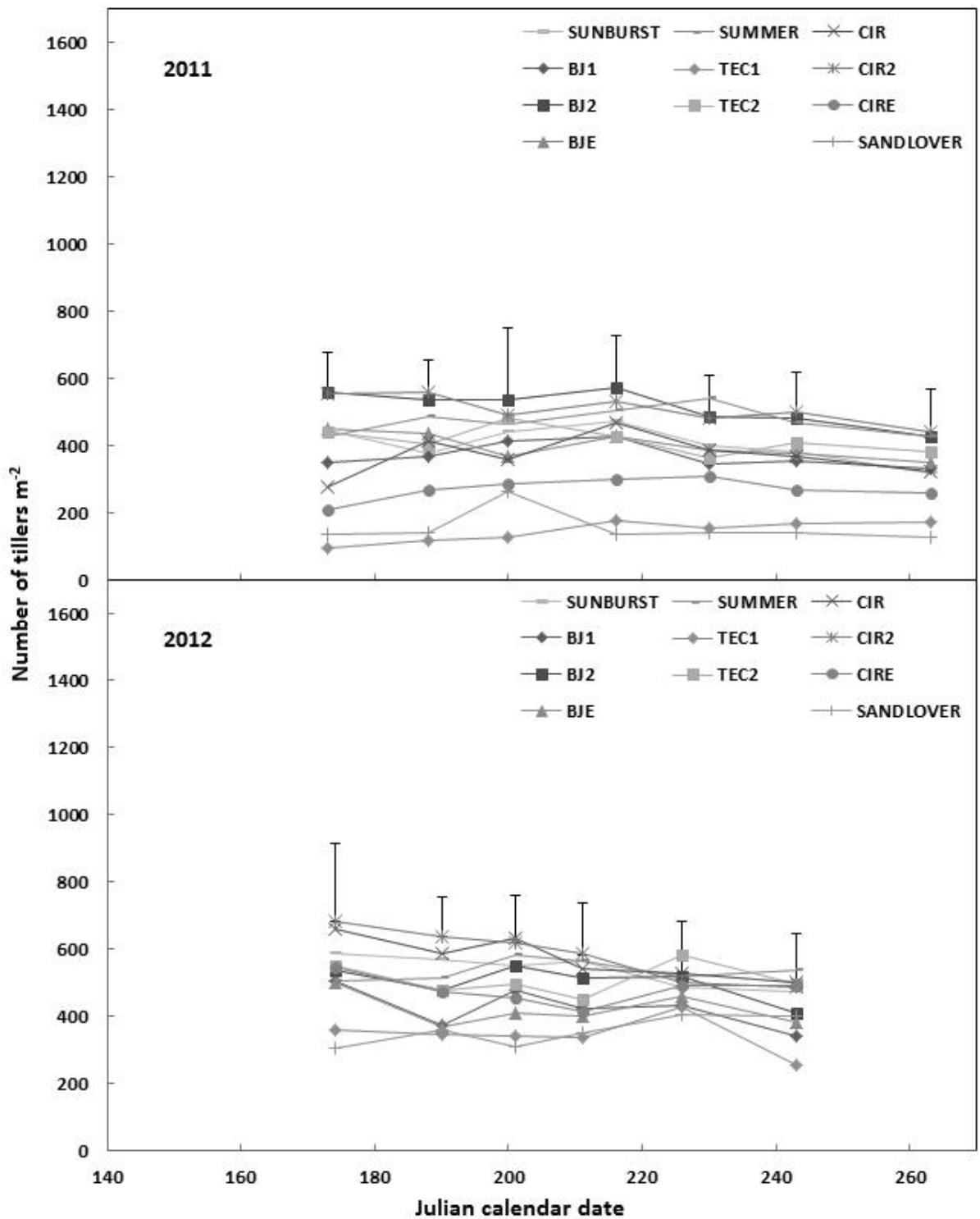


Figure 3.3: Number of tillers m⁻² of 11 selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.

Table 3.4: Fraction of reproductive tillers, average dry matter mass per reproductive tiller, and average dry matter mass per tiller overall of 11 selections of switchgrass in 2011 and 2012 grown at 2 sites in Southern Quebec and harvested in October.

Cookshire-Eaton						
Entry	Reproductive tiller fraction		Mass reproductive tiller ⁻¹ (g)		Mass tiller ⁻¹ (g)	
	2011	2012	2011	2012	2011	2012
Sunburst	0.27	0.27	1.9	1.8	1.2	0.9
BJ	0.48	0.44	2.2	1.7	1.4	1.2
BJ2	0.21	0.23	2.4	1.9	1.3	1.2
BJE	0.22	0.31	2.4	1.7	1.2	1.1
Summer	0.51	0.51	1.7	1.8	1.2	1.2
TEC	0.50	0.66	2.0	2.6	1.4	2.3
TEC2	0.49	0.56	2.0	2.1	1.3	1.7
CIR	0.25	0.47	2.2	2.4	1.2	1.7
CIR2	0.40	0.48	2.9	3.3	1.9	2.2
CIRE	0.16	0.34	1.8	2.3	0.9	1.4
Sandlover	0.14	0.41	1.2	2.5	0.8	1.5
Mean	0.33	0.42	2.1	2.2	1.3	1.5
P value	0.0008	<.0001	0.1244	0.0035	0.0424	0.0002
LSD	0.19	0.14	0.97	0.73	0.57	0.51
Ste-Anne-de-Bellevue						
Sunburst	0.45	0.27	2.4	2.6	1.5	1.2
BJ	0.52	0.37	2.5	2.7	1.7	1.5
BJ2	0.36	0.28	2.7	3.4	1.5	1.5
BJE	0.38	0.33	2.7	3.0	1.6	1.6
Summer	0.63	0.52	2.4	3.1	1.8	2.0
TEC	0.80	0.55	3.0	3.0	2.6	2.0
TEC2	0.59	0.47	2.7	2.9	1.9	1.8
CIR	0.53	0.52	2.8	2.9	1.9	2.0
CIR2	0.49	0.37	2.9	3.2	1.9	1.8
CIRE	0.53	0.36	2.7	2.5	1.8	1.4
Sandlover	0.59	0.39	2.7	2.2	1.9	1.2
Mean	0.53	0.40	2.7	2.9	1.8	1.6
P value	0.0034	<.0001	0.0891	<.0001	0.0019	0.0004
LSD	0.19	0.09	0.39	0.40	0.42	0.39

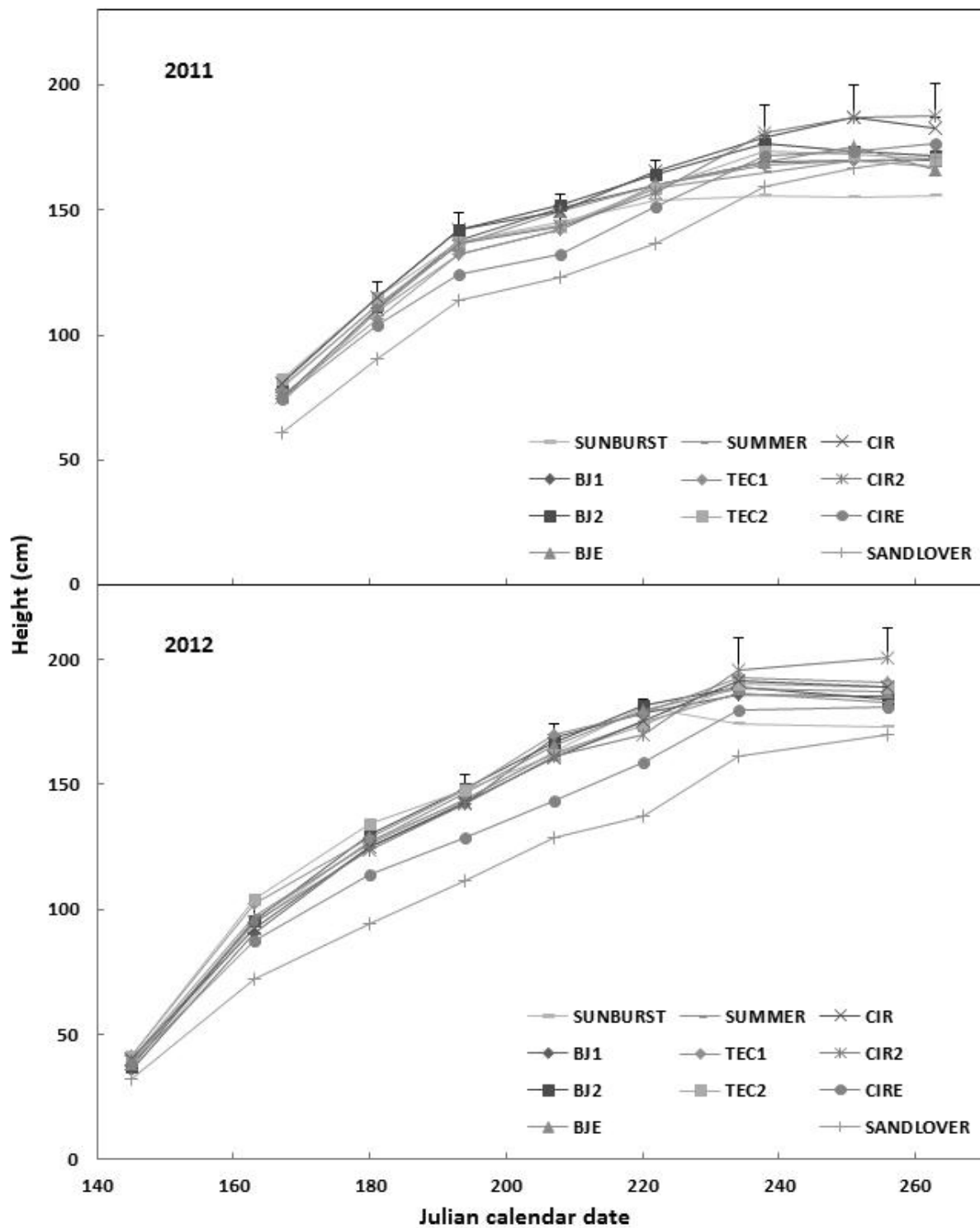


Figure 3.4: Height of 11 selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

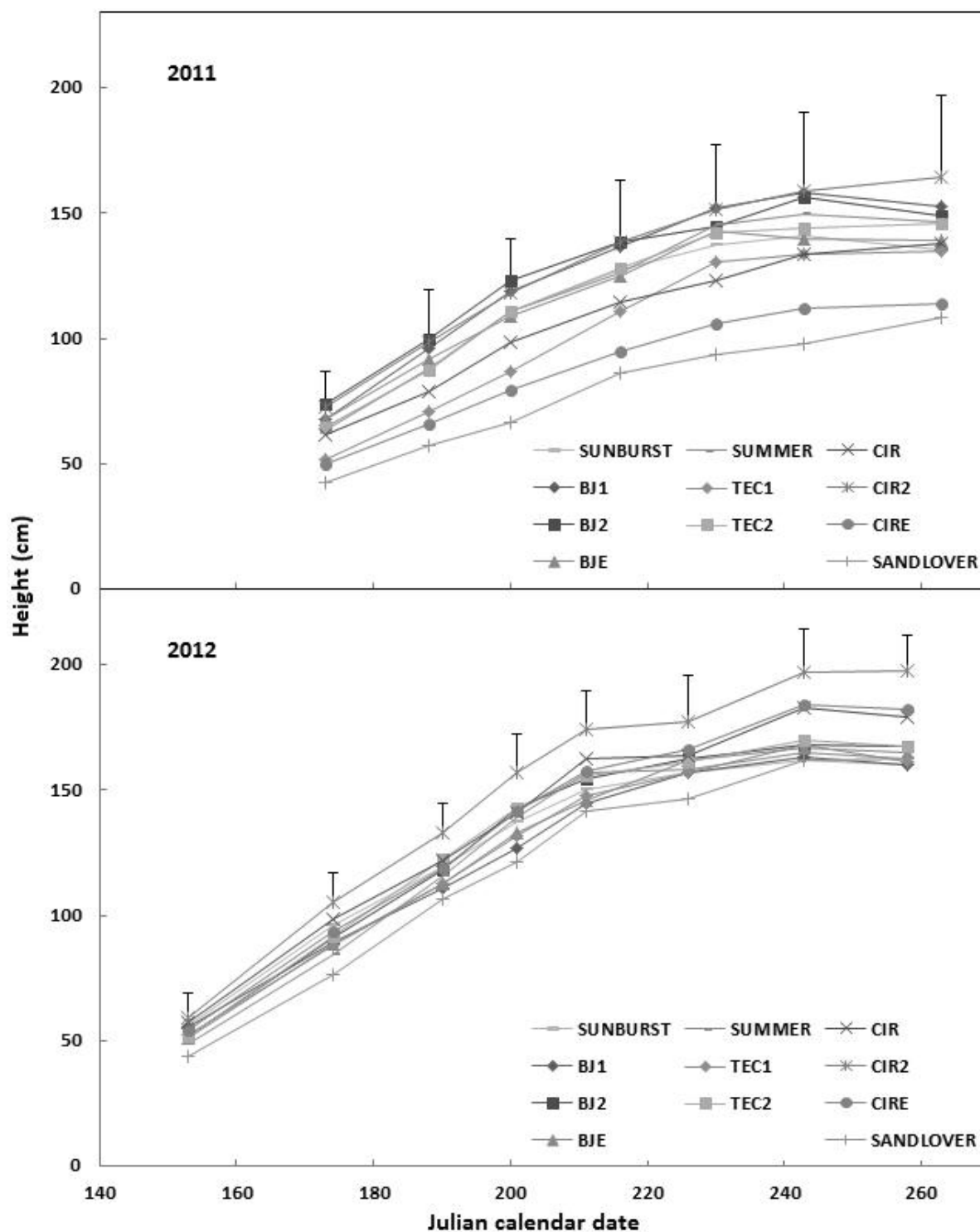


Figure 3.5: Height of 11 selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

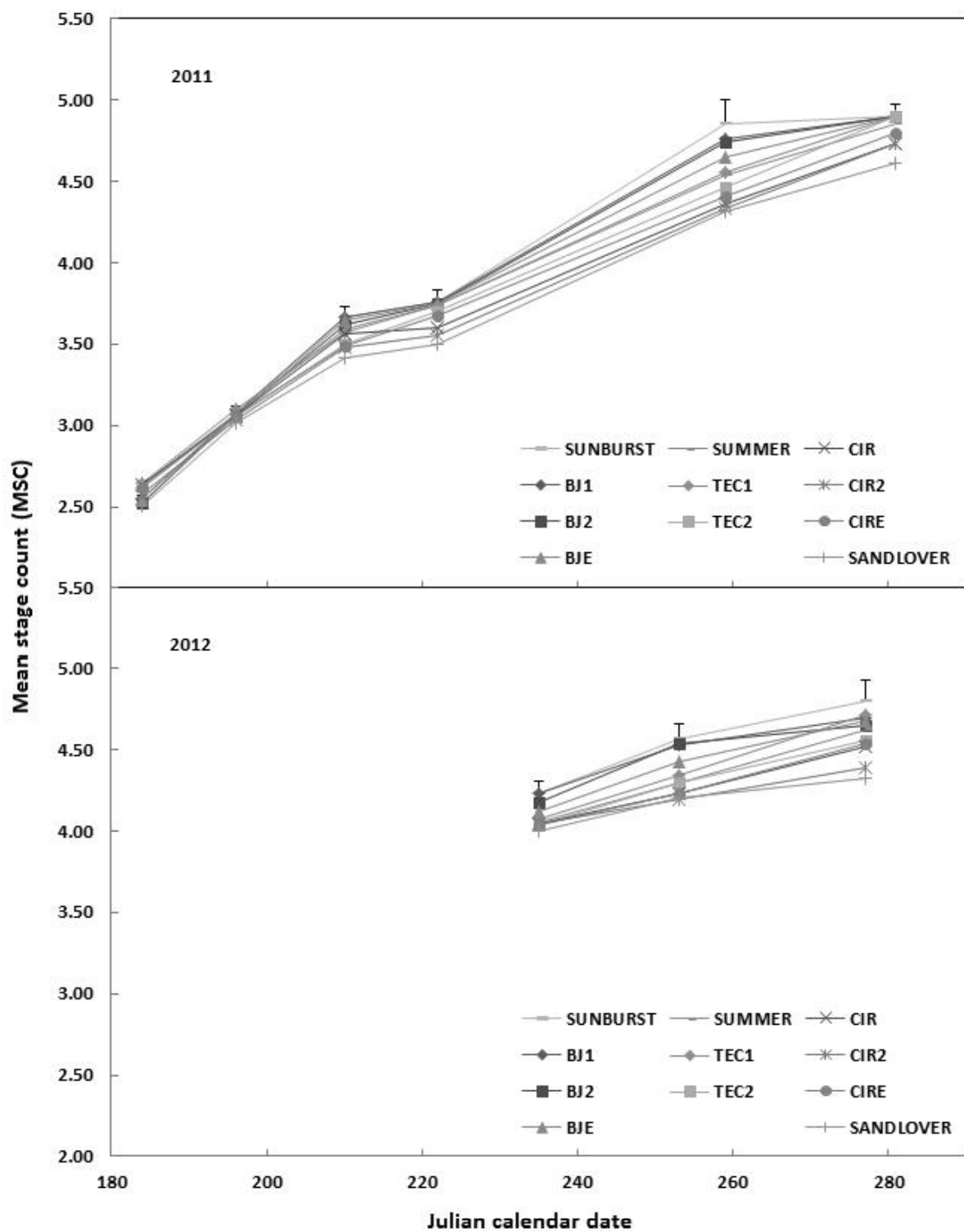


Figure 3.6: Mean stage count of 11 selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

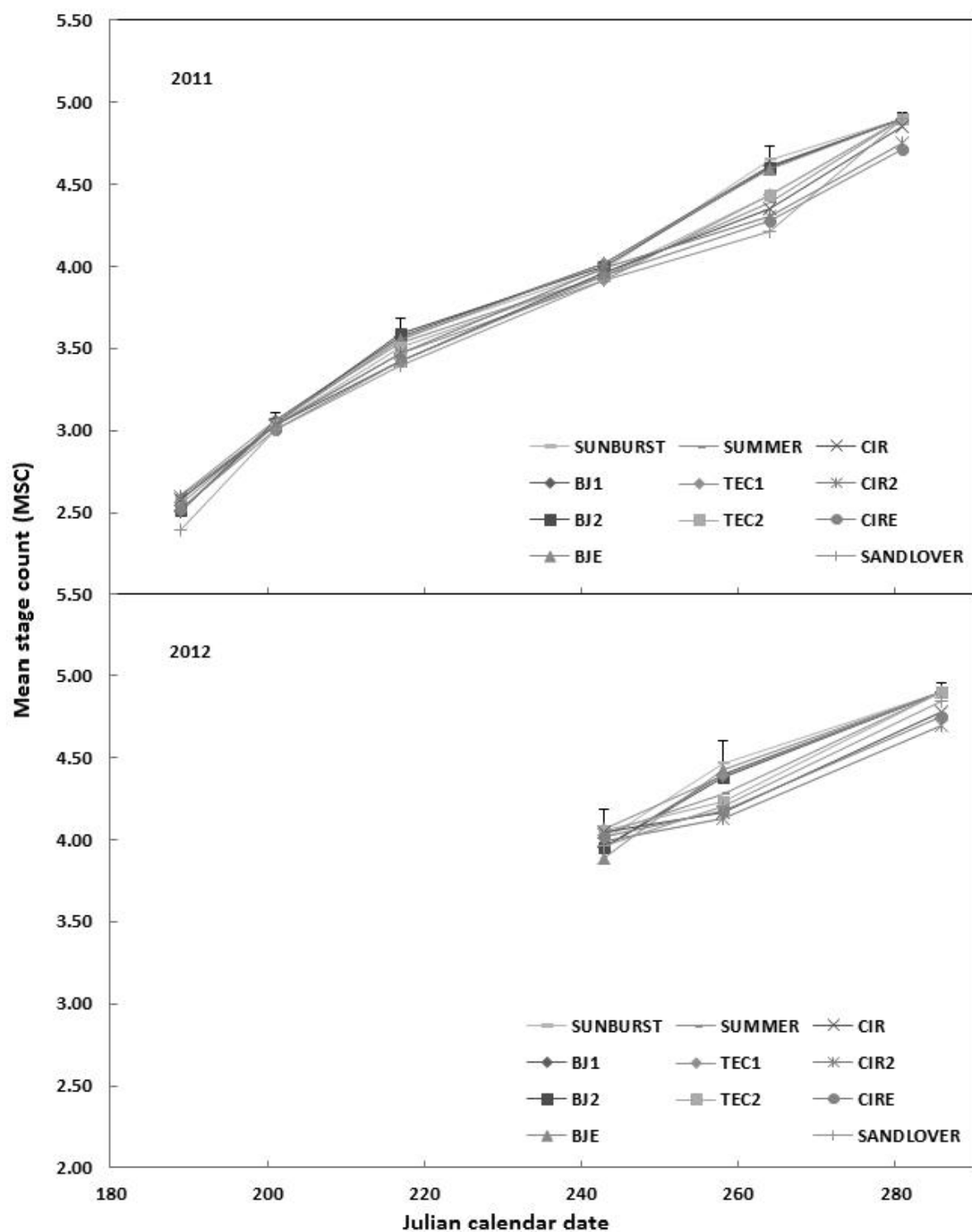


Figure 3.7: Mean stage count of 11 selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

Table 3.5: Fall 2011 and spring 2012 values for yield, moisture content, cell wall components, ash and higher heating value (HHV) of three selections of switchgrass and three selections of big bluestem in Ste-Anne-de-Bellevue, Quebec.

Entry		CIR	CIR2	CIRE	PV	PV2	PVE	P value	LSD
Moisture (%)	Fall	46.1	45.8	44.1	43.7	46.8	43.8	0.1843	3.076
	Spring	11.2	14	10.6	10.7	11.5	10.9	0.326	3.411
	% change†	75.7	69.4	76	75.4	75.4	75.1		
	P value	0.0001	<.0001	0.0002	<.0001	0.0002	0.0002		
	LSD	4.209	2.812	4.925	3.737	4.706	4.2826		
Yield (Mg ha ⁻¹)	Fall	10	10.2	8.8	6	5.6	6.6	<.0001	1.202
	Spring	6.2	6.1	6.6	3.8	2.8	3	<.0001	1.518
	% change†	38.3	40.4	25.2	37.9	49.5	55.1		
	P value	0.0052	0.0102	0.0493	0.053	0.0018	0.0034		
	LSD	1.67	2.253	2.205	2.341	0.8374	1.3616		
Cellulose (g kg ⁻¹)	Fall	419	414	389	439	431	438	0.0056	25.05
	Spring	456	438	429	476	449	447	0.001	17.59
	% change†	-8.8	-5.6	-10.4	-8.3	-4.2	-2.1		
	P value	0.0654	0.0004	0.0042	0.029	0.1361	0.0143		
	LSD	41.2	4.227	16.29	29.42	28.491	5.676		
Hemi-cellulose (g kg ⁻¹)	Fall	315	321	324	316	303	300	0.0593	17.42
	Spring	324	336	340	291	313	307	0.0005	18.97
	% change†	-2.9	-4.7	-4.9	7.9	-3.3	-2.3		
	P value	0.457	0.2528	0.0061	0.056	0.209	0.1208		
	LSD	34.56	35.1	7.173	26.219	20.403	10.329		
Lignin (g kg ⁻¹)	Fall	77	69	65.5	60.1	62.9	63.1	0.2727	14.94
	Spring	90.3	73.5	64.5	79.3	63.7	67.9	<.0001	8.647
	% change†	-17.3	-6.5	1.5	-31.9	-1.3	-7.6		
	P value	0.2283	0.3488	0.8434	0.0094	0.8919	0.2655		
	LSD	28.093	12.857	14.786	9.9	16.149	11.246		
Ash (g kg ⁻¹)	Fall	45.2	48.9	47.4	48.2	49.1	46.3	0.9689	11.14
	Spring	50.4	53.2	50.8	48.3	50.3	55.5	0.9601	17.25
	% change†	-11.5	-8.8	-7.2	-0.2	-2.4	-19.9		
	P value	0.5503	0.4563	0.2225	0.9562	0.7607	0.1186		
	LSD	24.9	16.041	6.897	9.329	11.929	13.538		
HHV (MJ kg ⁻¹)	Fall	18.71	18.96	18.78	18.73	19	18.94	0.5896	0.4463
	Spring	18.96	18.99	18.73	18.85	18.88	18.92	0.761	0.399
	% change†	-1.3	-0.2	0.3	-0.6	0.6	0.1		
	P value	0.4213	0.8845	0.7915	0.4774	0.4568	0.9009		
	LSD	0.887	0.5706	0.5966	0.4781	0.46	0.4408		

†Percent change of fall value remaining in spring; negative values indicate an increase in spring.
CIR, Cave-in-Rock; CIR2, Cave-in-Rock 2; CIRE, Cave-in-Rock early; PV, prairie view; PV2, prairie view 2; PVE, prairie view early

Table 3.6: Fall 2011 and spring 2012 values for yield, moisture content, cell wall components, ash and higher heating value (HHV) of three selections of switchgrass and three selections of big bluestem in Cookshire-Eaton, Quebec.

Entry		CIR	CIR2	CIRE	PV	PV2	PVE	P value	LSD
Moisture (%)	Fall	43.6	44.9	35.5	44.3	35.6	43.9	0.0962	8.8360
	Spring	8.5	8.8	7.3					
	% change	80.4	80.3	79.5					
	P value	0.0007	0.001	0.0106					
	LSD	3.8578	4.8707	12.587					
Yield (Mg ha ⁻¹)	Fall	3.5	5.9	2.5	2.1	1.9	1.6	0.0002	1.2830
	Spring	2.6	2.6	2.3	0.0	0.0	0.0		
	% change	26.2	56.3	8.5	100.0	100.0	100.0		
	P value	0.2539	0.0188	0.6558					
	LSD	2.5201	1.9826	1.7471					
Cellulose (g kg ⁻¹)	Fall	415	396	376	385	381	371	0.2719	40.852
	Spring	445	454	415				0.001	10.307
	% change	-7.2	-14.7	-10.3					
	P value	0.3218	0.1236	0.0132					
	LSD	98.893	97.169	19.321					
Hemi-cellulose (g kg ⁻¹)	Fall	352	317	339	316	301	290	0.0088	29.914
	Spring	345	304	333				0.0001	6.325
	% change	1.7	4.0	1.7					
	P value	0.3591	0.2353	0.2808					
	LSD	22.334	32.475	17.053					
Lignin (g kg ⁻¹)	Fall	73	77	61	55	70	66	0.1155	16.628
	Spring	72	89	63				0.0009	6.488
	% change	0.5	-16.2	-3.4					
	P value	0.9687	0.3272	0.5228					
	LSD	34.118	41.443	11.684					
Ash (g kg ⁻¹)	Fall	39	48	45	45	46	45	0.6675	11.274
	Spring	33	30	32				0.0179	1.539
	% change	17.5	38.0	30.2					
	P value	0.1626	0.0725	0.0625					
	LSD	13.665	22.343	15.498					
HHV (MJ kg ⁻¹)	Fall	19.15	19.09	19.31	19.22	19.20	19.27	0.7519	3.452
	Spring	19.35	19.36	19.25				0.6191	3.264
	% change	-1.0	-1.4	0.3					
	P value	0.158	0.4399	0.2681					
	LSD	0.387	1.2138	0.1836					

†Percent change of fall value remaining in spring; negative values indicate an increase in spring. CIR, Cave-in-Rock; CIR2, Cave-in-Rock 2; CIRE, Cave-in-Rock early; PV, prairie view; PV2, prairie view 2; PVE, prairie view early

CONNECTING TEXT FOR CHAPTER 4

The previous chapter evaluated the performance of several new selections of switchgrass in Southern Quebec. Significant differences were noted between selections for all characteristics studied, with these effects varying by site. In some cases local selections outperformed their parent populations, suggesting that local selection programmes have the capacity to produce improved varieties.

In the following chapter we examined the effects of various treatments on poorly established switchgrass fields.

The following chapter was written as a manuscript which will be submitted for future publication. The manuscript was co-authored by the candidate, Dr. Philippe Seguin, Department of Plant Science, McGill University, Dr. Arif Mustafa, Department of Animal Science, McGill University, and Roger Samson, REAP-Canada and Huguette Martel, MAPAQ, Direction régionale de l'Estrie. The candidate carried out the experiments and was the primary author of the manuscript. Dr. P. Seguin provided funds and assistance for this research, including supervisory guidance and reviewing of the manuscript. Mr. Roger Samson provided all germplasm and selections used in the experiment as well as technical assistance in field management and data collection. Huguette Martel provided field and technical support. All authors contributed to editing the manuscript.

CHAPTER 4

RENOVATION STRATEGIES FOR POORLY ESTABLISHED SWITCHGRASS FIELDS

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4.1 ABSTRACT

The development of switchgrass (*Panicum virgatum* L.) as a bioenergy crop has been hampered by producer concerns related to its slow and sometimes difficult establishment. An experiment was conducted in Southern Quebec to test the effects of no till re-seeding, herbicide application and nitrogen fertilization on switchgrass yield in poorly established fields. The re-seeding treatment initially negatively impacted switchgrass yields, while in the following year only the application of all three treatments resulted in a response, producing significantly higher yield than those with re-seeding and nitrogen application alone. In many cases switchgrass is likely able to achieve high yields given enough time to fully establish, even in cases when initial populations are low.

4.2 INTRODUCTION

A major hurdle identified in producer acceptance of switchgrass as a commercial crop is its slow and sometimes difficult establishment (Aiken and Springer 1995; Curran *et al.* 2011; Kludze, Deen, and Dutta 2011). The main causes of poor establishment are poor seed quality, inappropriate bed preparation and planting procedures, weed competition due to lack of proper weed control methods, and lack of rainfall throughout the establishment period (Mitchell and Vogel 2012; Smart and Moser 1999; Curran *et al.* 2011). With the exception of rainfall, all these problems can be addressed by modifying management techniques.

Management strategies for the improvement of switchgrass establishment include application of herbicides, fertilizers (USDA 1986), conservation tillage and no-till methods (Panciera and Jung 1984; Nyoka *et al.* 2007), planting in rotation with a ‘smother crop’ such as winter wheat (McLaughlin and Kszos 2005) and modified seeding rate (Panciera and Jung 1984; West and Kincer 2011).

Weed control in particular has been identified as a critical factor for promoting good establishment in new switchgrass fields (Curran *et al.* 2011; Parrish and Fike 2005; Sanderson *et al.* 1996; McLaughlin and Kszos 2005). To date there has been limited effort to register herbicides for use on switchgrass in Canada. Some success has been reported with Quinclorac, Sulfosulfuron (Curran *et al.* 2011) and preemergence Atrazine (Vogel 1987; McLaughlin and Kszos 2005) applications in the United States, where a range of

products have been evaluated for various broadleaf and grass weeds (Renz, Undersander, and Casler 2009). The use of atrazine for weed control in switchgrass has been well-documented since the 1980's, and switchgrass is often considered to be atrazine-tolerant, with good success in the Northern great plains (Parrish and Fike 2005; Nyoka *et al.* 2007; McLaughlin and Kszos 2005). Atrazine application has also been successfully employed to increase big bluestem yields (Masters 1997). Quinclorac in particular has demonstrated good broad weed control both pre and post-emergence in switchgrass, big bluestem and prairie cordgrass, but is currently not registered in Ontario or Quebec.

Key factors in renovation strategies include the level of suppression of resident vegetation, soil fertility, soil moisture, and type of seeding equipment used (Seguin 1998). No-till seeding into old grass pastures has been successful, but the authors reported that 6 weeks of suppression of the resident vegetation were needed for the new seedlings to become well established (Samson and Moser 1985). While the application of N in such situations is not recommended (Samson 2007), it has improved the growth of other slowly establishing species (Laberge *et al.* 2005). Nitrogen fertilization may indeed improve seedling vigor, however it is essential to achieve good control of the resident vegetation and weeds which also benefit from the treatment (Seguin 1998). The potential of using N fertilization in combination with herbicides when using switchgrass to renovate fields has never been investigated.

An experiment was performed on a commercial switchgrass farm in Cookshire-Eaton, QC in 2010-2012 to determine the effects of reseedling, nitrogen fertilization and herbicide application on poorly established switchgrass fields.

4.3 MATERIAL AND METHODS

4.3.1 Field management

A split-split plot design was used to evaluate a combination of treatments to renovate a poorly established field of switchgrass in Cookshire-Eaton (45°20'57.49"N, 71°47'08.40"W, 243 m elevation). Treatments were assigned to a randomized complete block design with 4 replication. Plot size was 4 x 5 m and the soil was an imperfectly-drained Magog stony loam. The main treatment was reseedling with a no-till seeder while the subtreatment was herbicide application and the sub-subtreatment was N fertilizer

application. Reseeding was done with a Great Plains no-till drill seeder. Plots were reseeded on June 3, 2011 at a rate of 10.5 kg PLS ha⁻¹. The herbicide treatment consisted of two products: atrazine and Option® applied in June 2011. As there are no herbicides presently registered for use on switchgrass in Ontario or Quebec, these two products were selected based on the advice and experiences of local experts. Option® (Foramsulfuron) is a commercial herbicide licensed for the control of annual and perennial grass and broadleaf weeds in field corn. Its use on warm-season grasses is experimental but seems to have produced favorable results in trials across Ontario and Quebec. Atrazine 480 [2-chloro-4-ethylamino-6-isopropylamine-s-triazine] is a commercial herbicide registered for use on field corn in Eastern Canada for the control of broadleaf weeds and wild oats, either as a pre-emergence or post-emergence treatment. The herbicide mix was applied using a backpack sprayer on June 7, 2011. Atrazine 480 was applied at the rate of 1L/ha and Option® at 1.5L/ha. The N treatment was applied to the plots on June 7 using granular urea (54-0-0) at a rate of 60 kg N ha⁻¹. There were thus a total of 8 treatments consisting of all combinations of re-seeding, herbicide and N application.

Plots were harvested both in the fall using a flail-type forage harvester (Fabro Enterprises, Swift Current, SK, Canada) with a cutting surface of 0.6 x 5 m and a cutting height of 8 cm. Plot yields were weighed and subsamples of approximately 500 g were collected from each plot to determine biomass yield on a dry matter basis. Subsamples were dried for 48 hours at 60°C in a forced-air oven and then reweighed to determine moisture content (AOAC 1990, method No. 934.01) and express yields on a dry matter basis.

4.3.2 Statistical analysis

All data were analyzed using the GLM procedure of the SAS statistical software program (SAS Institute, 2003). Unless otherwise noted, tests were considered significant at the 5% probability level. The experiment design was a split-split plot with four replications.

4.4 RESULTS AND DISCUSSION

The first attempt at the experiment was established in 2010 on an area which the producer had identified as problematic and poorly established. Later in the season it was apparent that plants in all plots had vigorously regrown and no differences were present between any of the treatments and plots (data not shown). For this reason the experiment was repeated in 2011 on an even more poorly drained and badly established area with an approximate plant density of 5 plant m⁻².

The results of the fall 2011 harvest indicated a re-seeding effect, with the re-seeded treatments performing more poorly than all others (See Fig. 4.1 and Table 4.1). A seeding x N interaction was also significant, with the reseeded and N fertilized treatments achieving lower yields than those receiving the N treatment alone. In 2012 this effect did not persist, with only the interaction effect between seeding, nitrogen and herbicide application being significant. Plots which received all three treatments produced the highest yields, with a mean of 5016 kg ha⁻¹. This was significantly higher than treatments receiving only the combination of re-seeding and nitrogen treatments.

The use of no-till drills has been endorsed in the Northern great plains, provided the packing wheels give good seed to soil contact (Nyoka *et al.* 2007). In this case it was noted that the furrows opened by the disks of the seeder did not close well in the heavy, humid soils of the experimental site (Fig. 4.3). Good soil contact with switchgrass seeds is a requisite for germination. Follow-up evaluations did not detect a significant difference between the number of seedlings between the seeded and unseeded treatments. In the absence of the germination of new plants, the compaction of the soil by this heavy equipment on the wet spring soils may in fact be a detriment to established plants. In some plots it was observed that the disks of the seeders had bisected the crowns of existing plants. This may explain the negative effect associated with seeding in the first year.

In 2012 the plants may have recovered enough from this treatment to overcome the previous year's setback. The only advantage noted in this period was in the plots which in the previous year received all three treatments; these plots outperformed those receiving only the seeding and nitrogen treatments but not the herbicide treatment. This may be explained by the noted disadvantages produced by applying nitrogen in the

absence of weed control. Due to the slow spring emergence of establishing switchgrass seedlings, spring N applications may nourish annual weeds, giving them a competitive advantage (Nyoka *et al.* 2007; USDA 1986). Soil compaction caused by the re-seeding treatment may exacerbate this effect, preventing the nitrogen from quickly infiltrating to the deeper layers of soil (Soanea and Van Ouwerkerk, 1995). Annual weeds tend to have a much shallower rooting zone than deep-rooted perennials such as switchgrass, which may give them a further advantage under these conditions.

The average yield over all treatments increased from 2663 to 3937 kg ha⁻¹ from 2011 to 2012, respectively, representing an increase of 48%. Research has shown that plots with as few as 10 plants m⁻² may result in adequate stands for biomass production, while as few as 1 plant m⁻² are required for areas under conservation plantings (Parrish and Fike 2005). Switchgrass is capable of self-seeding and can spread by rhizomes to fill in gaps in the canopy and a large degree of phenotypic plasticity in growth habit allow the plant to adapt to variable plant densities (Boe 2007; Boe and Casler 2005; Parrish and Fike 2005). This is supported by a wide range of previous studies which have indicated that stands with very different plant spacings can produce similar biomass yields (Vogel 1987; Muir *et al.* 2000; Sanderson *et al.* 1996).

The Canada Pest Management Regulatory Agency (PMRA) is the body responsible for pesticide registration in Canada. In 2012, switchgrass received a priority rating for butryl-L. The PMRA's priority rating implies that the trial data has already been collected and registration should proceed very soon. The advent of these new weed control tools for switchgrass may simplify establishment.

4.5 CONCLUSION

In the first year following establishment, none of the treatments displayed an effect on plot yield with the exception of the re-seeding treatment, which produced a negative effect. In the subsequent year the only significant difference was between the highest yielding treatment (that which received all three treatments) and the lowest yielding treatment (the plots which received seeding and fertilizer but not herbicide). These results suggest that spring re-seeding may not be an effective strategy for renovating apparently poorly established switchgrass fields, and may in fact have a

negative effect under the studied conditions. The application of herbicides visibly controlled weeds but did not result in a significant yield increase except in 2012 when compared to the herbicide-free treatment in the presence of the re-seeding and N treatments. Considering the large average increase in plot yield in the second year, the most significant factor in renovating poorly established fields may simply be time, however, the use of non-selective herbicides such as glyphosate early in the spring before switchgrass initiate its growth might be a good option to reduce competition from weeds.

Table 4.1: ANOVA test results for the effects of seeding, nitrogen and herbicide on the yield of switchgrass plots in Cookshire-Eaton, QC.

Source	2011	2012
	P value	P value
S	0.0481	0.9213
H	0.6657	0.0654
S*H	0.7554	0.183
N	0.3624	0.9781
S*N	0.0454	0.3613
H*N	0.7789	0.8412
S*H*N	0.2602	0.0482

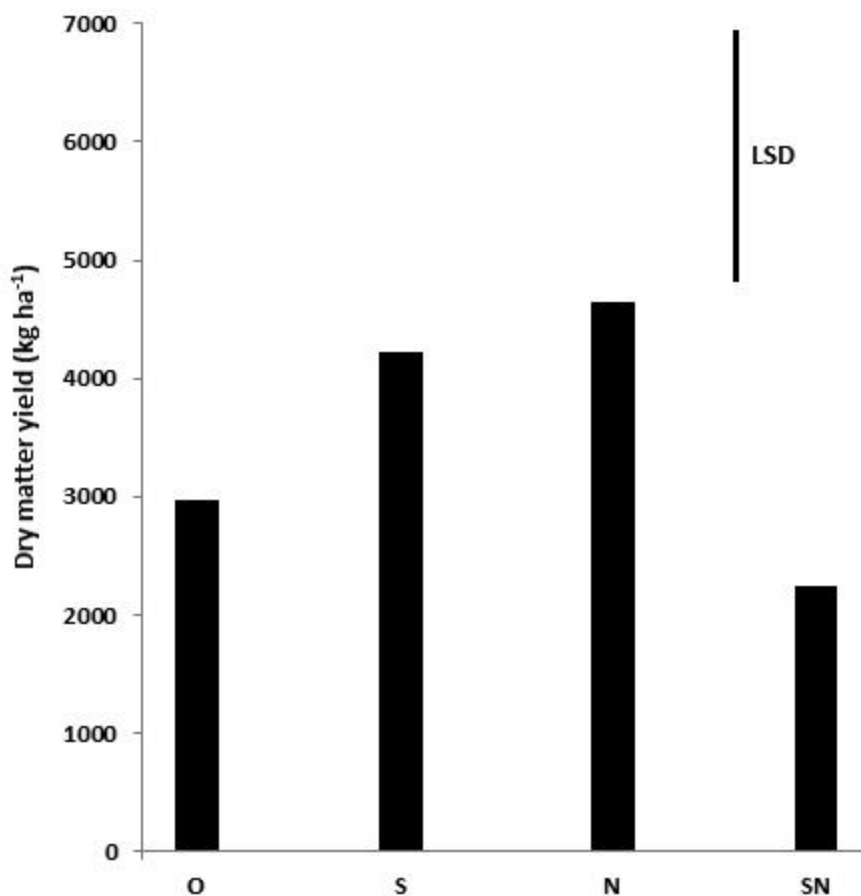


Figure 4.1: Average dry matter yield of switchgrass plots in Cookshire-Eaton, QC subject to various renovation treatments in the spring of the post-seeding year (O, no treatment, S, no-till seeding, N, nitrogen fertilizer). Results are for the renovation year and illustrate the re-seeding by nitrogen fertilizer interaction.

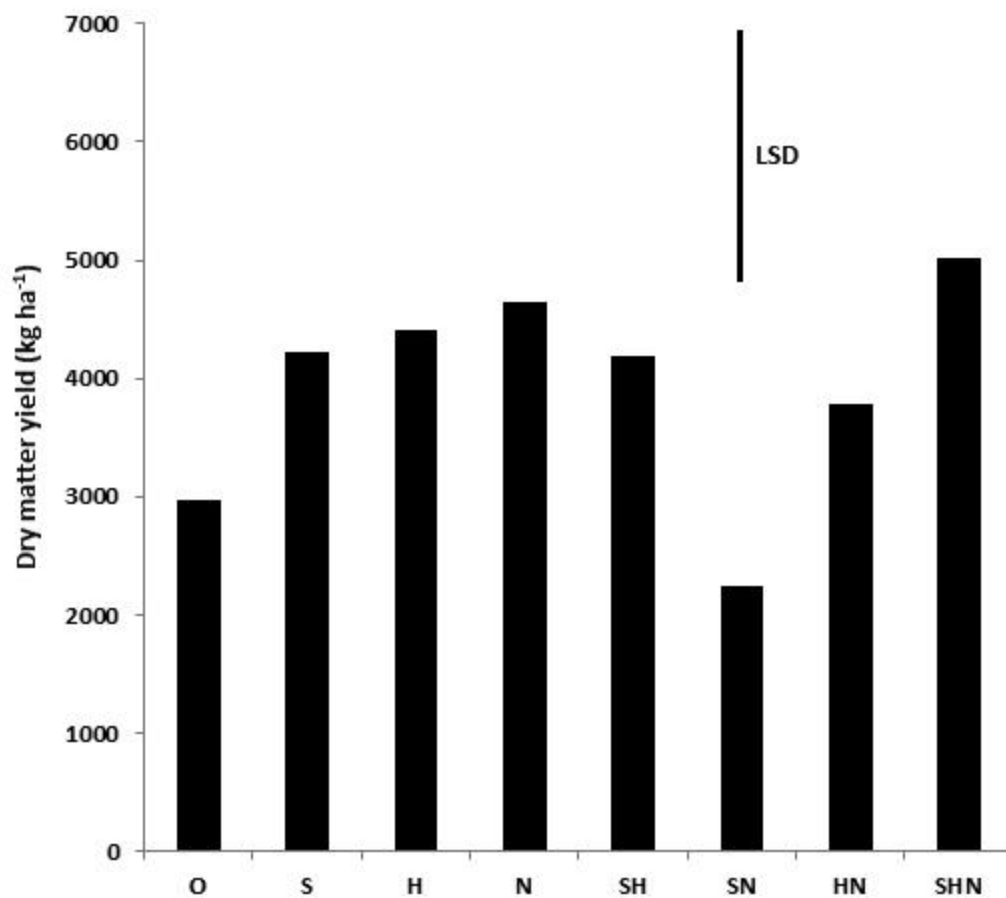


Figure 4.2: Average dry matter yield of switchgrass plots in Cookshire-Eaton, QC subject to various renovation treatments in the spring of the post-seeding year (O, no treatment, S, no-till seeding, H, herbicide, N, nitrogen fertilizer). Results are for the post-renovation year.



Figure 4.3: Experimental plot after seeding with a Brillion no-till disc seeder. Note the compacted soil, incomplete closing of the furrows and bisected plant on the right.

CONNECTING TEXT FOR CHAPTER 5

In the preceding chapter we examined the effects of several renovation strategies on switchgrass establishment. The results indicated that the most appropriate treatment option for somewhat poorly established switchgrass fields is likely often simply waiting for the stand to self-thicken. While this addresses the concerns of farmers starting production, much remains unknown about the relationships between the characteristics of producers' fields and the quality of biomass produced.

In the following study we examined the relationships between spring-harvested switchgrass and soil characteristics.

The following chapter was written as a manuscript which will be submitted for future publication. The manuscript was co-authored by the candidate, Dr. Philippe Seguin, Department of Plant Science, McGill University, Dr. Arif Mustafa, Department of Animal Science, McGill University, Roger Samson, REAP-Canada and MAPAQ, Direction régionale de l'Estrie. The candidate carried out the experiments and was the primary author of the manuscript. Dr. P. Seguin provided funds and assistance for this research, including supervisory guidance and reviewing of the manuscript. Laboratory analyses were conducted in the laboratory and under the supervision of Dr. Arif Mustafa. Mr. Roger Samson provided all germplasm and selections used in the experiment as well as technical assistance in field management and data collection. Huguette Martel provided field and technical support. All authors contributed to editing the manuscript.

CHAPTER 5

CORRELATIONS BETWEEN SOIL CHARACTERISTICS AND SPRING- HARVESTED SWITCHGRASS BIOMASS COMPOSITION

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5.1 ABSTRACT

Switchgrass (*Panicum viragatum* L.) is a promising bioenergy crop for Eastern North America and Europe. The development of combustion systems for the conversion of this feedstock into energy has identified that spring harvests result in biomass with more amenable qualities to the combustion process, however, little information exists about the relationships between soil characteristics and biomass quality. Samples were collected from 58 environments across southern Quebec and Ontario to determine possible relationships between soil characteristics and switchgrass biomass quality. Analysis of soil and biomass samples collected indicate that soil parameters explain 74% of the variation in biomass Si content as well as 45% of the variation in ash and 32% of the variation in Mg. For other important elements including K, little of the variation was explained by soil characteristics.

5.2 INTRODUCTION

The combustion of biomass feedstocks is affected by their varying chemical composition, which may lead to undesirable reactions occurring during the combustion process. Although not fully understood, recent research has increased our understanding of the effects the chemical composition of feedstocks have on combustion characteristics. Fouling, slagging, and corrosion occur as a result of a very complex set of chemical reactions and interactions (Johansson *et al.* 2003). Fouling refers to the deposition of compounds on boiler surfaces, while slagging indicates the formation of a glass-like layer in the ash due to the presence of substances causing a lowering in the ash melting point. Corrosion results from the interaction of combustion byproducts with the metal surfaces of the boiler or heat exchangers, accelerating the degradation of equipment and incurring higher maintenance costs. All three of these processes, and the degree of their severity, are regulated by the concentration and composition of the ash mineral fractions present in combusted feedstock (Jenkins *et al.* 1998).

Specific elements found in plant biomass have been identified as being particularly problematic during their combustion. Alkali metals are catalysts with chlorine in the production of highly corrosive hydrochloric acid. Potassium tends to be the most problematic of the alkali metals, as it is often present in far higher concentrations

(Cherney 2006). Potassium lowers the softening point of the ash, increasing fouling and slagging (Passalacqua *et al.* 2004). Alkali metals and alkali earth metals are both capable of forming molten low viscous melts, which contribute to boiler sintering and bed agglomeration in fluidised bed boilers (Skrifvars, Backman, and Hupa 1998). Finally, Si reacts with alkali metals in the ash, forming complexes which lower the melting temperature of the ash (Cherney 2006).

Higher concentrations of ash in herbaceous materials relative to wood can decrease the efficiency of combustion processes and result in added operating expenses related to ash removal and disposal. High levels of alkali metals such as K and Na, and inorganic elements including Cl and S can cause significant problems during thermochemical conversion, including the formation of deposits on boiler surfaces and corrosion of the equipment (Turn, Kinoshita, and Ishimura 1997; Miles *et al.* 1996). In fluidized bed reactors, K and Ca can also react with sand in the bottom of the boiler to produce substances with low melting temperatures (Hiltunen, Barišić, and Zabetta 2008). Herbaceous energy crops tend to be high in Si and K, leading to distinct challenges (Monti, Di Virgilio, and Venturi 2008).

Switchgrass (*Panicum virgatum* L.), is a promising warm-season grass native of North America, which shows significant potential as a dedicated biomass energy crop (Sanderson *et al.* 1996; Casler 2012; Vogel and Masters 1998; Vogel *et al.* 2011). In switchgrass, levels of S and Na are quite low, but levels of Si, K and Cl are generally higher than those found in woody biomass (Jenkins *et al.* 1998). In particular, the high K content of switchgrass has been shown to have negative effects on combustion equipment, leading to the formation of deposits and corrosion of metal surfaces (Miles *et al.* 1996). The chemical composition of switchgrass has been reported to vary significantly depending on variety, growth stage, and morphological fraction (Hu, Foston, and Ragauskas 2011; Hu *et al.* 2010). Information on the relationship between soil characteristics and switchgrass biomass quality however remains scarce. Soil characteristics have been shown to influence biomass quality in reed canarygrass (*Phalaris arundinacea* L.) (Burvall 1997), while Finell and Nilsson (2005) found that variation in soil qualities affect the ash component of combusted biomass. Greenhouse studies have indicated that Si concentrations in aboveground switchgrass biomass

increases with higher soil Si content, but N content is also negatively associated with Si uptake (Nabity *et al.* 2012). Silicates in straw can react with potassium to produce molten potassium silicate, which has a low melting point and can contribute to bed agglomeration in boilers (Hiltunen, Barišić, and Zabetta 2008). Di Virgilio *et al.* (2007) found variations in soil characteristics including N, P, pH and soil moisture to be correlated with switchgrass yield, while Lemus *et al.* (2009) found P, K, Ca, and pH not to limit yield. Additional factors such as rooting density are also affected by soil type and composition (Ma, Wood, and Bransby 2000).

Delayed harvest of standing grasses has been demonstrated to improve biomass quality. In reed canarygrass, a spring harvest system resulted in reduced moisture content and mineral concentration across a wide range of elements compared to harvest in the fall (Burvall 1997). Similar reductions have also been noted for ash and mineral content in overwintered *Miscanthus x giganteus* (Nolan *et al.* 2009; Meehan, Finnan, and McDonnell 2012; Lewandowski *et al.* 2003). In recent years this concept has also been applied to switchgrass; as well as lowering moisture content (Adler *et al.* 2006), a delayed harvest has been demonstrated to lower ash (Vermerris 2008; Kim *et al.* 2011) and concentration of elements including S, P, K, Cl and N (Vermerris 2008; Adler *et al.* 2006). To our knowledge the relationship between soil and biomass mineral composition in spring-harvested switchgrass has never been investigated. An experiment was thus conducted in Southern Quebec and Ontario to determine the relationships between soil and biomass characteristics. Samples were taken in the spring from 58 sites in Southern Quebec and Ontario; 27 sites in 2011 and 31 sites in 2012.

5.3 MATERIALS AND METHODS

5.3.1 Site details

Sites used in this study were all fields of fully mature (>3 years) ‘Cave-in-Rock’ switchgrass being actively managed for biomass production. The 58 fields sampled in the spring of 2011 and 2012 (March and April) were located in environments ranging from 45°01' to 47°31' N and 68°40' to 75°20' W (see appendix tables A5.6 and A5.7 for details). Above ground biomass and soils were sampled from locations at least 50 m from the edge of the field. The exact location of each sampling site was recorded by GPS.

Biomass collection at each site was done in the spring from three 1 m² quadrats within a 5 m radius. Fresh yield of the biomass collected was recorded. Samples were then dried in a forced air oven for 48 hours at 60 °C to determine moisture content and dry matter (DM) yields. Dry samples were ground with a Model 4 Thomas-Wiley Laboratory Mill forage grinder (Thomas Scientific 1654 High Hill Road, Swedesboro, NJ, USA) to pass through a 1 mm screen, homogenized and used for biomass analyses. Soil samples were collected at the same time as the biomass using a manual soil probe at 2 depths; 0-15 cm and 15-30 cm. At each sampling site 10 soil samples were collected at each depth within the quadrats used for the biomass sampling. Samples were then homogenized and used for laboratory analyses.

5.3.2 Elemental analyses

Elemental analyses (i.e. P, K, Ca, Mg, Al, B, Fe, Zn, Na, N and Si) on soil and biomass samples as well as soil pH and organic matter (OM) content were conducted and determined by the Institut de Recherche et de Développement en Agroenvironnement (IRDA) (Quebec, QC, Canada) while the biomass ash content was determined in the Crampton Laboratory of McGill University. All analyses were done according to standard procedures of the Association of Official Analytical Chemists (1990).

5.3.3 Statistical analyses

All statistical analyses were performed using SAS (SAS Institute, 2003). Many elements interact and may form complex associations in soil, resulting in covariation of their concentrations across a range of soil types. The correlation of two or more variables in a regression model is known as multicollinearity, which may result in redundant information confounding the analysis (Bowerman and O'Connell, 1990). Many studies examining soil element concentrations encounter this problem. Previous studies have noted the utility of using principal component analysis (PCA) to group soil variables for regression analysis (Kaspar *et al.* 2004). In the present study, the variables retained for PCA were limited to those of significant interest to biomass combustion applications; these were pH, organic matter content (OM), P, K, Ca, Mg, Al, B, Fe, Zn, Na, N and Si, all at both measured soil depths for a total of 26 variables. Principal component analysis

was performed using PROC FACTOR to develop principal components (PCs) made up of factor loadings for each variable. PCs represent groupings of highly correlated factors that account for much of the variance in the variables. PCs with eigenvalues >1.0 were retained and rotated orthogonally using the VARIMAX option of SAS, and the scoring coefficients obtained using the SCORE procedure of SAS. Stepwise regression was then conducted with PROC REG using the biomass variables as the dependent variables and the scoring coefficients of the retained PCs as the independent variables. The significance level for entry into the model was $P = 0.15$ and the significance level to stay in the model was $P \leq 0.05$.

5.4 RESULTS AND DISCUSSION

Means, maximum and minimum values and standard deviations for the 22 measured soil variables at both sampled depths are presented in tables 5.1 and 5.2. The concentrations of all minerals examined were extremely similar between the 2 measured soil depths with the exception of N, P and K which were 24, 27 and 36 % lower at the 15-30 cm depth, respectively. This is likely due to the widespread application of these elements in fertilizer. The minerals found in greatest concentrations were Ca and Al, which had the highest concentrations at both depths but also high variation between sites. The next grouping including Fe, Si and Mg, which were all present at or just below 200 mg kg⁻¹.

Means, maximum and minimum values and standard deviations for the 24 measured biomass variables are presented in table 5.3. Biomass ash levels varied by a factor of 5 with an average of 36.8 g kg⁻¹, higher than the 23.7 g kg⁻¹ value reported from spring-harvested fields in Pennsylvania (Adler *et al.* 2006). Silicon was present at higher levels than any of the other minerals measured in the biomass, and varied by a factor of 8. The large variation in the concentration of many elements in this study strongly suggests the possible contamination of some of the samples with soil. Significant lodging was noted at all sites in this study due to Quebec's high snow accumulation over winter. Flattening of straw is known to lead to contamination of biomass with soil (Bakker and Elbersen 2005), and lodging contributes to this soil contamination (Elbersen 2001). Soil contamination has been frequently cited as a major problem for energy grasses

(Lewandowski *et al.* 2003; Clarke, Eng, and Preto 2011; El-Nashaar *et al.* 2009; Bakker and Elbersen 2005). Heavy winter winds have previously been implicated in soil contamination problems with reed canarygrass in Finland, resulting in unusually high ash, Si and K in the harvested biomass (Saijonkari-Pahkala 2001), while overwintered materials are more prone to problems with lodging due to exposure to the winter elements. Departures from the concentrations found in previous studies in Quebec (Madakadze, Coulman, Mcelroy, *et al.* 1998) may also reflect the fact that to the best of our knowledge this is the first study to do elemental analyses on a wide range of commercial switchgrass plantations in Quebec, other studies having been restricted to a limited number of sites, largely from small plot research trials. Actively managed switchgrass environments may be subject to different sources of exposure to airborne dust and soil not present in research plots due to factors such as adjacent spring mowing, plowing or other agricultural activities.

PCA is a type of multivariate statistical method which summarizes and describes large sets of variables; by grouping closely associated factor loadings this type of analysis can produce independent variables which may represent underlying common factors. In the analysis of soil data, six factors with eigenvalues >1.0 were identified and retained (Table 5.4). Communalities of the 26 variables included in the analysis indicated that the six factors retained explained a large portion of the variation of most of the measured variables (Table 5.4). In all cases this represented $\geq 80\%$ with the exception of P at both soil depths as well as Al and Fe at the 0-15 cm depth, although in these latter cases the variation explained was still $\geq 70\%$. Highly negative or positive factor loadings for a given variable indicate a correlation between the observed variable and the associated PC. In the present study all the measured variables were retained for subsequent analyses.

For all PCs the variables with significant factor loadings were the same for both soil depths, indicating that in switchgrass fields in Quebec and Ontario the soil horizons did not vary significantly between those depths, and likely sampling at a single depth would have been sufficient. PC₁ had the largest eigenvalue and particularly high loadings for Ca, Mg, Na, and B. It was termed *Ca/Mg/Na* due to the importance of these three particular elements to combustion processes (Jenkins *et al.* 1998; Miles *et al.* 1996). This component also displayed highly negative loading for Al, which has been previously

demonstrated to inhibit Ca and Mg uptake in ryegrass (*Lolium multiflorum* Lam.) (Rengel and Robinson 1989). PC₂ had high loadings for organic matter content and N and was termed *organic matter*. Like other warm-season grasses, switchgrass has been reported to have low N requirements (Jung, Shaffer, and Stout 1988), and therefore is in practice a sparingly fertilized crop. This leads to organic matter being a significant source of N in the soil, which may explain the strong positive association between the two variables seen here. This factor had negative loadings for both Fe and Si, likely reflecting that those soils higher in organic matter had lower clay components and thus less available silicic acid to be taken up by plants. PC₃ was highly loaded for K at both depths and was termed the *K* factor. K is particularly problematic for combustion processes, acting as a fluxing agent in the ash fraction and enhancing the deposition of material on boiler surfaces (Nordin 1994). PC₄, PC₅, and PC₆ were highly loaded for P, Zn and pH, respectively, and were named accordingly.

The results of the stepwise regression using these factor loadings represent the associations between each of these soil PCs and 12 variables of biomass quality as well as yield. Significant proportions of the variation in Si and ash concentrations in the biomass (i.e., 74 and 45% respectively), were explained by the 6 PCs, with the organic matter component being negatively correlated with both (Table 5.5). Si levels were most strongly associated with OM content, with the negative association indicating that soils higher in organic matter resulted in biomass with lower Si content. This is in accordance with the results of previous studies conducted with reed canarygrass which reported higher Si and ash levels in soils with high clay and low OM content (Burvall 1997). Given the varying field sites, topography, local weather and other external factors present across field sites we sampled, these figures indicate that soil characteristics do have an important relationship with biomass quality, especially Si and ash content.

The 6 PCs explained 32 and 27 % of the variation in the biomass Mg and Ca concentrations, respectively (Table 5.5), while the model explained the least of the variation in K. The only PC which affected this latter variable was the soil K factor, but this accounted for only 5% of the variation present. This indicates that the final K concentration in spring harvested switchgrass is strongly affected by external factors other than the soil characteristics studied herein, which is of note given the importance of

mitigating the presence of this element for increasing biomass quality. For all other biomass variables examined in this study the model explained less than 25% of the variation present, suggesting that for these factors soil characteristics cannot be used to predict biomass quality.

Previous studies have found no correlation between soil P, K, Ca and plant tissue concentrations of those elements in reed canarygrass (Lemus, Parrish, and Wolf 2009), which may be a reflection of the complex soil and plant cycles and ecosystem processes which govern the availability and uptake of those nutrients. The low P demands of switchgrass have long been known, however, an increase in P concentration in switchgrass biomass has been reported when plots on low-P soils are fertilized (Morris, Fox, and Jung 1982), indicating that soil P concentrations may affect plant uptake when other variables are constant. Typical soil levels of P, K, Ca and pH have also been reported not to limit yield (Lemus, Parrish, and Wolf 2009). Di Virgilio *et al.* (2007) found that in switchgrass fields soil parameters varied notably over short scales within a single field, and were accompanied by variation in yield from 3 to 20 Mg ha⁻¹. This variability in switchgrass yield is present throughout the literature as well as in the present study (Table 5.3), and may suggest a need for larger-scale harvest methodologies to control for large small-scale variation. The sites used in this study may also have been subject to various amendments over their recent history, which may have affected the nutrient content of soil samples. The deep rooting patterns of switchgrass are well-documented, with studies finding increased soil organic C under switchgrass fields at depths up to 90 cm (Liebig *et al.* 2005). For this reason future studies should consider evaluating the influence of soil characteristics from greater depths on biomass quality.

5.5 CONCLUSION

The concentrations of Si and ash in spring-harvested switchgrass biomass samples appear to be strongly influenced by soil characteristics, notably organic matter and alkali metal / Mg content. The variation in the content of several additional elements important to combustion processes (Ca, Mg and S) showed weaker correlations with soil characteristics, while several other important biomass characteristics including yield, Cl and Na concentrations appeared to only be weakly related to soil characteristics. Notably

K, one of the most problematic factors for combustion of herbaceous biomass, did not appear to be correlated with any of the measured soil variables. The results also suggest that soil contamination was a significant problem with spring-harvested material, leading to very high levels of Si and ash.

These findings could be of importance to researchers evaluating Eastern Canada's biomass fuel potential and may be useful for facilitating site choice for producers wishing to maximize switchgrass quality. Future studies should consider sampling soil at a single 0-30 cm depth or evaluating soil characteristics at deeper levels in the switchgrass root zone. Further research should be conducted to pursue strategies for maximizing the quality of switchgrass as a biomass feedstock, especially with respect to strategies for mitigating soil contamination issues.

Table 5.1: Mean, maximum, minimum and standard deviation of soil characteristics at 0-15 cm in switchgrass fields at 58 Quebec and Ontario sites. All values are in mg kg⁻¹ unless otherwise noted.

Variable	Mean	Max	Min	SD
pH	6.17	7.43	4.93	0.59
pHSMP	6.59	7.23	5.76	0.38
OM (%)	5.00	9.25	1.63	1.87
Ca	1500.6	3545.0	150.0	649.6
Al	1160.9	1697.0	614.0	263.4
Fe	197.1	330.0	105.0	57.3
Mg	194.0	861.0	17.6	186.4
Si	187.6	452.0	70.5	104.5
K	123.2	336.0	36.1	73.9
P	59.8	178.0	7.24	40.6
Mn	33.5	146.0	5.18	29.5
N	21.9	38.4	7.10	7.54
Na	6.85	18.6	1.48	4.44
Zn	5.08	20.0	1.31	3.71
Pb	2.64	5.75	0.87	1.06
Cu	2.24	6.93	0.51	1.27
Ni	0.61	1.59	0.16	0.37
B	0.33	0.76	0.11	0.14
Co	0.28	0.83	0.06	0.20
Cr	0.18	0.39	0.07	0.08
Cd	0.10	0.20	0.03	0.04
Mo	0.03	0.05	0.01	0.01

Table 5.2: Mean, maximum, minimum and standard deviation of soil characteristics at 15-30 cm in switchgrass fields at 58 Quebec and Ontario sites. All values are in mg kg⁻¹ unless otherwise noted.

Variable	Mean	Max	Min	SD
pH	6.19	7.74	4.79	0.58
pHSMP	6.64	7.38	5.89	0.38
OM (%)	3.84	7.10	1.42	1.43
Ca	1352.8	3701.0	102.0	787.3
Al	1238.3	1957.0	597.0	348.9
Si	208.3	453.0	65.6	99.4
Fe	182.7	585.0	86.0	81.4
Mg	174.1	991.0	7.35	196.9
K	79.4	300.0	18.2	64.3
P	43.1	160.0	2.90	33.7
Mn	26.6	101.0	4.90	24.4
N	16.9	30.4	6.40	5.74
Na	8.14	29.4	1.60	6.64
Zn	3.31	18.4	0.66	2.88
Pb	2.27	7.18	0.09	1.25
Cu	1.76	5.26	0.38	1.07
Ni	0.56	1.45	0.09	0.36
B	0.27	0.59	0.08	0.13
Co	0.25	0.84	0.03	0.19
Cr	0.21	0.58	0.06	0.10
Cd	0.08	0.19	0.02	0.03
Mo	0.03	0.05	0.01	0.01

Table 5.3: Mean, maximum, minimum and standard deviation of spring-harvested Cave-in-Rock switchgrass samples from 58 sites in Quebec and Ontario. All values are in mg kg⁻¹ unless otherwise noted.

Variable	Mean	Max	Min	SD
Yield (kg ha ⁻¹)	5555	9674	1550	2072
Ash (g kg ⁻¹)	36.9	74.4	13.8	17.2
Si	6280.6	14677.0	1689.0	3725.4
Ca	3342.4	6909.0	1857.0	1077.5
K	980.4	2133.0	358.0	374.4
Mg	751.8	1284.0	256.0	174.1
P	534.7	1155.0	277.0	175.7
S	514.8	927.0	332.0	143.2
Fe	136.6	637.0	58.0	101.6
Al	120.5	569.0	50.7	92.2
Mn	61.4	253.0	12.8	54.3
Cl	33.7	82.0	17.8	13.8
Zn	27.7	71.3	11.1	13.9
Na	15.9	83.9	7.40	12.2
Ba	15.2	54.0	4.33	9.31
Sr	11.8	33.2	4.04	7.50
Cu	5.63	9.10	2.61	1.68
B	2.10	4.80	1.17	0.70
Cr	0.82	4.23	0.21	0.71
Mo	0.74	3.00	0.00	0.63
Ni	0.66	2.48	0.14	0.46
Pb	0.19	1.60	0.00	0.32
Cd	0.17	0.95	0.00	0.20
Co	0.14	0.79	0.00	0.14

Table 5.4: Rotated principal components (PCs) and Communality estimates (CE) from soil data across 58 environments in switchgrass fields in Southern Quebec and Ontario.

Soil depth	Parameter ^a	PC ₁	PC ₂	PC ₃	PC ₄	PC ₅	PC ₆	CE
		Ca/Mg/Na	OM	K	P	Zn	pH	
0-15	pH	0.27	-0.34	0.05	-0.14	0.11	0.80*	0.87
	OM	-0.12	0.92*	-0.16	0.03	0.01	-0.15	0.91
	P	-0.12	0.08	-0.01	0.76*	0.37	0.04	0.74
	K	0.06	0.04	0.91*	-0.06	-0.22	0.03	0.88
	Ca	0.87*	0.16	0.01	-0.11	0.03	0.32	0.89
	Mg	0.73*	-0.17	0.21	-0.36	0.27	0.12	0.83
	Al	-0.54	0.64	-0.13	-0.14	0.19	-0.08	0.78
	B	0.83*	0.03	0.13	0.12	0.05	0.34	0.84
	Fe	0.18	-0.53	0.45	0.14	0.29	-0.32	0.72
	Zn	-0.07	0.26	-0.16	0.21	0.85*	0.10	0.87
	Na	0.81*	-0.18	0.16	-0.36	-0.13	-0.23	0.91
	N	0.02	0.91*	-0.10	0.14	-0.02	-0.17	0.89
	Si	0.34	-0.49	0.36	-0.54	0.09	0.30	0.87
15-30	pH	0.45	-0.48	0.07	-0.04	-0.06	0.67	0.89
	OM	-0.03	0.88*	0	0.15	0.18	-0.07	0.84
	P	-0.07	0	0.12	0.79*	0.34	-0.07	0.76
	K	0.26	-0.13	0.90*	-0.03	-0.10	0.12	0.92
	Ca	0.89*	-0.08	0.04	0.01	-0.03	0.23	0.86
	Mg	0.82*	-0.24	0.20	-0.32	0.11	-0.03	0.88
	Al	-0.55	0.68	-0.12	-0.18	0.15	-0.01	0.84
	B	0.78*	-0.09	0.30	0.19	0.15	0.24	0.82
	Fe	0.24	-0.52	0.61	0.05	0.33	-0.10	0.81
	Zn	0.13	0.08	-0.06	0.36	0.84*	-0.01	0.86
	Na	0.80*	-0.10	-0.02	-0.27	-0.15	-0.34	0.87
	N	0.13	0.85*	0.10	0.27	0.19	-0.12	0.88
	Si	0.37	-0.44	0.30	-0.60	0.02	0.29	0.85
	Eigenvalues	6.71	5.64	2.78	2.73	2.23	2.00	

^a OM, organic matter

Table 5.5: Coefficients and multiple coefficient of determination for stepwise regression of switchgrass biomass dependent variables on rotated PCs of corresponding soil variables obtained through PCA.

Biomass variable	PC ₁ Ca/Mg/Na	PC ₂ OM	PC ₃ K	PC ₄ P	PC ₅ Zn	PC ₆ pH	R ²
Si	0.661	-0.770	0.468	-0.668		0.240	0.7436
Ash	0.396	-0.351	-0.197		-0.320	-0.163	0.4478
Mg	0.190	0.187	-0.181		0.406	0.223	0.3183
Ca		0.282	-0.365		-0.249		0.2748
S		0.469			-0.202		0.2608
B	0.281		-0.346		-0.210		0.2428
Zn			-0.261	0.240		-0.341	0.2422
Cl	0.213		-0.265		-0.311		0.2121
Na		-0.425					0.1809
P		0.424					0.1797
Fe		-0.412					0.1694
Yield			0.319		0.236		0.1682
Al		-0.407					0.1653
K			0.233				0.0541

CHAPTER 6

FINAL CONCLUSION AND SUMMARY

Switchgrass is the focus of great recent interest as a potential biomass energy crop. In Eastern North America switchgrass is capable of producing large amounts of cellulosic feedstock on marginal soils with few inputs. The experiments contained herein had the objective of addressing three major needs of current switchgrass producers in Eastern Canada.

The first objective was to determine the performance of new switchgrass selections in Southern Quebec and to determine the impacts of harvest date on switchgrass yield and quality. Significant differences were observed among selections for yield, tiller density, height, maturity, reproductive tiller fraction and tiller mass. These differences varied between sites. Big bluestem was slower to establish than switchgrass, requiring a longer establishment period to attain yield potential. Delayed spring harvest resulted in a large decrease in moisture content and a slight increase in cellulose content, however yield losses approached 40%. Unlike in previous studies the ash content was not affected by harvest date, nor was the HHV. The results of this study indicate that local selection programmes have the potential to modify characteristics including height and tiller density to produce adapted varieties of switchgrass for Southern Quebec.

The objective of the second study was to determine the most effective strategies for renovating poorly established switchgrass stands. Treatments included reseeded with a no-till drill, herbicide application and application of urea fertilizer. In the first year following establishment none of the treatments displayed an effect on plot yield with the exception of the re-seeding treatment, which produced a negative effect. In the subsequent year the only significant difference was between the highest yielding treatment (that which received all three treatments) and the lowest yielding treatment (the plots which received seeding and fertilizer but not herbicide). These results suggest that spring re-seeding may not be an effective strategy for renovating apparently poorly established switchgrass fields, and may in fact have a negative effect under the studied conditions. The herbicide treatment did not result in a significant yield increase except in

2012 when compared to the herbicide-free treatment in the presence of the re-seeding and N treatments. These results indicate that switchgrass stands are able to self-thicken over the course of several years after plantation and may often require little intervention to achieve adequate stands even in the event of fairly poor establishment.

The objective of the third experiment was to determine the relationships between soil parameters and spring-harvested switchgrass quality. The results indicated that concentrations of Si and ash in spring-harvested switchgrass biomass samples appear to be strongly influenced by soil characteristics, among these organic matter and alkali metal / Mg content. The variation in the mineral content of several elements important to combustion processes (Ca, Mg and S) showed weak correlations with soil characteristics, while other important biomass characteristics including yield, Cl and Na concentrations appeared to only be very weakly related to soil characteristics. The results also suggest that soil contamination was a significant problem with spring-harvested material, leading to very high levels of Si and ash.

CHAPTER 7

RECOMMENDATIONS FOR FUTURE RESEARCH

The results of this research point to several areas that warrant further investigation for the production of switchgrass in Eastern Canada. In particular further research could include:

1. The continuation of studies evaluating the progress of local switchgrass selection and breeding programmes.
2. The effectiveness of different herbicides on switchgrass establishment.
3. Strategies for maximizing the quality of switchgrass as a biomass feedstock, especially with respect to strategies for mitigating soil contamination issues.

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APPENDICES

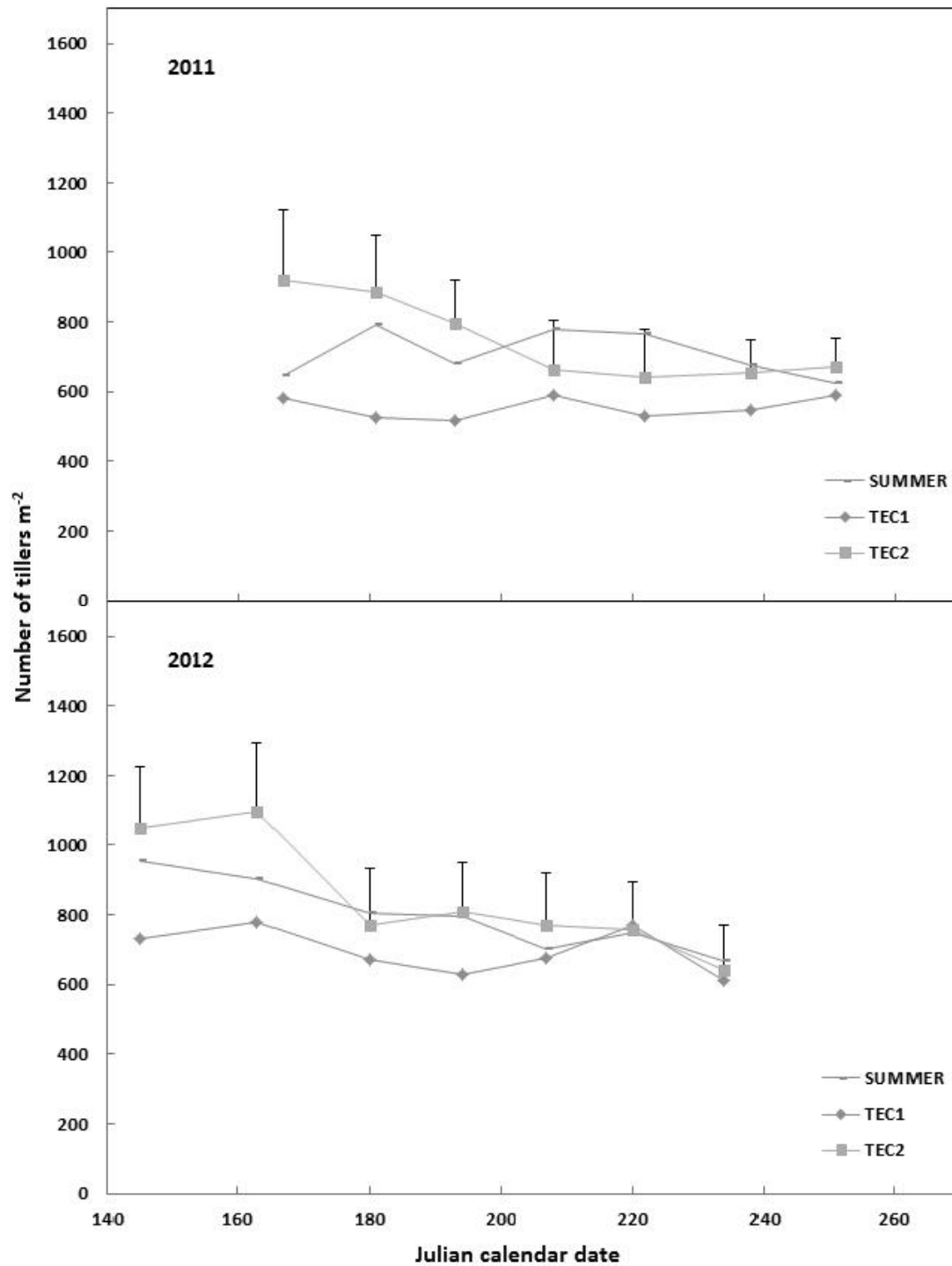


Figure A3.8: Number of tillers m⁻² of Summer lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.

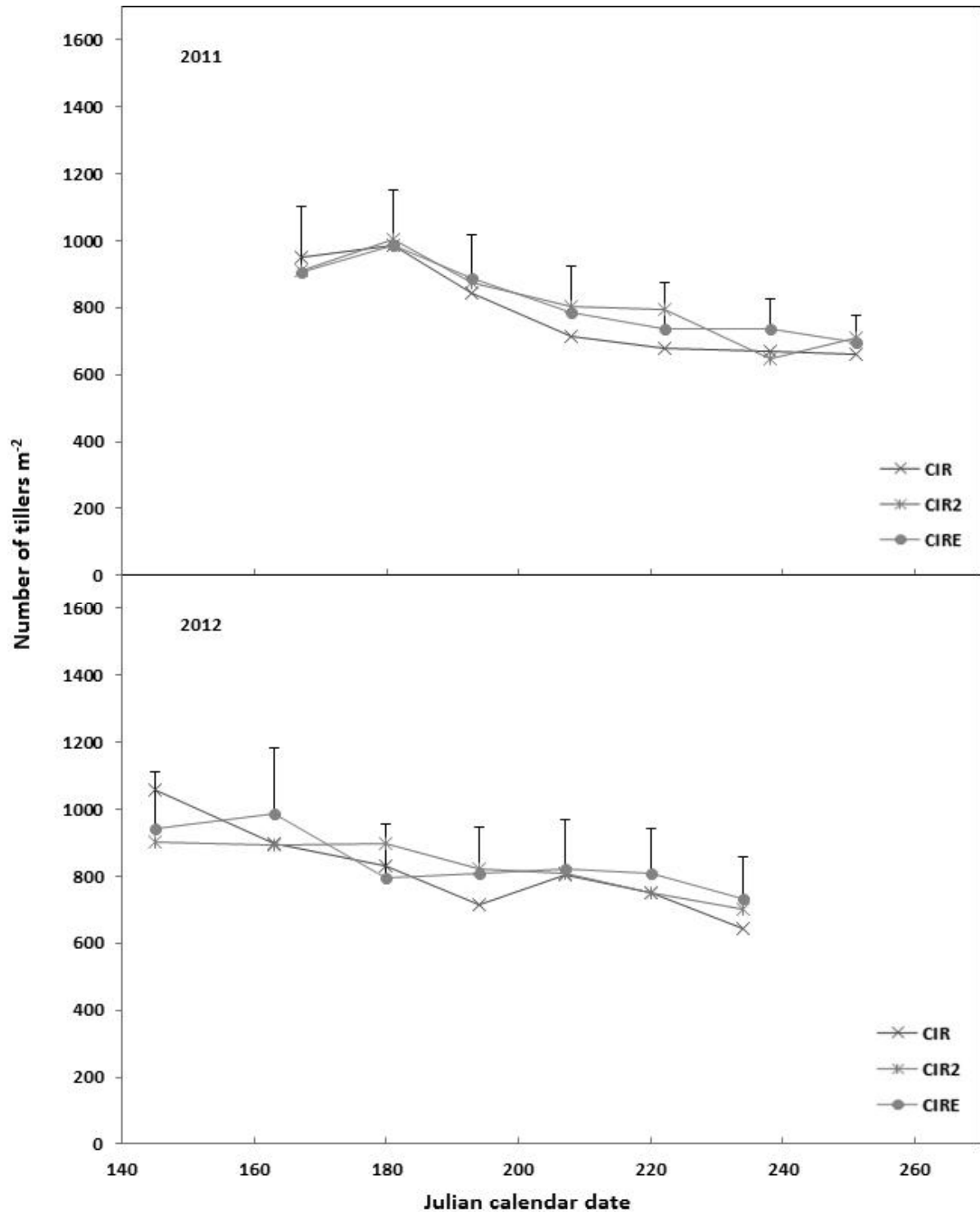


Figure A3.9: Number of tillers m^{-2} of Cave-in-Rock lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

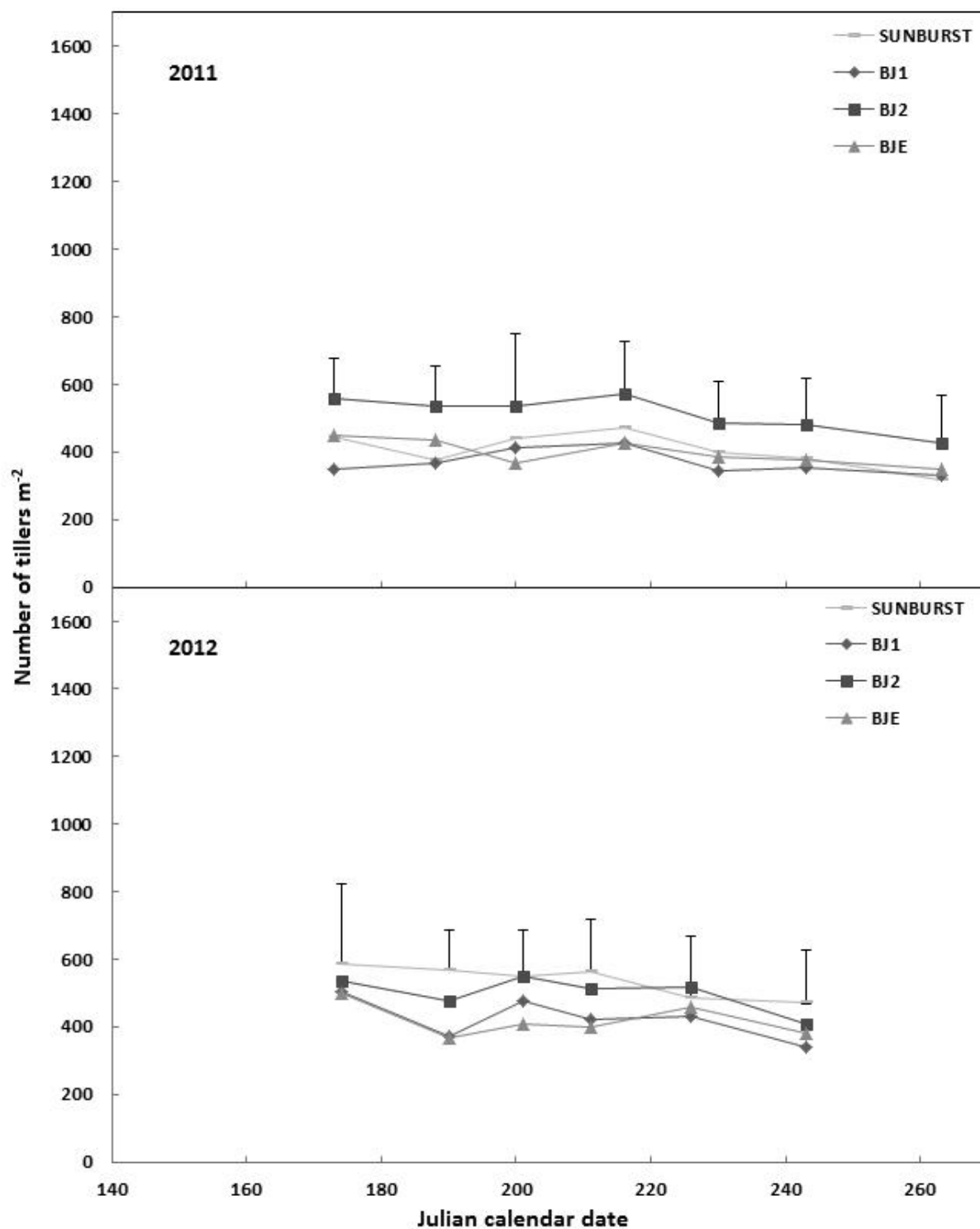


Figure A3.10: Number of tillers m^{-2} of Sunburst lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

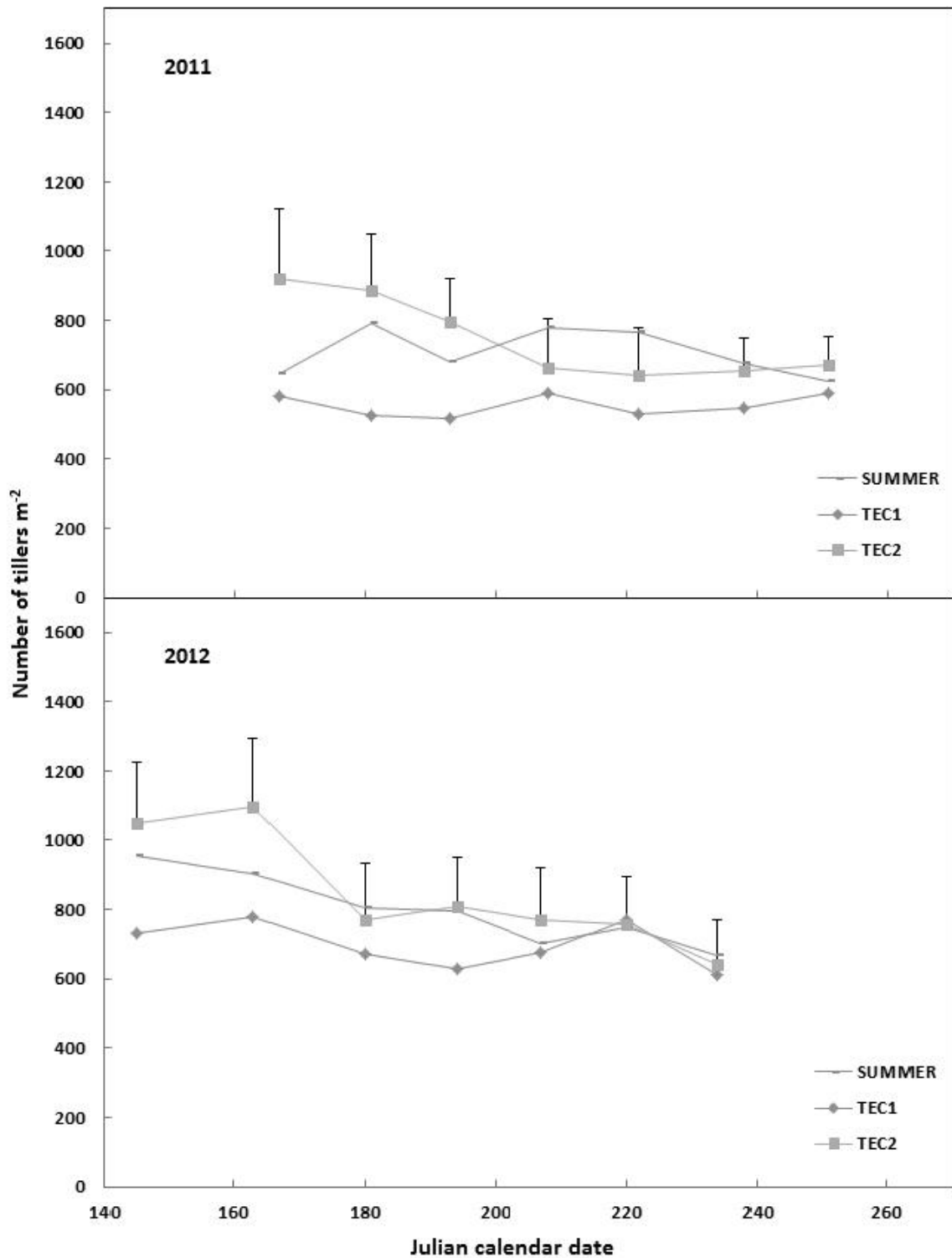


Figure A3.11: Number of tillers m^{-2} of Summer lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

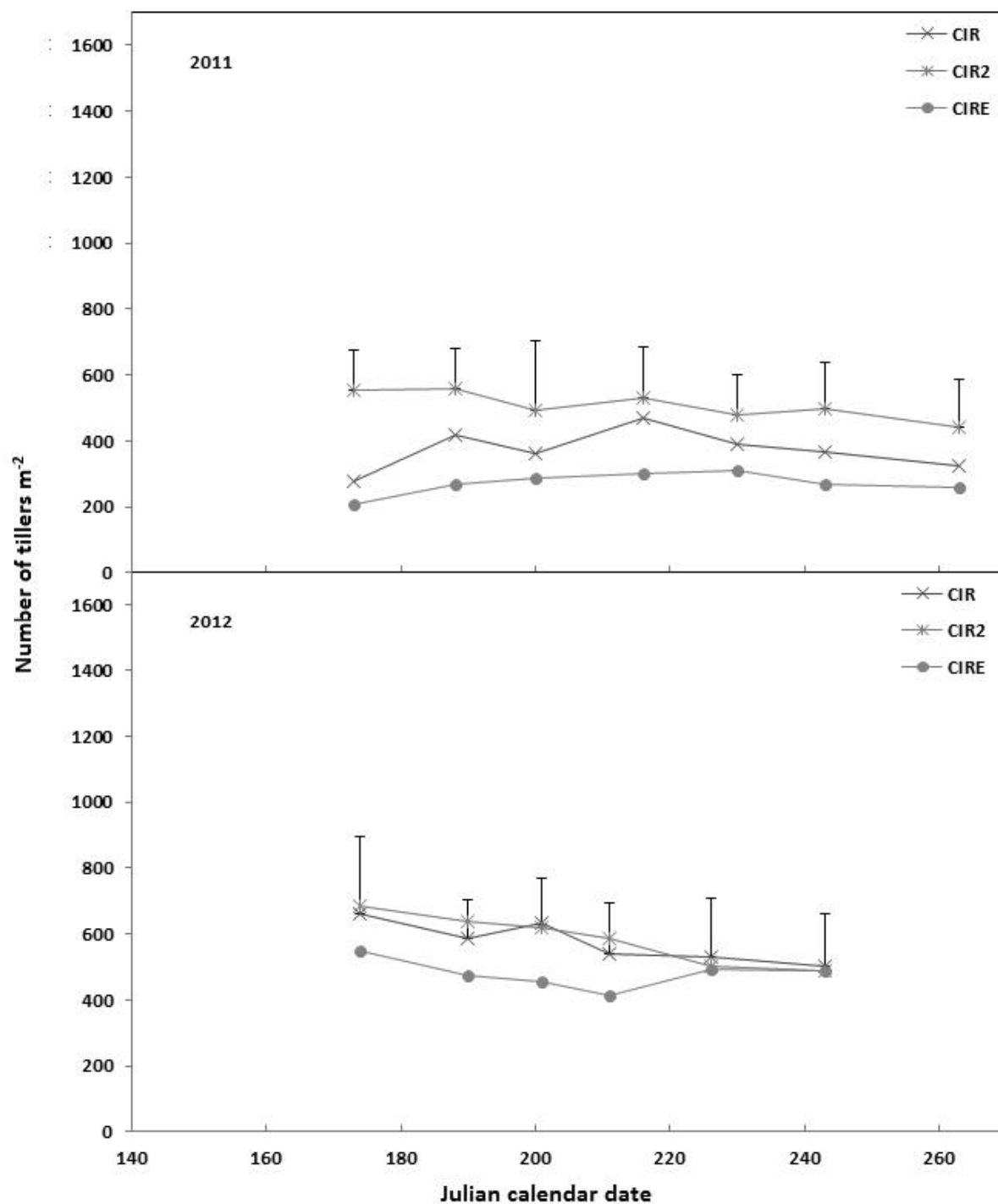


Figure A3.12: Number of tillers m⁻² of Cave-in-rock lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, p=0.05) at each sampling point throughout the season.

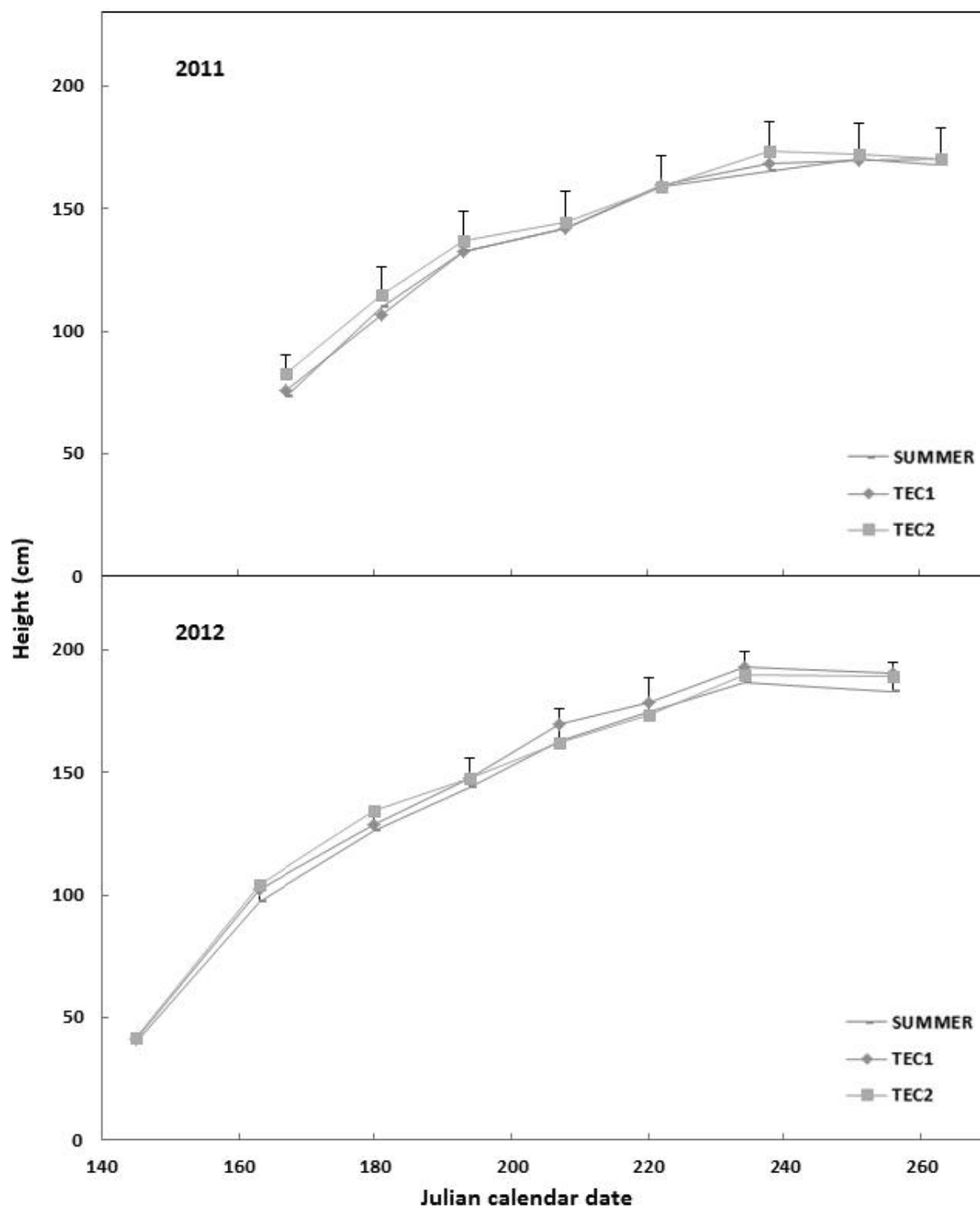


Figure A3.13: Height of Summer lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

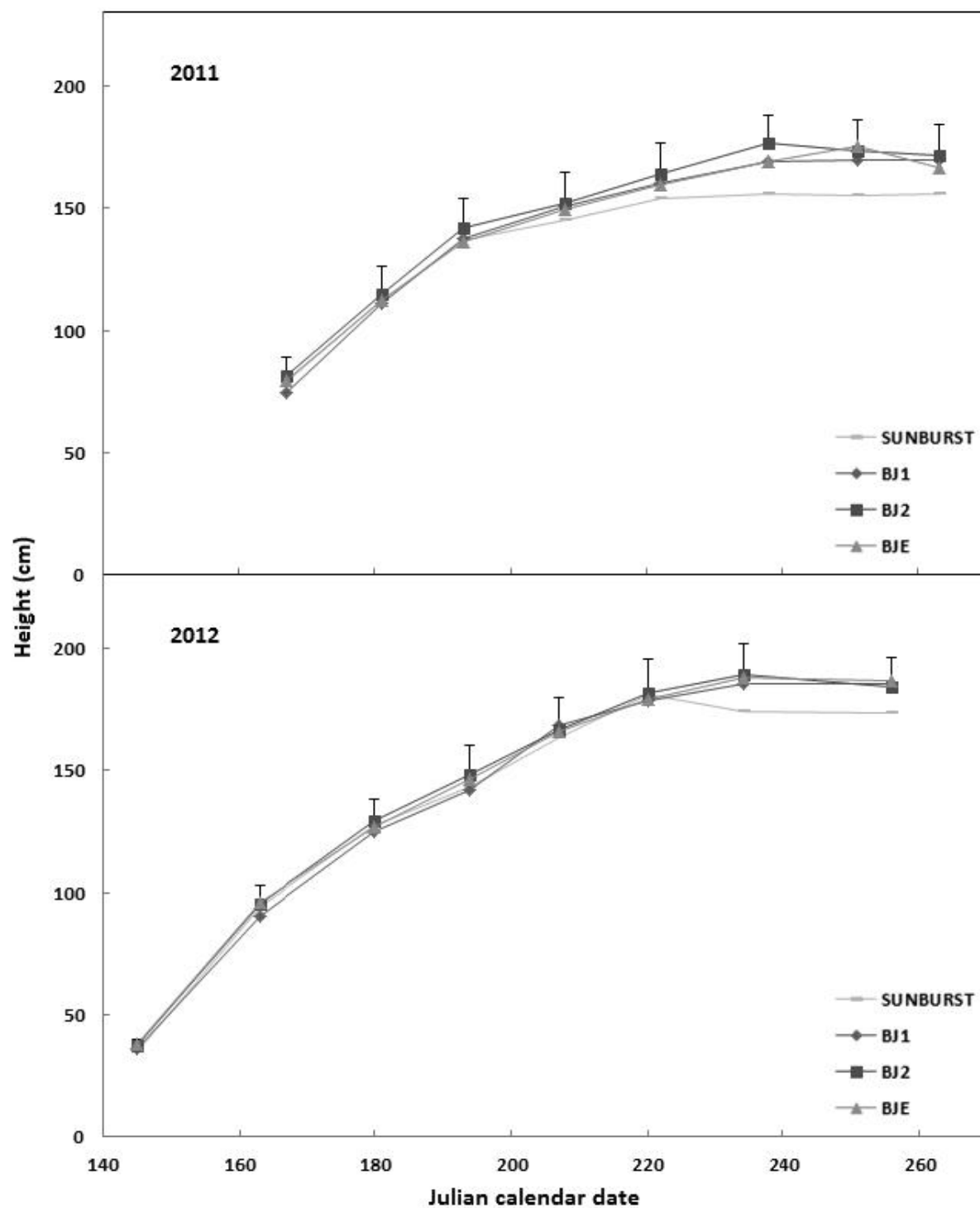


Figure A3.14: Height of Sunburst lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

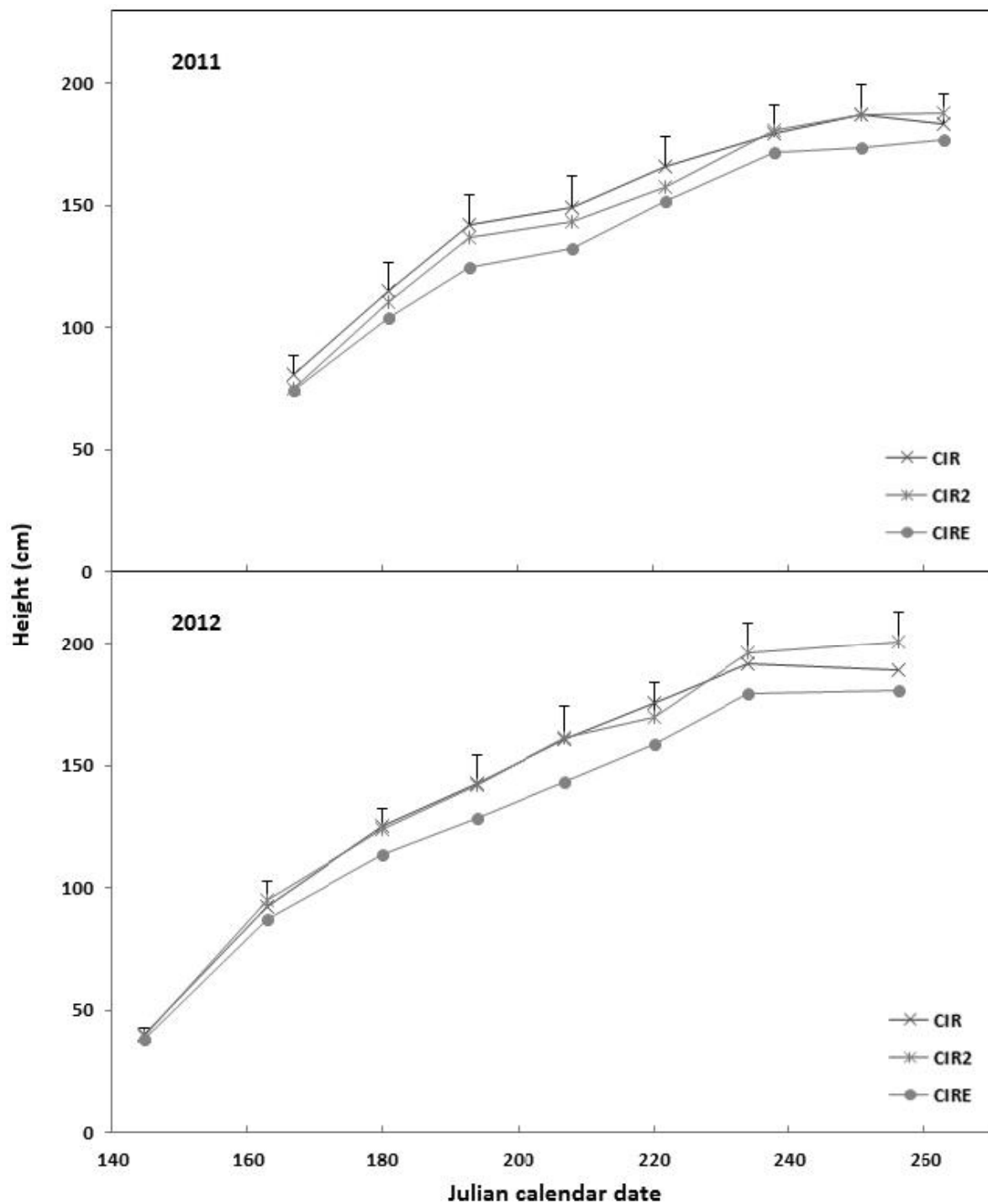


Figure A3.15: Height of Cave-in-rock selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

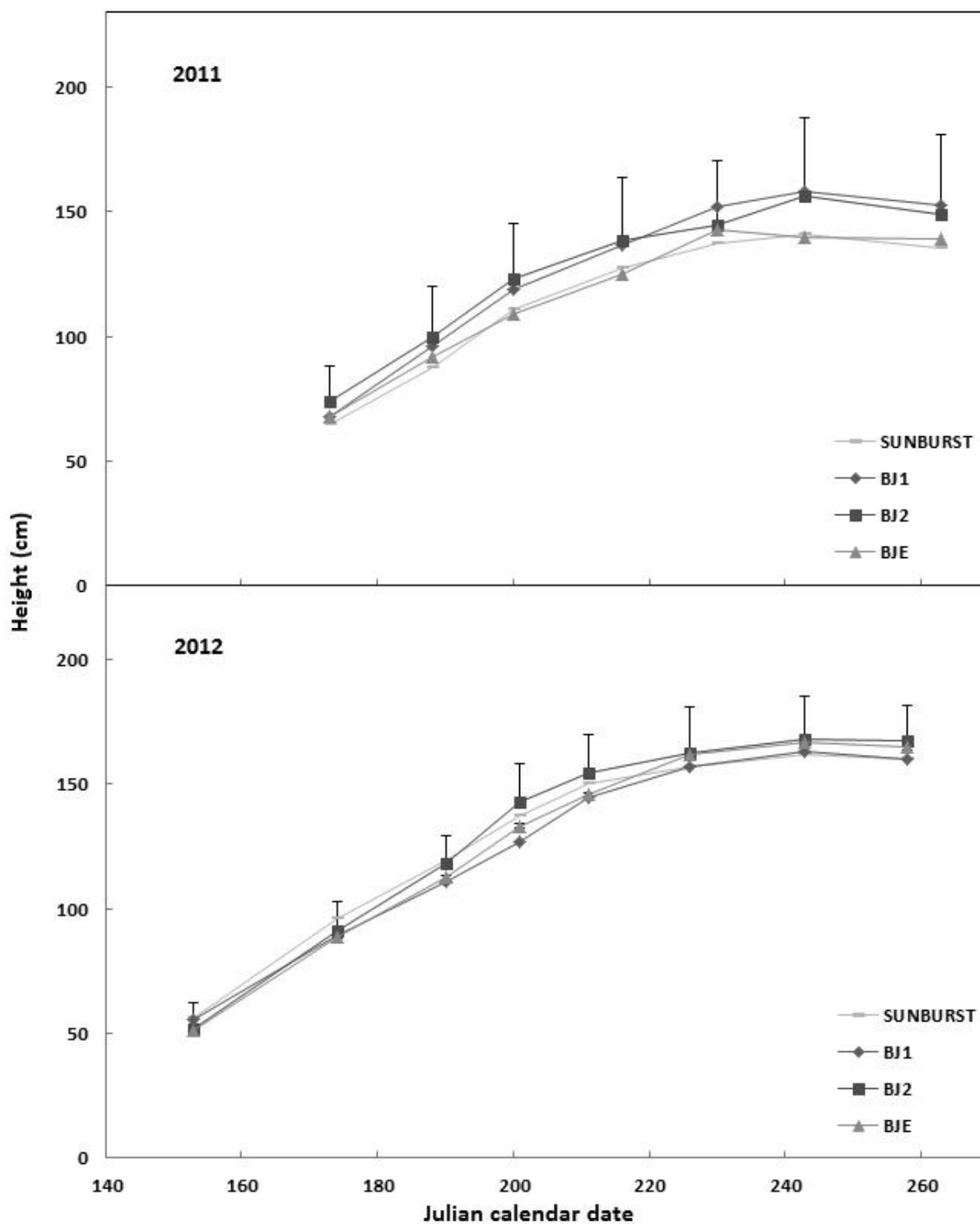


Figure A3.16: Height of Sunburst lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

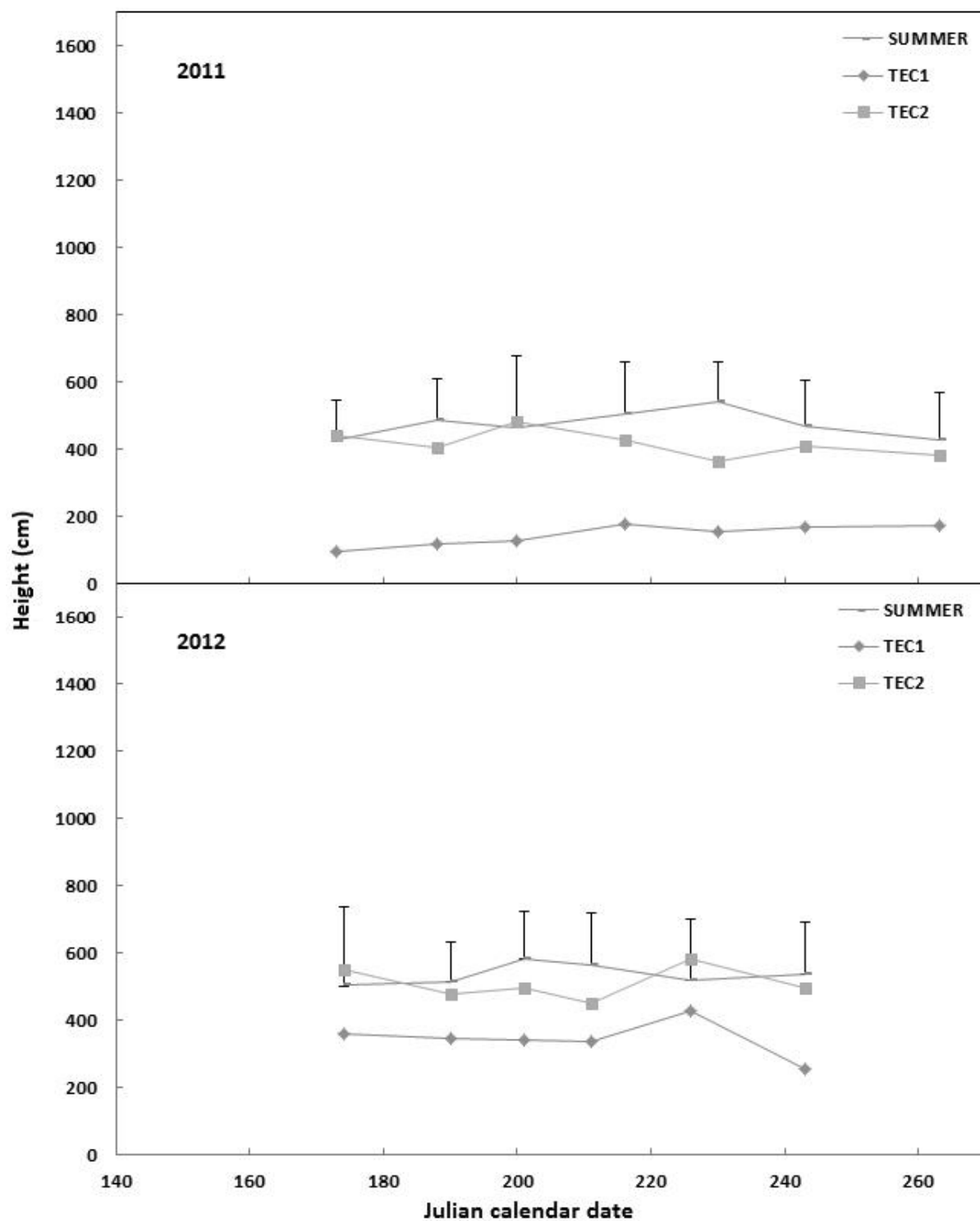


Figure A3.17: Height of Summer lineage of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

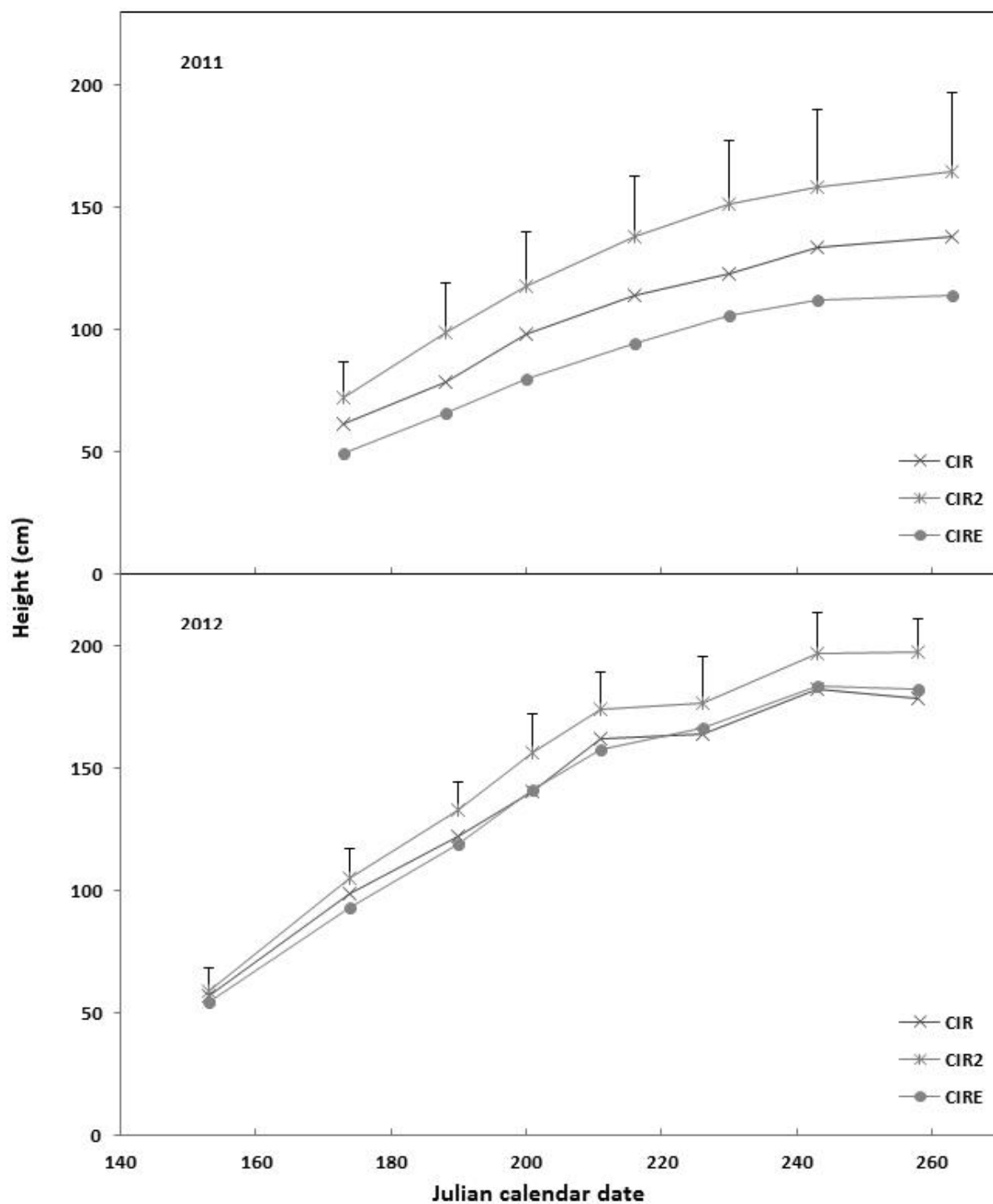


Figure A3.18: Height of Cave-in-rock lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

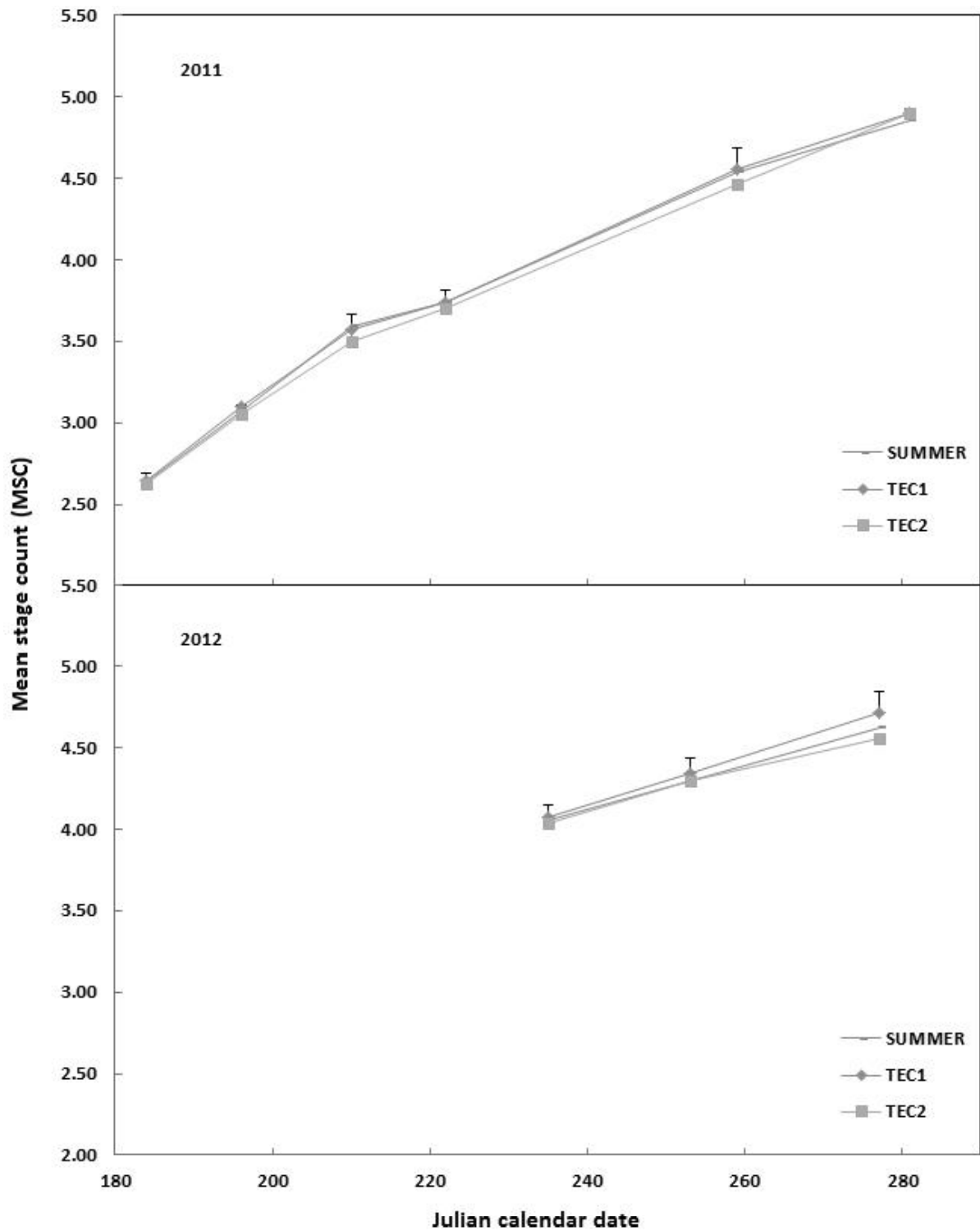


Figure A3.19: Phenology of Summer lineage selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

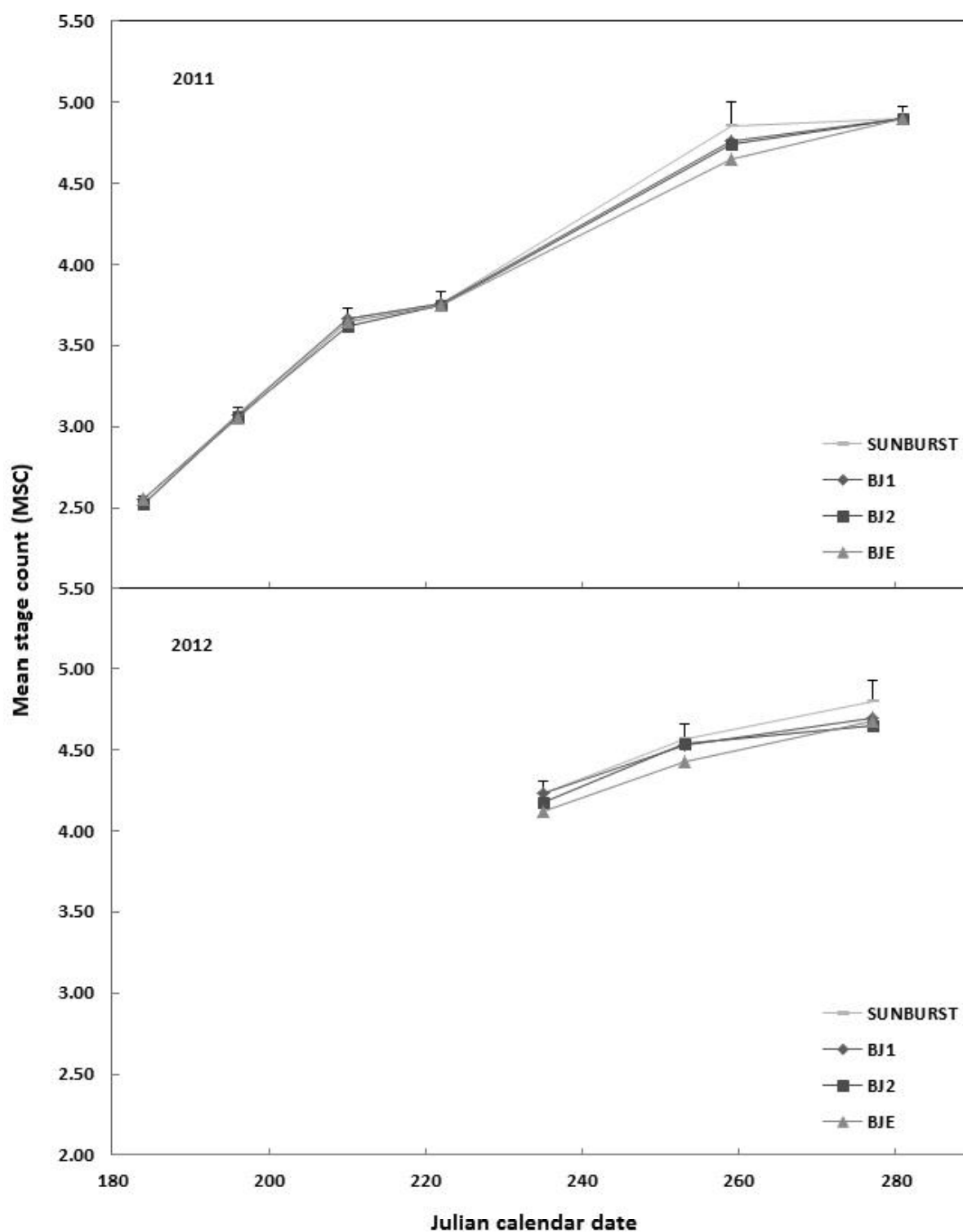


Figure A3.20: Phenology of Sunburst selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

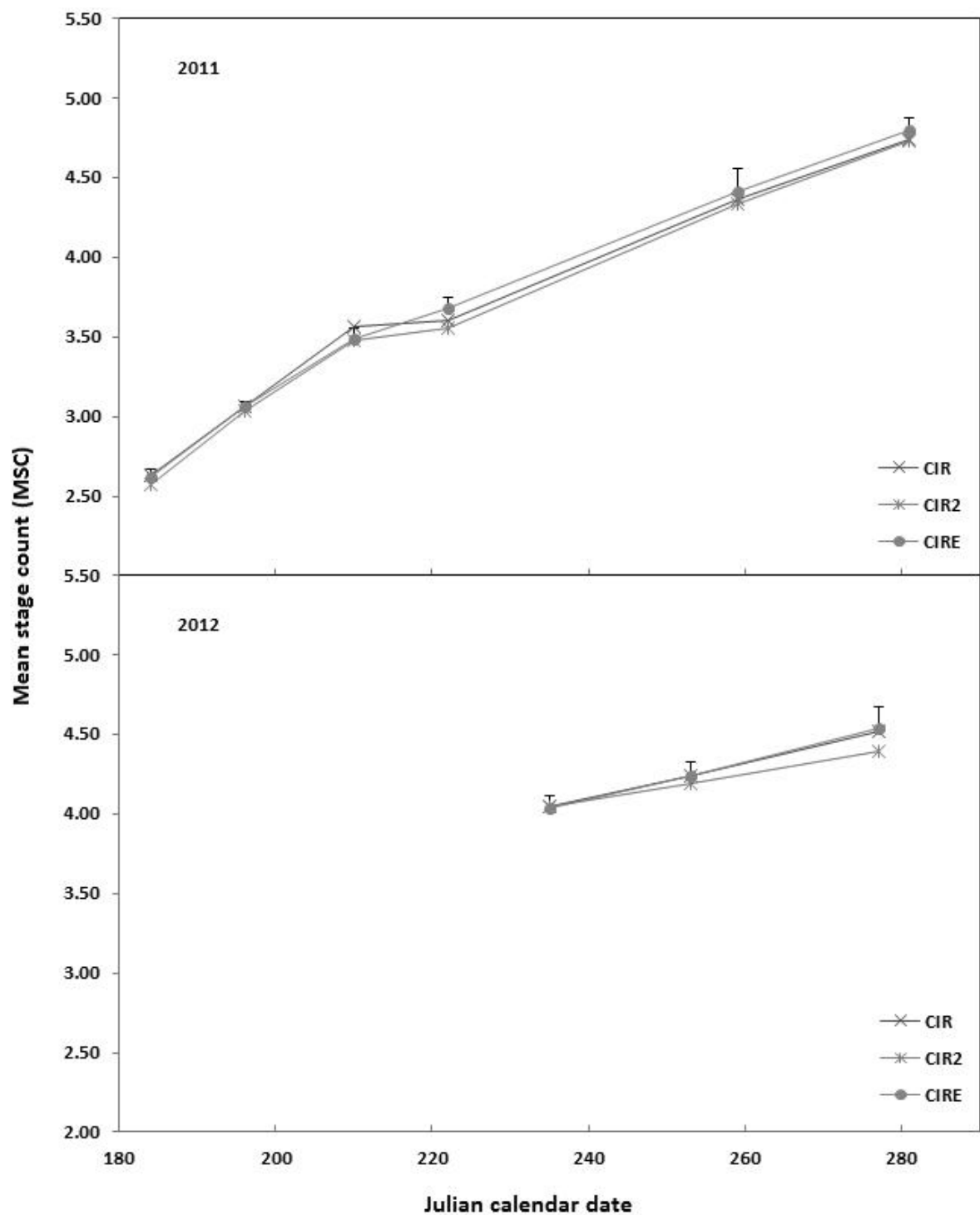


Figure A3.21: Phenology of Cave-in-rock selections of switchgrass at Ste-Anne-de-Bellevue, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

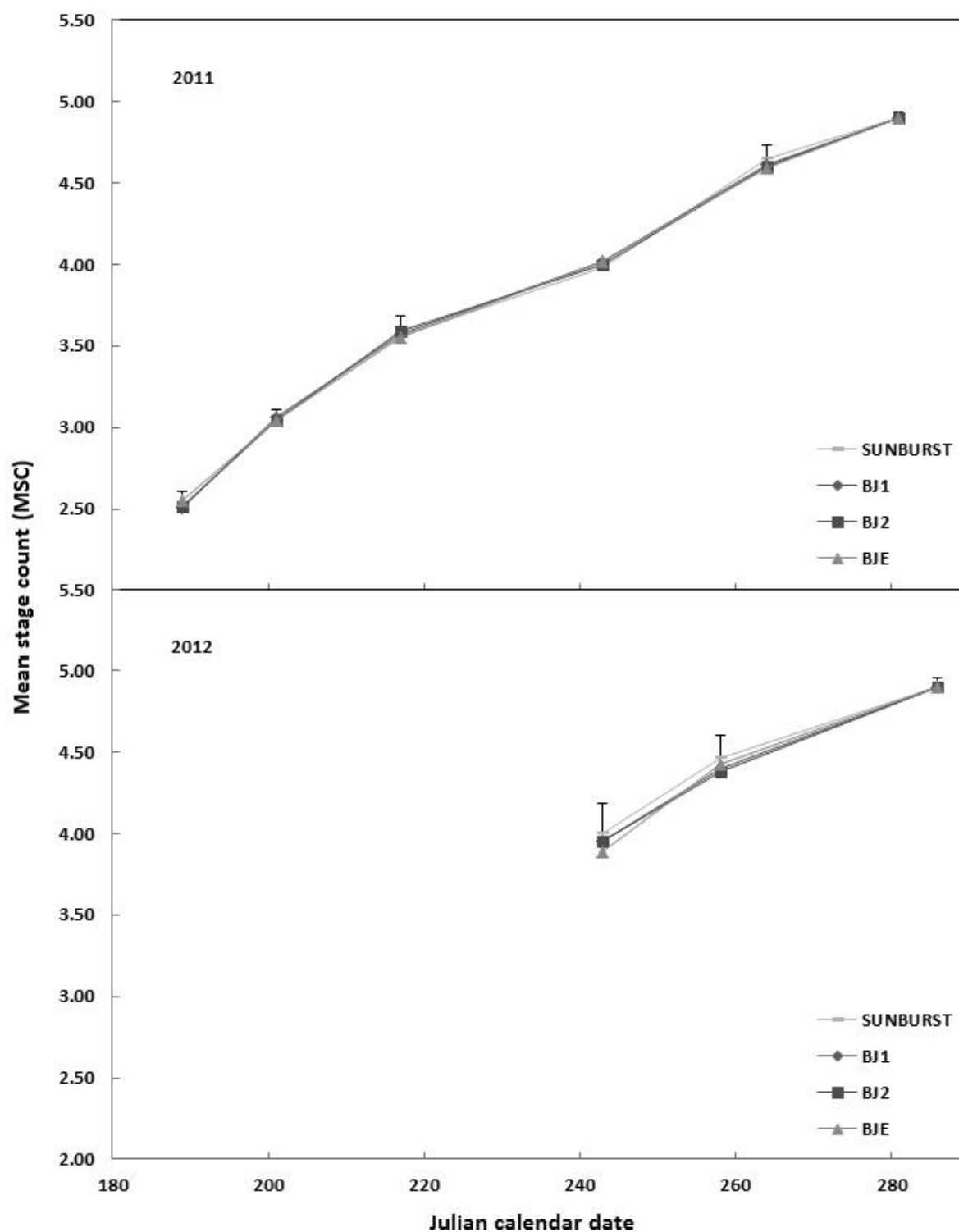


Figure A3.22: Mean stage count of Sunburst lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

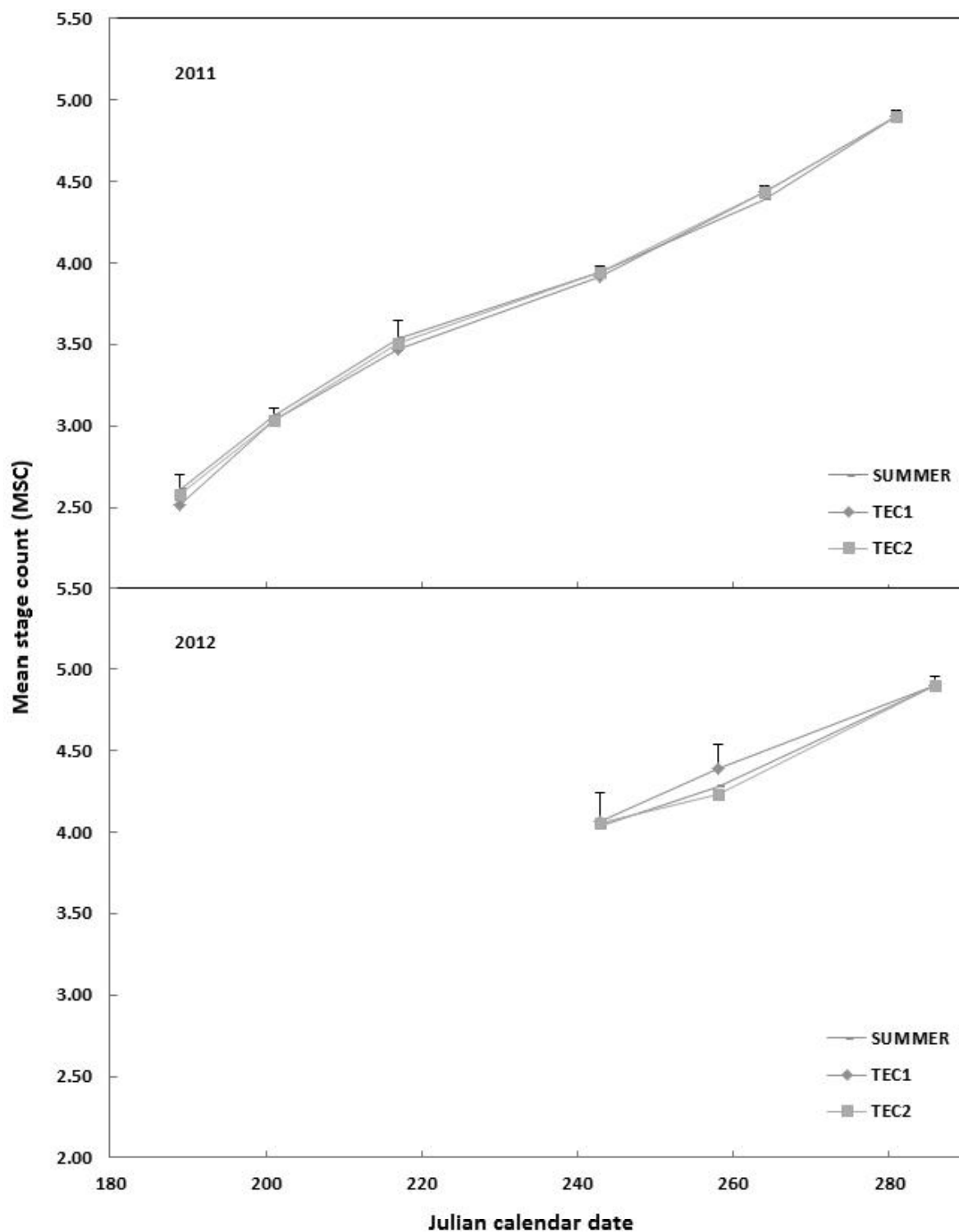


Figure A3.23: Mean stage count of Summer lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

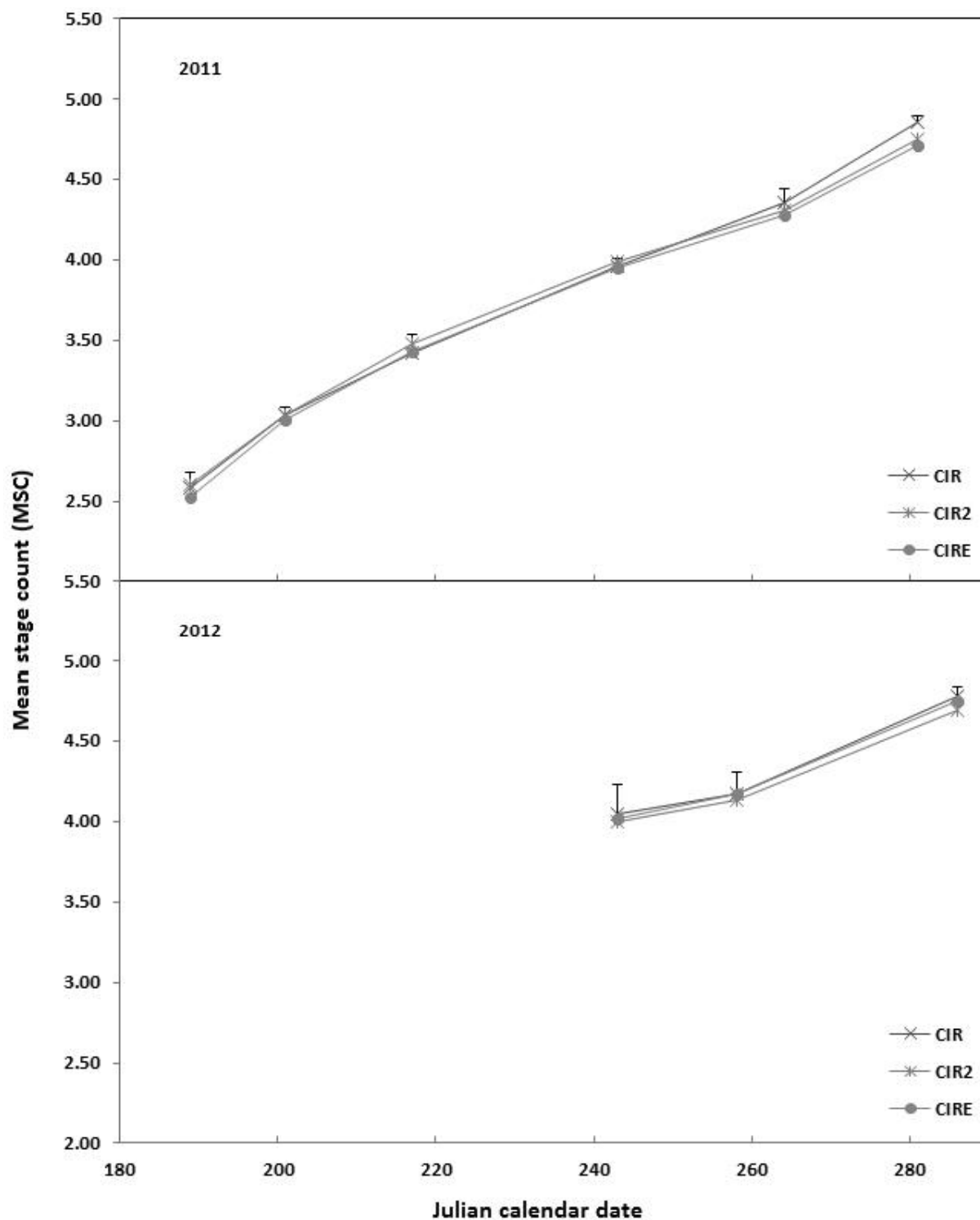


Figure A3.24: Mean stage count of Cave-in-rock lineage selections of switchgrass at Cookshire-Eaton, Quebec in 2011 and 2012. Vertical bars represent the least significant difference (LSD, $p=0.05$) at each sampling point throughout the season.

Table A5.6: Sampling locations for switchgrass biomass and soil samples collected in 2011 in Quebec and Ontario. Mowed column indicates fields that were mowed and left in windrows over winter.

Municipality	Latitude	Longitude	Mowed
Salaberry-de-Valleyfield	45°16.525'	74°00.470'	Y
Grande-île	45°16.430'	74°09.681'	N
Grande-île	45°16.366'	74°09.548'	N
Grande-île	45°16.333'	74°09.445'	N
Grande-île	45°17.103'	74°07.434'	N
Ormstown	45°01.586'	75°20.635'	Y
Ormstown	45°04.981'	73°59.476'	Y
Ormstown	45°04.999'	73°59.291'	N
Ormstown	45°05.043'	73°59.540'	Y
Ormstown	45°05.081'	73°59.682'	Y
Pointe-Lalonde	45°12.551'	73°19.758'	N
Pointe-Lalonde	45°12.427'	74°19.611'	N
Inkerman, ON	45°01.586'	75°20.639'	Y
St-Théodore-d'Acton	45°40.524'	72°34.601'	Y
St-Théodore-d'Acton	45°41.468'	72°34.587'	Y
St-Alexandre	45°14.358'	73°05.570'	Y
Bromont	45°19.271'	72°44.003'	N
Bromont	45°19.292'	72°44.127'	N
Ste-Mélanie	46°09.196'	73°34.694'	N
Ste-Mélanie	46°09.252'	73°34.670'	N
Baie-du-Febvre	46°08.091'	72°38.278'	N
Baie-du-Febvre	46°08.142'	72°38.232'	N
Notre-Dame-du-Lac	47°31.227'	68°40.576'	N
Lennoxville	45°21.833'	71°49.616'	Y
Bury	45°32.347'	71°31.929'	Y
Johnville	45°20.783'	71°47.383'	N
Johnville	45°20.655'	71°47.441'	N

Table A5.7: Sampling locations for switchgrass biomass and soil samples collected in 2012 in Quebec and Ontario. Mowed column indicates fields that were mowed and left in windrows over winter.

Municipality	Latitude	Longitude	Mowed
Ormstown	45°04.972'	73°59.462'	Y
Ormstown	45°05.024'	73°59.515'	Y
Bromont	45°19.278'	72°44.030'	N
St-Alexandre	45°14.280'	73°05.750'	Y
St-Alexandre	45°14.433'	73°05.472'	Y
Ste-Mélanie	46°09.285'	73°34.729'	N
Ste-Mélanie	46°09.249'	73°34.681'	N
Ste-Mélanie	46°09.230'	73°34.720'	N
Ste-Mélanie	46°09.169'	73°34.660'	N
Ste-Hénédine	46°29.986'	70°56.717'	N
Ste-Hénédine	46°30.068'	70°56.800'	N
Ste-Hénédine	46°30.136'	70°56.877'	N
Baie-du-Febvre	46°08.051'	72°38.338'	N
Baie-du-Febvre	46°08.084'	72°38.298'	N
Baie-du-Febvre	46°08.127'	72°38.239'	N
Baie-du-Febvre	46°08.183'	72°38.142'	N
Ste-Clotilde-de-Horton	45°58.807'	72°14.260'	N
Ste-Clotilde-de-Horton	45°58.982'	72°14.362'	N
Ste-Sophie-d'Halifax	46°07.733'	71°46.106'	N
Ste-Sophie-d'Halifax	46°07.679'	71°45.986'	N
Ste-Anges	46°28.730'	70°55.049'	N
Ste-Anges	46°28.704'	70°54.991'	N
Ste-Anges	46°28.449'	70°54.905'	N
Ste-Anges	46°28.402'	70°54.969'	N
Johnville	45°20.415'	71°47.385'	N
Johnville	45°20.485'	71°47.380'	N
Johnville	45°20.445'	71°47.506'	N
Lennoxville	45°22.005'	71°49.001'	Y
Lennoxville	45°23.013'	71°50.007'	Y
Bury	45°32.354'	71°31.937'	Y
Bury	45°32.348'	71°31.971'	Y