Assessing the Potential for Hybrid Vigour Within a Species: Disparate Population Breeding of Balsam Poplar

by

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Abstract

When two or more species are crossed to produce hybrid progeny, some of them can be expected to yield a growth performance far superior to that of either parent (i.e., hybrid vigour/heterosis). To date, there have been few attempts to examine whether hybrid vigour can be achieved by crossing disparate populations of the same species. Balsam poplar (*Populus balsamifera* L.) is a widespread tree species in North America ranging from Alaska to Newfoundland. This wide range makes it an ideal species to study within-species hybrid vigour for increasing genetic gain in growth.

In this thesis project, I tested the hypothesis that within species breeding of widely-spaced populations of balsam poplar leads to the expression of hybrid vigour through the following mechanisms: 1) A longer growing season due to phenology differences, which leads to increased growth; and 2) Differences in endogenous hormone levels that are linked to physiological performance. In addition, I explored epigenetic responses of hybrid balsam poplar to abiotic environmental stress.

In September 2009, three balsam poplar field trials (two in Alberta (AB) (Field AB1 and AB2 respectively) and one in Quebec (QC) (Field QC1)) were established. Five male parents from each province with five female parents from Quebec, and four female parents from Alberta were used for breeding, both for within-region and between-region crosses. Preliminary analysis on six-year height and diameter data from AB1 and AB2 indicated differences in family performance among the different cross-types (within- and between-region crosses). The results from this project showed that the AB♀ × QC♂ cross-type ranked first for height and diameter at breast height (DBH). From phenology study (Chapter 2), the increased growth of hybrid balsam poplar was due to phenological differences (earlier bud burst and often a later bud set). From the

hormone study (Chapter 3), results showed stem volumes calculated from height and diameter of 2-month-old rooted cuttings grown under optimal greenhouse conditions are positively and significantly correlated with stem volumes of 8-year old field-grown trees. Additionally, hybrid vigour is correlated with hormone levels and linked to photosynthetic performance. The epigenetics study (Chapter 4) suggested that fast growing (FG) progeny originating from sources in AB were more resistant to drought than the same genotypes originating from wetter locations. Additionally, cuttings of the same genotype grown in different locations showed different responses under well-watered versus drought conditions. Therefore, epigenetics at the phenotypic level of measurement was successfully detected.

My study demonstrated the potential of using disparate, native populations of balsam poplar to produce superior progeny with enhanced stem growth traits. However, future use of this material on crown land for reforestation or reclamation purposes may require additional field testing to meet policy regulations.

Preface

The overall study idea was conceived by Dr. Barb R. Thomas. Mr. Pierre Périnet (retired) was responsible for conducting the breeding protocols and field designs. The protocol for bud phenology was developed by me and David Kamelchuk based on Dr. Catherine Bastien and Dr. Raju Soolanayakanahally's work. The protocol for the extraction of hormones was devised by the late Dr. R.P. (Dick) Pharis and Mrs. Loeke Janzen (retired). Loeke Janzen also advised on data interpretation and thesis editing. Advice on data interpretation was also provided by Dr. Rong-Cai Yang and Dr. Barb R. Thomas. Mr. Steven Williams (retired) and Ms. Sarah Jespersen provided technical support in the greenhouse. Briana Ledic, Gregg Hamilton, Victoria Diederichs, Ho-Chun (Aaron) Chen, and Michael Thomson aided in the propagation, care and maintenance of plant material, and in the measurements undertaken in the greenhouse and field.

The field experiment was already in place when I started. I devised the greenhouse experiment, conducted gas exchange and growth measurements, collected and prepared samples for hormone analysis, and phenological scoring. I was also responsible for the data analysis and writing the thesis.

Chapter 2 of this thesis is in preparation to be submitted for publication (Plant, Cell & Environment) as Hu, Y., Yang, R-C., Thomas, B.R. Phenological factors responsible for heterosis in hybrid balsam poplar (*Populus balsamifera* L.). I was responsible for data collection and analysis as well as drafting the manuscript. B.R. Thomas was involved in developing the idea for the experiment and editing the manuscript. R-C. Yang was involved in designing the data analysis, editing the manuscript and data interpretation.

Chapter 3 of this thesis has been published as Hu, Y., Thomas, B.R. 2019. Hormones and heterosis in hybrid balsam poplar (*Populus balsamifera* L.). Forests 10: 143.

doi:10.3390/f10020143. I was responsible for performing the greenhouse experiment, data collection and analysis and drafting the manuscript. B.R. Thomas was involved in developing the idea for the experiment and editing the manuscript.

Chapter 4 of this thesis is in preparation to be submitted for publication (Tree Physiology) as Hu, Y., Thomas, B.R. An evaluation of epigenetic responses to drought stress through physiological parameters in hybrid balsam poplar (*Populus balsamifera* L.). I was responsible for performing the greenhouse experiment, data collection and analysis as well as drafting the manuscript. B.R. Thomas was involved in developing the idea for the experiment and editing the manuscript.

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I am grateful to Mr. Pierre Périnet (retired), who oversaw the breeding and designed the field set-up. I will never forget the conversations we had, often over beer. He really made me feel the passion of being a tree breeder. I am indebted to Mr. David Kamelchuk who helped me

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I appreciate Steven Williams (retired) at the Bioscience greenhouse for helping me arrange the space for my entire project and teaching me how to take care of cuttings in the early stage of my project. Thanks to Sarah Jespersen from the Bioscience greenhouse for providing me space and answering my questions. Special thanks to Loeke Janzen (retired) for the hormone analysis at University of Calgary, who taught me how to run hormone analysis for my project. I thank Nikki-Karyssa Scott in the Ag-For greenhouse at the University of Alberta for giving a lot of useful advice on my project.

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I thank Briana Ledic, Gregg Hamilton, Victoria Diederichs and Ho-Chun (Aaron) Chen for their assistance in collecting and processing plant material, and for their assistance with data collection. I know the work was boring, but when we worked together we always had fun. Special thanks to my assistant Michael Thomson during my entire Ph.D. career. Thank you for always listening to K-pop and sharing stories with me. With you, even repetitive work became enjoyable.

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Table of Contents

List of Tables

List of Figures

Chapter 1. General introduction

1.1 Biology of Poplars and Their Importance for Plantations

In Canada, forest land area is about 347 million hectares (ha), which represents nearly 9% of the world's forests (Natural Resources Canada 2020a). The forests not only reduced CO_2 emissions by 38% in 2018, they also contributed $28 billion to Canada's economy that year (Natural Resources Canada 2020a). The boreal forest in Canada represents 25% of the world's remaining intact forest (Natural Resources Canada 2020a). In Canada's boreal forest, species of the genus *Populus* are the most widespread deciduous trees, covering a total of 13.1% of the boreal region, and are second only to the genus *Picea* (spruce) (Natural Resources Canada 2020b). Poplars are a relatively short-lived, pioneer species with a dioecious breeding system. In addition, poplars are shallow-rooted and young poplars' roots are generally concentrated within the top 15 cm of the soil (Al Afas et al. 2008; Douglas et al. 2010). Because poplars have rapid leaf production and high net assimilation rates, they are a group of productive species (Rhodenbaugh and Pallardy 1993). The genus *Populus* was considered as one of the oldest contemporary angiosperms with about 30–35 poplar species world-wide, it has been categorized into six sections due to rapid allopatric speciation that the species population separated by geographic separation and subsequent evolution (for review, see Eckenwalder 1996). As poplars are a pioneering species, the migration of genes between sections within this genus can occur easily and quickly throughout most areas in the north temperate zone (Kaul 1995). In North America, *Populus* appeared as early as the late Paleocene, about 58 million years ago, according to the ancient fossil records (for review, see Collinson 1992; Manchester et al. 2006). Due to its wide distribution in different climatic zones and life history traits, the genetic variability within

the species allows for the selection of genotypes according to different habitats (Chen and Polle 2010; Brunner et al. 2004).

Poplars not only play a significant role in the agricultural landscape as agricultural shelterbelts (Arens et al. 1998), they provide ecological diversity for the boreal forest. Poplars and hybrids are grown worldwide due to their economic importance (Poplar Council of Canada 2012). They can be used for timber production (plywood, lumber, paper), woody biomass, and bioenergy production (for review, see Sannigrahi et al. 2010). Additionally, poplars are used for environmental protection, e.g. phytoremediation (Isebrands and Karnosky 2001). Poplars have a wide natural distribution in the northern hemisphere and are less common but still exist in small numbers in tropical Africa (Dickmann and Kuzovkina 2014). In the northern hemisphere, the genus *Populus* (poplars, cottonwoods and aspens) contains approximately 30 species of woody plants. Some poplars, such as balsam poplar (*Populus balsamifera* L.), are riparian species that grow along river margins and banks. Cottonwoods, such as eastern cottonwood (*Populus deltoides ssp. deltoides* Bartr. ex Marsh.), are pioneer species that typically establish on freshly exposed sandbars, streambanks, or river valley flood plains. Aspens, such as trembling aspen (*Populus tremuloides* Michx.), are generally upland species that grow in moist upland areas (Peterson and Peterson 1992). Cottonwoods and aspens usually tend to live no more than 150 years, although some of the trees may live for more than 200 years (Perala 1990; Van Haverbeke 1990).

Poplars are normally diploid (2n=38) with two sets of 19 chromosomes (Smith 1943). However, triploids and tetraploids have also been identified in this species (Esinspahr et al. 1963; Every and Wiens 1971). In 2004, the sequencing of the genome of black cottonwood (*P. trichocarpa* (*Populus balsamifera ssp. trichocarpa* (Torr et A. Gray) Brayshaw)) was completed

by the Joint Genome Institute in the United States of America (USA). *P. trichocarpa* became the first tree species to be sequenced (Tuskan et al. 2006). Poplar was selected as the model forest tree for genetic mapping and cloning because of its rapid growth, relatively small genome size for a tree, relative ease of experimental manipulation, and the favourable ratio (200 kb/centimorgan) between physical distance and genetic distance in chromosomes (for review, see Bradshaw et al. 2000). The genome size of poplar (about 485 Mb (megabases)) is six times smaller than the genome of maize, and 40 times smaller than the genome of conifers such as loblolly pine (Tuskan et al. 2006; Dickmann and Kuzovkina 2014). Moreover, the 200 kb/cM ratio in the poplar genome benefits the process of positional gene cloning (for review, see Bradshaw et al. 2000).

Poplars are considered to be drought-sensitive (Marron et al. 2003; Hennig et al. 2015). However, different poplar species can adapt to different drought levels. For example, aspen (*P. tremuloides*) can survive in places with low precipitation and periodic drought, whereas *P. deltoides* and *P. alba* are more drought-sensitive and are found in wetter lowland and riparian areas (for review, see Marron et al. 2008).

1.2 Balsam poplars

Balsam poplar (*Populus balsamifera* L.) is a medium-sized, dioecious species that spans about 110° in longitude (55° to 165° W.) and 26° in latitude (42° to 68° N.) with its wide distribution in North America ranging from Alaska to Newfoundland (Zasada and Phipps 1990). Since balsam poplar can live in both high (up to 44°C) and low temperatures (down to -62°C), it is widely distributed in its natural range (Richardson et al. 2014). Balsam poplars can be reproduced both sexually and asexually. The flower production of balsam poplar begins when the trees are about eight years old. Seeds are typically produced every year (Zasada and Phipps

1990). Seeds are dispersed through wind and most of them fall within 200 m of the parent tree. The seeds remain viable for two to four weeks and usually germinate immediately under suitable growing conditions, i.e. moist mineral soil. The germination rate of balsam poplar has been shown in the greenhouse to be between 98 to 100 percent in two to three days at temperatures ranging from 5 to 25 °C. However, the seedlings require at least one month of high moisture level to survive (Peterson and Peterson 1992; Poplar Council of Canada 2012). Additionally, balsam poplar can be regenerated through vegetative means such as root suckers, stump sprouts, stem sprouts, and buried branches (Zasada and Phipps 1990; Peterson and Peterson 1992). The root suckers of balsam poplar typically grow from roots of about one cm in diameter within the top two cm of the soil and their formation can be enhanced by soil disturbance. If the temperature of the disturbed soil is warmer than that of the undisturbed soil, the activity of balsam poplar's root suckers will increase (Rood et al. 1994; Kalischuk et al. 2001). Schier and Campbell (1976) used mean height of the tallest suckers per segment as a measure of sucker growth. They found that the root suckers of balsam poplar were larger than eastern cottonwoods (*P. deltoides*) and had superior rooting capacity than aspen (*P. tremuloides*) suckers (Schier and Campbell 1976).

Balsam poplar is one of the tree species that can regenerate from sprouts and suckers after fire in the boreal forest (Richardson et al. 2014). Krasny et al. (1988) found that regeneration of balsam poplar through root suckers following clearcutting or fire was a primary method of expansion in the landscape. The vegetative expansion process is very fast after establishment on more mesic sites, such that balsam poplar can expand onto drier, sandier sites adjacent to river floodplains. Similar to other pioneer species, balsam poplar requires full sunlight and no

competition to grow (shade-intolerant) and exhibits rapid juvenile growth (Zasada and Phipps 1990).

Balsam poplar can be attacked by several insects and is susceptible to several common pathogens. The insects that are the most dangerous to this species include poplar and willow borer (*Cryptorhynchus*), bronze poplar borer (*Agrilu*s), and poplar borer (*Saperda*). Other insects, such as the forest tent caterpillar (*Malacosoma*) and aspen leaf beetle (*Chrysomela*), can also attack trees of this species causing minor damage (Morris et al. 1975). The most common fungal pathogens affecting balsam poplar are *Phellinis*, *Pholiata*, *Corticium*, and *Bjerkandra*. These pathogens commonly cause decay of mature balsam poplars (Hiratsuka and Loman 1984). However, the damage linked to these diseases is highly dependent on the association between the site and environmental conditions (Zasada and Phipps 1990). In addition, foliage diseases such as *Septoria* leaf spot can reduce the aesthetic value and cause premature defoliation of balsam poplar (Ostry and McNabb 1995). *Septoria* stem canker caused by the fungal pathogen *Septoria musiva* Peck (*Mycosphaerella populorum* G.E. Thomps.) can easily infect a whole tree from a single stem infection and ultimately result in poplar plantation failure (Ostry and McNabb 1995). Balsam poplars' foliar buds have a high resin content that will reduce cellulose digestion of animals, so they are less attractive to animals than the internodes of twigs and stems (Zasada and Phipps 1990). The wood of balsam poplar is soft and light, so it is used for pulpwood, lumber, crates, boxes, plywood, tissues and to make other high-grade paper products (Peterson and Peterson 1992).

Balsam poplar is a tree species that underwent a massive range expansion at the end of the Pleistocene era (Williams et al. 2004). During this range expansion, boreal forest species including balsam poplar were largely displaced south of their current limits in North America

and migrated northward. As a result, balsam poplar also experienced changes in genome-wide genetic diversity and strong genetic drift through these events (Hewitt 1996). In a study conducted by Keller et al. (2010), they examined the effects of range expansion on the genetic diversity of balsam poplar, the authors then divided populations of balsam poplar in North America into a northern section (Alaska and Yukon), a central section (western and central Canada), and an eastern section (Ontario, Quebec, Maritimes) (Keller et al. 2010) based on their ancestral allele frequencies. Keller et al. (2010) found that the central section was the least distinguished from the refugial population and an unbalanced relocation occurred from the center to north and east. Proof of this is in marker diversity, migration models, and population size. The gradient in the direction of migration is influenced by environmental factors at different latitudes and altitudes, such as temperature, photoperiod, and precipitation. Pioneer species such as balsam poplar, with a large geographical gradient, are expected to exhibit phenotypic variation in traits and show local adaptation to new environments (Keller et al. 2011).

1.3 Hybrid poplars

Natural and artificial hybridization among poplar species has been well documented in the literature (Ceulemans et al. 1992; van den Driessche et al. 2007). On the one hand, hybridization/introgression occurs naturally where the geographic distribution of two species is sympatric. On the other hand, hybrids are also obtained through artificial techniques such as plant breeding. Hybrids can be obtained by crossing two distinct species or two disparate individuals within one species (Sinha and Khanna 1975). Hybrid poplars typically grow well on sites where moisture and nutrients are abundant such as fluvial floodplains (Thomas et al. 2000). Additionally, hybrids can tolerate periodic flooding during the winter when they are not actively growing. However, hybrids can be severely damaged by frost and winter cold (Dickmann et al.

2001). In terms of productivity of hybrid poplars, Truax et al. (2014) evaluated the mean annual growth increment (MAI) of five hybrid poplar clones (*P. trichocarpa* × *P. deltoides* (TxD-3230), *P. deltoides* × *P. nigra* (DxN-3570), *P. xcanadensis* × *P. maximowiczii* (DNxM-915508), *P. nigra* × *P maximowiczii* (NxM-3729) and *P. maximowiczii* × *P. balsamifera* (MxB-915311)) in southern Quebec. The authors found that the mean MAI of the five clones could reach 27.5 m^3 ha^{-1} yr^{-1} at the best site (high fertility and low elevation) which indicates the high productivity potential of these clones. Studies in Sweden found that an MAI of up to 25 m^3 ha^{-1} $year^{-1}$ with a rotation of about 25 years was achieved in selected clones of hybrid poplars on well-fertilized sites (Rytter and Stener 2014; Stener and Westin 2017).

Hybrid poplars have been increasingly cultivated as a source of fibre for various purposes including pulp for the paper industry; raw material for the composite wood industry such as oriented strand board, medium density fibreboard and plywood; and as a supplier of biomass for energy (Telenius 1999; Poplar Council of Canada 2012). Hybrid poplars can also be used for fuelwood, and the use of young poplar branches and foliage as alternative fodder can be very useful during periods of drought (Ball et al. 2005). Other benefits of planted hybrids involve various environmental and ecosystem services such as carbon sequestration, protection from soil erosion, increase of annual crop yield by field shelterbelts, wind reduction by farmstead shelterbelts, and the provision of wildlife habitat (Ball et al. 2005). Hybrids play an essential role in forest reclamation by rapidly stabilizing soils of degraded areas (i.e. abandoned forestry roads) (DesRochers et al. 2004) and are used for phytoremediation (Isebrands et al. 2000; Isebrands et al. 2014). From a review by Richardson et al. (2007), they indicated that it was possible to reduce the rotation length from 60 to 120 years for native aspen and balsam poplar to less than 25 years for hybrid poplar in the boreal forest, demonstrating the potential benefits of hybrid

poplars to Canada's forestry industry. Further benefits include restoring or improving riparian habitats by reducing run-off in annual agriculture crops (for review, see Schultz et al. 2004). Last but not least, Arnold et al. (2008) found that some superior genotypes occurring through hybridization would be better adapted under more severe extreme climatic conditions. Zanewich et al. (2018), found cottonwood hybrids perform better than their parental species at suboptimal temperatures which indicated phenotypic stability could allow hybrids to better adapt to a broader environmental range than their parental species.

1.4 Heterosis

Heterosis is defined as an increase in the size, vigour, and productivity of hybrid offspring over the average of both its parents (Sinha and Khanna 1975). In addition, the increase in vigour can contribute to many components such as carbon allocation patterns, water and nutrient use efficiency, and shoot growth phenology (Yu et al. 2001). Hybrid vigour, typically achieved through the controlled crossing of two species, or pure genetic lines of the same species, has long been exploited in agriculture (Shull 1909; Wehrhahn and Allard 1965; Meyer et al. 2004) and in some tree species including *Populus* (Stettler et al. 1996). For poplars, including the aspens, two or more species are typically crossed to produce hybrid progeny that are superior to their parents. Research conducted by Niemczyk et al. (2019) found that clones of *Populus tremula* x *Populus tremuloides* exhibited higher diameter at breast height (DBH), height, volume production and mean annual increment than the local wild aspen population of *P. tremula*. These interspecific hybrid aspen clones also showed better chemical and physical properties which made them more suitable for paper products than the pure aspen clones (Niemczyk et al. 2019). Results from this study also suggested that the use of hybrid aspen could reduce the stand rotation age from 40 to 20 years, providing the potential to increase pulp and paper production in

Poland (Niemczyk et al. 2019). In another study, Ceulemans et al. (1992) compared the performance of 12 poplar clones ((three clones of *P. trichocarpa* (black cottonwood) from northern latitudes (44-49°N), three clones of *P. deltoides* (plains cottonwood) from southern latitudes (30-39°N), and six F_1 hybrids (between the two species)) in a four-year field study. They found that all hybrids showed better growth performance (height, diameter and stem volume) and had a longer growing season than their parents. Since the progeny had larger leaves than their parents, the progeny might have also inherited the capacity for a greater number of cells and size of cells in their leaves, providing a larger surface area for photosynthesis, resulting in an increase in productivity which ultimately led to an increase in biomass (Ceulemans et al. 1992).

The practical application of intraspecific hybridization in plant breeding has been quite successful to improve agricultural or horticultural plant species through the development of hybrid cultivars with increased yields, biomass, oil, seed production, etc. However, the basic understanding of this phenomenon is not very advanced in forestry (Goulet et al. 2017). To date, there have been few attempts to examine whether hybrid vigour can be achieved by crossing disparate populations of the same species in forestry. For intraspecific hybridization, it can simply be defined as hybrids that are produced from two individuals with distinct characteristics within a species (Ford 2013). For a detailed definition, Stebbins (1959) explained the process as successful matings between individuals from distinct populations originally isolated from one another with different allele frequencies. If the F_1 hybrid individuals or later generation hybrids exhibited high productivity, the recombination from them would lead to novel genetic rearrangements which could allow hybrids to expand their ecological tolerance and occupy new environments (Stebbins 1959; Arnold 1997). Additionally, Pearson et al. (2010) found that

planted hybrids, which were highly productive, were usually more widely adaptable or tolerant of environmental extremes than the parents, which was attributed to both hybrid vigour and intensive cultural practices. Moreover, from a review study conducted by Lee (2002), the author indicated that intraspecific hybridization could lead to heterosis by masking the effects of recessive deleterious alleles, reinstalling the over-dominant loci, modifying epistatic interactions, increasing genetic variance, and transferring favorable genes. Alternatively, some artificial hybrids might have lower vigour and productivity when compared to their parents. This is due to physiological or developmental irregularities that occur through the hybridization process (Burke and Arnold 2001). Additionally, outbreeding depression from intraspecific hybridization of plant species, such as sunflower species *Helianthus annuus*, was primarily caused by disrupting coadapted gene complexes and local adaptation (for review, see Arnold 1997).

The biological basis of heterosis has been of primary interest for biology researchers for many years due to its scientific and practical significance. Complete clarification of the mechanism for this phenomenon requires knowledge at three levels: genetic, molecular, and physiological (Ma et al. 2011). However, researchers (Yu et al. 2001; Soolanayakanahally et al. 2009) have found that in addition to the aforementioned three levels, phenology might also play an important role in explaining the phenomenon.

1.4.1 Genetic basis of heterosis

The genetic basis of heterosis currently relies on information collected from quantitative trait loci analyses of yield-related traits in various plant species (Stuber et al. 1992; Yu et al. 1997; Kusterer et al. 2007). There are currently three key possible competing explanations that exist to explain the genetic basis of heterosis: 1) the dominance hypothesis demonstrating that deleterious recessive alleles of one parent is complemented in the F_1 hybrid by the dominant

alleles of the other parent (Davenport 1908); 2) the overdominance hypothesis illustrates that the heterozygous combination of the alleles at a locus is better than either of the two possible homozygous combinations (East 1908; Shull 1908); and 3) the epistasis hypothesis explains that the combined effect of the interaction between two loci results in superior F_1 progeny (Williams 1959).

Various studies have shown that overdominance (Hoecker et al. 2008; Wang et al. 2016), epistasis (Li et al. 2020), and dominance (Yang et al. 2017) provided evidence of why hybrid maize outperforms non-hybrid maize. Similar findings were also found in genetic studies that examined the genetic basis in hybrid rice (Yu et al. 1997; Huang et al. 2016). In a recent study conducted by Huang et al. (2016), they found that incomplete dominance and overdominance effects played primary and secondary roles, respectively, in rice heterosis via an integrated genomic approach by using 17 F_2 populations. On the other hand, Yu et al. (1997) identified the importance of epistasis in influencing the kernel number and grain weight in hybrid rice.

The discussion about the genetic basis of heterosis has continued for over 100 years. Recent genomic studies have suggested that in most cases, multiple genetic mechanisms, including dominance, overdominance, epistasis, and epigenetics, act simultaneously in producing heterotic phenotypes in F_1 hybrids (Flint-Garcia et al. 2009; Shen et al. 2014; Shang et al. 2015). This finding also implies that heterosis across hybrids is mainly trait-specific as most traits are likely to be controlled by multiple genes (for review, see Kaeppler 2011). For example, the genes such as H*d3a,* which relate to key traits in hybrid vigour of rice, regulate the pathways involved in flowering time, plant architecture and panicle development (Huang et al. 2016). Although genetic studies can help us understand heterosis, the genetic components of heterosis are still unclear (Li et al. 2018). Heterosis for shoot growth is typically through the complementation of

two or more independent traits (Pearce et al. 2004). From a study conducted by Stettler et al. (1988), they found that hybrids of *P. deltoides* × *P. trichocarpa* exhibited higher stem basal area than that of either parent but with similar height of the taller parent. This finding confirmed that stem growth including stem basal area and height are controlled by different developmental processes. For stem basal area, it is the secondary growth involving the cambium, on the other hand, height is the primary growth at the main shoot apex (Pearce et al. 2004). Overall, it is critically important to use phenotypic assessments of hybrid vigour rather than hybrid sterility in natural and controlled environments to determine the contribution of heterosis in plant evolution (for review, see Goulet et al. 2017).

1.4.2 Molecular basis of heterosis

In addition to the traditional explanations describing the root causes of heterosis, recent studies have revealed a new approach that epigenetic regulation might also contribute to heterosis in the hybrids produced within and between species (for review, see Greaves et al. 2015). Epigenetic modifications of crucial regulatory genes which induce cascade changes in downstream genes and physiological pathways in hybrids can ultimately alter complex regulatory networks of physiology and metabolism, leading to changes in the programming of genes that promote the growth, biomass, stress tolerance, and fitness of hybrids (for review, see Chen 2007). From a study conducted by Ni et al. (2009), F_1 hybrids of *A. thaliana* and *A. arenosa* had better growth than their parents. Ni et al. (2009) attributed the increase in growth to epigenetic modifications in the circadian clock genes in *Arabidopsis*, which ultimately increased the chlorophyll and starch content by changing the downstream gene expression. Additionally, epigenetics might act as a mechanism in affecting the growth and fitness in hybrids of *Arabidopsis thaliana* by changing the gene expression in their metabolic pathways (for review,

see Chen et al. 2013). Moreover, another study by Wang et al. (2015) showed F_1 hybrids of *Arabidopsis* exhibited gene expression levels outside of the parental range for abiotic stress and hormone response pathways, partially due to epigenetic regulation. Another popular explanation is that small RNAs, including microRNAs and small interfering RNAs, may contribute to heterosis by regulating F_1 hybrids' target genes, especially in controlling the auxin signaling pathway (for review, see Ng et al. 2012). To better understand the molecular basis of heterosis, quantitative trait loci (QTL) analyses has been used in the past (for review, see Lippman and Zamir 2007). However, these earlier studies were only able to demonstrate that heterosis was defined by a limited number of individual genes inherited in a complicated way (for review, see Hochholdinger and Hoecker 2007). The challenge in developing a molecular model for heterosis is to make the correct connections between phenotype and any relevant molecular events that occur in hybrids. Studies that detected heterosis at the molecular level could be classified into three categories (for review, see Hochholdinger and Hoecker 2007). First, different inbred lines of maize have been analyzed at various loci where the expression of several genes in genome organization has been tested; second, different developmental stages and tissues in different species (maize, rice, and *Arabidopsis*) have had their transcriptome-wide gene expression profiles examined. Finally, a selected number of genes have been tested to determine the allele-specific contribution to gene expression (for review, see Hochholdinger and Hoecker 2007). Large numbers of differentially regulated genes were discovered in several plant species through high-throughput expression profiling of heterotic crosses to explore the molecular basis of heterosis (Guo et al. 2006; Swanson-Wagner et al. 2006; Zhang et al. 2008). Although the same results have not emerged in maize from the review conducted by Springer and Stupar (2007), it is thought that allelic variants at a large number of loci provide favorable combinations, although partial to complete dominance would result in superior hybrid phenotypes (for review, see

Springer and Stupar 2007). In addition, Springer and Stupar (2007) indicated that other factors such as DNA methylation, allelic variations in genetic sequences, and histone chromatin also contribute to the molecular basis of heterosis. For poplars, several studies have been conducted to explore the molecular basis of heterosis via genetic mapping and gene expression profiling (Zhuang and Adams 2007; Jiang et al. 2016; Han et al. 2020). In a study conducted by Zhuang and Adams (2007), they studied the allelic variation in gene expression using *Populus trichocarpa* × *Populus deltoides* interspecific F_1 hybrids for 30 genes and concluded that hybridization within poplars had extensive effects on allelic expression patterns which might lead to phenotypic changes.

1.4.3 Physiological basis of heterosis

Information on the physiological basis of heterosis is limited and mainly focused on specific traits, such as freezing tolerance in *Arabidopsis* (Korn et al. 2010). With the development of technologies for high throughput metabolic profiling, it is now possible to predict heterosis using both genetic and physiological metabolic markers (i.e., markers that are used in plants to examine the relationship between relative levels of particular metabolic compounds and biomass (Gärtner et al. 2009; Andorf et al. 2010), which will be of considerable value for finding physiological clues to heterosis. Gibberellic acids (GAs) are a group of more than 100 tetracyclic diterpenes, some of which are essential endogenous regulators that influence growth and development processes throughout the plant life cycle, including shoot elongation, the expansion and shape of leaves, flowering, and seed germination (for review, see Gupta and Chakrabarty 2013). Ma et al. (2011) used combined analyses of fluctuations of endogenous GA content and expression levels of GA-related genes to show GAs played a regulatory role in heterosis for rice seedling growth at the physiological level. For broad-leaved trees, such as

poplars, gibberellins (GAs), particularly the 3β-hydroxylated, growth-active GA_1 and GA_4, are causal for stem elongation (Bate et al. 1988). Rood et al. (1994) found that GA_1 played a promoting role in the primary growth of poplar stems after GA was applied to *Populus* hybrids. Ectopic overexpression of a GA 20-oxidase gene in hybrid aspen (*Populus tremula* × *P. tremuloides*) resulted in trees with faster growth in height and diameter, larger leaves, more numerous and longer xylem fibers, and increased biomass (Eriksson et al. 2000). In other poplar hybrids (*Populus deltoides* × *P. nigra*), an increase in the GA_1 concentration in the cambial region tissues has been linked to hybrid vigour for stem radial growth (Bate et al. 1988). Various studies (Eriksson et al. 2000; Reinecke et al. 2013) have also found that the concentration of growth-active GAs in growing stems is controlled by transcriptional regulation of genes encoding for both biosynthetic (*GA20ox* and *GA3ox*) and catabolic (*GA2ox*) enzymes, and that modifying the expression of these genes can alter plant growth rate. The relationship between the expression of the GA biosynthetic genes and hybrid vigour supported evidence that GAs played a role in the regulation of heterosis for shoot growth in trees (for review, see Hedden 2016). Furthermore, studies showed that metabolism of $[^3H]GA_{20}$ was faster in heterotic hybrids than parental genotypes in maize and sorghum, indicating faster oxidative metabolism in fast-growing hybrids, which proved that GAs have a role in regulating heterosis (Rood et al. 1983; Rood et al. 1994).

In addition to GAs, indole-3-acetic acid (IAA), has been causally linked to tree stem radial growth, thus contributing to heterosis (Uggla et al. 1996; Tuominene et al. 1997; Groszmann et al. 2015; Li et al. 2019). IAA is the most common plant hormone of the auxin class and it controls several phases of plant growth and development such as cell division, elongation and differentiation (for review, see Fu et al. 2015). Recent work in *Arabidopsis* by

Groszmann et al. (2015) found that there was upregulated gene expression in the auxin biosynthesis pathway in F1 hybrids, and that hybrids have larger cells than the parents due to increased levels of auxin. Li et al. (2019) found that an auxin signaling gene *BnaA3.IAA7* (AUX/IAA protein) contributed to yield heterosis by improving plant architecture, which might be useful for breeding superior and high-yield rapeseed (canola; *Brassica napus*) hybrid cultivars of *Brassica* crops.

Abscisic acid (ABA), also called a "stress hormone," is another important hormone that regulates many physiological processes in plants, including seed maturation, seed germination and dormancy, and adaptive responses to drought, waterlogging, and other adverse environmental conditions (for review, see Lim et al. 2015). ABA also regulates IAA biosynthesis and activity (for review, see Fu et al. 2015). In several maize studies, the endogenous levels of ABA were neither positively nor negatively correlated with hybrid vigour, which indicated ABA did not appear to be involved in the process of heterosis (Sarkissian et al. 1964; Rood et al. 1983). Additionally, Pearce et al. (2004) conducted a study by crossing *Populus deltoides* (Clone 'ILL-129') and *P. trichocarpa* (Clone 93-968) to examine the associations between concentrations of GAs, ABA and IAA, and shoot extension. In the F_1 family, Pearce et al. (2004) found no evidence of a consistent promoting or inhibiting effect of ABA or IAA within the F1 family. However, proteomic analysis of different hybrid combinations during maize seed germination conducted by Fu et al. (2011) found that ABA and gibberellin regulation networks were involved in seed germination heterosis. Overall, other hormones (such as ABA, IAA) may be involved independently or in association with GAs.

1.4.4 Phenology

Phenology examines reoccurring events such as spring bud flush, growth cessation, bud set and leaf senescence and how these activities can be affected under different climatic conditions (Leith 1974). Phenology is primarily affected by two environmental factors: temperature and photoperiod (day length) (Ford et al. 2017). As *Populus* species are always distributed widely, individuals can live in a diversity of habitats following the environmental gradients (McKown et al. 2014). As variation exists in temperature and photoperiods over a geographic gradient, the optimal phenotype of a tree species is also often found to be different through the geographical range (Farmer 1993; Beaubien and Hamann 2011). Various studies (Howe et al. 2003; Alberto et al. 2013; Lutter et al. 2016; Richards et al. 2020) have found that phenology traits are highly related to growth and development under local selection for species with large geographic gradients. Richards et al. (2020) used a quantitative genetic approach to assess the phenotypic variation in a clonally replicated population of *Populus trichocarpa* to determine this species capacity for climate adaptation. The results suggested that *P. trichocarpa* populations had the ability to adapt their phenology in response to climatic change without having any negative impacts on growth.

Research related to phenology has shown different results in reflecting the importance of genetic differences or local adaptation. For example, a study conducted by Chuine et al. (2000) with nine European tree species found little difference in climatic requirements among populations and suggested that local adaptation was not crucial to modelling phenological responses. This finding was confirmed by Vitasse et al. (2009), whose research compared the phenology of three European woody species across an elevational gradient. Other studies have found different amounts of cooling or thermal requirements for bud flush among populations of

different tree species when individual plants were grown in a common garden (Olson et al. 2013; Zohner and Renner 2014). These findings taken together confirm the importance of local adaptation and show genetic differences in phenology (Olson et al. 2013; Zohner and Renner 2014).

Strong genetic differentiation in bud set and leaf senescence across geographic ranges has been found throughout the literature (Wüehlisch et al. 1995; Howe et al. 2003; Ingvarsson et al. 2006; Aitken et al. 2008; Fracheboud et al. 2009; Soolanayakanahally et al. 2009; Holliday et al. 2010 Soolanayakanahally et al. 2013) as the critical photoperiod of many forest tree species tends to increase with latitude of origin. Howe et al. (1995) examined the bud set of northern (54°N) and southern ecotypes (34°N) of black cottonwood (*P. trichocarpa*) and showed that northern ecotypes not only require a longer critical photoperiod to set bud, but are more sensitive to photoperiod than the southern ecotypes in the greenhouse. The authors found that these differences were due to the northern ecotypes had shorter response time to photoperiod. Here, the ecotype referred to the population's ability to adapt to specific photoperiodic conditions (Howe et al. 1995). Research by Yu et al. (2001) suggests that the increased growth of hybrid aspen progeny, when compared with the local pure species, was due to a longer period of summer growth as a result of combined, earlier bud-burst in spring and a later bud-set in fall. Parental origin played a significant role in determining growth patterns, with the more northern parent (*Populus tremula*) having stronger genetic control over growth cessation than the more southern parent (*Populus tremuloides*). In addition, Li and Wu (1996, 1997) determined that it was the female aspen parent (*P. tremuloides*) that primarily influenced growth patterns and phenology in the progeny (*P. tremuloides* × *P. tremula*) at the juvenile growth stage.

The influence of climatic factors on phenology is well documented (Hunter and Lechowicz 1992; Rood et al. 2007; Fu et al. 2014; Yun et al. 2018). In temperate regions, temperature plays a dominant role in phenology where moisture is not limiting (Delpierre et al. 2016). Additionally, for most temperate and boreal tree species, the initiation of spring phenology (bud burst) is primarily controlled by temperature whereas the fall phenophases (i.e., bud set, leaf senescence) are mostly affected by photoperiod (Way and Montgomery 2015). Hunter and Lechowicz (1992) concluded that there was a strong correlation between the accumulation of chilling during the winter and heat during the winter/early spring and the timing of bud flush and leaf emergence for both individual plants and forest plantations. Cooke et al. (2012) found that the bud flush of poplar was mostly initiated by temperature changes when the dormancy was broken by accumulative heat sums. In addition to temperature and photoperiod, other studies have found pre-season precipitation to have an influence on spring phenology (Fu et al. 2014; Yun et al. 2018).

1.5 Epigenetic regulation during drought stress in plants

Plants can cope with biotic and abiotic stresses in the natural environment through biochemical and molecular changes including epigenetic regulation of gene expression (for review, see Chinnusamy and Zhu 2009). Abiotic stresses such as drought, high salinity, extreme temperatures, anoxia, and nutrient deficiencies usually significantly affect plant growth, productivity, and survival (for review, see Zhu 2016). Barber et al. (2000) found that many forest species could lose up to 45% in radial growth through decreased stem hydraulic conductance and aboveground biomass production due to drought stress. Epigenetics refers to heritable gene expression changes that do not involve changes in the DNA sequence (for review, see Peschansky and Wahlestedt 2014). Epigenetic mechanisms are widely involved in the plant

abiotic stress response by regulating stress-responsive genes at the transcriptional and posttranscriptional levels by altering the chromatin status of genes in various ways including DNA methylation, histone modifications, small interfering RNAs (siRNAs), some long non-coding RNAs (lncRNAs), and small RNA-mediated RNA silencing genes (Liu et al. 2018; Zhao et al. 2018). Additionally, in a review by Friedrich et al. (2019), they summarized that the formation of stress memory, which might be inherited by the offspring, was significantly correlated with epigenetic mechanisms.

In plants, the NAC (no apical meristem (NAM), *Arabidopsis* transcription activation factor (ATAF) and cup-shaped cotyledon (CUC) transcription factors (TFs)) have been reported to be involved in regulating plant development, and biotic and abiotic stress responses (Wang and Dane 2013). *Populus* contains 163 full-length NAC genes (Hu et al. 2010). In plants, ABA is the key phytohormone that relates to drought stress (for review, see Shinozaki and Yamaguchi-Shinozaki 2007). ABA promotes drought resistance while drought stress induces the synthesis of ABA in plants (for review, see Shinozaki and Yamaguchi-Shinozaki 2007). Several studies that examined the epigenetic mechanisms during drought in poplar suggested the chromatin status shaped by histone modifications and DNA methylation played crucial roles in the drought stress response (Liang et al. 2014; Li et al. 2019). Liang et al. (2014) found drought stress treatment induced alterations in the DNA methylation level, which modified the expression patterns of many drought stress-responsive genes in *Populus trichocarpa*. In addition to DNA methylation, regulation of histone dynamics can affect the drought stress response. Research conducted by Li et al. (2019) examined the relationship between genome-wide acetylated lysine residue 9 of histone H3 (H3K9ac) enrichment and transcriptomes in *Populus trichocarpa* under drought stress. The authors concluded that the combined function of the ternary proteins established a

coordinated histone modification and transcription factor-mediated gene activation for drought response and tolerance in *Populus* species (Li et al. 2019).

1.6 Research objectives

Balsam poplar is a transcontinental species that occupies a wide range of climatic and site conditions. It often grows in mixed forest stands with conifers or other broadleaf trees, contributing to stand and landscape level diversity (Zasada and Phipps 1990). The wide range of this species makes it a model system to study species hybrid vigour as a tool for increasing genetic gain for volume through increased genetic diversity. Because of the clonal nature of balsam poplar and its ease of propagation, any gain in growth achieved through hybrid vigour can be rapidly exploited in a tree improvement program.

In order to test the hypothesis that within-species breeding will lead to the expression of hybrid vigour, a series of controlled crosses were conducted including local x local and local x distant parental types from both Alberta (AB) and Quebec (QC) sources of balsam poplar.

This project tested a series of hypotheses by assessing physiology, plant growth, phenology, and hormone levels, exploring if hybrid vigour was detectable. In cases where hybrid vigour was present, we used field and greenhouse assessments to determine the underlying mechanisms. Hypotheses tested included: 1) Hybrid vigour is due to endogenous hormone levels and linked to physiological performance; 2) Phenology resulting in a longer growing season explains any increase in growth; and 3) Cuttings of the same clone grown in different locations show the same physiological responses under drought conditions.

1.7 General design of the field experiment

In September 2009, three field trials (Figure 1.1) were established with trees cloned from seedlings, planted in four-tree family plots, with 10 blocks at 2.5x2.5m spacing at two field sites (Field AB1 and Field AB2) in Alberta, at the Alberta-Pacific Forest Industries Inc. (Al-Pac) millsite (54º N, 112º W, 575 m), and a single site in Quebec (Field QC1) located at Trécesson (48º N, 78 º W, 348 m). Five male parents from each province (Abitibi, Quebec and Athabasca, Alberta regions), five female parents from Quebec, and four female parents from Alberta were used for breeding, both for within-region and between-region crosses. The seedlings were cultivated in several facilities in Quebec including DRF greenhouses, Duchesnay Centre, and Lotbinière poplar nursery. Seeds were sown directly in containers (Figure A1 in Appendix A) and cultivated in greenhouses at the Complexe Scientifique in Sainte-Foy, QC during winter and spring 2004. They were transferred outside (Figure A1 in Appendix A) during the summer 2004 at the Duchesnay Centre (Centre d'expérimentation et de greffage de Duchesnay, Quebec). Containers were stored for winter 2004 in an outside concrete root cellar in Duchesnay, QC. In April 2005, the containers were moved to the Lotbinière experimental nursery where the plants were uprooted one-by-one, sorted by family and prepared for planting in the field in Trécesson, QC for the 2006 season. In November 2005, seedlings were uprooted, sorted and stored in a freezer at -2 °C in Lotbinière. Then the plants were established in 2006 as a progeny test in Trécesson, QC. In autumn 2008, 1 cutting per tree was collected from the trees of progeny test, to establish another test in Trécesson in 2009. The progeny test planted in 2006 with the original seedling population is still in place in Trécesson, QC except for blocks 1 and 2 which were removed during major irrigation pipe work. The cuttings used in the Alberta sites were collected from the original seedlings planted in the 2006 progeny trial, in the fall of 2009 in Trécesson, QC

and sent to Alberta as dormant cuttings (P. Pėrinet, personal communication, May 12, 2021). Each progeny is represented once in each trial across the 10 blocks at each site.

1.8 Thesis structure

Chapter 1 provides a general introduction for the thesis. Chapter 2 and Chapter 3 were developed by exploring the underlying mechanisms of hybrid vigour by assessing physiology, plant growth, phenology, and hormone levels. In Chapter 2, the field growth data, phenology measurements and least square means analysis are used to determine if the length of growing season is linked to an observed increase in growth. The first greenhouse experiment (Chapter 3) was conducted in 2016 to determine the role of endogenous hormones in enhanced growth, including links to whether physiological performance addressing hybrid vigour is due to endogenous hormone levels and linked to physiological performance. Chapter 4 reports on an experiment conducted in 2018, which tested whether the drought response of a given hybrid balsam poplar genotype could be shaped by its site origin of that clone under drought stress. Chapter 5 ties together the results from the three previous data chapters, answers the questions and shows advancements that this research has made to the greater body of literature in this field.

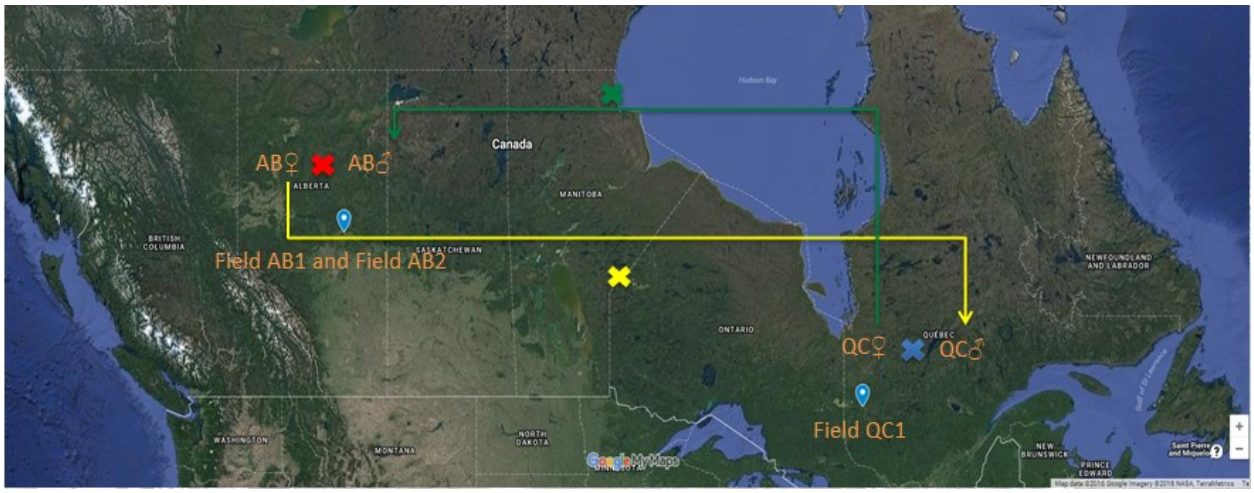

Figure 1.1. Trial information (including filed sites' location, cross-types, and parents). Note: progeny are from four cross-types planted in the field: AB♀ (mother from Alberta (AB)) × AB♂ (father from AB), AB♀ × QC♂ (father from Quebec (QC)), QC♀ (mother from QC) × AB♂, QC♀ × QC♂; Field AB1 and Field AB2: field sites at AB; Field QC1: field site at QC.

Chapter 2. Phenological factors responsible for heterosis in balsam poplar (*Populus balsamifera*) hybrids

2.1 Introduction

Hybrid vigour, or heterosis, represents individuals that display superior growth than either parent when two or more species are crossed or hybridized (for review, see Crow 1998). The utilization of hybridization is common in crop plants and some trees (Shull 1909; Wehrhahn and Allard 1965; Stettler et al. 1996; Meyer et al. 2004). Moreover, interspecific hybridization has been used as a tool for genetic improvement through plant breeding over the past century, especially in poplar breeding (Stettler et al. 1980). For this reason, artificial hybridization is commonly used in forestry to improve productivity and wood quality, as well as reduce rotation age (Poplar Council of Canada 2012). To date, however, there have been few attempts to examine whether hybrid vigour can be achieved by crossing disparate populations of the same species (intraspecific hybridization) except for crop plant species such as maize, rice and canola. Balsam poplar (*Populus balsamifera* L.) is a transcontinental species in North America ranging from Alaska to Newfoundland (Zasada and Phipps 1990). This large geographical distribution makes it an ideal species to study within-species hybrid vigour and plasticity. Because of the large clonal differences in phenology of balsam poplar and its ease of propagation (Ceulemans et al. 1992), any gain achieved through hybrid vigour can be rapidly exploited in a tree improvement program. As the growth of poplars is often highly correlated with traits such as leaf and stomatal morphology, photosynthetic capacity, and phenology (Michael et al. 1990; Ceulemans et al. 1992), it is very important to examine the relationship between growth and phenology to determine if the length of a growing season is linked to increased growth of hybrids.

Phenology is the study of the periodic changes of natural events in relation to environmental fluctuations including bud burst, growth cessation, bud set and leaf senescence (Leith 1974). Bud phenology, including bud burst and bud set, are highly related to plant growth as they determine the length of the growing season (Chuine and Beaubien 2001). The timing of bud burst and bud set is crucial to plant development as it can be used to explain the tradeoffs between plants' survival and growth and their adaptations to the natural environment (Li et al. 2003; Ingvarsson et al. 2006; Luquez et al. 2008). Temperature and photoperiod are two major environment factors that affect phenology (Ford et al. 2017). Temperature is the main driver that affects the initiation of bud burst of poplar and plays a significant role in releasing the dormant period of plants (Cooke et al. 2012). The most favorable timing for bud burst is that which is early enough to allow trees to increase the net ecosystem and gross primary production of forest stands (White et al. 1999; Keskitalo et al. 2005). Moreover, the timing must also be late enough to reduce the risk of trees being damaged by late frost (Hänninen 1990) and keep their competitive ability and growth potential (Frewen et al. 2000). In addition to temperature, photoperiod controls the phenological responses in poplars including growth cessation, bud set and development of bud dormancy (Way and Montgomery 2015). Various studies have found that growth cessation and development of low temperature tolerance are mainly controlled by shortening photoperiod in most woody species in temperate regions (Welling et al. 1997; Li et al. 2003). In the case of bud set, a similar scenario was found where if bud set occurs too early, it results in a shortened growing season that reduces trees' competitive ability and if it is too late, the tree tissues may be damaged since a sufficient level of frost hardiness does not develop prior to the first fall frost (Ingvarsson et al. 2006). Winter chilling was found to be the most significant environmental factor for releasing the dormancy in all northern trees as the buds will remain dormant until enough chilling accumulates (Rinne et al. 2001). In terms of the relationship

between temperature and photoperiod in regulating the bud phenology processes, Rohde et al. (2011a) conducted research to evaluate bud set in 52 clonally replicated poplar genotypes (genotypes were crossed from *P. deltoides* × *P. trichocarpa*, *P. deltoides* × *P. nigra*, *P. deltoides* × *P. trichocarpa*, *P. nigra* × *P. nigra,* and *P. deltoides* × *P. nigra*) across field sites at different latitudes to test the impact of photoperiod and other environmental factors on growth cessation and bud formation. They found that for hybrid poplars, elevated temperature adjusted the sensitivity to photoperiod signals for growth cessation and bud set.

In addition to photoperiod and temperature, bud phenology is affected by genetic variation (Chuine et al. 2000). For instance, Pellis et al. (2004) found that bud burst, as an adaptive trait, explained genetic variation within poplar clones due to differences in latitude and elevation. Additionally, spring leaf phenology, including bud burst and leaf expansion in *Populus,* is mainly controlled by genetic factors as an estimate of broad-sense heritability (including non-additive genetic effects; H^2=98.0%). Spring leaf phenology showed that 98% of the total phenotypic variation was explained by genetic effects and only 2% by environmental effects (Bradshaw and Stettler 1995). Frewen et al. (2000) also found mean clonal heritability of 91% for bud set and 94% for bud burst in *Populus* spp. growing in Washington State, suggesting very strong genetic control of both traits.

The potential relationship between phenology and heterosis has been examined in various studies (Li et al. 1998; Yu et al. 2001). Li et al. (1998) found that interspecific aspen hybrids (*P. tremuloides* × *P. tremula*) grew faster than intraspecific hybrids at the juvenile stage due to their internode number and length (internode length = stem height/number of internodes) and leaf number. They found the intraspecific hybrids had more internodes and larger internode length, and more leaves. Li et al. (1998) suggested this might be caused by delayed bud set, which

lengthened the duration of height growth for the interspecific hybrids. Research by Yu et al. (2001) suggested that the increased growth of hybrid aspen progeny, when compared with the local pure species, was due to a longer period of summer growth as a result of combined, earlier bud burst in spring and a later bud set in fall. Parental origin played a significant role in determining growth patterns, with the more northern female parent (*P. tremula*) having stronger genetic control over growth cessation. Li and Wu (1996, 1997) determined that it was the female aspen parent that primarily influenced growth patterns, bud burst and bud set in the progeny (*P. tremuloides* × *P. tremula*) at the juvenile growth stage.

The objective of this chapter is to test the hypothesis that within-species breeding leads to the express of hybrid vigour. Specifically, we investigated whether the length of a growing season is linked to increased growth of hybrids from local × local and local × distant crosses. We did this by measuring the growth (height and DBH) over eight years and phenology (bud burst and bud set) at two field sites (Field AB1 and Field AB2) over two years. In this way, we examined the relationship between phenology and growth.

2.2 Materials and Methods

2.2.1 Study sites and plant material

A series of controlled crosses were used including local x local and local x distant parental types from both Alberta (AB) and Quebec (QC) sources of balsam poplar. Five male parents from each province (Abitibi, Quebec and Athabasca, Alberta regions), with five female parents from QC, and four female parents from AB were used for within-region crosses (AB♀ × AB♂ and QC♀ × QC♂) and between-region crosses (AB♀ × QC♂ and QC♀ × AB♂). Parent trees were identified in AB and QC and crossed in the winter of 2005 in QC after branches were

shipped to the QC Ministry of Forests, Wildlife and Parks (QCMFWP). After crossing was complete, the seedlings were grown in stool-beds in QC. In September 2009, sufficient one-year-old dormant cuttings were taken in order to establish three field trials. The trials were established with cuttings planted in four-tree family plots, with 10 blocks at 2.5 x 2.5 m spacing on two sites (Field AB1 and Field AB2) in Alberta (Alberta-Pacific Forest Industries Inc. (Al-Pac) millsite (54° N, 112° W, 575 m, mean annual precipitation of 30-year average (1982-2012) at 458mm (Climate Edmonton 2020)), and a single site (Field QC1) located at Trécesson, Quebec (48° N, 78° W, 348 m, mean annual precipitation of 30-year average (1982-2012) at 890mm) (Climate Trécesson 2020). These crosses produced a total of 33 families of AB x AB, AB x QC, QC x AB, and QC x QC cross types respectively. Each progeny from each family is represented once in each trial across the 10 blocks (see Figure A2 in Appendix A).

2.2.2 Phenology

Starting in spring 2016, the bud burst and bud set scores were completed for the two AB field sites (AB1, AB2) by repeated observation over approximately a one-week period in the spring and fall. Bud burst was also assessed in May 2017 and 2018 (exact dates: May 12, May 18 and May 25 in 2017; May 9, May 11, May 14 and May 16 in 2018), and divided into five phenophases based on a visual observation of terminal bud development as follows (Turok et al. 1996): Stage 0: dormant bud completely enveloped by the scales; Stage 1: bud swelling with scales slightly diverging showing a narrow yellow margin; Stage 2: bud sprouting, with tips of the small leaves emerging out of the scales; Stage 3: buds completely opened with leaves still clustered together and scales still present; Stage 4: leaves diverging with their blades still rolled up; Stage 5: leaves completely unfolded and lengthening of the axis of the shoot evident.

Bud set on leaders (last to set bud) was assessed in August 2017 and 2018 (exact dates: Aug. 2, Aug. 8, Aug. 11, Aug. 16, and Aug. 24 in 2017; in 2018, Aug. 7, Aug. 9, Aug. 11, and Aug. 13) and divided into four phenophases based on the visual observation of bud and foliage presence (Rohde et al. 2011): Stage 3: two rolled-up young leaves, no bud structure; Stage 2: transition to bud structure; Stage 1: apical bud fully closed, color between green and red; Stage 0: apical bud red-brown.

2.2.3 Growth

The height and basal diameter of all trees from each plot and site were measured at planting (spring 2009 (Year-0)) and at the end of each growing season until autumn 2017 (Year-8). In 2011, the original site (established in 2009) in QC was accidently damaged during installation of the irrigation system, so the cuttings were replanted in 2011. Therefore, the growth data that is presented here is Year-4 (2015) and Year-6 (2017) growth for the QC site, and Year-6 (2015) and Year-8 (2017) growth for the two AB sites.

Stem volume (V) was estimated with the equation:

$V = A_b \times H/3;$

where V: stem volume (cm^3), A_b: basal area = π x DBH^2(diameter at breast height)/4 (cm^2) and H: height (cm) (Brown and van den Driessche 2002).

2.2.4 Statistical analysis

All of the growth data (height, DBH and stem volume) and phenology data (bud burst and bud set) were analyzed using PROC MIXED of SAS 9.4 (SAS Institute 2001). Following significant main effects, multiple comparisons among means were completed using the Student-Newman-Keuls test. A $p \leq 0.05$ was considered significant. Quantitative analysis using least squares means (LSM) were estimated for each trait (height, DBH, stem volume, bud burst and bud set) of each year using a mixed-effects model to compare the performance of the four cross-types.

2.3 Results

2.3.1 Heterosis and Field Growth

In order to detect the intraspecific hybrid vigour, intra-regional crosses (AB♀ × AB♂ and QC♀ × QC♂) were used as a control to compare with inter-regional crosses (AB♀ × QC♂ and QC♀ × AB♂). Preliminary analysis on six-year and eight-year height, DBH and stem volume from Field AB1 and Field AB2 (Table 2.1, 2.2) indicated differences in family performance among the different cross-types. In general, progeny from the AB × AB cross-type was the slowest growing, while progeny from the AB × QC crosses ranked first and significantly better than intra-regional crosses in terms of growth performance (height, DBH and stem volume). Additionally, a similar trend was observed at the Quebec site (QC1), where progeny from the AB × QC crosses displayed superior growth over the other cross-types and the progeny from the QC × AB cross-type had the slowest growing progeny (Table 2.3).

Differences in heterosis between the two intraspecific crosses were found for height, DBH and stem volume (Table 2.1-2.3). Heterosis was detected in the AB × QC progeny which

consistently had larger values for height, DBH and stem volume than did progeny from the other crosses. However, no heterosis was detected in the progeny from the QC × AB cross-type.

Table 2.1 shows that tree growth was slower with an average of an 840 cm^3 increase in stem volume from Year 4 to Year 6 (Table 2.3). However, from Year 6 to Year 8 (Table 2.1, 2.2), the stem volumes of all cross-types were dramatically increased by an average of 4300 cm^3 (Field AB1) and 6360 cm^3 (Field AB2) respectively. For progeny from the AB × QC cross-type, heterosis and stem volume increased by 5050 cm^3 and 7100 cm^3 in field sites AB1 and AB2, respectively.

2.3.2 Bud burst and Bud set

In 2017, the bud burst observations started on the 132nd day of the year (DOY), when it was already observed that the average bud burst score was approximately three, indicating that bud burst had begun prior to DOY 132 in both fields (Field AB1 and Field AB2) (Figure 2.1a, b). However, the AB × QC cross-type progeny still had significantly higher scores (p <0.05) than the progeny from the other crosses which might suggest that an earlier bud burst would have occurred for this cross-type. In 2018 (Figure 2.1c, d), with an earlier observation day (DOY 129), the whole bud burst process was captured and recorded. Even though progeny from all cross-types had completed the bud burst process between day 129 and day 136, the bud burst scores (1.64 for Field AB1 and 1.55 for Field AB2) for the progeny from the AB x QC crosses were significantly higher (p <0.05) than those recorded for the other three crosses in both fields, confirming the early bud burst for progeny from the AB × QC cross-type.

The observation for the bud set was completed from DOY 214 to DOY 236 in 2017. During this period of 22 days of observation, progeny from all cross-types except for AB × QC

finished the bud set process. On the last day of observation (DOY 236), the bud set score (0.85) of progeny from the AB × QC cross-type was significantly higher (p <0.001) than those from the other crosses, indicating the longer bud set duration for the progeny from the AB × QC crosses (Figure 2.2a, b). In 2018, with fewer days of observation (DOY 219 to 225), a similar trend was obtained from both AB1 And AB2 (Figure 2.2c, d). In general, the progeny from cross-type AB × QC were the last trees to set bud whereas progeny from the other cross-types showed a similar pattern to each other in terms of bud set.

2.3.3 Comparison of hybrid performance

Table 2.4 to Table 2.6 show significant differences (p< 0.05) between progeny from the AB × QC cross-type was determined by least square means and the progeny from the QC x AB cross-type for all three traits (height, DBH and stem volume) (except for Field AB2 at Year-8), which indicates that this cross-type's progeny showed clear advantages in terms of growth, exhibiting heterosis in the progeny from the AB × QC cross-type. For height (Table 2.4), a significant difference as determined by least square means was also found between progeny from the AB × AB cross-type and progeny from other cross-types (QC × AB and QC × QC) in Field AB1 and Field AB2 at Year-6, but a significant difference was only found between progeny from QC × AB and QC × QC cross-types in Field QC1 for height. Similar patterns were found for stem volume (Table 2.6) at Year-6 for progeny from all cross-types at the three field sites, Field AB1, Field AB2 and Field QC1. There were significant differences between the two AB sites and the QC site in terms of environmental conditions that affect the growing seasons of the progeny.

For DBH, the significant differences (p < 0.05) among the progeny from each cross-type did not show the same pattern as height (Table 2.5). For Field AB1 at Year-6, additional

differences in DBH were found between the progeny from AB × AB and AB × QC cross-types, and progeny from AB × AB and QC × QC, and progeny from QC × AB and QC × QC cross-types. However, only progeny from AB × QC crosses were bigger than progeny from AB × AB crosses for Field AB2 at Year-6 (Table 2.5).

In order to explain the differences among progeny from the four cross-types for bud burst and bud set at the two AB sites, pairwise least squares means comparisons following ANOVA were conducted (Table 2.7, 2.8). The first bud burst observations (DOY 132 for 2017; DOY 129 for 2018) and last bud set observations (DOY 236 for 2017; DOY 225 for 2018) were used to determine if the progeny from a particular cross-type showed heterosis with an earlier bud burst (high bud burst score) and later bud set (high bud set score). Table 2.7 and Table 2.8 show that progeny from the AB × QC cross-type were significantly different than progeny from the other cross-types for all traits in both years for Field AB1 and Field AB2. In addition, the progeny from the AB × QC cross-type had a higher bud burst score than progeny from the other cross-types at the first observation, indicating these progeny (from the AB × QC cross-type)'s early bud burst behaviour (Table 2.7). On the other hand, the highest bud set score at the last observation also indicates that the bud formation process for progeny from the AB × QC cross-type was the slowest among progeny from all other cross-types (Table 2.8).

2.4 Discussion

Populus has been selected as a model to study heterosis in forest trees due to its fast growth, genetic variation, and ease of hybridizing (Isebrands et al. 1988; Hinckley et al. 1989; Drew and Chapman 1992). Traits such as the size of leaf area, canopy photosynthesis, volume growth and hormone concentration were all found to contribute to heterosis (Hinckley et al. 1989). Since both height and diameter growth are important components of volume growth, they

are used to evaluate heterosis in our research. From our study, the height and diameter growth superiority of the progeny from the AB × QC cross-type was evident in the first growing season measured and persisted until Year-8 (2017) in the field. Progeny from the AB × QC cross-type had greater growth performance than did progeny from the local crosses (Table 2.1 to Table 2.3). We found that the intraspecific hybrids of AB × QC parents displayed over 11-30% more volume growth than local crosses (AB × AB or QC × QC) during eight years of growth across all sites (Table 2.1 to Table 2.3).

The crossing of parents from AB and QC (Table 2.1 to Table 2.3) showed superior growth and heterosis (hybrid vigour), the phenomenon by which hybrids outperform their parents in yield, biomass, and other traits. Although we did not have the parents for a direct comparison, crosses made by local parents (AB × AB and QC × QC) acted as a control to compare with inter-regional (AB × QC and QC × AB) crosses. In general, the progeny from AB × QC crosses ranked first and significantly better than intra-regional crosses in terms of growth performance (height, diameter at breast height (DBH), and stem volume) in all three field site locations (Table 2.1 to Table 2.3). Additionally, our findings showed that intra-specific hybridization between geographically distant populations (Alberta × Quebec) may lead to heterosis.

Genetic factors play a critical role in regulating plant growth as they influenced various morphological, physiological, and biochemical factors that involved in the growth process (Li and Wu 1997). From our results, there were significant differences in height, DBH and stem volume between the AB sites (Field AB1 and Field AB2) and QC site (Table 2.1 to Table 2.4) for the same progeny, which indicated that there were genotype (G) × environment (E) effects in our study. G × E interaction demonstrated that some genotypes responded differently to various

environments (Isik and Kleinschmit 2003). Additionally, tree species played a very important role in responding to environmental changes through genetic adaptation and phenotypic plasticity (for review, see Nicotra et al. 2010), which ultimately affected the ecosystem functioning and resilience by influencing the genetic composition and dynamics of local populations. In breeding programs, well studied G × E interactions could help researchers select right hybrids better adapted to various environments and determine test environments (Jong and Brewbaker 2005). Yu and Pulkkinen (2003) evaluated G × E interaction and stability in the growth of hybrid aspen clones and found significant G × E interactions in height and basal diameter in three- and four-year-old trees. Additionally, the author expected 17% of the genetic gain for height growth increment during the third year by selecting the right clone for the right site.

Trade-offs between photosynthetic assimilation rates, growth and phenology were observed in *P. balsamifera* (Soolanayakanahally et al. 2009). Wareing (1956) found that spring bud burst of poplar is not controlled by photoperiod and only occurs upon the accumulation of a sufficient heat sum after bud dormancy has been broken by winter chilling. From our observation, AB parental genotypes may have lower chilling or heat sum requirements, or, because of an earlier bud set in the preceding year, they fulfil these requirements in advance of QC parental genotypes. This may lead to the earlier bud burst of progeny from the AB × QC cross-type than the other cross-types. Northern trees used a conservative growth strategy under stable conditions to minimize the risk of being damaged by either late spring or early autumn frost (for review, see Guy 2014). Therefore, northern provenances maximized their growth by having better photosynthetic performance with a shorter growing season (Gornall and Fellow 2007). However, there were no significant differences between the progeny from the QC × AB

cross-type compared with the local crosses, and other intra-regional crosses may indicate that the maternal effect played a role in the growth of the F_1 hybrids, with offspring from AB mothers exhibiting greater fitness (i.e., growth) than those from QC mothers (Kirk et al. 2005; Bräutigam et al. 2017). Kirk et al. (2015) found that maternal effects significantly improved the reproduction of F1 hybrids between *S. jacobaea* and *S. aquaticus*. From a review by Jaenisch and Bird (2003), the authors also concluded that mother plants control nuclear gene expression in the offspring through cues passed via the cytoplasm. Furthermore, Campbell and Waser (2001) studied naturally hybridizing *Ipomopsis aggregata* and *I. tenuituba* and suggested that the survival of hybrids was strongly depended on the maternal parent. This fitness difference between reciprocal hybrids appeared only in the parental environments, suggesting cytonuclear gene interactions that are environment specific. This may explain why the AB × QC cross-type showed better performance than the progeny from the corresponding QC × AB families.

Our results showed that progeny from AB × QC with an earlier bud burst and the latest bud set formation led to a longer growing season which promoted greater growth in the progeny (Figure 2.2 and Figure 2.3), which is consistent with previous work on poplar bud phenology (Yu et al. 2001). Since all progeny were planted in a similar environment at each trial site, minimizing environmental variation, the differences in bud burst and bud set among the cross-types is likely due to genetics. Several researchers have investigated genetic variation in bud phenology on different species and offered possible explanations. Seiwa (1999) mentioned that bud burst phenology may be affected by the age and height of trees. In general, bud burst is driven by degree days in continental climates and bud set is determined by photoperiod. The genotype of a species can also affect the timing of bud burst (Rötzer et al. 2004; Wesolowski and Rowinski 2006). Moreover, climatic factors such as temperature, humidity, rainfall (Wielgolaski

2001), snow fall (Inouye et al. 2002), as well as non-climatic factors such as soil characteristics and nutrients can also change the timing of bud burst (Wielgolaski 2001; Rötzer et al. 2004). However, the timing of bud set at each site was more similar than the timing of bud burst (Figure 2.2), probably because the fall phenophase is mainly triggered by photoperiod (<10 h) and not temperature (Howe et al. 1996; Rohde et al. 2011). In our study, progeny from the most productive cross-type set bud later than the other crosses. The different length/occurrence of phenology processes between the two years in our study might be evidence that bud burst and bud set are not completely independent processes, as Heide (2003) found that warmer temperatures during fall phenophases in boreal regions delayed the bud burst dates the following year for *Betula pendula* Roth, *Betula pubescens* Ehrh. and *Alnus glutinosa* (L.) Moench. Another critical issue to consider is the effect of climate change on phenology. For example, Morin et al. (2009) found that an earlier occurrence of spring phenophases (i.e., bud burst) might also be caused by climate change. Climate change not only increased the temperature but decreased the differences between day and night temperature which was expected to have a distinct effect in altering bud set and bud burst (Beaubien and Hamann 2011). The shifts in phenology can also negatively affect the ecosystems; they can disrupt species interactions, and affect global nutrient cycles (Norby et al. 2010; Scranton and Amarasekare 2017). A study by Scranton and Amarasekare (2017) found that high-latitude species (poplars) might adapt to low mean temperatures and high-amplitude fluctuations under the increase in seasonal fluctuations caused by climate change.

Since no parent information was available in our study, it is very hard to use traditional biometric methods to estimate the genetic effects of heterosis. In our study, least squares means were used to directly compare the differences between intra and local crosses for all growth traits

and bud phenology, and estimate the difference in the phenotypic means. The results from quantitative analysis confirmed the existence of hybrid vigour in progeny derived from the AB × QC cross-type (Table 2.4 to Table 2.8). The phenology data supported that the existence of hybrid vigour in progeny from AB × QC crosses was due to an extended growing season (Figure 2.2 and Figure 2.3). The results presented in the next chapter reveal that gibberellic acids play a regulatory role in heterosis for hybrid balsam poplar at the physiological level. This suggests that in this study, phenology and endogenous hormone levels are likely mechanisms that led to the heterosis of intraspecific hybrids in the progeny from the AB × QC cross-type.

2.5 Conclusion

Poplar has always been used as a forest model tree due to its ability to grow quickly and its ease of propagation and genetic variation within and among species and populations (Stettler et al. 1996). The results presented in this research confirm that the widespread occurrence of heterosis typically found in interspecific crosses (Drew and Chapman 1992; Li and Wu 1996; Li and Wu 1997) can also be achieved with disparate population breeding (intraspecific hybridization) in balsam poplar. Breeding disparate and native populations of balsam poplar can result in superior progeny with enhanced stem growth traits (height and DBH). Moreover, the increased growth of hybrid balsam poplar (AB × QC), when compared with the local crosses (AB × AB or QC × QC parents), appears, at least in part, to be due to phenological differences (earlier bud burst and often a later bud set). With the effects of climate change on phenology mentioned above, it is crucial to examine how natural systems may be altered by the shift of plant phenology. The full impact of this extended growing season on tree phenological processes as well as on tree growth and productivity needs to be investigated to determine whether increased tree growth is a phenology- or temperature-driven (climate change) process. In the

absence of parental information, it is difficult to determine what makes hybrids so superior. Future work should include the original parents' information for better estimation of heterosis. Since G × E effects were detected in our study, a combined analysis of variance will be needed to explicitly assess the impact of G × E. However, the combining of data over different sites, must be done for the same age of trees.

Table 2.1 Field AB1 mean height (±SE), diameter at breast height (DBH) (±SE), and stem volume (±SE) for the four cross-types in balsam poplar at ages six and eight (N=947) from two Alberta sites (AB1 and AB2) indicated by Alberta (AB) and Quebec (QC) with the female parent listed first. Significant differences between cross-types means are indicated by different letters.

Cross-types	**Year-6**		
	Height (m)	**DBH (mm)**	**Stem volume (cm^3)**
AB♀ × AB♂	3.69±0.07^{b}	34.18±1.04^{b}	1410.80±102.66^{b}
AB♀ × QC♂	3.95±0.06^{a}	36.73±0.73^{a}	1653.49±82.29^{a}
QC♀ × AB♂	3.71±0.04^{b}	33.04±0.60^{b}	1267.97±50.45^{b}
QC♀ × QC♂	3.78±0.07^{b}	34.91±0.87^{ab}	1460.28±84.68^{b}
	Year-8		
	Height (m)	**DBH (mm)**	**Stem volume (cm^3)**
AB♀ × AB♂	5.57±0.10^{b}	59.30±0.75^{b}	5458.70±230.04^{bc}
AB♀ × QC♂	6.02±0.06^{a}	63.05±0.77^{a}	6707.20±223.16^{a}
QC♀ × AB♂	5.59±0.05^{b}	56.96±0.55^{b}	5074.20±128.03^{c}
QC♀ × QC♂	5.88±0.08^{a}	58.78±0.88^{b}	5750.50±227.12^{b}

Table 2.2 Field AB2 mean height (±SE), diameter at breast height (DBH) (±SE), and stem volume (±SE) for the four cross-types in balsam poplar at ages six and eight (N=947) from two Alberta sites (AB1 and AB2) indicated by Alberta (AB) and Quebec (QC) with the female parent listed first. Significant differences between cross-types means are indicated by different letters.

Cross-types	**Year-6**		
	Height (m)	**DBH (mm)**	**Stem volume (cm^3)**
AB♀ × AB♂	3.74 ± 0.05^{c}	37.94 ± 0.57^{b}	1513.18 ± 68.00^{c}
AB♀ × QC♂	4.14 ± 0.05^{a}	40.65 ± 0.61^{a}	1984.08 ± 78.51^{a}
QC♀ × AB♂	3.92 ± 0.04^{b}	37.84 ± 0.50^{b}	1658.50 ± 55.66^{bc}
QC♀ × QC♂	3.99 ± 0.07^{b}	38.68 ± 0.84^{b}	1835.77 ± 93.32^{ab}
	Year-8		
	Height (m)	**DBH (mm)**	**Stem volume (cm^3)**
AB♀ × AB♂	5.89 ± 0.10^{c}	63.89 ± 0.10^{b}	6861.36 ± 297.50^{c}
AB♀ × QC♂	6.66 ± 0.07^{a}	69.36 ± 0.96^{a}	9092.86 ± 300.22^{a}
QC♀ × AB♂	6.44 ± 0.05^{b}	67.48 ± 0.69^{a}	8258.72 ± 210.11^{b}
QC♀ × QC♂	6.32 ± 0.09^{b}	67.76 ± 0.99^{a}	8220.96 ± 295.04^{b}

Table 2.3 Field QC1 mean height (±SE), diameter at breast height (DBH) (±SE), and stem volume (±SE) for the four cross-types in balsam poplar at ages four and six (N=947) from one QC sites indicated by Alberta (AB) and Quebec (QC) with the female parent listed first. Significant differences between cross-types means are indicated by different letters.

Cross-types	**Year-4**		
	Height (m)	**DBH (mm)**	**Stem volume (cm^3)**
AB♀ × AB♂	2.84±0.05[a]	23.36±0.76[b]	513.30±45.86[b]
AB♀ × QC♂	2.95±0.05[a]	25.30±0.55[a]	597.00±41.09[a]
QC♀ × AB♂	2.66±0.03[b]	20.37±0.45[c]	367.21±23.40[b]
QC♀ × QC♂	2.84±0.07[a]	23.19±0.70[b]	504.48±47.52 [b]
	Year-6		
	Height (m)	**DBH (mm)**	**Stem volume (cm^3)**
AB♀ × AB♂	3.39±0.08[b]	35.79±0.83[b]	1325.26±101.79[b]
AB♀ × QC♂	3.60±0.07[a]	36.62±0.76[a]	1491.12±95.28[a]
QC♀ × AB♂	3.37±0.06[b]	33.39±0.56[c]	1171.52±65.71[c]
QC♀ × QC♂	3.49±0.08[b]	34.92±0.83[bc]	1341.23±103.20[b]

Table 2.4 Pairwise Least Squares Means comparisons following ANOVA of height of balsam poplar progeny (N=2841) among cross-types at different fields (Field AB1, Field AB2 and Field QC1) in different years (2017 and 2018).

Cross-types Comparison		Year-6			Year-8		
		Differences between Means			Differences between Means		
		Field AB1	Field AB2	Field QC1	Field AB1	Field AB2	Field QC1
AB♀ × AB♂	AB♀ × QC♂	-0.23*	-0.37*	-0.14	-0.41*	-0.71*	-0.24*
AB♀ × AB♂	QC♀ × AB♂	-0.08	-0.17	0.13	-0.08	-0.58*	-0.04
AB♀ × AB♂	QC♀ × QC♂	-0.21*	-0.29*	-0.08	-0.45*	-0.51*	-0.21*
AB♀ × QC♂	QC♀ × AB♂	0.15*	0.20*	0.27*	0.32*	0.13	0.20*
AB♀ × QC♂	QC♀ × QC♂	0.02	0.07	0.06	-0.04	0.21	0.04
QC♀ × AB♂	QC♀ × QC♂	-0.13*	-0.13	-0.21*	-0.37*	0.08	-0.16

*Significant at $p \leq 0.05$

Table 2.5 Pairwise Least Squares Means comparisons following ANOVA of diameter at breast height (DBH) of balsam poplar progeny (N=2841) among cross-types at different fields (Field AB1, Field AB2 and Field QC1) in different years (2017 and 2018).

Cross-types Comparison		Year-6			Year-8		
		Differences between Means			Differences between Means		
		Field AB1	Field AB2	Field QC1	Field AB1	Field AB2	Field QC1
AB♀ × AB♂	AB♀ × QC♂	-2.47*	-2.35*	-2.82*	-3.36	-4.72*	-1.25
AB♀ × AB♂	QC♀ × AB♂	-0.14	-0.05	2.10	1.58	-3.91*	-1.68*
AB♀ × AB♂	QC♀ × QC♂	-2.98*	-1.22	-0.80	-1.33	-4.81*	-0.10*
AB♀ × QC♂	QC♀ × AB♂	2.61*	2.31*	4.92*	4.94*	0.81	2.93*
AB♀ × QC♂	QC♀ × QC♂	0.51	1.13	2.02	2.02	-0.09	1.15
QC♀ × AB♂	QC♀ × QC♂	-3.12*	-1.18	-2.90*	-2.92	-0.90	-1.78

*Significant at $p \leq 0.05$

Table 2.6 Pairwise Least Squares Means comparisons following ANOVA of stem volume of balsam poplar progeny (N=2841) among cross-types at different fields (Field AB1, Field AB2 and Field QC1) in different years (2017 and 2018).

Cross-type Comparison		Year-6			Year-8		
		Differences between Means			Differences between Means		
		Field AB1	Field AB2	Field QC1	Field AB1	Field AB2	Field QC1
AB♀ × AB♂	AB♀ × QC♂	-235.7*	-422.8*	-116.5	-1145.9	-1986.4*	-211.5*
AB♀ × AB♂	QC♀ × AB♂	27.7	-159.0	-100.7	150.5	-1501.9*	59.0
AB♀ × AB♂	QC♀ × QC♂	-272.2*	-404.9*	-42.0	-804.4	-1654.6*	-168.4*
AB♀ × QC♂	QC♀ × AB♂	262.7*	263.9*	217.2*	1296.5*	484.5	270.5*
AB♀ × QC♂	QC♀ × QC♂	-36.6	17.9	74.5	341.5	331.7	43.1
QC♀ × AB♂	QC♀ × QC♂	-299.3*	-246.0*	-142.7	-954.9	-152.8	-227.4*

*Significant at $p \leq 0.05$

Table 2.7 Pairwise Least Squares Means comparisons following ANOVA of bud burst of balsam poplar progeny (N=1894) among cross-types at different fields (Field AB1 and Field AB2) in different years (2017 and 2018).

Cross-types Comparison		2017 DOY=132		2018 DOY=129	
		Differences between Means		Differences between Means	
		Field AB1	Field AB2	Field AB1	Field AB2
AB♀ × AB♂	AB♀ × QC♂	-0.28*	-0.36*	-0.38*	-0.37*
AB♀ × AB♂	QC♀ × AB♂	-0.06	0.04	-0.08	-0.12
AB♀ × AB♂	QC♀ × QC♂	-0.02	0.07	0.03	0.15
AB♀ × QC♂	QC♀ × AB♂	0.22*	0.31*	0.30*	0.26*
AB♀ × QC♂	QC♀ × QC♂	0.26*	0.43*	0.41*	0.52*
QC♀ × AB♂	QC♀ × QC♂	0.04	0.11	0.11	0.26*

*Significant at $p \leq 0.05$

Table 2.8 Pairwise Least Squares Means comparisons following ANOVA of bud set of balsam poplar progeny (N=1894) among cross-types at different fields (Field AB1 and Field AB2) in different years (2017 and 2018).

Cross-types Comparison		2017 DOY=236		2018 DOY=225	
		Differences between Means		Differences between Means	
		Field AB1	Field AB2	Field AB1	Field AB2
AB♀ × AB♂	AB♀ × QC♂	-0.55*	-0.29*	-0.39*	-0.37*
AB♀ × AB♂	QC♀ × AB♂	0.11*	-0.02	-0.10	-0.12
AB♀ × AB♂	QC♀ × QC♂	0.04	0.06	-0.07	-0.13
AB♀ × QC♂	QC♀ × AB♂	0.67*	0.27*	0.30*	0.25*
AB♀ × QC♂	QC♀ × QC♂	0.60*	0.35*	0.32*	0.34*
QC♀ × AB♂	QC♀ × QC♂	-0.07	0.08	0.02	-0.01

*Significant at $p \leq 0.05$

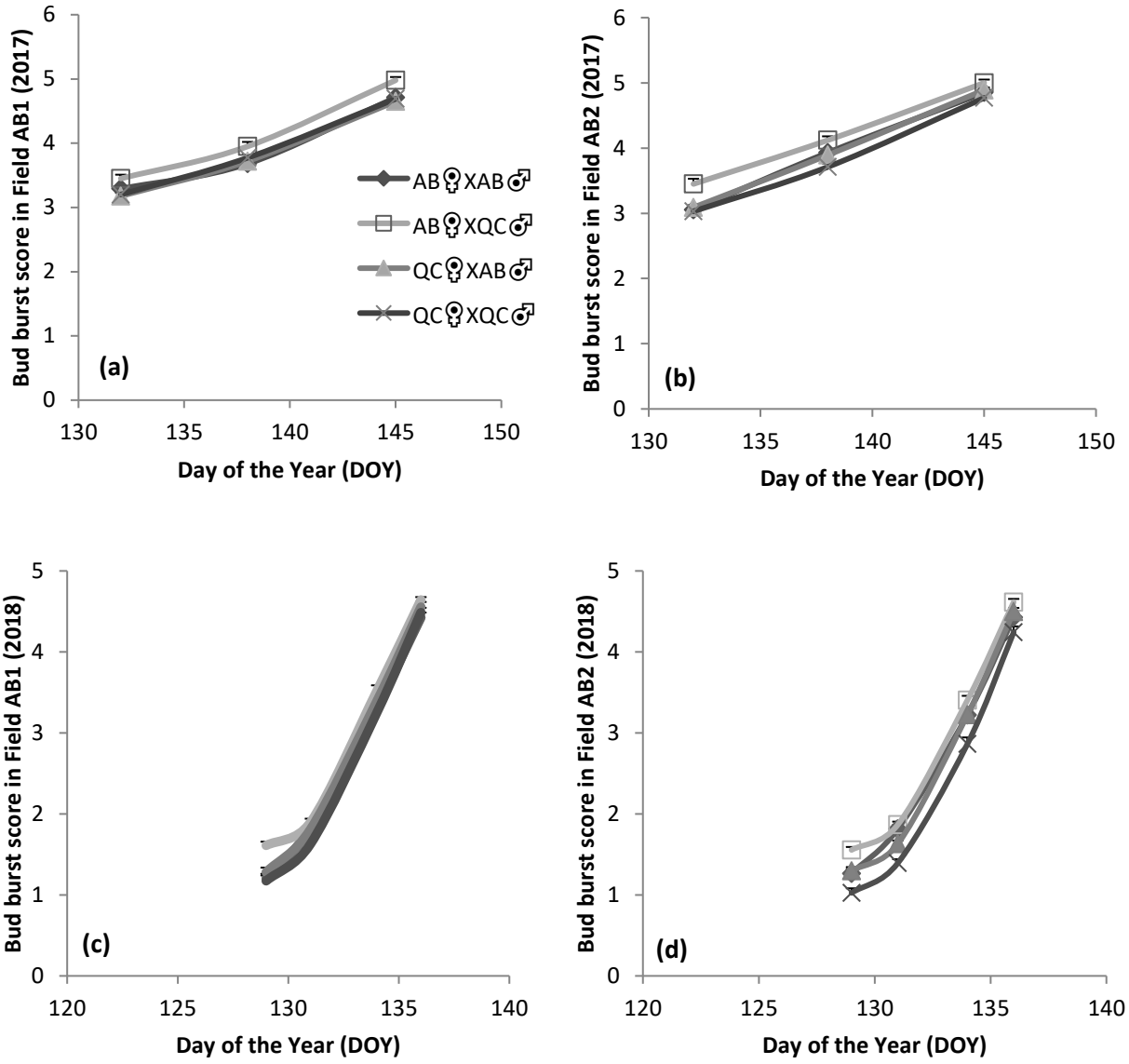

Figure 2.1. Mean bud burst (+SE) of balsam poplar progeny (N=947) for two Alberta sites (Field AB1 and Field AB2) in two observation years (2017 and 2018) for all cross-types as follows: a) Field AB1, 2017; b) Field AB2, 2017; c) Field AB1, 2018; d) Field AB2, 2018. DOY: Day of the year. Note: symbols indicated different cross-types.

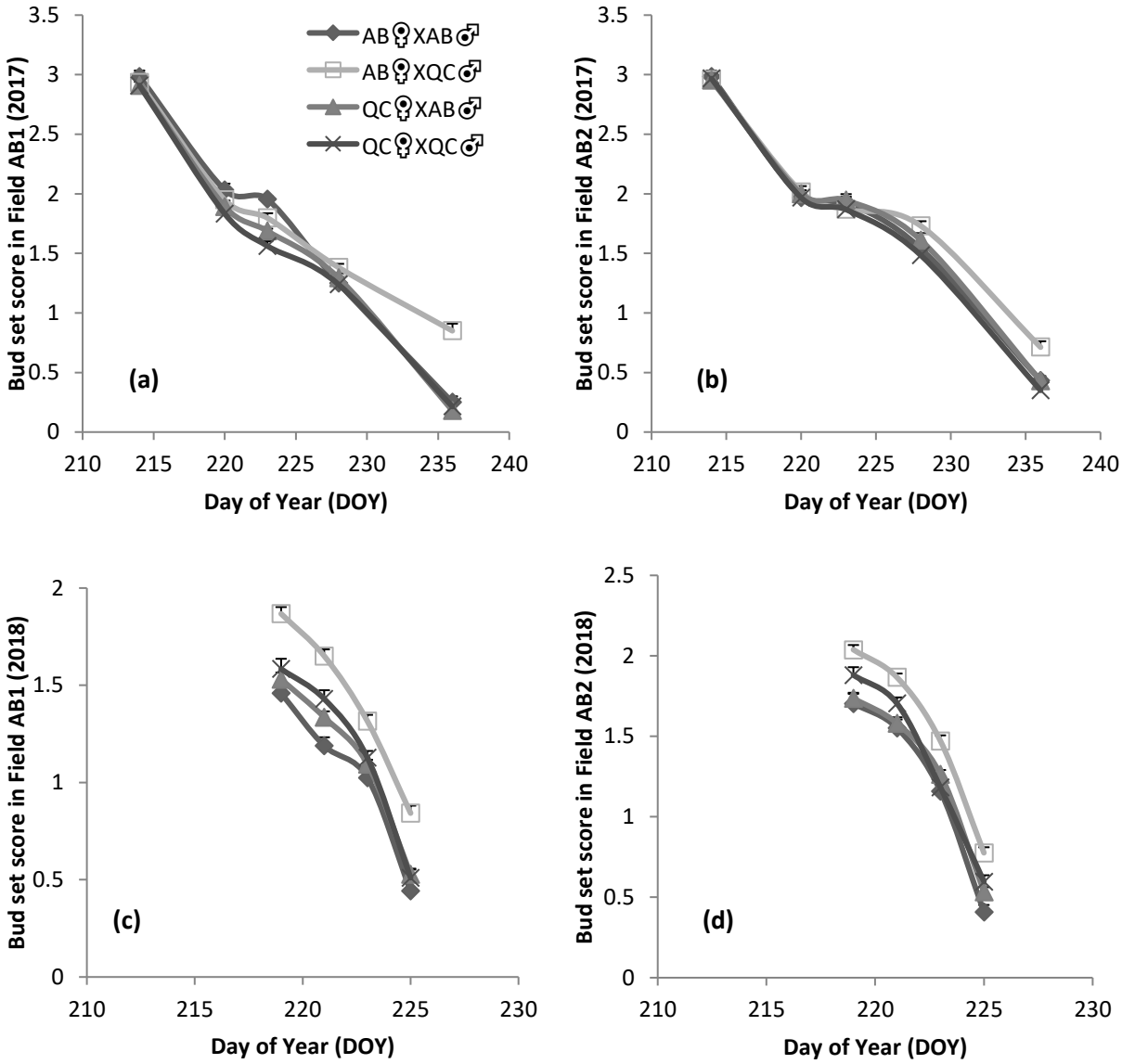

Figure 2.2. Mean bud set (+SE) of balsam poplar progeny (N=947) between two Alberta sites (Field AB1 and Field AB2) in two observation years (2017 and 2018) for all cross-types as follows: a) Field AB1, 2017; b) Field AB2, 2017; c) Field AB1, 2018; d) Field AB2, 2018. DOY: Day of the year. Note: symbols indicate different cross-types.

Chapter 3. Hormones and heterosis in hybrid balsam poplar (*Populus balsamifera* L.)[1]

3.1 Abstract

Balsam poplar (*Populus balsamifera* L.) is a transcontinental tree species in North America, making it ideal as a subject to study intraspecific hybrid vigour as a tool for increasing genetic gain in growth. We tested the hypothesis that intraspecific breeding of disparate populations of balsam poplar would lead to the expression of hybrid vigour and we determined the role of endogenous hormones linked to ecophysiological and growth performance.

In September 2009, three field trials were established in Canada (two in Alberta (AB), Fields AB1 and AB2, and one in Quebec (QC), Field QC1)). Five male parents from each province with five female parents from QC, and four female parents from AB were used for breeding intra-regional and inter-regional crosses. Based on a significant difference at Year 6 for height and diameter, from the AB1 and AB2 field trials, the AB × QC cross-type was selected for further study. Cuttings from the AB × QC cross-type were grown in a randomized complete block design under near-optimal greenhouse conditions. Families were identified as slow- or fast-growing and the relationship between hormone levels and growth performance of the genotypes within the families was examined. In late June, after 34 days of growth, internode tissue samples were collected from each progeny and analyzed for gibberellic acids, indole-3-acetic acid and abscisic acid content. The stem volume of two-month-old rooted cuttings grown under optimal greenhouse conditions was positively and significantly correlated with net photosynthesis rate, greenhouse growth and stem volumes of eight-year old field-grown trees (Fields AB1 values: $r = 0.629$ and $p = 0.012$; AB2 values: $r = 0.619$ and $p = 0.014$; and QC1

values: $r = 0.588$ and $p = 0.021$, respectively). We determined that disparate, native populations of balsam poplar can be bred to produce superior progeny with enhanced stem-growth traits.

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3.2 Introduction

Heterosis, or hybrid vigour, refers to the phenomenon in which hybrids outperform their parents in yield, biomass, biotic and abiotic stress tolerance, and other traits (Sinha and Khanna 1975). Hybrid vigour, typically achieved through controlled crossing of two species, or pure genetic lines of the same species, has long been exploited in agricultural crops such as maize and wheat (Wehrhahn and Allard 1965; Stuber 1994) and in some tree species including *Populus* (Stettler et al. 1996). Interspecific breeding has been successfully applied in a variety of tree species to improve their productivity. These species include birch (Atkinson et al. 1997), pine (Bradley and Will 2017), and poplar (Wullschleger et al. 2005). For poplars, including the aspens, two or more species are typically crossed to produce hybrid progeny, some of which can be expected to yield growth performance far superior than either parent (i.e., hybrid vigour/heterosis). Balsam poplar (*Populus balsamifera* L.) is a transcontinental species in North America with a wide distribution from Alaska to Newfoundland (Zasada and Phipps 1990). This wide range makes it an ideal species to study within-species hybrid vigour as a tool for increasing genetic gain through increased genetic diversity. Additionally, with its clonal nature and ease of propagation, any gain achieved through hybrid vigour can be rapidly exploited in a tree improvement program.

Plant growth and development is a complicated process that is driven by multiple factors, including genetics, physiology, and phytohormones (for review, see Fujimoto et al. 2008). In terms of phytohormones, several studies with hybrid poplar found that gibberellic acid (GA) was involved in heterosis for poplar shoot growth as hybrids with hybrid vigour contained higher levels of endogenous GAs (Bate et al. 1988; Pharis et al. 1991; Pearce et al. 2004). In plant species, the majority of GA's metabolism genes have already been identified and characterized

(for review, see Olszewski et al. 2002). Gibberellins, a group of tetracyclic diterpenoid compounds, function as plant hormones that play a crucial role in the regulation of many aspects of plant growth and development, such as seed germination, stem elongation, leaf expansion, flower and fruit development, and wood formation (Kurepin et al. 2014). In order to be synthesized via the terpenoid pathway, gibberellins residing in three different cellular compartments (plastid, endoplasmic reticulum, and cytoplasm) require terpene synthase (TPSs), cytochrome P450 mono-oxygenase (P450s), and 2-oxoglutarate-dependent dehydrogenase (2 ODDs) to biosynthesize bioactive GA from geranylgeranyl diphosphate (GGDP) in plants (Ueguchi-Tanaka et al. 2000). The GA 20-oxidases (*GA20ox*) and GA 3-oxidases (*GA3ox*) convert the early GA structures (GA_{12} or GA_{53}) to the growth-active structures, GA_4 and GA_1, via the early non-13 hydroxylation pathway (leading to GA_4) and the early 13-hydroxylation pathway (leading to GA_1), respectively (Ma et al. 2011). The inactivation of these growth-activating GAs and many of their early precursors is achieved by GA 2-oxidases (*GA2ox*) (Rieu et al. 2008).

Recent work in rice has revealed evidence that endogenous gibberellins play a key role regulating heterosis (Ma et al. 2011). Rood et al. (1988) analyzed the differences in responsiveness to the exogenous application of GA_3 and endogenous levels of GAs between F1 hybrids and their inbred parents of diallel combinations in maize (*Zea mays*). They concluded that the increased endogenous concentration of GA in the hybrids could provide a phytohormone basis for heterosis in shoot growth. Additionally, Park et al. (2014) compared growth of young conifer seedlings under optimal conditions with the field performance of the same seedlings via a retrospective approach and found that endogenous GA levels might explain the natural variations seen in tree stem size in even-aged pine forests.

Indole-3-acetic acid (IAA), the most common plant hormone of the auxin class, was found to be linked to tree stem radial growth both for conifers and deciduous trees ((Uggla et al. 1996; Tuominen et al. 1997). IAA not only regulated various aspects of plant growth and development, but also acted as a positional signal to control the cambial growth rate by adjusting the radial number of dividing cells in the cambial meristem. As IAA determined the cambial growth rate, it was also an important component contributing to heterosis (Uggla et al. 1998; Zhu et al. 2020). Zhu et al. (2020) found that auxin was a key phytohormone regulating plant development, promoting cell expansion and cell proliferation. Earlier activation of auxin biosynthesis might contribute to heterosis in the early development of plants. In addition to GAs and IAA, abscisic acid (ABA) is another important hormone in regulating seed development and maturation. It induces dormancy in buds, underground stems, and seeds (for review, see Zeevaart and Creelman 1988). ABA also regulates IAA biosynthesis and activity (Fu et al. 2015). It is also considered a stress hormone because the production of hormones is stimulated by drought, water logging, and other adverse environmental conditions by regulating stomatal closure, inducing the expression of stress responsive genes and the accumulation of osmo-compatible solutes (for review, see Lim et al. 2015). To date, few studies have been conducted to examine the role of endogenous hormones including links to physiological and growth performance in balsam poplar produced from disparate populations. Therefore, a greenhouse study was designed to grow progeny from selected families under near-optimal growing conditions. The stem tissue was harvested, prior to any senescence or corresponding hormone degradation, for GA, IAA and ABA analysis in order to examine whether there is a causal relationship between hormone concentration in the elongating stem (internodes) tissue and the growth rate of the vegetatively propagated progeny from selected families of balsam poplar.

3.3 Materials and Methods

3.3.1 Selection of Families and Progeny

In order to test the hypothesis that within-species breeding will lead to the expression of hybrid vigour, a series of controlled crosses were completed including local x local and local x distant parental types from both Alberta (AB) and Quebec (QC) sources of balsam poplar. Five male parents from each province (Abitibi, QC and Athabasca, AB regions) as well as five female parents from QC, and four female parents from AB were used for breeding, both for within-region and between-region crosses. Parent trees were identified in AB and QC and bred in the winter of 2005, the seedlings were grown in stool-beds, and cuttings were taken to establish three field trials in September 2009. In each field trial trees were planted in four-tree family plots, with 10 blocks at a 2.5 x 2.5 m spacing on two sites (Field AB1 and AB2) in AB (Alberta- Pacific Forest Industries Inc. (Al-Pac) millsite (54^{o} N, 112^{o} W, 575 m, mean annual precipitation at 458mm (Climate Edmonton 2019)), and a single site in QC (Field QC1) located at Trécesson in QC (48^{o} N, 78^{o} W, 348 m, mean annual precipitation at 890mm (Climate Trécesson 2019)). The crosses produced a total of 33 families of AB × AB, AB × QC, QC × AB, and QC × QC cross-types, respectively. Each progeny from each family is represented once in each trial across the 10 blocks. In summer 2016, a greenhouse trial using families identified as slow- and fast-growing in cross-type AB × QC were selected in order to use extremes of performance for examining the relationship between hormone levels and growth performance of the selected genotypes within specific families.

Stem volumes were calculated using six-year-old tree data from AB1 and AB2 field sites. The relationship between individual progeny and stem volume for the AB × QC cross-types was then plotted (Figure 3.1). Across the eight families in this cross-type, there were distinctly

different patterns of stem volume in the progeny. Three groups with three progeny per family were selected as follows: 1) a fast-growing (FG) group (families AP5396, AP5402 and AP 5416); 2) a slow-growing (SG) group (families AP5401, AP5411 and AP5414); and 3) three slow-growing progeny selected from the fast-growing families (SFG) (families AP5396, AP5402 and AP 5416). Three alive and well-growing individual progenies were then selected from each of the families. Another three slow-growing individual progenies from the FG group were also used. The selection of these progeny and families allowed for the following comparisons: slow-growing vs. fast-growing within the same family group, and slow-growing vs. slow-growing between different family groups. To demonstrate the selection process, AP5396 is used as an example. In order to select the individual progeny with consistent performance in both field AB1 and field AB2, the ranking system based on mean stem volume (cm^3) at Year-6 (2015) was used (see Table A2 in Appendix A). The mean stem volume was calculated to consider both height and DBH for the individual progeny. According to the data (see Table A2 in Appendix A), individual progeny 147072, 147083 and 147071 were selected as fast-growing progenies in the fast-growing group (FG), and 147041, 147051 and 147043 were selected as slow-growing progenies in the fast-growing group (SFG). This selection process was applied to all the families for all individual progenies within the group types. In total, 27 individual progeny were selected for study within each family and group type within the AB x QC cross-type (Table 3.1).

3.3.2 Greenhouse Propagation

Dormant branch cuttings that were 40-50 cm long were collected on March 31, 2016 from the selected 27 progeny in the AB1 field at the Al-Pac mill site, placed in black plastic bags and stored in the fridge for eight days at -4 °C, until the greenhouse experiment started. Cuttings were soaked in cold water for two days at room temperature in the lab without the use of any

additional rooting hormone (DesRochers and Thomas 2003), with water replaced daily with fresh cold water. On April 11, eight stem cuttings six to nine centimeters (cm) in length with a minimum of two buds for each of the 27 progeny were rooted in Format 360 Hillsons Rootrainer trays (Beaver Plastics Ltd., Acheson, AB, Canada) filled with Sunshine Mix #3 (Sun Gro Horticulture, Vancouver, BC, Canada). Budburst was scored and recorded three times over the course of one week. Upon bud flush, and after height growth of approximately 10 cm was reached (~Day 40 after striking), cuttings were transplanted directly into 2 L pots filled with Sunshine Mix #4 (Sun Gro Horticulture, Vancouver, BC, Canada). The rooted cuttings (stecklings) were grown in the greenhouse at the University of Alberta. Day 1 of the experiment was May 24, 2016. The stecklings were placed under natural light supplemented by cool-white fluorescent lamps to provide a 21 h long photoperiod and a minimum photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1} at plant level. Maximum day and night temperatures were maintained at approximately 25 °C and 18 °C, respectively, throughout the experimental period. The stecklings were kept well-watered and fertilized using a 20-20-20 commercial water-soluble fertilizer (20:20:20 plus micronutrients (Fe 0.1%; Mn 0.05%; Zn 0.05%; Cu 0.05%; B 0.02%; Mo 0.0005%)) (Plant Products Co. Ltd. Brampton, ON, Canada) at a pH of 5.8–6.3, adjusted by adding phosphoric acid (H3PO4). The greenhouse was well-ventilated. PPFD, humidity, and air temperature were continuously monitored and recorded using a HOBO U12-012 data logger (Onset Computer Cooperation, Pocasset, MA, USA). The potted stecklings were rotated weekly to minimize position effect in the greenhouse (Drewes et al. 2009).

3.3.3 Measurements, Harvest and Selection for Hormone Analysis

Caliper, measured at the base of the new stem, and height growth were measured every 10 days after transplanting, starting on Day 1 of the experiment (May 24, 2016). Gas exchange

measurements including net photosynthetic rate (A), stomatal conductance (g_s), and intrinsic water-use efficiency (iWUE) were made using a CIRAS-3 infrared gas analyzer (IRGA) (PP Systems, Amesbury, MA, USA) and a broad leaf cuvette (PLC4 (B) Broad Leaf Cuvette, PP Systems, Amesbury, MA, USA). Just prior to harvesting, on Day 34, June 27, 2016 (near the longest day of the year), the cuvette window was 18 mm in diameter with a total area of 2.5 cm^2. In all, 15 progeny were selected (six from FG, six from SFG, and three from SG) from three families for further hormone analysis (See Table A1 in Appendix A).

At harvest, eight ramets of each of the 15 progeny (genotypes) were grouped based on a visual assessment of vigour, which in turn was based on height and diameter, to select: a) the largest, b) the second largest, and c) the third largest ramet for use in the hormone level analysis in the elongating internode stem tissue (Kurepin et al. 2014).

For each of the selected ramets, leaves were snipped off at the base of the petiole, along the chosen length of stem while the tree was still intact. The upper 20–30% (all internodes which were still elongating plus two lower internodes which had ceased to elongate) of the stem was harvested, wrapped in a double-layer of aluminum foil forming a package, and placed onto dry ice for storage prior to preparation for the hormone analysis. The roots were then carefully washed and put into paper bags. The biomass components (leaves, remaining stem tissue, and roots) were stored in paper bags before drying for two days at 65 °C, then measured using a model AV53 scale (readability 0.001g, OHAUS Adventurer Pro, Melrose, MA, USA). After drying, the leaves were ground in a ball grinder (Model MM200, Retsch Inc., Haan, North Rhine-Westphalia, Germany) and stored in 20 mL plastic scintillation vials (Fisher Scientific, Hampton, NH, USA) in preparation for $\delta^{13}C$ analysis at the University of Alberta's Natural Resources Analytical Laboratory (NRAL). After five days in the -80 °C freezer, the stem

samples, which had been harvested earlier for hormone analysis, were freeze-dried in a FreeZone® 2.5 L Benchtop freeze dry system (Labconco Corporation, Kansas City, MO, USA) for three days.

3.3.4 Analysis of GAs, IAA, and ABA

One gram dry weight (DW) of each tissue sample was ground with liquid N_2 and washed sea sand (Fisher Scientific, Fair Lawn, NJ, USA), then extracted in 80% MeOH (H_2O:MeOH=20:80, v/v). Following this, 250 ng [$^{13}C_6$] IAA (a gift from Dr. J. Cohen, available from Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA), 200 ng [$^{2}H_6$] ABA (a gift from Drs. L. Rivier and M. Saugy, University of Lausanne, Lausanne, Switzerland), and 20–40 ng each of [$^{2}H_2$] GA_{15}, GA_{24}, GA_9, GA_{20}, GA_4, GA_1, GA_8, and GA_{34} (deuterated GAs were obtained from Professor L.N. Mander, Research School of Chemistry, Australian National University, Canberra, Australia) were added to the aqueous MeOH extraction solvent as internal standards. Subsequent purification, separation, and stable isotope dilution analysis by gas chromatography–mass spectrometry (GC-MS)-selected ion monitoring (SIM) were accomplished as described by Kurepin et al. (2007).

3.3.5 Data Analysis

All the growth data (height, caliper, and stem volume) were analyzed by ANOVA using SAS 9.4 (SAS 2013). Following significant main effects, multiple comparisons among means were completed using the Student–Newman–Keuls test. A result of $p \leq 0.05$ was considered significant. The correlation coefficient (r) and probability (p) were determined by Pearson's correlation analysis using the Statistical Package in SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, USA).

3.4 Results

3.4.1 Hybrid Vigour in Intra-Specific Hybrids

Preliminary analysis of the six-year height, diameter and stem volume from the Al-Pac field sites, AB1 and AB2 only, indicated differences in family performance among the different cross-types. In order to detect the intraspecific hybrid vigour, intra-regional crosses (AB♀ × AB♂ and QC♀ × QC♂) were used as a control to compare with inter-regional (AB♀ × QC♂ and QC♀ × AB♂) crosses. In general, AB♀ × AB♂ (the female is described first) families were the slowest growing, while AB × QC crosses ranked first and significantly better than intra-regional crosses in terms of growth performance (height, DBH and stem volume) (Table 3.2). These results were used to make further selections for families and progeny based on cross-type for the greenhouse experiment.

3.4.2 Greenhouse Growth at Two Months (34 Days after Transplanting)

Initial growth of cuttings after striking, for all families in the FG, SG and SFG groups, showed similar heights during the first seven days of growth early in the growing season. However, after the emergence of primary leaves, cuttings of the fast-growing group (FG) grew much faster than cuttings of the intermediate (SFG) or the SG group selected from the slow growing families. By Day 34 after transplanting (about two months old), the FG group performed better than both the SG and SFG groups in stem volume (Figure 3.2) and biomass (Figure 3.3). Additionally, within the same family (i.e. family AP5416), fast-growing progeny (272084) performed better than slow-growing progeny (272023 and 272024) under near-optimal greenhouse conditions, thus indicating the wide range of performance variability within a single family (Figure 3.4).

3.4.3 Greenhouse Gas Exchange

Table 3.3 shows that higher rates of photosynthesis were correlated with growth (height, caliper and stem volume) at Day 34 (after transplanting, about two months old), just prior to harvest. Additionally, increased photosynthetic demand was supported by an increased supply of CO_2 through an increase in stomatal conductance (g_s). It was also found that $\delta^{13}C$ in the leaf tissue showed a significant negative correlation with g_s results, indicating that the stomates were open, promoting an increase in gas exchange and carbohydrate production, resulting in an increase in height growth.

3.4.4 Comparisons of greenhouse growth at Day 34 and Field Growth Performance at Age Eight Years

The Pearson's correlation analysis showed that stem volumes calculated from height and caliper of Day 34 stecklings grown under near-optimal greenhouse conditions are positively and significantly correlated with stem volumes of six- and eight-year-old field-grown trees of the same genotypes (Figure 3.5). Additionally, positive and significant correlations were also obtained when Day 34 stem dry biomass and stem volumes of Day 34 stecklings grown under near-optimal greenhouse conditions (Table 3.4) were regressed against each of six- and eight-year old stem diameters, and stem volume of field-grown trees of the same genotypes.

3.4.5 Hormone Analysis

Since the gibberellin (GA) profiles are likely to be very different between the active growth phase and growth cessation (bud set) phase, we grew the poplar trees under near-optimal conditions and harvested the tissue near the longest day of the year to avoid the deficiency and degradation of bioactive GAs (Zawaski and Busov 2014).

The concentrations of three endogenous plant hormone classes, namely, ABA, IAA and GAs, were quantified in stem tissues of 15 selected FG, SFG and SG progeny. Stem IAA concentration in Day 34 stecklings was positively and significantly correlated with stem biomass and stem volume (Figure 3.6a, b). In contrast, stem ABA in Day 34 stecklings was negatively and significantly correlated with stem volume and stem dry biomass (Figure 3.6c, d).

3.4.6 Endogenous Plant Growth Hormone in Greenhouse-grown Stecklings versus Field Growth Performance

The relationships between stem GA levels and stem volume were analyzed through Pearson's correlation (Figure 3.7). Our results showed that stem GA_{19} and GA_{20} content were all significant and negatively correlated with greenhouse stem volume, which indicates that GA_{19} and GA_{20} serve as precursors for GA_8 (Figure 3.7a, b). In addition, we confirmed that both GA_{19} and GA_{20} play an important role in the early *GA20ox* portion of the GA biosynthesis pathway as found by Ma et al. (2011). In the elongated stems of the Day 34 stecklings, a significant, positive correlation between stem volume and the content of GA_8 was detected, with an r of 0.840 ($p < 0.001$) (Figure 3.7c). Moreover, a positive, significant correlation was also detected between field growth (Fields AB1 and AB2) trees and the content of GA_8 in the greenhouse grown trees for the 15 selected progeny (r = 0.749 ($p = 0.001$); and r = 0.734 ($p = 0.002$), respectively (Figure 3.8)).

3.5 Discussion

Obvious trade-offs have been identified between net photosynthesis rate (A), growth and phenology in *P. balsamifera* (Soolanayakanahally et al. 2009). Phenology studies the timing of the annual cycles of plants and is extremely sensitive to changes in temperature and photoperiod

(Scranton and Amarasekare 2017). Trees from the AB genotypes had higher A but accomplished far less growth than the trees from the QC genotypes because of the latitude differences that drive the photoperiod differences when grown at the same site. Intrinsic physiological constraints might prevent the combination of high A (from the AB test sites) and the longer growing period of QC test sites. However, if there are no such constraints, the progeny of intra-specific crosses between AB and QC populations may accomplish more growth than local crosses (i.e. heterosis). From our results, the crossing of AB and QC *P. balsamifera* (Table 3.2) showed superior growth and heterosis (hybrid vigour), the phenomenon by which hybrids outperform their parents in yield, biomass and other traits (Sinha and Khanna 1975). Additionally, our findings showed that intra-specific hybridizations between geographically distant populations may lead to heterosis (Schmidtling and Nelson 1996; Harfouche et al. 2000) through bringing together a new combination of alleles.

Several past studies have indicated that morphological measures of young conifer trees grown under near-optimal conditions can be predictive of inherently rapid stem growth at the family level, at older ages (i.e., nine years or 32 years) (Pharis et al. 1991; Park et al. 2014). However, our study is the first to show a significant and positive correlation between greenhouse-grown (Day 34) and field-grown (age eight) balsam poplars, while similar findings have been found in the literature for conifers, namely, 12 open-pollinated families of *Pinus densiflora* Sieb. et Zucc., where seedling stem volume at six months was significantly correlated with field performance at age 32 years (Park et al. 2014). In additional, Pharis et al. (1991) found that full-sibling families *of P. radiata*, that is, seedling stem volume at age 138 days gave reliable estimates of field performance at any age measured over nine years. Additionally, six-month heights of full-sibling families of black spruce (*P. mariana* Mill.), which were grown in a

greenhouse environment, were significantly correlated with age 13 field heights (Williams et al. 1987). From the above discussion, two common characteristics were identified for the retrospective approaches. First, the cuttings were grown under near-optimal environmental conditions in the greenhouses. Second, morphological measurements were made prior to the setting of the terminal bud. These findings implied that the early stem and shoot growth of young rooted cuttings (two months) that were raised under near-optimal conditions could be a useful trait for identifying inherently rapid stem growth in mature balsam poplar.

Leaf $\delta^{13}C$ is largely related to the ratio of CO_2 partial pressure inside the leaf and ambient air (ci/ca) (for review, see Farquhar et al. 1989), which is driven by stomatal conductance and photosynthetic processes. Several studies have shown a strong positive correlation between $\delta^{13}C$ and plant water-use efficiency (WUE) via ci/ca (Körner et al. 1991; Monclus et al. 2006; Chamaillard et al. 2011), which suggests that leaf $\delta^{13}C$ can be measured as a proxy for plant WUE. Water-use efficiency reflects the balance between carbon fixation and the amount of water released by plants. Water-use efficiency trends were assessed through $\delta^{13}C$ values of leaf tissue, with higher $\delta^{13}C$ values generally associated with greater WUE, while more negative $\delta^{13}C$ values are linked to reduced WUE and greater water loss (Guy and Holowachuk 2001; Diefendorf et al. 2010). On one hand, high WUE has been observed in trees with high productivity, and these gains are mainly associated with photosynthetic capacity (Sun et al. 1996). On the other hand, trees generally grow more slowly with higher stomatal conductance and exhibit lower WUE (Patterson et al. 1997). In conifer species, positive correlations between growth traits and $\delta^{13}C$ values have been found consistently (Guy and Holowachuk 2001; Eilmann et al. 2013), and our results, shown in Table 3.3, also indicate the same trend in a deciduous species, suggesting that variation in WUE is driven primarily by photosynthetic capacity. The superior growth of hybrids

is due to an increase in the photosynthetic rate, which results in more positive $\delta^{13}C$ values and higher WUE (Pointeau and Guy 2014).

High IAA levels have been associated with enhanced levels of expression of two GA biosynthesis genes, *GA20ox* and *GA3ox* (Wolbang and Ross 2001; Ozga et al. 2003; Nemhauser et al. 2006). Thus, biosynthesis of the GAs required for rapid stem growth may depend on an adequate supply of the auxin IAA. In our study, stem tissue ABA concentrations in Day 34 stecklings showed a significant negative correlation with stem volume and dry biomass (Figure 3.6c,d); this suggests that an ABA:IAA "balance" may control very early height growth in rooted cuttings. Recent evidence suggests that IAA acts downstream of the ABA response (Rinaldi et al. 2012) and there is also potential for high ABA concentrations to negatively influence the biosynthesis of GAs (Kurepin et al. 2010), including inhibition at the gene expression level.

Elevated levels of bioactive GA usually suppress the expression of *GA20ox* and *GA3ox* while stimulating the expression of *GA2ox*; conversely, a drop in the bioactive GA level usually up-regulates the expression of *GA20ox* and *GA3ox* and down-regulates the expression of *GA2ox* (for review, see Hedden and Philips 2000). The concentration of growth-activating GAs in growing tissues is regulated by transcriptional control of biosynthetic (*GA20ox* and *GA3ox*) or catabolic (*GA2ox*) genes (for review, see Thomas and Hedden 2012). Modifying the way in which these genes are expressed alters plant growth, a phenomenon that has been successfully demonstrated in various plant species including poplars (Eriksson et al. 2000; Busov et al. 2003; Reinecke et al. 2013). Figure 3.8 shows the trend for using endogenous GA levels to accelerate the early selection of balsam poplars that possess traits for inherently rapid stem growth. Moreover, the correlations found between stem volume and GA content in our study confirm the findings of others such as Yao et al. (2007). They found that GA content was correlated with

heterosis in plant height in wheat hybrids, and reported that increased elongation of the uppermost internode contributed most significantly to heterosis for plant height. Our findings also confirmed the review summarized by Zanewich and Rood (2020) that the GAs are primarily involved in heterosis for shoot growth.

In case of *Populus*, fiber and vessel lengths increase in the transition zone between juvenile and mature wood (Park et al. 2015). The time it takes for a cell to mature within the different differentiation zones will ultimately determine the size and cell wall thickness. It is possible that the action of GAs like GA_1 and/or GA_4 will extend this transition time, and thereby increase the fiber length in hybrid aspen (*Populus tremula* × *P. tremuloides*) compared to pure *P. tremula* plants (Uggla et al. 1996). These results also provide new insights into the mechanisms whereby GAs control growth and development in trees. Overexpression of *Arabidopsis* GA 20-oxidase (*AtGA20ox1*) in transgenic hybrid aspen (*Populus tremula* × *P. tremuloides*) resulted in a substantial increase in plant height and fiber length, nearly doubling stem dry weight relative to the wild-type control (Eriksson et al. 2000). Additionally, *GA20ox* appears to play an important role in wood development through tree growth. Ko et al. (2012) showed that poplar GA20-oxidase (*PtGA20ox*) has the highest expression in developing xylem. In that study, tissue-specific transcriptome analysis was used, which resulted in several GA receptors being highly expressed in the tissue.

3.6 Conclusion

In conclusion, the data presented in this research—based on disparate population breeding of intra-specific hybrids in balsam poplar—confirms the widespread occurrence of heterosis typically found in interspecific crosses. The combined analyses of endogenous GA content and growth data revealed that GAs play a regulatory role in heterosis for hybrid balsam

poplar at the physiological level. Larger scale investigations of multiple plant hormones at more developmental stages of hybrid balsam poplar are anticipated to confirm and extend these findings. Moreover, the results obtained from selected progeny were validated by retrospective comparisons with the stem growth performance of the corresponding field-grown trees at eight years of age. These results with *P. balsamifera* also point toward the successful early selection of progeny from genetic crosses of other deciduous tree species, that is, the identification of progeny which are inherently fast growing and have high stem biomass production. The relationship between GAs and growth performance (both field and greenhouse) may lead to new ways to manipulate trees to grow faster and produce more biomass by increasing endogenous GA levels. Moreover, screening for high levels of expression of *GA20ox* genes may be a useful technique for future tree-breeding programs. Future RT-PCR work should be used to test *GA20oxs*, *GA3oxs*, and *GA2oxs,* which are the main targets of regulation by GA signaling to establish homeostasis.

Table 3.1 Family codes and selected balsam poplar progeny for each group type, fast-growing (FG), slow-growing (SG) and slow-fast growing (SFG), based on six-year-old growth from two field sites (AB1 and AB2).

Family	Fast-growing Progeny	Group (Fast-growing (FG))	Slow-growing Progeny	Group (Slow-growing progeny in Fast growing Group (SFG) or Slow-growing (SG) Group)
AP5396	147071	FG	147043	SFG
	147083	FG	147051	SFG
	147072	FG	147041	SFG
AP5401			178092	SG
			178073	SG
			178104	SG
AP5402	180071	FG	180093	SFG
	180103	FG	180063	SFG
	180081	FG	180094	SFG
AP5411			255081	SG
			255011	SG
			255102	SG
AP5414			270051	SG
			270101	SG
			270061	SG
AP5416	272084	FG	272024	SFG
	272102	FG	272071	SFG
	272091	FG	272023	SFG

Table 3.2 Mean height (±SE), diameter at breast height (DBH) (±SE), and stem volume (±SE) (N=1894) at age six from two Alberta sites (AB1 and AB2) for the four cross-types in balsam poplar as indicated by Alberta (AB) and Quebec (QC) with the female parent listed first. Significant differences between cross-type means are indicated by different letters.

Cross-types	Height (m)	DBH (mm)	Stem volume (cm^3)
AB♀ × AB♂	3.67±0.05^{c}	33.70±0.76^{b}	1375.94±65.88^{b}
AB♀ × QC♂	4.06±0.04^{a}	37.77±0.54^{a}	1786.78±59.48^{a}
QC♀ × AB♂	3.78±0.03^{bc}	34.73±0.40^{b}	1389.78±35.27^{b}
QC♀ × QC♂	3.80±0.05^{b}	34.52±0.63^{b}	1433.09±55.63^{b}

Table 3.3 Pearson's correlations (r) analysis among physiological variables under optimal growth condition in greenhouse for selected balsam poplar progeny (N=216) growth at two months.

	Height (cm)	**Caliper (mm)**	**Stem volume (mm^3)**	**A**	**g_s**	**iWUE**	**$\delta^{13}C_{leaf}$ (‰)**
Height (cm)	1	0.933**	0.861**	0.751*	0.532*	0.606*	0.545**
Caliper (mm)		1	0.890**	0.743*	0.614*	0.517*	0.502
Stem volume (mm^3)			1	0.556*	0.327	0.344	0.495
A				1	0.596*	0.796**	0.461
g_s					1	-0.205	-0.563*
iWUE						1	0.505*

*Significant at $p \leq 0.05$: 1) **Significant at $p \leq 0.01$.A: Net photosynthetic rate (µmol CO_2 m^{-2} s^{-1}); g_s: stomatal conductance (mol H_2O m^{-2} s^{-1}); iWUE: intrinsic water-use efficiency (µmol CO_2 $mmol^{-1}$ H_2O); $\delta^{13}C$ leaf: carbon isotope composition.

Table 3.4 Pearson's correlation (r) analysis between phenotypic characteristics of 8-year old field-grown hybrid *P. balsamifera* trees and Day 34 greenhouse-grown stecklings for the same 15 genotypes. AB = Alberta and QC = Quebec.

Field AB1		
Stem parameters of 8-year-old trees at FieldAB1	**Day 34 stem volume (mm^3)**	**Day 34 stem dry weight (g)**
Height (cm)	0.550*[1]	0.516*
DBH (mm)	0.608*	0.584*
Stem volume (cm^3)	0.629*	0.601*
Field AB2		
Stem parameters of 8-year-old trees at	**Day 34 stem volume (mm^3)**	**Day 34 stem dry weight (g)**
Height (cm)	0.654**	0.686**
DBH (mm)	0.537*	0.604*
Stem volume (cm^3)	0.619*	0.671**
Field QC1		
Stem parameters of 6-year-old trees at	**Day 34 stem volume (mm^3)**	**Day 34 stem dry weight (g)**
Height (cm)	0.758**	0.739**
DBH (mm)	0.666**	0.705**
Stem volume (cm^3)	0.588*	0.684**

[1]All values represent the correlation coefficient (r) from Pearson's correlation analysis. DBH = diameter at breast height (1.3 m). **Significant at $p \leq 0.01$. *Significant at $p \leq 0.05$.

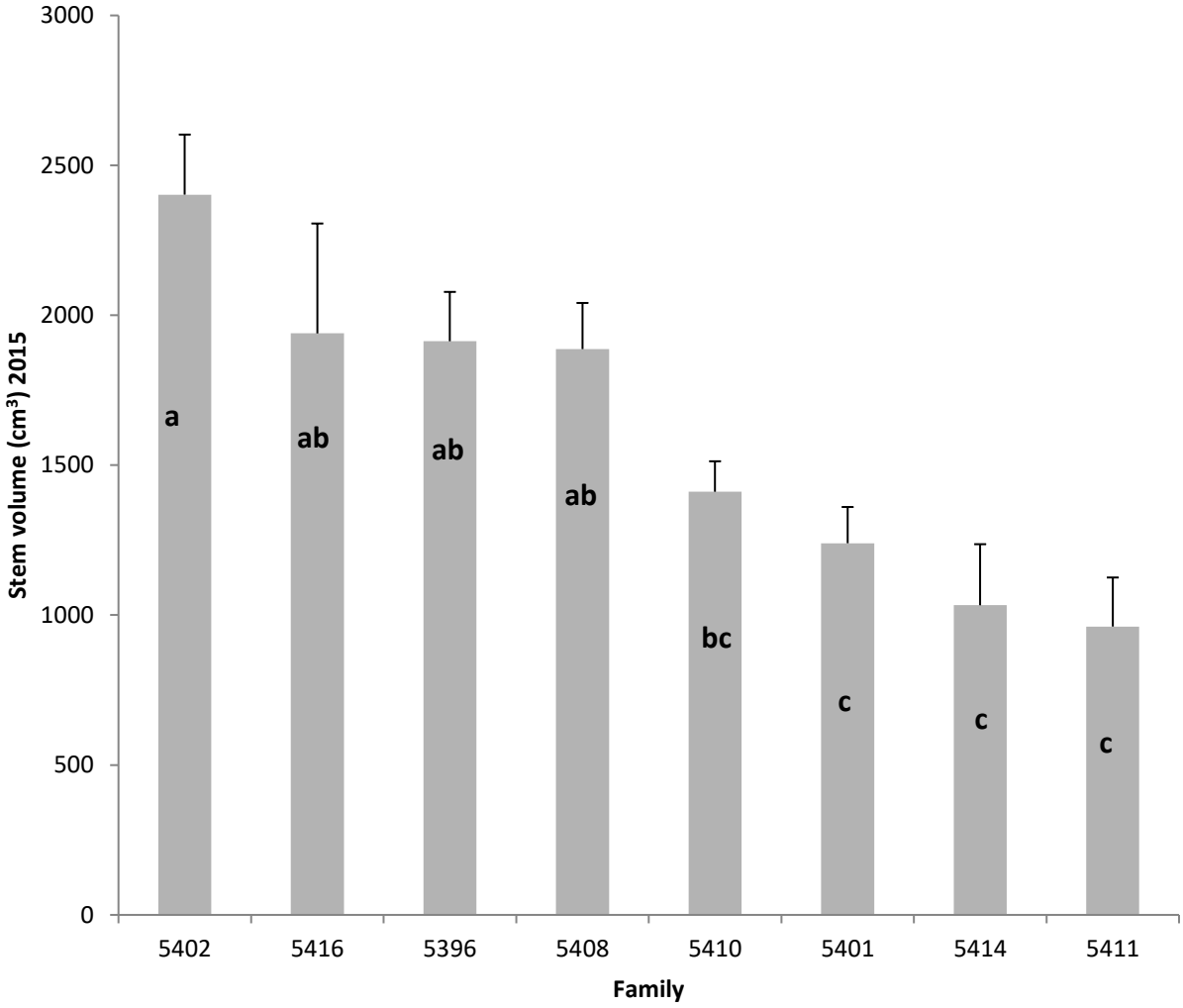

Figure 3.1. Mean stem volumes (cm^3) (+SE) for 6-year old (N=245 for each field) Alberta × Quebec cross-type families in balsam poplar grown at two Alberta sites (AB1 and AB2). Significant differences between family means are indicated by different letters at a $p \leq 0.05$.

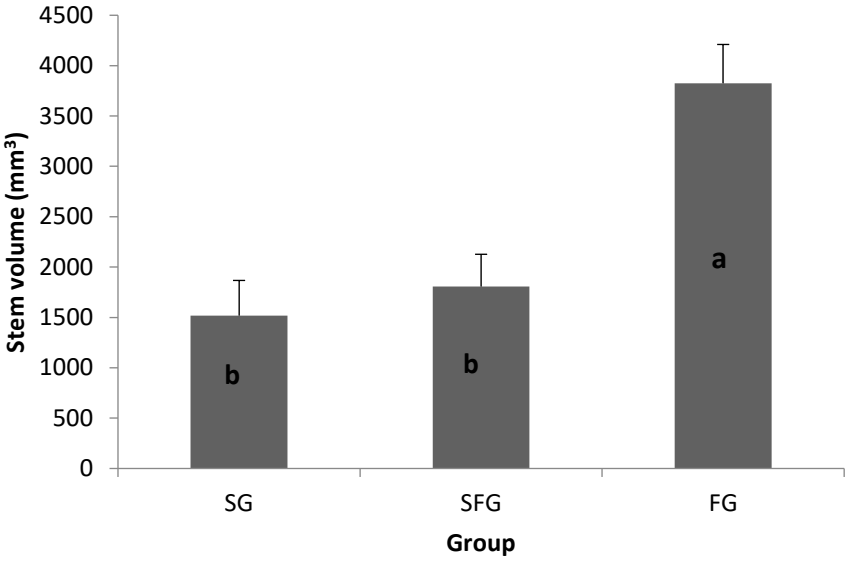

Figure 3.2. Mean stem volume (mm^3) (+ SE) of three groups (N=216) in balsam poplar at Day 34 under near-optimal greenhouse conditions. Note: SG = slow-growing progeny; SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny. Significant differences between group means are indicated by different letters at $p \leq 0.05$.

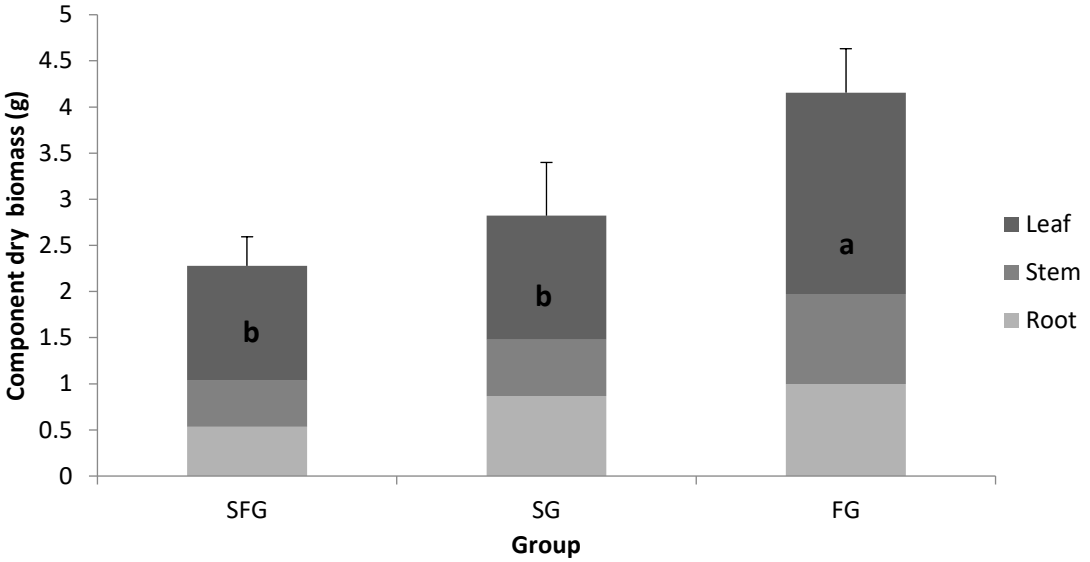

Figure 3.3. Mean component dry biomass (g) (+SE) of three groups (N=216) in balsam poplar at Day 34 under near optimal greenhouse conditions. Note: SG = slow-growing progeny; SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny. Significant differences between group means of total dry mass are indicated by different letters at $p \leq 0.05$.

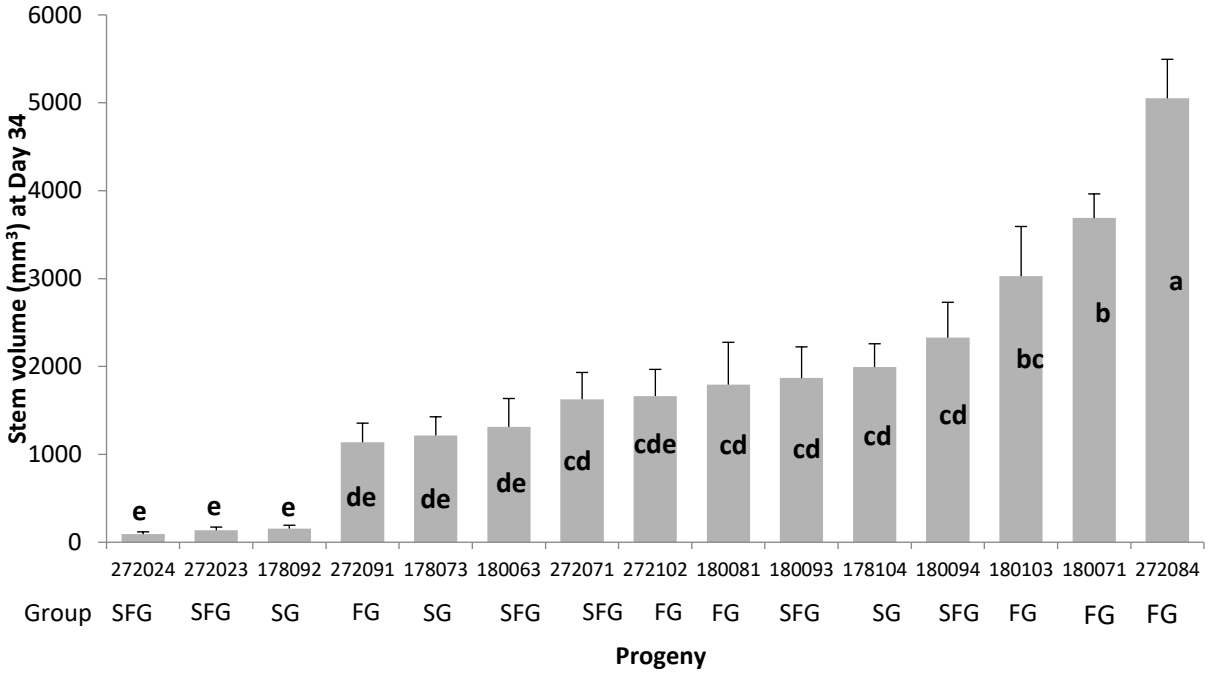

Figure 3.4. Mean stem volume (mm^3) (+ SE) of 15 selected balsam poplar progeny (N=120) at Day 34 under near-optimal greenhouse conditions. Note: SG = slow-growing progeny; SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny. Significant differences between progeny means are indicated by different letters at $p \leq 0.05$.

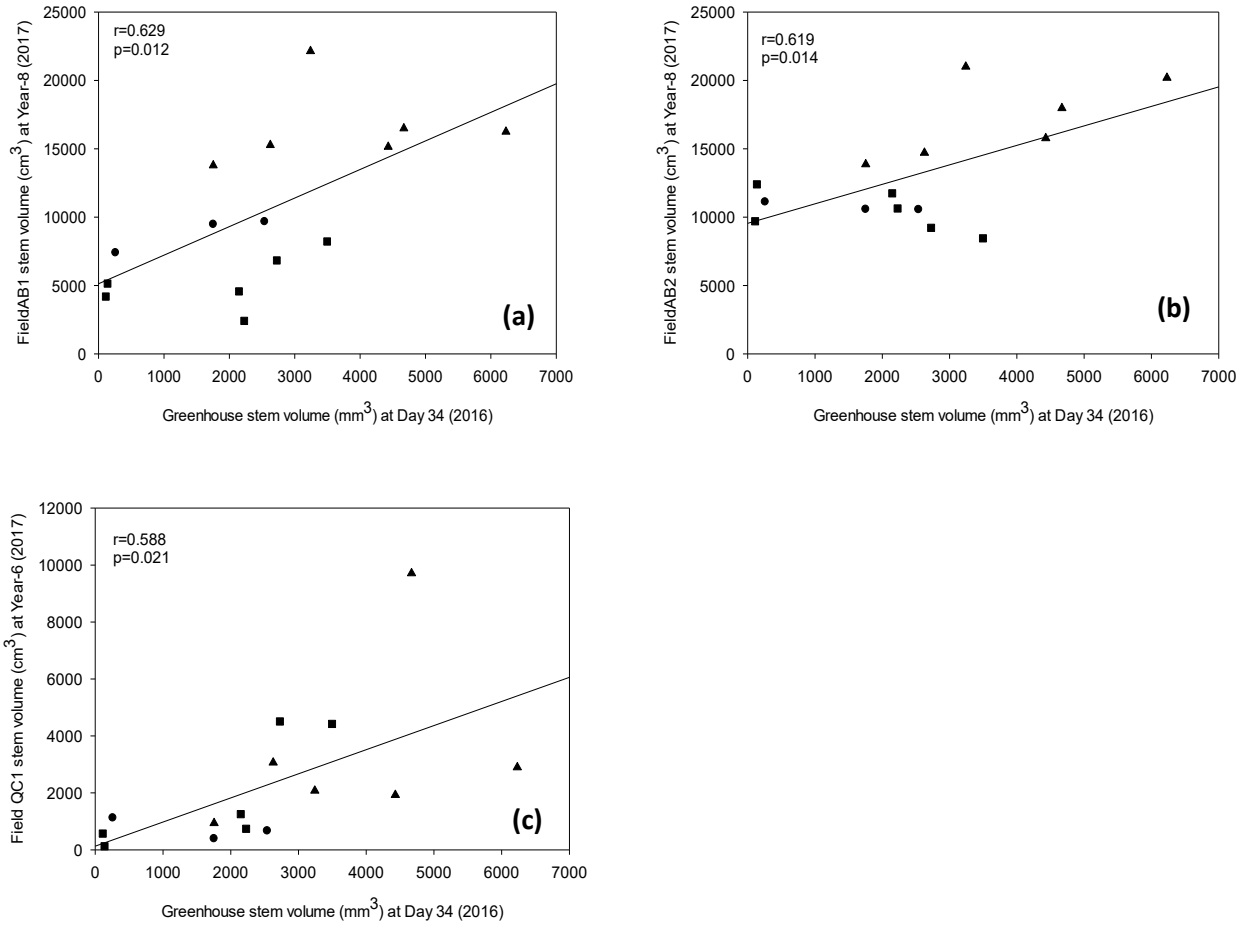

Figure 3.5. Pearson's correlation (r) between field stem volume and greenhouse growth at Day 34 (after transplanting) for 15 selected balsam poplar progenies (N=45) in 2017 for: a) Year-8, Field AB1, AB; b) Year-8, Field AB2, AB; and c) Year-6, Field QC1, QC. Symbols represent individual progeny that had been previously grouped with FG (▲), SFG (■) and SG (●). Note: SG = slow-growing progeny; SFG = slow-growing from a fast-growing family; and FG = fast-growing progeny. AB = Alberta and QC = Quebec.

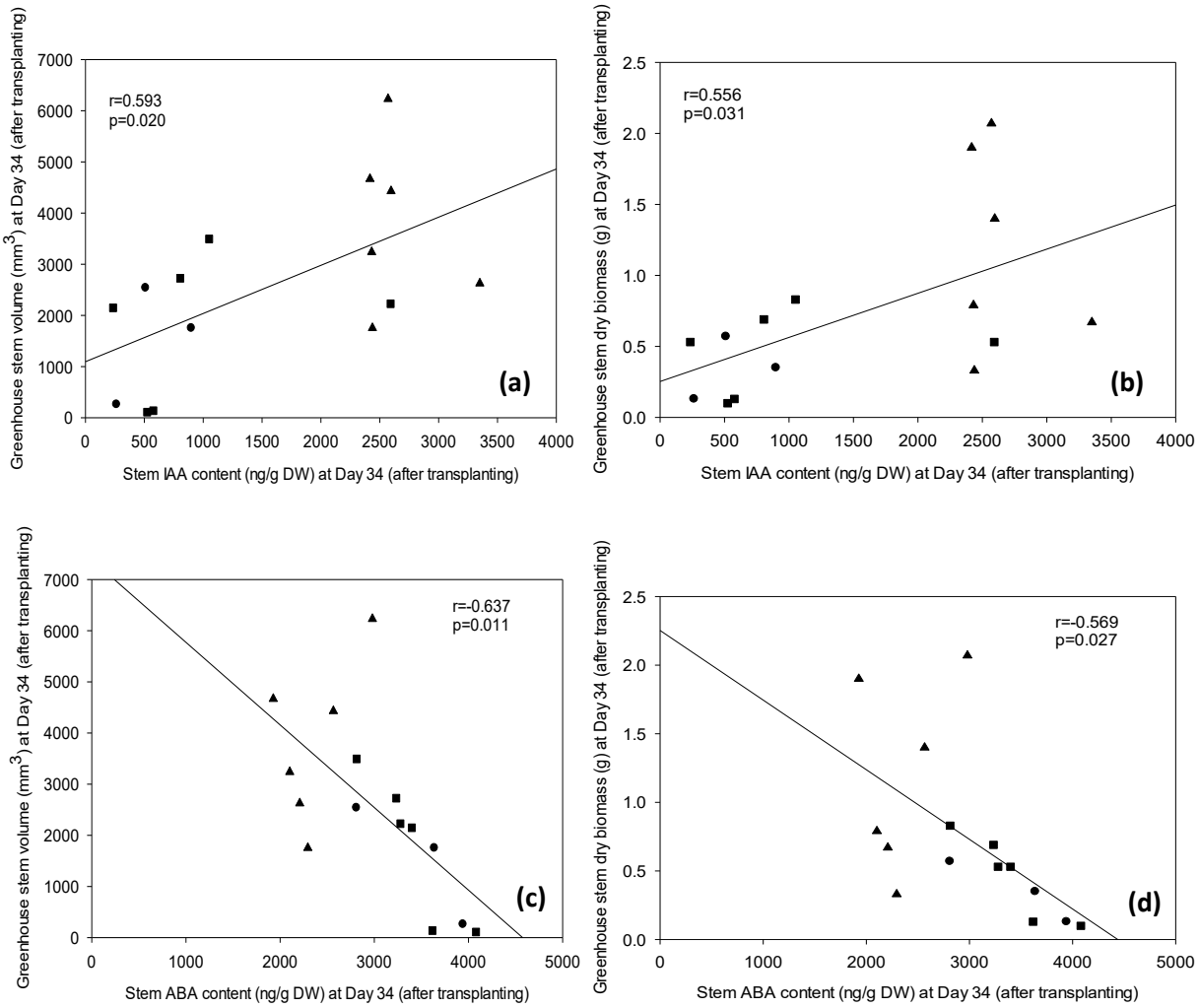

Figure 3.6. Pearson's correlations (r) of tissue concentrations of indole-3-acetic acid (IAA) and abscisic acid (ABA), *versus* stem dry biomass or stem volume of nursery-grown balsam poplar cuttings (new growth) age Day 34 (after transplanting) (N=45). a) New growth stem volume at Day 34 *versus* IAA in stem tissue at Day 34; b) New growth stem dry biomass at Day 34 *versus* IAA in stem tissue at Day 34; c) New growth stem volume at Day 34 *versus* ABA in stem tissue at Day 34; d) New growth stem biomass at Day 34 *versus* ABA in stem tissue at Day 34. Symbols represent individual progeny that had been previously grouped with FG (▲), SFG (■) and SG (●). Note: SG = slow-growing progeny; SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny.

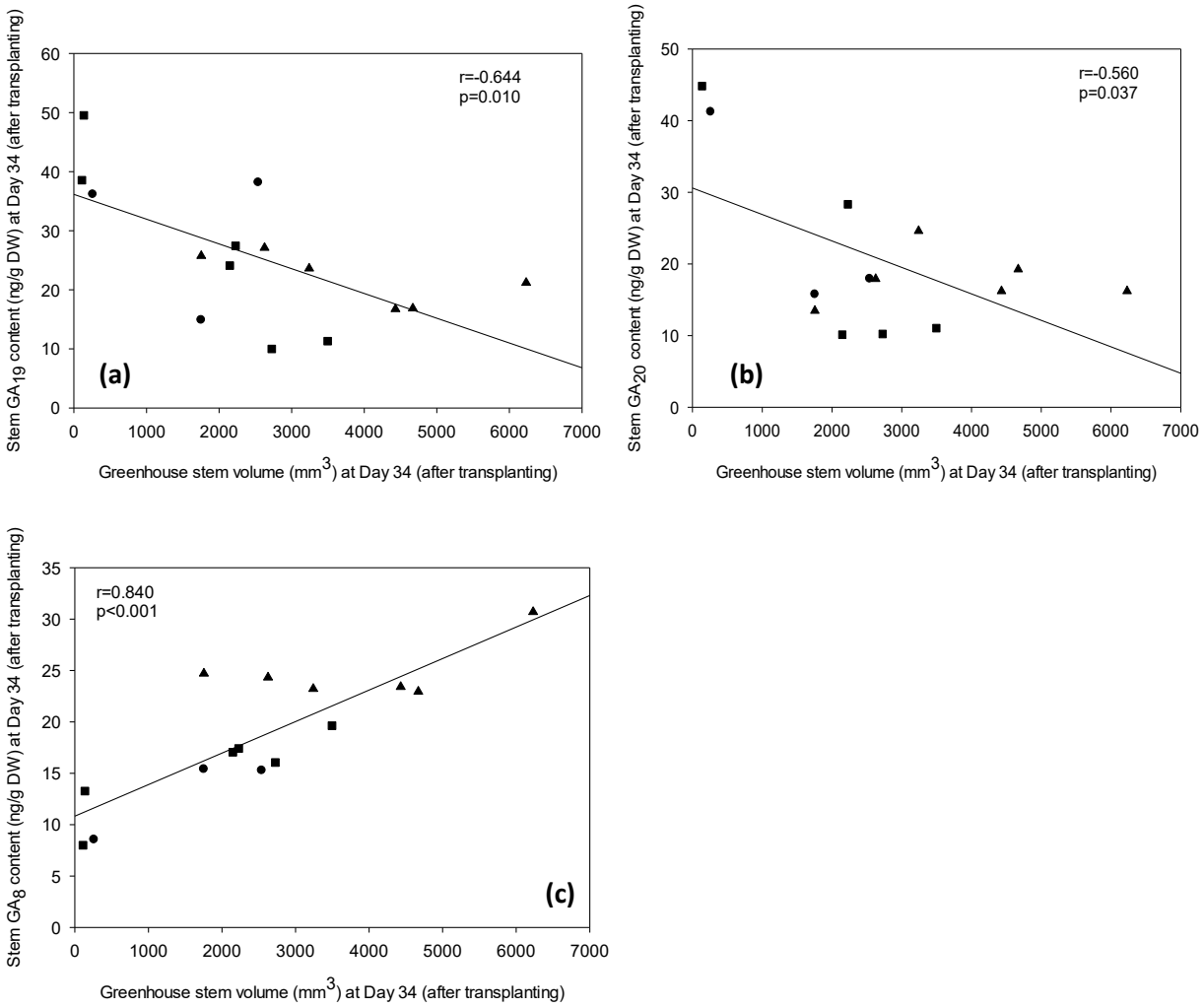

Figure 3.7. Pearson's correlation (r) between greenhouse stem volume at Day 34 (after transplanting) and plant hormones for 15 selected balsam poplar progenies (N=45); a) GA_{19} (ng/g DW); b) GA_{20} (ng/g DW); and c) GA_8 (ng/g DW). Symbols represent individual progeny that had been previously grouped with FG (▲), SFG (■) and SG (●). Note: SG = slow-growing progeny; SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny.

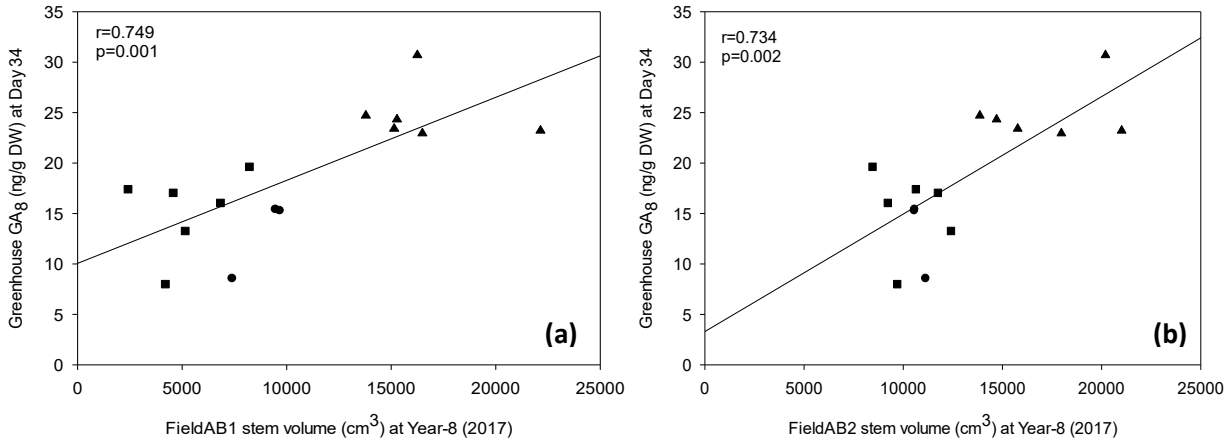

Figure 3.8. Pearson's correlation (r) between greenhouse GA_8 (ng/g DW) at Day 34 (after transplanting) and field stem volume for 15 selected balsam poplar progenies (N=45); a) FieldAB1, AB; b) Field AB2, AB. Symbols represent individual progeny that had been previously grouped with FG (▲), SFG (■) and SG (●).Note: SG = slow-growing progeny; SFG = slow-growing from a fast-growing family; and FG = fast-growing progeny.

Chapter 4. An evaluation of epigenetic responses to drought stress through physiological parameters in hybrid balsam poplar (*Populus balsamifera*)

4.1 Introduction

Plants are constantly challenged by biotic and abiotic stresses, from which they protect themselves by regulating their physiological and developmental strategies through changes in genome-wide gene expression (Wang et al. 2011). The process involved in how the environment and other external factors affect plant genes and phenotype can be studied through epigenetics (Kim et al. 2008). Epigenetic modifications remain as cells divide, and in some cases the changes can be stably transmitted through generations. Epigenetic regulation interacts with the genetic material modifying the expression of genes, but does not alter the underlying DNA sequence (Lin et al. 2005). Additionally, this mechanism provides a way for species to cope with severe environmental changes (Crowley 2017).

In trees, one of the first reports of this phenomenon was in Norway spruce (Johnsen et al. 2005a; Johnsen et al. 2005b) where researchers found that epigenetics enabled trees to adapt. The results from these studies found that trees have an internal regulator that is set by epigenetic mechanisms when the tree is still a seed (Johnsen et al. 2005a; Johnsen et al. 2005b). Trees such as Norway spruce responded each season as if they were biologically preprogrammed, like an epigenetic memory. During the development of the embryo seed, epigenetic marks were established on the Norway spruce's DNA in response to the ambient temperature (Yakovlev et al. 2016). The majority of the differently expressed genes were related to DNA and histone methylation. Also, sRNA pathway and signaling genes might be responsible for formulating the epigenetic memory in the spruce embryos (Johnsen et al. 2005a; Johnsen et al. 2005b). Additionally, the study demonstrated that these trees might be more adaptable to climate change

than previously expected (Yakovlev et al. 2016). For deciduous trees, Raj et al. (2011) tested drought responses of three interspecific hybrid poplar clones (DN34 (*Populus deltoides* × *Populus nigra*), Walker [*P. deltoides* var. *occidentalis* × (*Populus laurifolia* × *P. nigra*)], and Okanese [Walker × (*P. laurifolia* × *P. nigra*)]) from three different locations (Alberta, Manitoba and Saskatchewan) by using a common garden approach. They concluded that genetically identical clones would display varied physiological response under drought conditions in relation to their sites of origin.

Poplars are the woody plants that are most sensitive to water deficit (Larchvéque et al. 2011). Their productivity depends strongly on water availability (Tschaplinski et al. 1994). Therefore, they always distribute according to water availability (Larchvéque et al. 2011). The wide distribution of the 29 species of the *Populus* genus led the species to develop several drought resistance strategies that are possible to adapt to different types of ecosystems, including dry areas and deserts (for review, see Marron et al. 2008). Under drought conditions, recent studies showed that plants could alter their physiology to suppress shoot growth and stomatal conductance but enhance their drought resistance abilities in low water availability status (Reynolds-Henne et al. 2010; Aasamaa and Sõber 2011). Poplars can exhibit several drought resistance strategies including adjustments of stomatal closure, root architecture, water-use efficiency (WUE), and hydraulic conductivity (Mazzoleni and Dickmann 1988; Roden et al. 1990; Monclus et al. 2006; DesRochers et al. 2007). Hydraulic conductivity in the xylem is related to xylem water potential and vessel diameter. Decreases in water potential caused by water stress can lead to xylem cavitation (Tyree and Sperry 1989). Xylem cavitation occurs when xylem vessels are blocked by air bubbles or embolisms, which reduces hydraulic conductivity and productivity (Tyree et al. 1992).

The global warming expected at the end of the 21st century will produce an increased probability of drought stress, and in general more frequent and more severe extreme climatic conditions (Allen et al. 2015; Gray et al. 2016). For instance, Mbogga et al. (2009) found that Alberta recently experienced a temperature rise of 0.8°C and a precipitation reduction of almost 10% over a 25-year period. With global climate change likely further worsening environmental conditions, a major challenge will be to produce improved trees that can adjust to grow in novel climates (Pascual et al. 2014). Loustau et al. (2005) indicated that forest productivity would mainly be affected by climate change. They found that short rotation forestry, such as poplar plantations, would suffer severe decreases in productivity under the counterbalancing effects of drought and rising CO_2 concentrations. Climatic conditions have been notably drier than normal across large areas of the western Canadian interior, leading to widespread impacts on the forests of this region since 2001 (Hogg et al. 2017). Thus, it is very important to find desirable fast-growing clones that will combine satisfactorily high productivity and high water-use efficiency in this climatic change context where temperature and precipitation patterns have become so uncertain (Andalo et al. 2005; Monclus et al. 2006). However, the selection of drought-resistant and productive clones is not easy. Since the variability of the clones within the hybrids is very high, the high productivity clones may have strong drought tolerance while others will be more drought sensitive and less productive (Tschaplinski and Blake 1989; Chen et al. 1997; Brignolas et al. 2000; Zhang et al. 2004). The relationship between productivity and drought tolerance is hard to simplify within-species or hybrids; Zhang et al. (2004) found that trees from the same species would exhibit different water conservation strategies depending on their provenance from wet or dry areas.

In this study, we evaluated epigenetic responses to drought stress by using progeny from intraspecific hybridization. We also examined the physiological response behind the role of epigenetic regulation in a plant's adaptation to a changing environment. The same progeny were collected from two different locations (Alberta (AB) and Quebec (QC)) and then grown under common, controlled environmental conditions. The drought responses of the same progeny originating from different locations were assessed and the following questions asked: 1) Are fast-growing progeny more resistant to drought than slow-growing progeny?; and 2) Do cuttings of the same genotype grown in different locations show the same morphological and physiological response under controlled *versus* drought conditions?

4.2 Materials and Methods

4.2.1 Greenhouse Propagation

In February 2018, 40-50-cm-long dormant branch cuttings of six progeny (Table 4.1) (three from the fast-growing progeny (FG) group and three from the slow-growing progeny (SFG) group from family 5402 (based on the results from Chapter 3) were collected from two sites, one from an AB (FieldAB1) site (at Year-8) (Alberta- Pacific Forest Industries Inc. (Al-Pac) millsite (54ºN, 112ºW, 575 m, mean annual precipitation at 458mm (Climate Edmonton 2019)), and a single site in QC (Field QC1) (at Year-6) located at Trécesson (48ºN, 78ºW, 348 m, mean annual precipitation at 890mm (Climate Trécesson 2019)). The cuttings were placed in black plastic bags and stored in the fridge for eight days at -4 °C, until the greenhouse experiment started. On February 21st 2018, the cuttings were soaked in cold water for two days at room temperature in the lab, with water replaced daily with fresh cold water. On February 23rd, 2018, 12 stem cuttings that were six-nine cm in length with a minimum of two buds for each of the six progeny were rooted in Format 360 Hillsons Rootrainers trays (Beaver Plastics Ltd.,

Acheson, AB, Canada) filled with Sunshine Mix #4 (Sun Gro Horticulture, Vancouver, BC, Canada) without the use of any additional rooting hormone (DesRochers and Thomas 2003). Upon bud flush, and after height growth of about 10 cm was reached (~ Day 50 after striking), the cuttings were transplanted directly into 2L pots filled with Sunshine Mix #4 (Sun Gro Horticulture, Vancouver, BC, Canada) on May 22nd, 2018 (Day 0 after transplanting). The rooted cuttings (stecklings) were grown in the greenhouse under natural light supplemented by cool-white fluorescent lamps to provide a 21-hour-long photoperiod and a minimum photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1} at plant level. Maximum day and night temperatures were maintained close to 25 °C and 18 °C, respectively, throughout the nine-week experimental period. The stecklings were kept well-watered and fertilized using a 20-20-20 commercial water-soluble fertilizer (20:20:20 plus micronutrients (Fe 0.1%; Mn 0.05%; Zn 0.05%; Cu 0.05%; B 0.02%; Mo 0.0005%)) (Plant Products Co. Ltd., Brampton, ON, Canada) at a pH adjusted to 5.8-6.3 by adding phosphoric acid (H_3PO_4). The greenhouse was well-ventilated and PPFD, humidity and air temperature were continuously monitored and recorded using a HOBO U12-012 data logger (Onset Computer Cooperation, Pocasset, MA, USA). The experiment was a three-factorial completely randomized block design with two growth groups (FG and SFG) x two drought treatments (Drought (D) and well-watered (W)) x two collection sites of origin (AB and QC). Each treatment combination was applied to one tree and replicated in six blocks designed to remove possible environmental gradients in the greenhouse. Cuttings were watered daily to field capacity until the beginning of the drought experiment 80 days after the initial planting. On the first day of the drought experiment (Day 0 after transplanting (50 days after initial striking)), half of the trees (six) from each progeny and location of origin were selected for the well-watered (W) treatment and the other half were submitted to progressive drought (D) by reducing the water supply to each tree within each block. During the experiment,

pot weights were measured daily using a model GBK 16a bench check weighing scale (readability 0.1g, Adam Equipment Inc., Oxford, CT, USA). Soil volumetric water content (SVWC, the fraction of the total volume of the soil sample occupied by the water contained in the soil, %) was measured using a ProCheck soil moisture meter with a 10-cm long probe (Decagon Devices Inc., Pullman, WA, USA). Trees in the W treatment were individually watered to field capacity (100%) and calibrated with the ProCheck. The SVCW at the field capacity was approximately 50%. Therefore, trees in the W treatment maintained a SVWC of approximately 50%. In the D treatment, SVWC was maintained between 10 and 20% through the drought cycles by measuring pot weight and soil moisture. In total, there were three drought cycles over the whole experiment. For the first (Day 0 to Day 19) and second cycle (Day 23 to Day 38), the SVWC of the D treatment was kept at approximately 50% for one week before drought imposition, and thereafter reduced to 20%. The SVWC was kept at 20% for one week and then the trees were re-watered to 50% SVWC. For the third cycle (Day 38 to Day 62), the re-watered D treatment was reduced to 10% of the SWVC and kept for two weeks until the end of the experiment when all of the plants were harvested on Day 62. In addition, four days of recovery period (during which the plants were re-watered and the cuttings maintained at well-watered conditions) was applied between the dry-down cycles, i.e. Day 19 - Day 23 for the first and second; and Day 38- Day 42 for the second and third. Pot weights were measured, and the water supply adjusted daily to maintain 10-20% of the SWVC to meet the required drought level.

4.2.2 Measurements and Harvest

Diameter and height growth were measured immediately after the plants were transplanted to the 2L pots. After that, those measurements were taken bi-weekly. Seven times throughout the experiment, gas exchange measurements, including the photosynthetic rate (A,

μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), and intrinsic water-use efficiency (iWUE, μmol CO_2 $mmol^{-1}$ H_2O and the formula = A/g_s (for review, see Farquhar et al. 1989)), were made using a CIRAS-3 infrared gas analyzer (IRGA) (PP Systems, Amesbury, MA, USA) using a broad leaf cuvette (PLC4 (B) Broad Leaf Cuvette, PP Systems, Amesbury, MA, USA). Measurements were made on one fully expanded mature leaf per cutting with a fixed CO_2 level set at 390 ppm, a supplied (saturating) light level PAR (photosynthetic active radiation) of 1000 μmol m^{-2} s^{-1} , relative humidity of approximately 40% - 50%, and flow rate of 400 cc min^{-1}.The seven measurements were taken as follows: Day 0: start of the experiment (85 days after initial growth from dormant cuttings); Day 19: completion of the first drought cycle, re-watered to well-watered conditions (SVWC~50%); Day 23: start of the second drought cycle; Day 38: completion of the second drought cycle, re-watered well-watered conditions (SVWC~50%); Day 42: start of the third drought cycle; Day 52: drought level reached at third cycle; Day 62: end of the experiment. All plants were harvested on July 31, 2019 (Day 62 after transplanting). The night before harvesting, pre-dawn leaf water potentials (Ψ_{plwp}, MPa) were measured. One mature leaf was removed from the middle of the plant stem, immediately installed in a pressure chamber PMS Model 1000 (PMS Instrument Company, Albany, OR, USA) and exposed to increasing pressure until the xylem sap appeared at the cut surface of the petiole. At harvest, the stecklings were separated into leaves, stem and roots. The roots were carefully washed clean of all rooting medium and the total leaf area of each plant was measured using a LI-3100C area meter (LI-COR Inc., Lincoln, NE, USA). All separated parts were dried at 80 ^{0}C for two days, then weighed using a model AV53 scale (readability 0.001g, OHAUS Adventurer Pro, Melrose, MA, USA).

4.2.3 Data Analysis

The growth data were statistically analyzed with the General Linear Model procedure of SAS (SAS, 2001). Gas-exchange measurements were analyzed in a repeated measures analysis of variance (PROC MIXED, SAS). A significance level of $p \leq 0.05$ was chosen. The correlation coefficient (r) and probability (p) were determined by Pearson's correlation analysis using the Statistical Package in SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, USA).

4.3 Results

4.3.1 Growth and Biomass Production

Cuttings of the FG grew much faster (in height and diameter) and outperformed cuttings of the SFG (Table 4.2) under well-watered and drought conditions ($p<0.001$) as determined by multiple means comparisons. Additionally, progeny from the FG group that originated in AB showed superior growth potential in well-watered conditions with the largest diameter and height than cuttings from the same clone that originated in QC. For the well-watered treatment, within the same growing group (FG or SFG), there were significant differences in growth performance between the AB progeny and QC progeny (Table 4.2). The drought treatment significantly decreased the height and diameter for all progeny, and those from the SFG group from QC were affected more than all other groups including the SFG progeny from AB (Table 4.2).

At the end of the experiment, the FG progeny performed better than the SFG progeny in total new growth biomass, root dry biomass and root:shoot ratio (R/S) under different treatments regardless of origin (Figure 4.1) as determined by multiple means comparisons. Additionally, the same progeny groups from different locations showed significant differences in total biomass, root dry mass and R/S under well-watered treatment (Figure 4.1). Under well-watered

conditions, the FG progeny showed the greatest R/S ratio compared with SFG progeny ($p<0.001$). Under drought, all progeny (FG and SFG) performed similarly as total biomass, root dry biomass and R/S ratio decreased. The SFG progeny from both sites of origin were the most affected (Figure 4.1). However, for the R/S ratio, there were no significant differences between SFG progeny that originated in QC under the well-watered treatment and FG progeny that originated in AB and QC under the drought treatment.

4.3.2 Pre-dawn Leaf Water Potential

Overall, mean pre-dawn leaf water potential was significantly lower in drought-treated trees (Ψ_{plwp} = -0.554± 0.007 MPa) than in well-watered trees (Ψ_{plwp} = -0.310±0.004 MPa) as determined by multiple means comparisons (Figure 4.2). For both treatments, there were significant differences in pre-dawn leaf water potential for FG progeny originating in different locations. However, no significant differences were observed in the SFG progeny group.

4.3.3 Water Relations and Leaf Gas Exchange

At the end of the experiment, just prior to harvest on Day 62, higher rates of photosynthesis were correlated with growth (height, diameter, biomass) (Table 4.3). Additionally, increased photosynthetic demand was supported by an increased supply of CO_2 through an increase in stomatal conductance (g_s). A significant positive correlation through Pearson's correlation was found between iWUE and both height and diameter (r=0.658, 0.651, respectively), which may suggest that increased intrinsic water use efficiency promoted an increase in net photosynthetic rate (A) and carbohydrate production, resulting in an increase in growth. Furthermore, specific leaf area (SLA) had a significant and positive correlation with net photosynthesis and growth (Table 4.3).

SVWC (%) was measured nine times (at Day 0, 12, 19, 23, 30, 38, 42, 52 and 61 of the experiment) through all three drought cycles and confirmed that the desirable drought and water levels were achieved (Figure 4.3a). g_s, A and iWUE were measured seven times throughout the experiment to examine the overall trend of the physiological responses in all progeny (Figure 4.3b, c, d). Similar trends were observed for all progeny regardless of their location of origin, such that both FG and SFG progeny tended to partially recover from the re-watering process (measurements at Day 23 and Day 42) and decreased again through the drought period (Figures 4.3b, 4.4). However, full recovery was not observed after three cycles of dry-down in our study. In addition, the severe drought level (20% of SVWC from Day 52 to Day 61) resulted in a significant decrease in physiological performance for all progeny.

All progeny tended to perform better in the control treatment. However, the measurements of physiological parameters (g_s and A) showed a slight decrease during the whole experiment. In addition, all progeny responded similarly to the drought treatments with an increase in iWUE and a corresponding decrease in g_s (Figures 4.3a, 4.4b). In particular, for the FG progeny from Quebec, the iWUE was increased from 0.047 μmol CO_2 $mmol^{-1}$ H_2O (at Day 52) to 0.061 μmol CO_2 $mmol^{-1}$ H_2O (at Day 62) (Figure 4.4b).

Overall, drought level dramatically affected all physiological variables (stomatal conductance, net photosynthesis and iWUE) for both groups (Figure 4.5). For the well-watered treatment, FG progeny performed better than SFG progeny. Under drought treatment, there were significant differences in net photosynthesis for both FG and SFG progeny originating in different locations (Figure 4.5b). For the well-watered treatment, significant differences were detected in stomatal conductance and iWUE for FG progeny originating in different locations (Figures 4.5 a, c).

4.4 Discussion

For the site conditions, trees grew in QC and developed under much wetter conditions than the AB cuttings (Government of Canada 2019). Both FG progeny and SFG progeny from the same family, originating in the two source locations, reacted similarly in all traits measured throughout the experiment when submitted to drought. However, FG progeny were affected more than the SFG progeny under the drought treatment with a greater decrease in total biomass and root biomass. Root:shoot ratio (R/S) is often very useful in determining if plants have healthy root systems relative to their above-ground biomass (Landhäusser et al. 2003) and studies indicate that a higher R/S ratio would be desirable in dry environments as increased root growth will likely increase performance under drought conditions (Zalesny et al. 2005; Feng et al. 2012). From a review by Wilson (1988), the author indicated that biomass allocation could be affected by several factors including deficits of water and major inorganic nutrients, light and carbon dioxide, defoliation and root pruning. Additionally, changes in R/S ratio indicate the distribution of carbohydrates between shoots and roots. Because drought often limits root penetration and inhibits root growth, clones that have high root growth are likely going to have increased performance in drought environments (for review, see Brunner et al. 2015). Therefore, higher R/S ratios would be desirable. However, one must be careful not to look at R/S ratio as a single trait for selection as it gives no indication of the actual growth performance of the overall plant. The increase in the R/S ratio of plants under water stress may also be caused by enhancing a plant's root system under drought condition (Bogeat-Triboulot et al. 2007). In our study, however, under extreme soil drought, no R/S ratio increase was observed, indicating that the allocation of plant biomass could be limited under extreme levels of drought stress (Xu and Zhou 2005). Plant growth depends on the assimilation of carbon by photosynthesis (Hampp et al.

1994). Water stress can lead to an increase in the allocation to roots (Sack and Grubb 2002). Root growth is less sensitive than stem growth to a decrease in soil-water potential, leading to an increase in the R/S ratio that is commonly observed in plants exposed to moderate water stress (Younis et al. 2008). Xu and Zhou (2005) found that extreme water stress markedly reduced carbon partitioning to the roots, but increased the partitioning to the leaves and stems, indicating that root demand for carbon decreases under very high water stress. Thus, the regulation capacity of the plants might be lost when the plants are subjected to extreme soil drought. Ideally, progeny that have greater total above-ground biomass with an above average R/S ratio would be considered desirable. Our study showed that from this R/S ratio approach, the fast-growing progeny that originated in AB would be considered the best progeny to grow under drought conditions.

During photosynthesis, plants must open their stomata to take in CO_2 and draw up water and minerals from the ground through roots. Oxygen is released as a waste product. An unfortunate side effect of the stomata opening is that it allows for water loss (for review, see Farquhar et al. 1989). Pre-dawn water potential was measured when the water within the plant is at equilibrium as the stomata of plants remain closed during the night. According to review by Deloire et al. (2004), several levels of water stress were categorized by pre-dawn leaf water potential for common grape vine, including no water stress (0 MPa $\geq \Psi_{plwp} \geq$ -0.2 MPa), mild-to-moderate water deficit (-0.2 MPa $> \Psi_{plwp} \geq$ -0.4 MPa), moderate-to-severe water stress (-0.4 MPa $> \Psi_{plwp} \geq$ -0.6 MPa), and severe water stress (-0.6 MPa $> \Psi_{plwp}$). In my study, all progeny subjected to the drought treatment experienced moderate-to-severe water stress whereas the well-watered progeny were in a mild-to-moderate water deficit condition. In addition, a decrease in water availability resulted in a negative pre-dawn water potential in the drought trees, which

confirmed that the more negative the water potential, the higher the water stress experienced by the plants, as they were unable to reach equilibrium (Figure 4.2). A mild-to-moderate water deficit condition (-0.2 MPa > $\Psi_{plwp} \geq$ -0.4 MPa) was observed in the trees under the well-watered treatment. Several studies argue that water loss could occur at night (nighttime transpiration), which might affect the plant-soil water potential, even under well-watered conditions (Donovan et al. 2001; Dawson et al. 2007). In both the stems and leaves of woody plants, nighttime water flux occurs when leaf water potential declines over daytime transpiration and leads to a water potential gradient between leaf and soil after transpiration stops (Hinckley 1971). These findings from previous studies might be used to explain the current observations. However, the occurrence of nighttime transpiration cannot be assumed as incomplete nighttime stomatal closure is not found in all species (Dawson et al. 2017).

Specific leaf area (SLA), defined as the ratio of total leaf area to total leaf dry mass (Gunn et al. 1999), has been shown to be one of the leaf traits best reflecting whole plant growth (Cheng et al. 2016). SLA plays an important role in linking plant carbon and water cycles because it describes the distribution of leaf biomass relative to leaf area, and thus refers to carbon gain relative to water loss, within a plant canopy (Pierce et al. 1994; Liu et al. 2017). Our study showed the positive and significant correlation between SLA and net photosynthesis which may suggest that higher A increased SLA, and resulted in better whole plant growth.

Stomatal conductance estimates the rate of gas exchange (i.e., carbon dioxide uptake) and transpiration (i.e., water loss) through the leaf stomata as determined by the degree of stomatal aperture (for review, see Farquhar et al. 1989). More open stomata allow for greater conductance, and indicate that photosynthesis and transpiration rates are potentially higher. For the well-watered treatment, both the FG and SFG progeny showed higher A and g_s rates.

Stomatal closure as measured through g_s is one of the earliest responses to shoot or root dehydration; therefore, g_s was used as a measure of stomatal aperture regulation in leaves following Chaves et al. (2003). The plant requires the control of stomatal aperture to regulate the rates of photosynthesis/transpiration, which is the entry and exit of water and gases from a leaf (Araújo et al. 2011). Variable stomatal behaviour has been observed in intraspecific hybrids of poplars originating in different locations (Raj et al. 2011). The results of our study, which were obtained without measuring transcriptomics, showed that genetically identical FG progeny had varied stomatal behaviour under drought conditions (Figure 4.5a) in relation to their site of origin. This supports the findings of Raj et al. (2011).

Changes in water conditions can significantly affect plant growth and its biomass allocation (for review, see Chaves et al. 2003). Several other studies have also confirmed that plants overcompensate for growth upon being rewatered after a drought (Liu et al. 2002; Siopongco et al. 2006; Xu and Zhou 2007). Our results also showed that the biomass in plants subjected to severe drought could not reach the level of the control treatment trees, highlighting that whether plants fully recover following re-watering may depend on drought intensity and duration. Drought limitations to photosynthesis have been reported in many studies (Grassi and Magnani 2005; Xu et al. 2009; Sperlich et al. 2016), some of which examined photosynthetic responses to repeated drought cycles. The full recovery of A has been observed when drought stress was eliminated following re-watering in *Leymus chinensis* (Xu and Zhou 2007). In a study conducted by Fortunati et al. (2008), the *Populus nigra* L. leaf photosynthesis rate recovered completely to control levels after 15 days of re-watering. The plants were re-watered to field capacity after the leaf photosynthesis rate fell to almost zero at the lowest soil moisture fraction of available soil water (FASW), which was 25%. In this case, the FASW occurred 35 days after

starting the drought treatment. In our study, with only four days allowed for recovery (between each drought cycles) after re-watering, our results suggest that both net photosynthesis and stomatal conductance recovered only slightly for both the FG and SFG progeny originating from different locations after the re-watering. Had there been a longer recovery period (e.g. at least 15 days), the plants might have reached a state of full recovery in all gas exchange measurements, matching the level of the well-watered control trees.

The impact of clone history on epigenetic responses to stress can provide guidance for those who manage natural forests and tree plantations (Raj et al. 2011). Clonal individuals grown at different environmental locations can have different "histories" (Kemperman and Barnes, 1976; Mitton and Grant 1996; Raj et al. 2011) due to differences in abiotic and biotic factors such as water availability, soil conditions, and exposure to pests or pathogens. Consequently, individuals within these clonal groups express epigenetic responses to environmental stress including drought (Boyko and Kovalchuk 2008). Epigenetic regulation plays a very important role in modulating the expression of nuclear genes by switching on/off mechanisms in plant tissue to cope with environmental stresses (Lin et al. 2005). Epigenetic mechanisms including histone modifications and DNA methylation have been shown to play crucial roles in the drought stress response in poplars (Liang et al. 2014; Li et al. 2019). DNA methylation is defined as the process by which a methyl group is added and attached to the fifth carbon position of a cytosine ring in the DNA molecule. Plants exposed to different biotic and abiotic stresses have shown an increase in gene methylation (Steward et al. 2002). In contrast, lower methylation was found when plants were under favorable growth conditions and in an absence of stress (Labra et al. 2002). Epigenetics can also allow plants to adapt to environmental changes, which can be successfully transmitted to the progeny over long life cycles (Boyko and Kovalchuk 2008). Our

results have confirmed that the same progeny grown in different locations show different response under well-watered and drought conditions in a controlled, common environment, indicating that the drought response of a given poplar genotype can be regulated by epigenetics.

4.5 Conclusion

The response of plants to changing water conditions involves a number of factors, including molecular, genetic and physiological processes, from the individual to the population level. For long-lived organisms like forest trees, it is very important to understand the mechanisms necessary to adapt to a novel environment (Kvaalen and Johnsen 2008). Our findings support the hypothesis that drought response of a given hybrid balsam poplar genotype can be shaped by its site of origin. The same progeny originating in different locations showed different behaviour under well-watered and drought conditions in a controlled, greenhouse environment, exhibiting the phenomenon called epigenetics (Table 4.2). In an applied context, foresters should be aware that taking clones from existing plantation material or different nurseries means that they are drawing on stock that may exhibit greater phenotypic diversity than anticipated through genotype alone. In doing so, these clones may respond differently to existing conditions due to their origin histories.

Despite predicted warmer and drier conditions in the future (Overpeck and Udall 2020), the results of my study show that selections can be made to identify progeny that can maintain both photosynthetic capacity and growth under reduced water conditions. Additionally, growers are typically given the opportunity to select plants based on shoot height and diameter to meet nursery specifications (Thomas et al. 2016). To optimize out-planting success, growers should also consider root mass and R/S ratios (Landhäusser et al. 2012). From this current study, I found that FG progeny grown in Alberta are preferable because they showed an increase in growth and

an ability to withstand a major drought event—as part of our study—at the juvenile stage. Also, the AB progeny (mean annual precipitation of 30-year average (1982-2012) is 458mm) (Climate Edmonton 2020) were grown in a drier condition than the QC progeny (mean annual precipitation of 30-year average (1982-2012) is 890mm) (Climate Trécesson 2020), and appeared to be pre-conditioned to withstand the drought conditions in the greenhouse trial. This knowledge may help nursery managers pre-program plants to ensure that they are properly acclimated when designated for certain locations, sites and climates.

In conclusion, results from my study showed that FG progeny originating in sources in AB were more resistant to drought than the same genotypes originating from a wetter location. Cuttings of the same genotype grown in different locations showed different behaviour under controlled versus drought conditions. Therefore, epigenetics, at the phenotypic level of measurement, was successfully detected through growth and physiological assessments in my study (Table 4.2; Figures 4.2 and 4.5). Since the hybrid genotypes from this study were influenced by unique genetic backgrounds, genetic analysis including transcript abundance analysis and global methylation analysis should be added in future studies to provide evidence of the divergence in transcriptome-level and DNA methylation information about epigenetic responses (Eichten et al. 2013).

Table 4.1 Selected FG and SFG balsam poplar progeny for the 2018 greenhouse drought experiment based on the 2016 greenhouse experiment (refer to selection process (3.3.1) in Chapter 3 and Table 1A in Appendix A)

Family	Fast-growing progeny	Group ID (Fast-growing progeny in fast -growing family)	Slow-growing progeny	Group ID (slow growing progeny in fast growing family)
AP5402	180071	FG	180093	SFG
	180103	FG	180063	SFG
	180081	FG	180094	SFG

Table 4.2 Mean height and diameter (±SE) (N=127) at the end of the experiment (Day 62) for each group (FG and SFG), location (AB and QC), and watering treatments (W and D) in balsam poplar. Note: SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny; D =drought; W =well-watered; AB=Alberta; QC=Quebec. Significant differences between group means are indicated by different letters.

	Treatment							
	W				D			
	FG-AB	FG-QC	SFG-AB	SFG-QC	FG-AB	FG-QC	SFG-AB	SFG-QC
Height (cm)	50.2± 2.0^{a}	44.1± 2.5^{bc}	46.5± 1.3^{ab}	40.2± 1.2^{cd}	32.2± 1.5^{ef}	35.9± 2.5^{de}	30.0± 1.3^{fg}	25.9± 0.9^{g}
Diameter (mm)	5.4± 0.1^{a}	4.5± 0.2^{c}	5.0± 0.1^{b}	4.6± 0.1^{bc}	4.09±0 .11^{d}	3.9± 0.1^{d}	3.8± 0.1^{d}	3.0± 0.1^{e}

Table 4.3 Pearson's correlation (r) among physiological variables for all six balsam poplar genotypes (N=127) at the end of the experiment (Day 62) (those that are significant are in bold ($p<0.05$)).

	Diameter	A	g_s	iWUE	TDM	RDM	SLA
Height	**0.916**	**0.561**	**0.642**	**0.658**	**0.892**	**0.896**	**0.390**
Diameter	1	**0.555**	**0.623**	**0.651**	**0.790**	**0.811**	**0.438**
A		1	**0.841**	**0.740**	**0.534**	**0.561**	**0.364**
g_s			1	**-0.709**	**0.627**	**0.672**	**0.406**
iWUE				1	0.251	0.294	**0.286**
TDM					1	**0.973**	**0.367**
RDM						1	**0.335**
SLA							1

Note: Height (cm); Diameter (mm); A, photosynthesis rate (µmol CO_2 m^{-2} s^{-1}); g_s, stomatal conductance (mol H_2O m^{-2} s^{-1}); iWUE, intrinsic water-use efficiency (µmol CO_2 mol^{-1} H_2O); TDM, total dry mass (g); RDM, root dry mass (g); SLA, specific leaf area (cm^2 g^{-1}).

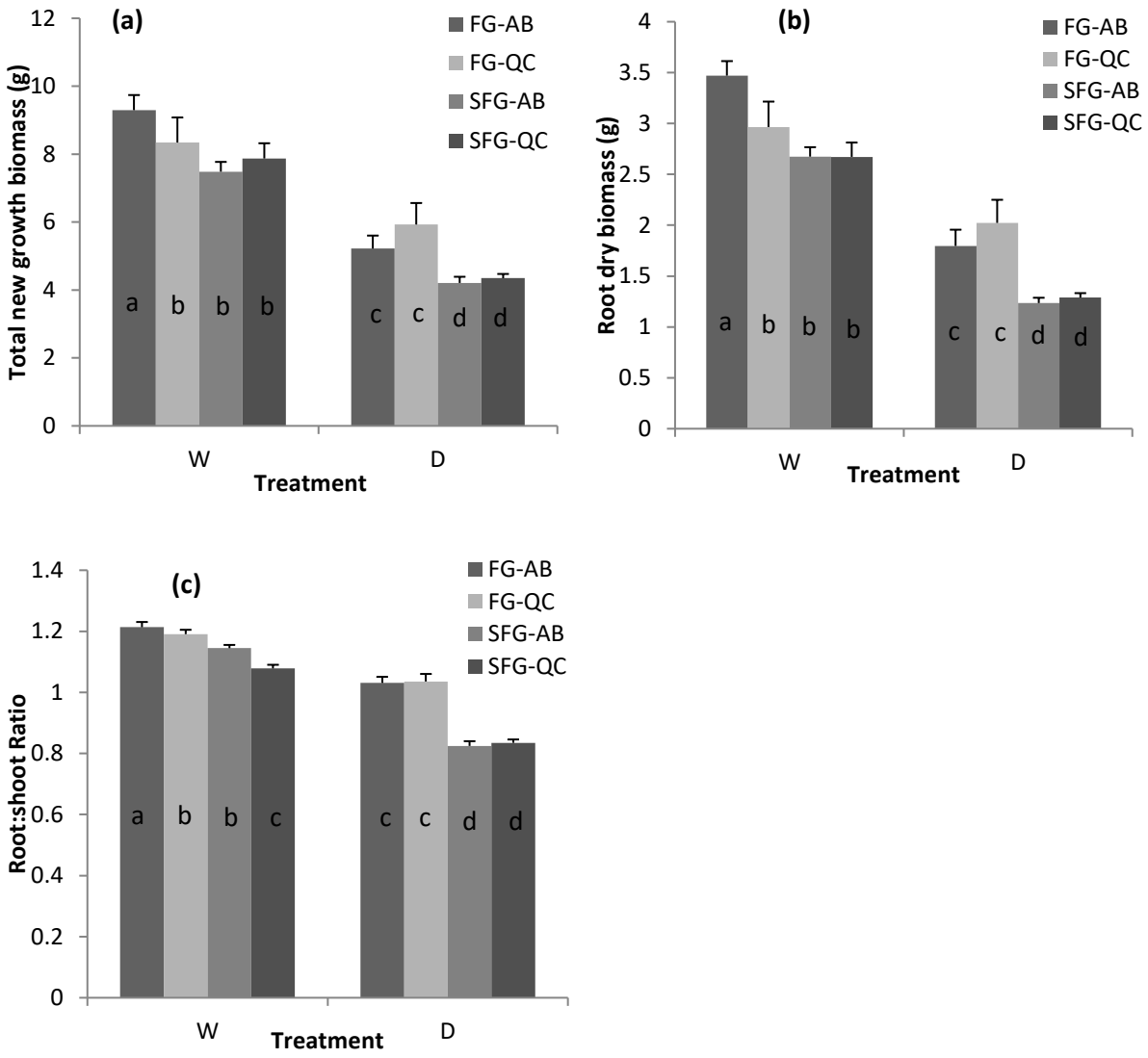

Figure 4.1. Mean total new growth biomass (+SE) (a), root dry biomass (+SE) (b) and root: shoot ratio (R/S) (+SE) (c), for each group (FG and SFG), location or cutting origin (AB and QC) and water treatment (W and D) in balsam poplar (N=127) at the end of the experiment (Day 62). Note: SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny; D =drought; W =well-watered; AB=Alberta; QC=Quebec. New growth biomass= Final biomass – Initial biomass. Significant differences between group means are indicated by different letters at $p \leq 0.05$.

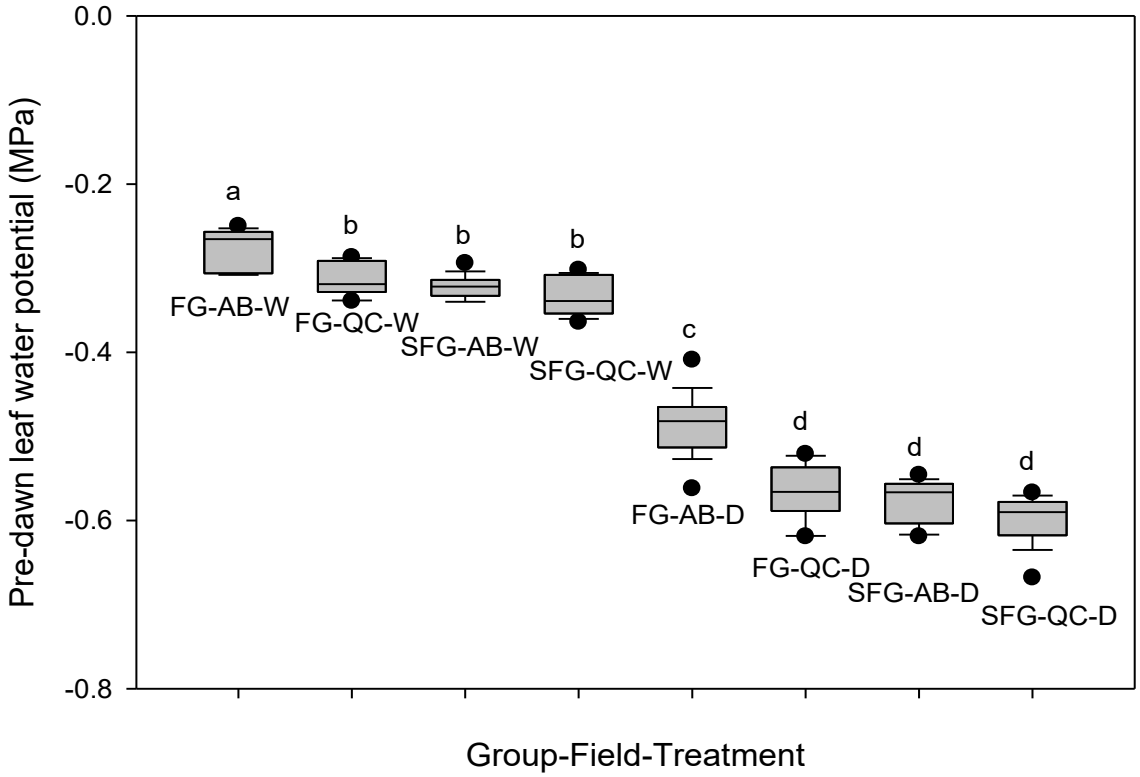

Figure 4.2. Mean pre-dawn leaf water potential (MPa) for each group (FG and SFG), location (AB and QC) and water treatment (W and D) in balsam poplar (N=127) at the end of the experiment (Day 62). Note: SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny; D =drought; W =well-watered; AB=Alberta; QC=Quebec. Significant differences between group means are indicated by different letters at $p \leq 0.05$.

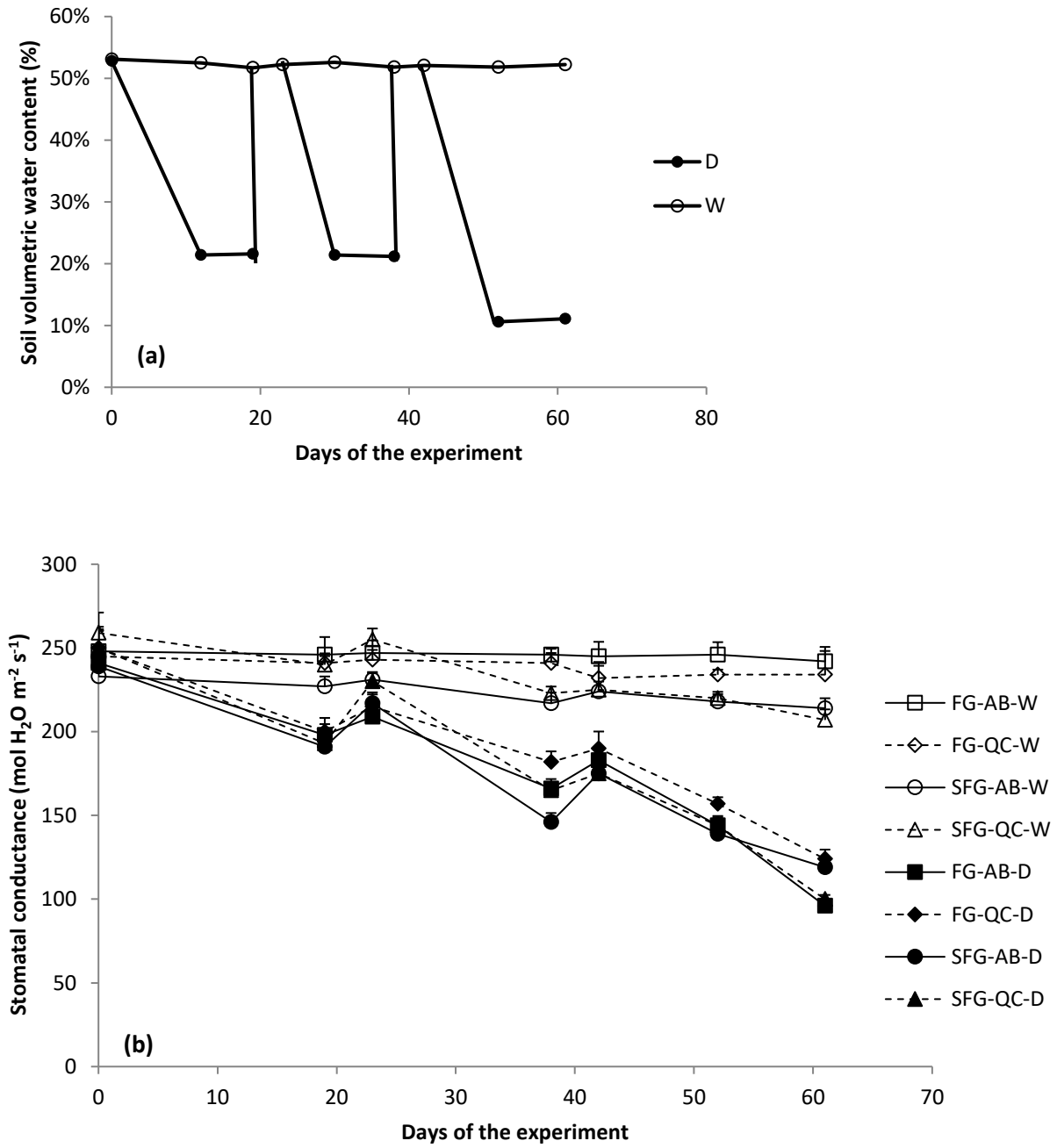

Figure 4.3. Evolution of (a) soil volumetric water content (SVWC) (%) (measured at days 0, 12, 19, 23, 30, 38, 42, 52, and 61 of the experiment) (N=127) and (b) mean stomatal conductance (g_s) (+SE) (measured at days 0, 19, 23, 38, 42, 52, and 61 of the experiment) in balsam poplar along with the moisture schedule for all balsam poplar progeny. Note: Day 0 - start of the experiment; Day 12 - drought level reached at 1st cycle; Day 19 - complete the first drought cycle, re-watered; Day 30 - drought level reached at 2nd cycle; Day 38 - complete the 2nd drought cycle, re-watered; Day 52 - drought level reached at 3rd cycle; Day 61 - end of the experiment. Note: SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny; D =drought; W =well-watered; AB=Alberta; QC=Quebec.

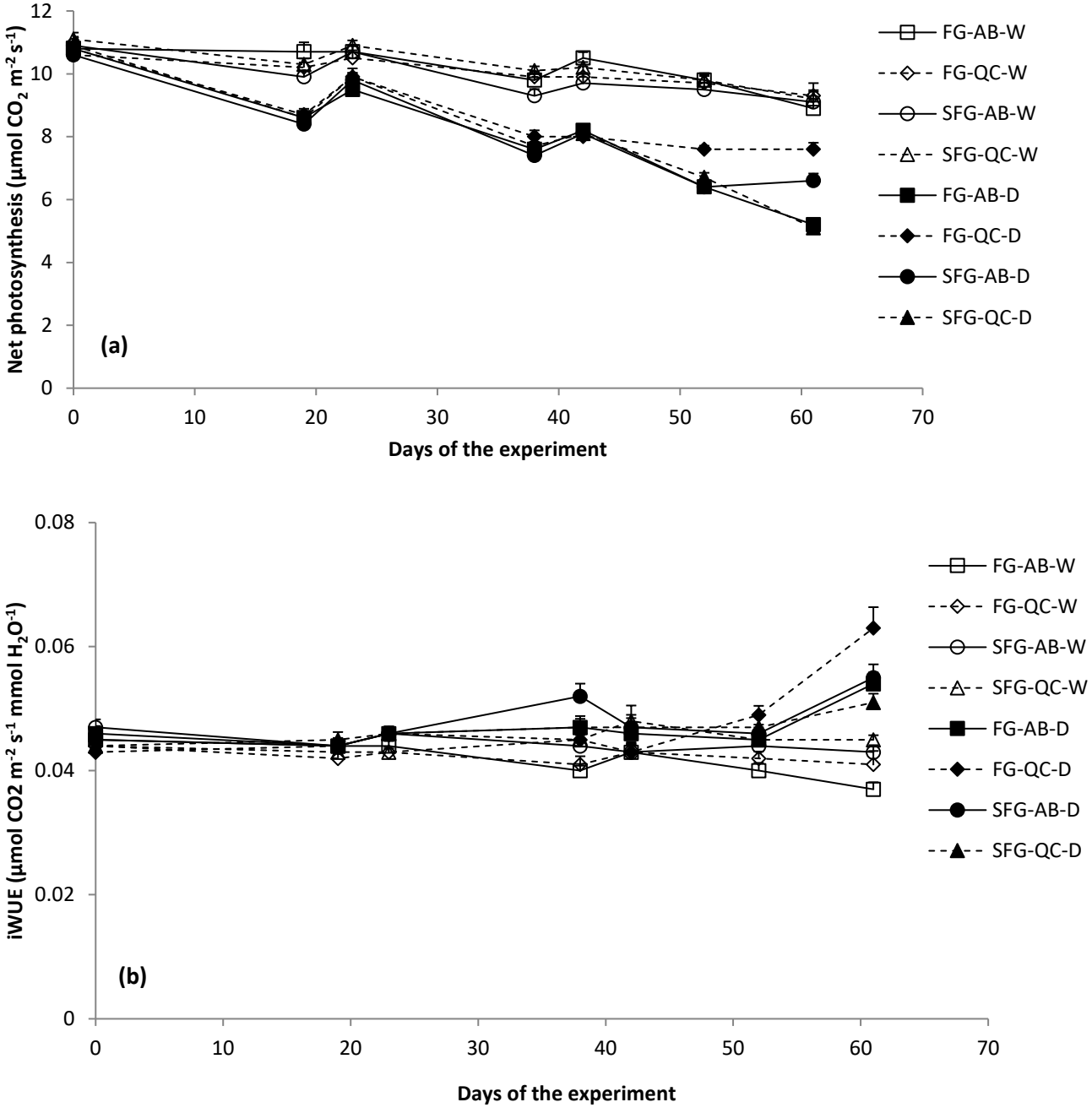

Figure 4.4. Evolution of (a) mean net photosynthesis rate (A) (+SE) (measured at days 0, 19, 23, 38, 42, 52, and 61 of the experiment) and (b) mean instrinc water-use efficiency (iWUE) (+SE) (measured at days 0, 19, 23, 38, 42, 52, and 61 of the experiment) in balsam poplar along with the moisture schedule. Note: Day 0 - start of the experiment; Day 12 - drought level reached at 1st cycle; Day 19 - complete the first drought cycle, re-watered; Day 30 - drought level reached at 2nd cycle; Day 38 - complete the 2nd drought cycle, re-watered; Day 52 - drought level reached at 3rd cycle; Day 61 - end of the experiment. Note: SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny; D =drought; W =well-watered; AB=Alberta; QC=Quebec.

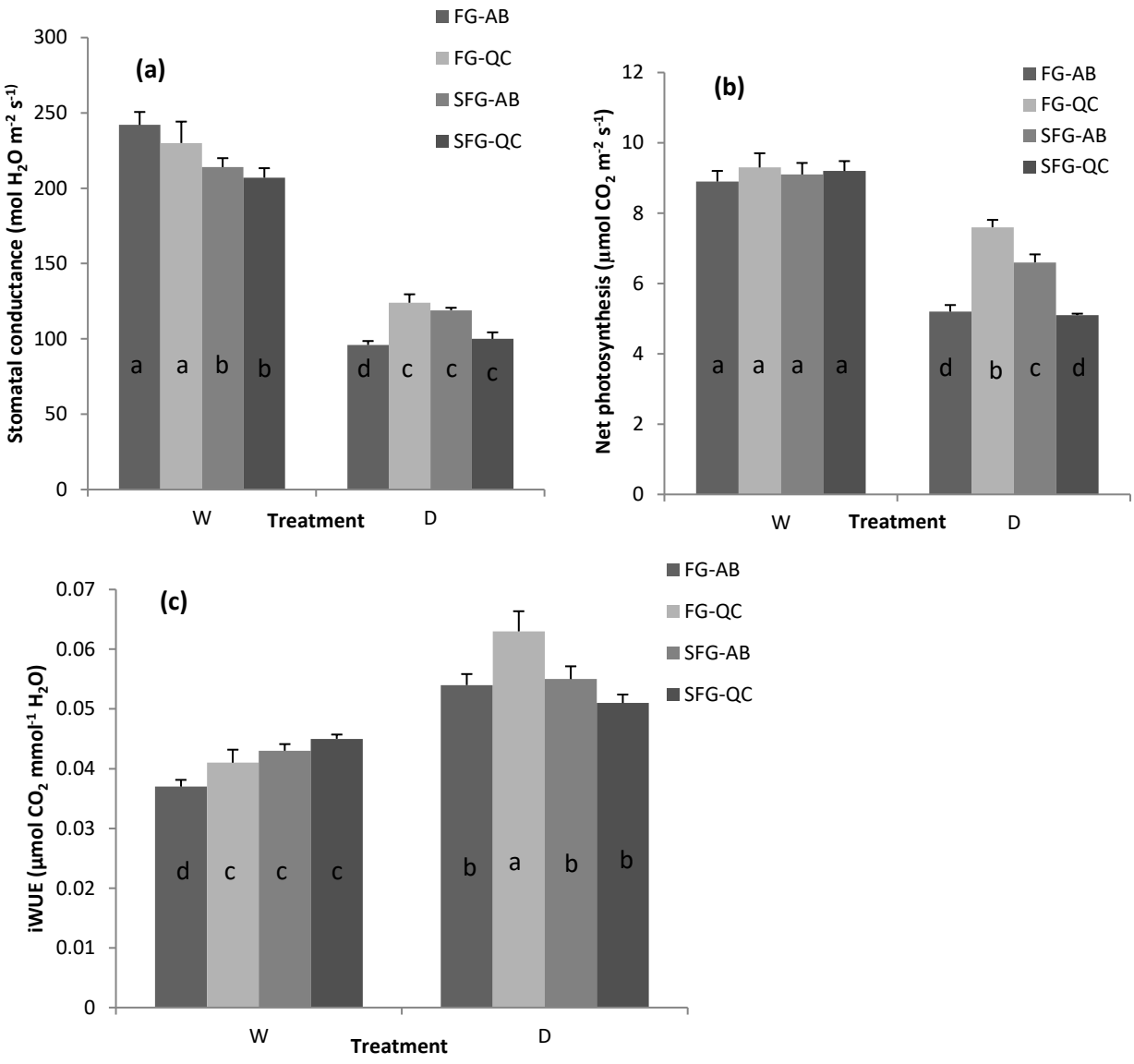

Figure 4.5. Mean stomatal conductance (g_s) (+SE) (a), net photosynthesis (A) (+SE) (b) and intrinsic water-use efficiency (iWUE) (+SE) (c) for each group (FG and SFG), location (AB and QC) and water treatment (W and D) in balsam poplar (N=127) at the end of the experiment (Day 62). Note: SFG = slow-growing progeny from a fast-growing family; and FG = fast-growing progeny; D =drought; W =well-watered; AB=Alberta; QC=Quebec. Significant differences between group means are indicated by different letters at $p \leq 0.05$.

Chapter 5. General Discussion and Conclusion

5.1 Summary and Synthesis

The main goal of my thesis was to increase our knowledge about within-species breeding of balsam poplar to determine if it would lead to the expression of hybrid vigour and, if so, to explore potential underlying mechanisms through field and greenhouse assessments. To this end, I tested the hypothesis that "hybrid vigour" could be obtained via crossing parents located across the range of the species (Alberta and Quebec), from disparate populations. I explored potential causal mechanisms through phenotypic, physiological, and hormonal analysis. All field data presented in this thesis was collected from two Alberta (AB) test sites (Field AB1 and Field AB2) located at the Alberta-Pacific mill site near Athabasca, and a single test site in Quebec (QC) (Field QC1) located at Trécesson. The greenhouse experiments were all conducted in the Bioscience greenhouse at the University of Alberta.

In the following paragraphs, I will summarize and synthesize the main findings of this dissertation, suggest management implications, briefly describe the limitations of the present work, and make some recommendations for further research.

In my first research chapter (Chapter 2), I examined if the length of growing season is linked to increased performance. I did this by measuring growth (height and DBH) and phenology (bud burst and bud set) at both the Field AB1 and AB2 test sites in 2017 and 2018. The hypotheses tested were:

a) Intraspecific hybridization of balsam poplar leads to the express of hybrid vigour; and
b) Increased growth of intraspecific hybrid balsam poplar is linked to a longer growing season.

Our results showed that progeny from the AB × QC parental cross-type exhibited an earlier bud burst and later bud set than the other cross-type progeny, resulting in a longer growing season which allowed the progeny to have greater growth (Figure 2.2 and Figure 2.3), which is consistent with previous work on poplar bud phenology (Yu et al. 2001). In our study, least squares means was used to directly compare the differences between intra- and local- parental cross-types for all growth traits and bud phenology and estimate the difference in the phenotypic means.

My second research chapter (Chapter 3) focused on testing the physiological basis of heterosis in intraspecific hybridization. We determined the role of endogenous hormones linked to ecophysiological and growth performance. The hypothesis for my second research chapter was to study:

a) There was a causal relationship between hormone concentration in the elongating stem (internodes) tissue and the growth rate of the vegetatively propagated progeny from selected families of hybrid balsam poplar; and
b) Hybrid vigour is due to endogenous hormones linked to ecophysiological and growth performance.

The results presented in Chapter 3 confirmed the widespread occurrence of heterosis typically found in interspecific crosses with disparate population breeding of intraspecific hybrids in balsam poplar (Hu and Thomas 2019). The combined analyses of endogenous gibberellic acid (GA) content and growth data revealed that GAs play a regulatory role in heterosis in hybrid balsam poplar at the physiological level. The results obtained from selected progenies were validated by retrospective comparisons with the stem growth performance of the corresponding field-grown trees at eight years of age (Hu and Thomas 2019).

My first two data chapters showed within-species breeding of balsam poplar can lead to the expression of hybrid vigour for growth. Previous research has suggested that genetically identical poplar clones performed differently under drought conditions in relationship to their site of origin (Raj et al. 2011). Trees are particularly sensitive to climate change as they are relatively long-lived compared to other organisms and have limited adaptive capacity to respond to rapid environmental change (Lindner et al. 2010). Due to rapid climate change, where precipitation patterns on a regional scale may become uncertain, it is crucial to have trees tested and selected under these conditions, allowing them to make physiological adjustments and improve their ability to grow in novel environmental conditions (e.g. drought) (Pascual et al. 2014). Since we had three test sites (AB1, AB2 and QC1) with the same clonal progeny planted, it provided a great opportunity to examine phenotypic epigenetic responses to drought stress by using cuttings from the same progeny from selected intraspecific hybrids grown under different environmental conditions at these different test site locations. The same clones were collected from two different locations, Field AB1 and Field QC1, and grown under common, controlled environmental conditions. The drought responses of the same progeny originating in different locations were assessed and the following hypotheses tested:

a) Fast-growing group (FG) are more resistant to drought than slow-growing group (SG) progeny; and
b) Cuttings of the same genotype grown in different locations show the same behaviour under controlled *versus* drought conditions in a common greenhouse trial.

The findings of this study were presented in my third data chapter (Chapter 4). The response of plants to changing water conditions involved many aspects, from the molecular genetic, biochemical and physiological process levels to the whole individual and community

levels (Kvaalen and Johnsen 2008). Mechanisms that lead to adaptation to a changing environment are of key importance for plants, and especially for long-lived species such as forest trees (Kvaalen and Johnsen 2008). Our results addressed the hypothesis that cuttings of the same genotype grown in different locations showed the same behaviour under controlled versus drought conditions in a common garden greenhouse trial and found that cuttings of the same genotype grown in different locations actually showed different behaviour under controlled *versus* drought conditions. Our results also showed that drought responses of a given hybrid balsam poplar genotype can be shaped by the history of that clone, indicating existence of epigenetics. Our results also suggested that the fast-growing progeny originating in AB should be preferred as they showed an increase in their growth ability while withstanding a severe drought event at the juvenile stage.

The main limitation for Chapter 4 was a lack of molecular genetic analysis including transcript abundance analysis and global methylation analysis. These types of results could have provided evidence of the divergence in transcriptome-level and DNA methylation information about epigenetics (Eichten et al. 2013). The genetic analysis at the molecular level should be added in future studies to confirm the divergence in transcriptome-level and DNA methylation information to explain the phenomenon that we showed based on our results.

5.2 Management implications

Poplar is considered a good choice for a plantation tree owing to its fast growth, ease of propagation, and high genetic variation in traits of interest (Stettler et al. 1996). To our knowledge, this is the first study that provides evidence of hybrid vigour in young, intraspecific hybrid balsam poplar.

The results presented in this research confirmed that the widespread occurrence of heterosis typically found in interspecific crosses (Drew and Chapman 1992; Li and Wu 1996; Li and Wu 1997) can also be achieved with disparate population breeding of intra-specific hybrids in balsam poplar. To date, there have been few attempts to examine whether hybrid vigour can be achieved by crossing disparate populations of the same species except for crop plant species such as maize (for review, see Birchler et al. 2010). In our study, we found that disparate and native populations of balsam poplar can be bred to produce superior progeny with enhanced stem growth traits (Chapter 3). Phenological differences (earlier bud burst and often a later bud set) might also contribute to the superior growth of progeny from hybrid cross-type AB × QC, when compared with progeny from the local cross-types (AB × AB or QC × QC) (Chapter 2). In other words, the progeny of intra-specific crosses between AB and QC populations may accomplish more growth than local crosses due to a longer growth period (Chapter 2) and higher endogenous hormone content (Chapter 3) which contributed to heterosis in growth. However, there were no significant differences between the QC × AB cross-type compared with the local crosses and other intra-regional crosses for growth and bud phenology, which may indicate that the maternal effect played a role in the growth of the F_1 hybrids, with offspring from AB mothers exhibiting higher fitness than those from QC mothers (Kirk et al. 2005; Bräutigam et al. 2017). This result was consistent with that of Yu et al. (2001), who concluded that parental origin played a significant role in determining growth patterns, with the more northern parent having stronger genetic control over growth cessation. Northern populations have been shown to adjust to a temperature-controlled delay in bud burst in spring and a period to promote photoperiod-sensitive bud set to avoid frost damage (Way and Montgomery 2015). In research conducted by Luquez et al. (2008), in a reciprocal transplant experiment, by using European aspen (*Populus tremula*) in Sweden, they found genotypes from southern areas consistently performed better

than northern genotypes in terms of height and diameter in both northern and southern common gardens due to the population-level differences in growth potential and genetic variation within the tested populations. This may explain why the AB × QC cross-type showed better performance than the corresponding QC × AB families. Li and Wu (1996, 1997) also determined that it was the female aspen parent that primarily influenced growth patterns (i.e. phenology) in the progeny (*P. tremuloides* × *P. tremula*) at the juvenile growth stage and the female parent was from 10 degrees latitude further north than the male parent.

The results obtained from Chapter 3 indicate that measurements of young balsam poplar progeny (Day 34 after transplanting) grown under near-optimal conditions can be predictive of inherently rapid stem growth at the clonal level, at older ages (age eight). These findings can be used to help breeders identify inherently fast-growing progeny of balsam poplar at an early nursery stage. Additionally, differences in stem volume at an earlier stage can be achieved by using the selected progeny according to our results and significantly reduce the rotation age of local plantations. The relationship between GAs and growth performance (both field and greenhouse) may open up new ways to manipulate trees to grow faster and produce more biomass by increasing endogenous GA levels (Eriksson et al. 2000).

Chapter 4 supported the hypothesis that drought response of a given hybrid balsam poplar genotype can be shaped by the history of that clone. In an applied context, foresters should be aware that sourcing plantation material from different nurseries, even when the genetic identity is known, means that they are drawing on stock that may exhibit greater phenotypic diversity than would be suggested by genotype alone, and which may respond differently to prevailing conditions. In nursery operations, growers typically use shoot height and shoot diameter as the main selection criteria (Thomas et al. 2016). Based on our study, the fast-growing progeny

should be preferred because they showed an increase in growing ability while withstanding a severe drought event at the juvenile stage. Epigenetics generally means that there has been a change in phenotype without a change in genotype. It is the study of heritable changes in gene expression that does not involve changes to the DNA sequence (for review, see Waterland 2006). Some epigenetic responses enable plants to acclimate to environmental fluctuations which can be successfully transmitted to the progeny over long life cycles, while other responses are non-heritable changes (for review, see Chinnusamy and Zhu 2009). This knowledge may help nursery managers to understand that some changes from epigenetic regulation can be carried from parent to offspring, and clone to clone, which makes it possible to select the best plants for specific conditions.

Overall, the above approaches determined the potential of using disparate, native populations of balsam poplar to produce superior progeny with enhanced stem growth traits. Future use of this material on crown land for reforestation or reclamation may require additional field testing to meet policy regulations.

5.3 Recommendations for Further Research

The main limitation for this study is the absence of data on the parents used to produce the crosses and subsequence crosses and cross-types that were examined. Heterosis is normally measured based on relative performance of the F_1 offspring as compared to their parents. For my study, the heterosis was evaluated by comparing the growth performance of progeny produced from inter- (AB × QC and QC × AB) and intra- (AB × AB and QC × QC) regional crosses which may not have had the same performance as the specific parents. In Chapter 2, I explained that the underlying causes of the superiority of hybrids are still unclear due to the absence of parental information. Despite considerable effort to locate the original parents in the field, it was not

possible. In the future, parental material used for breeding should be collected and propagated in a clone bank. Future work can then include the original parents' information for a better estimation and evaluation of hybrid vigour. In Chapter 3, even though the combined analyses of endogenous GA content and growth data revealed that GAs play a regulatory role in heterosis for hybrid balsam poplar at the physiological level, larger scale investigations of multiple plant hormones at more developmental stages will extend these findings. The important role of GA 20-oxidase expression to control both stem length and radial stem growth in transgenic hybrid aspen has been observed in past studies (Eriksson et al. 2000, Han et al. 2011). Therefore, screening for high levels of expression of *GA20ox* genes may be a useful technique for future tree-breeding programs to select fast-growing trees at an early stage. It will also create a way to genetically engineer trees to grow faster and produce more biomass simply by increasing endogenous GA levels. To establish homeostasis, future RT-PCR work should be used to test *GA20oxs*, *GA3oxs*, and *GA2oxs,* which are the main targets of regulation by GA signaling. The response of plants to changing water conditions involves the processes from the molecular genetic level, biochemical level, and physiological level. Boyko and Kovalchuk (2008) found that methylation level contributed greatly to a plant's ability to respond to stress. Therefore, genetic analysis including transcript abundance analysis and global methylation analysis should be added in future studies to provide evidence of the divergence in transcriptome-level and DNA methylation information underpinning the potential causes of epigenetics.

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Appendix A. Supplementary Information

Table A1. Family codes, and selected progeny for each group type, fast growing (FG), slow growing (SG) and slow-fast growing (SFG) based on six-year-old growth from two field sites in Alberta (AB1 and AB2) for hormone analysis. The selection of progeny for hormone analysis was based on the field representations and budget for the project.

Family	Fast-growing Progeny	Group (Fast-growing (FG))	Slow-growing Progeny	Group (Slow-growing in Fast growing Group (SFG) or Slow-growing (SG) Group)
AP5401			178092	SG
			178073	SG
			178104	SG
AP5402	180071	FG	180093	SFG
	180103	FG	180063	SFG
	180081	FG	180094	SFG
AP5416	272084	FG	272024	SFG
	272102	FG	272071	SFG
	272091	FG	272023	SFG

Table A2. Ranking based on mean stem volume (cm^3) at two Alberta sites (Field AB1 and AB2) for selected fast growing family (AP5396) in balsam poplar at Year-6 (2015)

Progeny	Ranking in Field AB1	Ranking in Field AB2	Mean Stem Volume (cm^3)
147023	33	33	399.12
147044	29	37	410.19
147032	32	31	461.57
147102	27	36	551.05
147024	31	28	568.69
147053	34	23	596.89
147042	28	32	603.05
147012	26	34	736.69
147092	21	38	760.32
147011	24	35	793.92
147021	30	20	887.28
147043	23	26	1041.96
147051	25	24	1059.36
147041	17	29	1113.92
147103	11	27	1369.16
147062	14	21	1510.25
147061	10	22	1624.38
147052	22	13	1670.44
147073	18	14	1774.65
147084	6	25	1901.64
147101	8	18	2009.89
147013	19	11	2104.87
147063	16	6	2554.15
147074	13	8	2560.01
147093	7	12	2641.37
147031	20	4	2678.51
147082	4	17	2703.61
147094	9	9	2750.96
147091	12	5	2835.08
147054	15	3	3040.87
147071	3	10	3284.93
147083	2	7	3547.93
147072	1	1	5467.47

Figure A1. Pictures that showed the original breeding process in Quebec in 2004. Images courtesy of Mr. Pierre Périnet. a) seeds from balsam poplar crosses were sown onto the surface of containers; b) seeds germinated in the DRF greenhouse c) cuttings being placed outside in summer 2004; d) containers with seedlings were at Duchesnay Centre, QC.

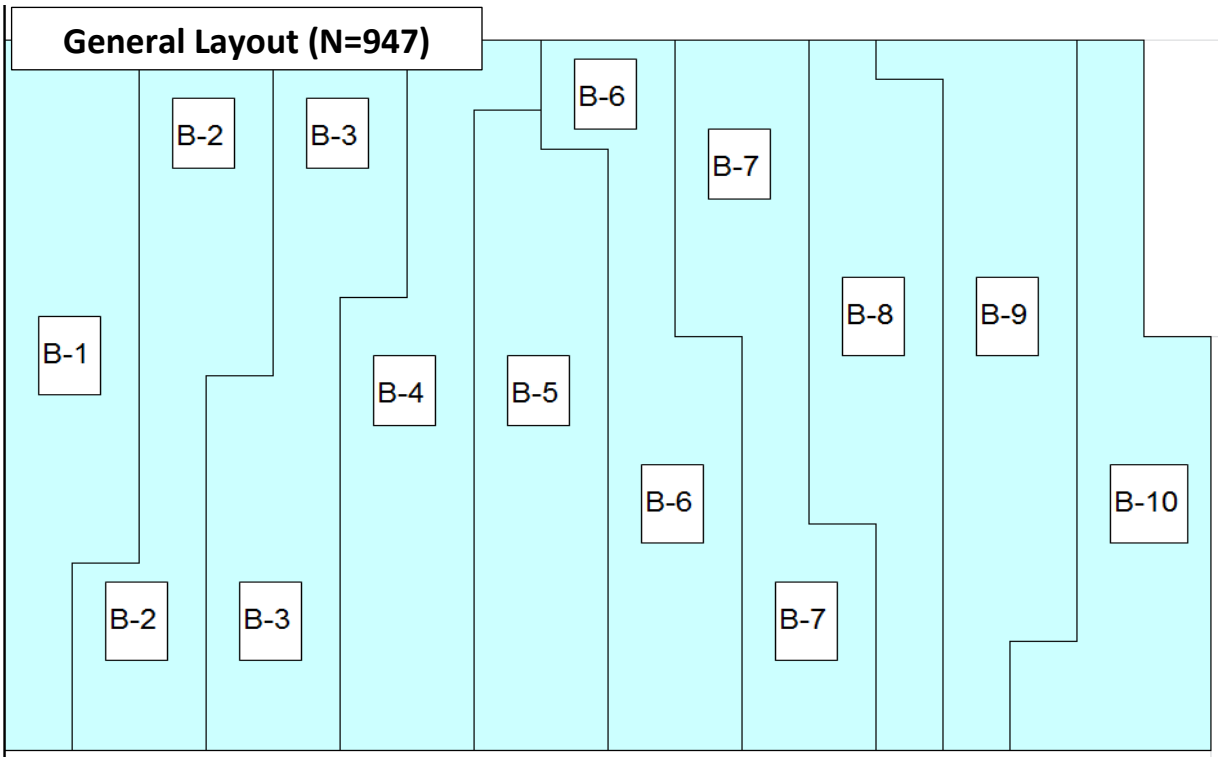

Figure A2. Details of the field layout for all sites (Field AB1, Field AB2 and Field QC). The general layout (or map) is 36 m wide by the length of all the blocks put together. There are 10 blocks representing 144 m long. B stands for Block. N stands for total number of trees that planted in the field.