University of Alberta

Adaptation of trembling aspen and hybrid poplars to frost and drought: implications for selection and movement of planting stock in western Canada

by

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Abstract

This study contains a series of experiments to evaluate growth performance and survival of hybrid poplars (*Populus* spp.) and trembling aspen (*Populus tremuloides* Michx.) in boreal planting environments in western Canada. Ecophysiological traits related to drought resistance and winter survival were studied and compared with growth in long-term field trials, within and between these two plant groups. The results showed that trembling aspen is more resistant to drought stress and more water-use efficient than hybrid poplars, suggesting that these two groups employ different water-use strategies. Tree height was negatively correlated with branch vessel diameter in both plant groups and was highly conserved in aspen trees from different geographic origins. Hybrid poplars with wider xylem vessel were also more prone to freezing-induced embolism, suggesting that smaller vessel diameters may be an essential adaptive trait to ensure frost tolerance and long-term productivity of hybrid poplar plantations in boreal planting environments. For aspen, provenances ranging from northeast British Columbia to Minnesota were tested in a series of reciprocal transplant experiments across western Canada. The analysis found pronounced increases in productivity as a result of long-distance transfers in northwest direction. Commonly reported trade-offs between freezing tolerance and growth rate were not found in this study. Seed transferred from Minnesota to northeast British Columbia (2,300 km northeast and 11° latitude north), still outperformed local sources by 17 % in height had more than twice the biomass at age ten. Increased productivity as a result of northwest transfers was not associated with reduced survival. The results suggest that the potential benefits of northward movement of aspen populations in forestry operations outweigh the potential risks, especially in the context of climate change.

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List of Symbols and Abbreviations

ABf Alberta foothills. 89, 98

$'$ arcminute. 18, 52

a.s.l. above see level. 52

BC British Columbia. 89, 93, 98

cAB central Alberta. 89, 93, 95, 98

$CaCl_2$ calcium chloride. 55

$^{\circ}C$ degree Celsius. 2, 5, 6, 16, 19, 20, 22, 28, 50, 53, 54, 56, 57, 61, 90, 92, 93, 95–98

L cell lysis. 54, 55, 57, 61, 93, 95

cm^3 cubic centimeter. 22

cm centimeter. 20, 22, 54, 57, 92

CO_2 carbon dioxide. 3, 49

DBH diameter at breast height. 18–20, 24, 25, 27, 52

° degree. 52, 87, 88, 93, 94, 98, 119

df degrees of freedom. 56

DL daylength. 91

DoY day of year. 91

g gram. 22

KCl potassium chloride. 55

kg kilogram. 90

K_h hydraulic conductivity. 17, 20, 21, 54, 55

K_L leaf-specific hydraulic conductivity. 21, 23

km^2 square kilometer. 1

km kilometer. 93, 94, 119

kPa kilopascal. 55

K_S xylem-specific hydraulic conductivity. 21

LAT latitude. 91

$\delta^{13}C$ leaf carbon isotope composition. 20, 23, 24, 26, 29, 117

ψ*-Leaf* leaf water potential. 20, 21, 23–26, 117

A_L:A_S leaf to sapwood area ratio. 23

mM millimolar. 55

min minute. 54, 55, 93

ml milliliter. 54, 92

mm millimeter. 2, 28, 54, 55, 92

MN Minnesota. 89, 93, 98

MPa megapascal. 23, 24, 26, 60

Mt megatonnes. 87

μm micrometer. 22, 60

m meter. 19, 52, 53, 56

nAB northern Alberta. 89, 98

OSB oriented strand board. 87

P_{50} pressure causing 50 % loss of hydraulic conductivity. 21, 23, 24, 26, 117

P_e air entry pressure. 21, 23–25

PLC_N percent loss hydraulic conductivity / Native embolism. 23, 54, 55, 59, 60, 118

P' vapour pressure of water. 3

P_x xylem pressure. 3, 5

P probability. 27, 52, 56, 59, 93, 95

R^2 coefficient of determination. 95

REL relative amount of electrolyte leakage. 54, 93

r Pearson product-moment correlation coefficient. 27, 52, 56, 59

SK Saskatchewan. 89, 98

TAV daily average temperature. 53

TMAX daily maximum temperature. 53

TMIN daily minimum temperature. 53

D_V vessel diameter. 15, 22, 24–27, 29, 55, 117, 118

d_W wood density. 24, 26, 27

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CHAPTER 1

General Introduction

Contents

1.1 Background

Canada is the second largest country in the world with 9,984,670 km^2. Of this, 9,093,507 km^2 is land and 891,163 km^2 is fresh water (`http://www.nrcan.gc.ca`). Canada's predominant ecosystem is the boreal forest, representing 10 % of the world's forest cover and 30 % of the boreal forest worldwide (Canadian Forest Service, 2011). Given these vast amounts of forest, the forest industry plays an important role for the people and the wealth of Canada. In 2009 the forest industry sector yielded revenue of over 50.8 billion dollars (Canadian Forest Service, 2011). However, current and predicted climate change scenarios pose a threat to the vegetation in the prairie provinces of western Canada. In central Alberta for instance, it became 25 % drier during the last two decades (Mbogga *et al.*, 2009). Massive diebacks have already been recorded and efforts are being made to understand the physiological causes that kill trees (Hogg & Bernier, 2005, Hogg *et al.*, 2008, McDowell *et al.*, 2008, Allen *et al.*, 2010, Anderegg *et al.*, 2011, Plaut *et al.*, 2012).

Although drought stress is one of the major abiotic factors influencing growth and survival in western Canada, freezing injury, due to extreme minimum temperatures, as well as late spring frosts, can also severely harm trees (cf. Sakai & Larcher, 1987). However, in large scale common garden experiments it has also been noted that some genotypes grow surprisingly well whilst others perform rather poorly. This is a very interesting observation and builds the foundation for the present work since it allows one to study phenotypic variability in a controlled experimental design and may help to identify adaptive traits related to drought and winter survival.

Identifying physiological characteristics of what makes some trees survive, and even thrive, in boreal environments and kills others builds the framework of this PhD thesis.

1.2 The Role of Poplars for the Prairie Provinces

Besides conifers, poplars (*Populus* spec.) play an important ecological and economical role in western Canada (Peterson & Peterson, 1992, Richardson *et al.*, 2007). *Populus balsamifera* and *Populus tremuloides* hold a vital position in the boreal mixedwood forest and the parklands of the prairie provinces Alberta, Saskatchewan and Manitoba. The climate is relatively dry (mean annual precipitation 400 to 500 *mm*) with long and cold winters (mean January temperature -10 to -24 °C), and short mild summers (mean July temperature 15 to 20 °C). The prairie provinces are characterized by two major biomes: the boreal forest in the north and the prairies in the south. The aspen parklands, a transitional biome (or ecotone) lies in between.

The genus *Populus* is tightly linked to human history. In fact, the name may translate from Latin to *people*, acknowledging its close relationship to humans (Dickmann, 2001). Poplars are deciduous trees and widespread across the northern hemisphere. Poplars are pioneer species and very adaptable to various environmental conditions. Poplars exhibiting superior growth characteristics, are easy to propagate sexually as well as asexually from branch or root cuttings, and share the ability to hybridize (Peterson & Peterson, 1992, Cooke & Rood, 2007). All these features highlight why poplars represent a very attractive renewable forest resource for various wood products such as oriented strand boards, paper and pulp, and biofuels. Poplars are also known for their CO_2 sequestration potential, which led to the Canadian federal *Forest 2020* afforestation initiative to help meet greenhouse gas reduction targets (Dominy *et al.*, 2010).

Hybrid poplar and aspen plantations are established throughout western Canada, either operational or as research plots to study questions related to ecology, physiology, genetics, and forest management. For the most part these plots are neither irrigated nor fertilized making them excellent study objects.

1.3 Tree-Water Relations and the Problem of Cavitation

Water is the most abundant molecule on earth and vital for all forms of life we know. Plants use water in biochemical reactions and other processes, but most of the water taken up by roots is transpired to the atmosphere (Jackson *et al.*, 2000). According to the *Cohesion-Tension theory* water ascends plants in a metastable state under negative hydrostatic pressure through a water conducting tissue called xylem (Tyree & Zimmermann, 2002). Water in the xylem is under metastable conditions when xylem pressures (P_x) are below the vapour pressure of water (P') and once broken will not rejoin until P_x rises to values above that of P' (Tyree, 1997).

The water movement is initiated at the plant-atmosphere interface located in the stomata. It is generated by surface tension due to the evaporation of water from the mesophyll cells. The resulting negative hydrostatic pressure is transmitted via a continuous water column to the roots and water uptake occurs when the water potential of the roots is lower than the water potential of the soil (Tyree, 1997).

Xylem is an interconnected network of water conducting pipes (vessels and tracheids) stacked beside and on top of each other. To move between adjacent vessels, water flows through bordered pits. Bordered pits are small bowl-shaped openings in the secondary walls. At the center of each bordered pit lies the pit membrane formed by the primary walls and the thin middle lamella (Zwieniecki & Holbrook, 2000, Sperry & Hacke, 2004).

If the negative hydrostatic pressure exceeds a species-specific threshold the water column may break as a consequence of cavitation (Tyree & Sperry, 1989). Cavitation is the process when air bubbles are formed in a liquid. These bubbles are a consequence of the metastability of water in the xylem. Cavitation refers to a sudden phase change of water from liquid to vapour at a nucleation site, i.e. on surfaces that are in contact with the liquid. If the water column cavitates, the affected xylem vessel becomes air-filled (embolized) and dysfunctional.

According to the air-seeding hypothesis (Sperry & Tyree, 1988, Tyree & Zimmermann, 2002) the embolized vessel may act as the source for further spreading of air along the xylem pathway. This happens when air-bubbles of embolized vessels are sucked into adjacent water filled vessels via the pits. Since xylem is an interconnected network of myriads of water conducting pipes, a few cavitation events will not significantly impair overall conductivity. Although, excessive cavitation within the xylem pathway may eventually result in hydraulic failure and desiccation of the affected leaves or branches (Tyree *et al.*, 1994b, Anderegg *et al.*, 2011).

However, cavitation is not only a result of excessively negative pressures in the xylem, it can also be induced by frequent freeze-thaw events. When water freezes, air is forced out of solution and air bubbles are formed in the ice (Sperry *et al.*, 1994, Davis *et al.*, 1999, Hacke & Sperry, 2001, Mayr *et al.*, 2003a, Pittermann & Sperry, 2006). When water thaws, these bubbles may resolve or expand, which leads to cavitation. Whether or not a vessel becomes embolized depends on the xylem pressure and the radius of the bubble (cf. Davis *et al.*, 1999, Hacke & Sperry, 2001). Susceptibility to freezing induced cavitation increases with more negative xylem pressures and larger bubbles. Larger bubbles can be formed in larger vessels and hence, the vessel size may play an important role for freezing-induced embolisms to occur. In a scenario in which frequent freeze thaw events are present, air bubbles can accumulate over winter and may expand in spring due to increased negative P_x when buds break and transpirations starts. As a consequence, insufficient water supply to the leaves may lead to decreased photosynthetic rates and eventually reduced growth (Wang *et al.*, 1992, Castro-Diez *et al.*, 1998, Cavender-Bares *et al.*, 2005), resulting in a potential selective force for smaller vessels in cold climates (Tyree *et al.*, 1994a).

1.4 The Impact of Frost on Living Plant Tissues

Freeze-thaw events, besides inducing embolisms, may also significantly contribute to frost injury and shoot-dieback over winter. When water changes its phase from liquid to crystalline it also expands in volume. These volume changes result in mechanical wear of cell components and may amplify the extent of frost injury. Intracellular ice formation may also result in plasma membrane disruption and cell death (Sakai & Larcher, 1987).

According to Burke *et al.* (1976) plants may show little or no freezing tolerance (tender annual plants), limited frost hardiness (biennial or perennial herbaceous plants), or full hardiness before frost occurs (woody plants). Plants with little or no freezing tolerance survive by means of dehydrated seeds that are very hardy. Biennial and perennial plants often survive under the insulating snow cover or as roots in the soil. Woody plants can be separated into two categories (Burke *et al.*, 1976): 1) woody plants which deep supercool and hence avoid freezing and 2) woody plants which do not deep supercool but are able to tolerate freezing. Supercooling is the process by which water can cool down to -40 °C without ice crystals being formed, which sets a lower limit to freeze-avoidance for plants and their organs (Pearce, 2001). These types of woody plants are mostly temperate deciduous trees, whereas trees which do not supercool but survive extreme freezing are more abundant in the boreal forest where temperatures can frequently drop below -40 °C. The hardening process for trees which do not supercool is initiated when water freezes in extracellular spaces creating a vapour pressure deficit, which withdraws water from the protoplast making cell contents highly viscous and may form a glass (vitrify) rather than freeze (Franks, 1985). This avoids freezing of the cell contents and minimizes the risk of plasma membrane disruption. Freezing tolerance by means of vitrification has been demonstrated in deeply frozen poplar cells (Hirsh *et al.*, 1985). This may explain why a fully hardened plant may survive even extreme experimental freezing as low as -196 °C (Sakai, 1966).

1.5 Phenology

Since plants are fully exposed to their native environment and are incapable of migrating, they have to acclimate to their local habitat in order to survive. Particularly, the winter months in which freezing can severely harm the living tissues are critical and need to be overcome. Most woody species accommodate this by entering a rest or dormancy period before freezing occurs and ending it when frost risks become sufficiently low again.

During dormancy trees stop active growth to minimize metabolic activity in order to overcome unfavourable growing conditions. The initiation of the dormancy period is primarily triggered by daylength (photoperiod) resulting in growth cessation, bud set, leave senescence and cold acclimation (Howe *et al.*, 1995, Horvath *et al.*, 2003, Howe *et al.*, 2003, Fracheboud *et al.*, 2009). In spring when temperatures rise, buds set in the previous summer, will be released from dormancy after sufficient chilling and a genetically fixed and species-specific amount of accumulated heat-sum is acquired. Heat-sum is measured in degree days and represents the accumulated sum of heat above a chosen temperature threshold (Hunter & Lechowicz, 1992, Li *et al.*, 2010, Beaubien & Hamann, 2011).

Taken together, timing and initiation of dormancy and dormancy release play an important role in protecting trees against the unfavourable winter season and secure their survival. Bud set, leave senescence and budbreak are adaptive traits which are under strong genetic control and attuned to a species' native habitat. Such traits have to be considered when moving planting stock for reforestation programs particularly under the context of current and predicted climate change scenarios.

1.6 Thesis outline and objectives

The present Ph.D. thesis consists of three individual research chapters (thesis chapter 2, 3 & 4). The main objective was to evaluate growth performance and survival of hybrid poplars (*Populus* spp.) and trembling aspen (*Populus tremuloides* Michx.) in the prairie provinces of western Canada. The goal was to provide information for future poplar breeding programs based on ecophysiological data that may help to optimize reforestation efforts under the assumption of current and predicted climate change.

In my *first research chapter*, I analyzed genetic differences in hydraulic and wood anatomical traits in seven hybrid poplar (*Populus* spec.) clones as well as six aspen genotypes (*Populus tremuloides* Michx.) from different provenances. The objectives were to:

(a) determine which plant group is better adapted to drought,

(b) identify intra- and interspecific differences in the measured traits, and

(c) identify key physiological and anatomical parameters, which can be linked to growth performance.

In my *second research chapter*, I evaluated the impact of freeze-thaw events and winter minimum temperature on growth performance of hybrid poplars. This study represents a follow-up on a key result of the previous research chapter. The objectives were to:

(d) test whether smaller branch vessel diameters also show an increased risk of freezing-induced embolism and how this may explain observed differences in tree height, and

(e) determine differences in cold hardiness between seven hybrid poplar clones with contrasting growth performances.

In my *third research chapter*, I assessed the potential of long-distance seed transfers of trembling aspen in a series of reciprocal transplant experiments with provenances ranging from Minnesota to northeast British Columbia. The objectives were to:

(f) quantify trade-offs among growth performance, frost hardiness, and timing of leaf senescence and budbreak, and

(g) discuss the potential of long-distance seed transfer for forestry operations in the context of climate change.

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CHAPTER 2

Genetic variation of hydraulic and wood anatomical traits in hybrid poplar and trembling aspen[1]

Contents

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2.1 Summary

Intensive forestry systems and breeding programs often include either native aspen or hybrid poplar clones, and performance and trait evaluations are mostly made within these two groups. Here we assessed how traits with potential adaptive value varied within and across these two plant groups. Variation in nine hydraulic and wood anatomical traits as well as growth were measured in selected aspen and hybrid poplar genotypes grown at a boreal planting site in Alberta, Canada. Variability in these traits was statistically evaluated based on a blocked experimental design. We found that genotypes of trembling aspen were more resistant to cavitation, exhibited more negative water potentials, and were more water-use efficient than hybrid poplars. Under the boreal field test conditions, which included major regional droughts, height growth was negatively correlated with branch vessel diameter (D_V) in both aspen and hybrid poplars and differences in D_V were highly conserved in aspen trees from different provenances. Differences between the hybrid poplars and aspen provenances suggest that these two groups employ different water-use strategies. The data also suggest that vessel diameter may be a key trait in evaluating growth performance in a boreal environment.

2.2 Introduction

Trembling aspen (*Populus tremuloides* Michx.) and other poplars (e.g.*Populus balsamifera* L.; *Populus deltoides* Bartr. ex Marsh.; *Populus trichocarpa* Torr. & A. Gray) play an important role in North American ecosystems, particularly in the boreal forest and the aspen parklands of the prairie provinces (Alberta, Saskatchewan, Manitoba) in western Canada (Richardson et al., 2007). Poplars (*Populus* ssp.) are among the fastest growing temperate trees and are considered to be vegetational pioneers (Eckenwalder, 1996, Bradshaw *et al.*, 2000). Poplars also represent an attractive and valuable forest resource since they grow fast and are easy to propagate from both seed and vegetative propagation (Peterson & Peterson, 1992, Cooke &

Rood, 2007). For instance, tree breeders in western Canada carry out intensive selection and breeding programs for poplars, searching for trees that produce high quality wood for pulp and for oriented strand board production, but are also able to withstand the dry cold climate of the Canadian prairies. Tree improvement programs often include either native aspen or non-native hybrid poplar clones in their breeding programs, and performance and trait evaluations are mostly made within these two groups, as reflected by a large number of studies conducted on either aspen or hybrid poplars. However, a comprehensive comparison between these two groups is still lacking (Lieffers *et al.*, 2001), even though it may become very valuable information for species selection in the context of climate change. When selecting suitable genotypes for a particular location, the concept *local is best* is normally applied, where nearby seed sources are selected for reforestation. Using locally adapted planting material reflects physiological adaptations of numerous tree generations to the local climate and site conditions. However, an accelerated trend in global warming (Houghton, 2005) may require a human-based relocation of certain genotypes from their southern distribution limits up to places where natural migration through seed dispersal would not be sufficient, given the magnitude of current and predicted climate change (Aitken *et al.*, 2008). In addition, hybrids among North American and Eurasian species of poplar are widely used for their superior growth characteristics. In both cases, physiological and field testing are required prior to large-scale deployment of this often non-local or novel plant material. These tests are typically common garden experiments that can differentiate environmental and genetic differences among genotypes in a shared environment (Gornall & Guy, 2007). In central Alberta, it may be particularly beneficial to facilitate the introduction of aspen genotypes from more southern latitudes since climate warming and decreases in precipitation for this region over the last 25 years have been very pronounced. The province of Alberta, for instance, has experienced warming of approximately 0.7 °C and a reduction of mean annual precipitation by 20 % over the last 25 years (Mbogga *et al.*, 2009). In 2002, a severe regional drought led to massive aspen dieback and mortality in the aspen parklands of southern Alberta (Hogg *et al.*, 2008). Historically, droughts have always been part of the climate in the Canadian prairies (Roberts *et al.*, 2006, Bonsal & Regier, 2007). However, more frequent and more severe droughts have been recorded in the recent past (including another exceptional drought in 2009), and this poses a serious threat for local vegetation. Since most poplar species are known to be sensitive to water deprivation (Blake *et al.*, 1996, Shock *et al.*, 2002), the question of how aspen and hybrid poplars will respond to drier conditions is becoming an important issue. Although poplar species are among the most susceptible trees to drought, considerable genotypic variability exists in water use efficiency, growth performance, hydraulic traits, and tolerance to moderate water deficits, particularly in hybrid poplar clones (Morrison *et al.*, 2000, Monclus *et al.*, 2006, DesRochers *et al.*, 2007, Fichot *et al.*, 2009, Silim *et al.*, 2009). Even greater differences are likely to exist between hybrid poplars and aspen as a group, but a comprehensive comparison of hydraulic traits between these two groups has, to our knowledge, not been conducted. Xylem traits, along with root and soil properties, can play an important role in limiting canopy water supply (Sperry *et al.*, 2002, McDowell *et al.*, 2008). Xylem properties may be especially important in riparian cottonwoods (Rood *et al.*, 2003) and hybrid poplars, which are known to be highly vulnerable to cavitation (Fichot *et al.*, 2010). As a result of cavitation and subsequent embolism, hydraulic conductivity in the xylem (K_h) declines as the xylem pressure becomes more negative. This dependence of K_h on xylem pressure is often referred to as a vulnerability curve (Sperry *et al.*, 2002). Comparisons of more or less distantly related taxa have shown that, at the interspecific level, cavitation resistance is often correlated with the water potential range that plants experience in their natural habitat (Hacke *et al.*, 2000, Pockman & Sperry, 2000). Interspecific comparisons have also linked differences in cavitation resistance with trends in xylem structure and transport efficiency (Maherali & DeLucia, 2000, Hacke *et al.*, 2006, Jacobsen *et al.*, 2007, Jansen *et al.*, 2009). However, such correlations may not be found when comparing closely related genotypes (Cochard *et al.*, 2007) or populations of a single species (Martinez-Vilalta *et al.*, 2009). For instance, a trade-off between xylem safety and

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xylem transport efficiency was absent across eight hybrid poplar genotypes (Fichot *et al.*, 2010), although it was found in a survey of 29 angiosperm species of diverse growth form and family affinity (Hacke *et al.*, 2006). In this present study, we measured genetic differences in hydraulic and wood anatomical traits of six aspen genotypes and seven hybrid poplar clones growing at a boreal planting site in Alberta, Canada. Aspen genotypes represented three provenances (Alberta, British Columbia, and Minnesota, *Table 2.1*). We assessed how traits varied within and across these two plant groups. We asked whether relationships between hydraulic traits seen in broad interspecific surveys would also be resolvable at a finer phylogenetic scale, i.e., across the studied genotypes of the genus *Populus*. We also evaluated the potential of linking differences in xylem traits with growth performance. Growth was measured as height and diameter at breast height (*DBH*), integrated over 16 and 11 years in hybrid poplar and aspen trial data respectively. A long-term goal is to identify easily accessible traits that can serve as predictors of growth performance under field conditions in this boreal environment. Finally, we assessed which of the measured traits in aspen were conserved by geographic source (provenance) and which varied independently. The plantations were designed as long-term field experiments and represent a good opportunity to investigate the previously outlined issues in a common garden setting.

2.3 Materials and Methods

2.3.1 Plant material

The hybrid poplar and aspen plant material used in this study came from field trials located at the Alberta-Pacific Forest Industries Inc. (Al-Pac) pulp mill site near Boyle (54′ 49′N, 113′ 31′W), Alberta, Canada. The clonal hybrid poplar trial was established in 1993, whereas the aspen trial is part of a common garden experiment with open pollinated single tree seed sources from Minnesota, Alberta, and British Columbia, planted in 1998. Both trials were planted in a randomized complete block design with five (hybrid poplar trial) and six (aspen provenance trial) replications per clone or seed source in five-tree row plots. The aspen trial is also surrounded by two rows of border trees to minimize error due to environmental effects. For this study we sampled eight trees (if not mentioned differently in the text) from each clone and provenance. The same trees were used for all analyses including growth measurements. The common garden trials contain a large amount of plant material, and we selected a representative sample of genotypes with contrasting performance for this study (*Table 2.1*, *Table 2.2*). Growth performance was evaluated by tree height (*m*) and *DBH*, measured 16 and 11 years after trials were established for the hybrid poplars and aspen, respectively. Since height and *DBH* were closely correlated, correlations seen with height could also be seen for *DBH* and vice versa. In addition to high, average, and poorly performing hybrid poplars, we added the Walker clone as a reference because it is well tested and widely used in shelterbelts and plantations in western Canada (Morrison *et al.*, 2000, Silim *et al.*, 2009) (*Table 2.2*). A total of 104 samples were collected over a period of seven weeks in June and July. The sampling was carried out once a week and the material was processed within the next four days. In order to minimize time effects, hybrid poplar and aspen provenances were sampled so that differences due to different sampling times were superimposed on spatial blocks of the experimental design. This undesired potential source of error could therefore be accounted for in the analysis as a block effect. In order to minimize destructive sampling, and for practical reasons, all hydraulic and wood anatomical measurements (*Table 2.3*) were conducted on branch segments. Samples were from two- to three-year-old branches, which were taken from sun-exposed areas within the canopy using a telescope pruner. The material was packed in plastic bags with moist tissues and stored at 4 °C in a walk-in refrigerator. The leaves from each branch segment and all remaining leaves distal to the segment were collected and stored in separate bags to determine leaf area and carbon isotope composition.

2.3.2 Leaf related measurements and growth

Leaf carbon isotope composition ($\delta^{13}C$) was used as an integrated measure for stomatal control and water use efficiency (Farquhar *et al.*, 1989). The analysis was conducted by the Stable Isotope Laboratory in the Department of Renewable Resources at the University of Alberta. The collected leaves were dried in an oven at 80 $^{\circ}C$ for a minimum of 48 h and were ground with a ball grinder until a fine powder was yielded. Leaf water potentials (ψ-*Leaf*) were measured during midday on a cloudless hot summer day (August 21, 2009; maximum daily temperature, 27 $^{\circ}C$ on a subset of three trees per hybrid poplar clone and aspen provenance. The measurements were carried out using a pressure chamber (Model 1000, PMS Instrument Company, Albany, OR, U.S.A.). Transpiring leaves were cut, bagged, and ψ-*Leaf* was immediately measured in the field. Tree height and *DBH* were measured in the fall when all leaves were shed. Height was measured with a laser hypsometer and *DBH* was measured using a digital caliper.

2.3.3 Hydraulic measurements

Branches were harvested in the field in lengths of at least 1m and brought to the laboratory in plastic bags. Segments were cut from the center of these branches under water to avoid blocking additional vessels with air and to avoid including vessels that were embolized during harvesting. Hydraulic conductivity (K_h) was measured on 14.2 *cm* long branch segments using a tubing apparatus (Sperry *et al.*, 1988) and a methodology thoroughly described in Hacke & Jansen (2009). Silicone injections (Hacke *et al.*, 2006) on branches of four of the hybrid poplar clones showed that less than 1 % of vessels were open in the 14.2 *cm* long segments. Hydraulic conductivity was calculated as the quotient of flow rate through the segment and pressure gradient. The tubing apparatus consisted of an elevated water reservoir connected to an electronic balance (CP225D; Sartorius, Göttingen, Germany) via Tygon tubing. The balance was interfaced with a computer using Col-

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lect 6 software (Labtronics, Guelph, Canada) and logged K_h every 10 seconds. Each branch segment was inserted in the tubing system and its native conductivity was measured. Subsequently, segments were flushed to remove native embolism and to obtain the maximum conductivity for a given segment. All segments were spun in a centrifuge to increasingly negative xylem pressure, and K_h was re-measured on the conductivity apparatus after spinning (Li *et al.*, 2008). The percentage loss in conductivity from the original value was plotted versus the negative pressure, and curves were fit with a Weibull function. The xylem pressure corresponding to 50 % loss of K_h (P_{50}) was calculated for each segment based on the Weibull fit. Values of P_{50} were then averaged for each genotype. The threshold xylem pressure at which loss of conductivity begins to increase rapidly was determined according to the method of Domec & Gartner (2001). This air entry pressure (P_e) is less frequently reported than the P_{50}, but it is a useful parameter when linking vulnerability curves with stomatal control of xylem pressure (Sparks & Black, 1999, Meinzer *et al.*, 2009). In this present study, the P_e was compared with ψ-*Leaf*. The difference between these two parameters was used to assess the degree of safety against the onset of cavitation. Specific conductivity (K_S) was measured by dividing the maximum K_h of a stem segment by its cross sectional sapwood area. The sapwood area was measured with a stereomicroscope (MS5, Leica, Wetzlar, Germany). Specific conductivity is a measure of the transport efficiency of the xylem. Leaf specific conductivity (K_L) was calculated by dividing the maximum K_h of a stem segment by the leaf area distal to the base of the segment, i.e., leaves attached to the segment were included in the measurements. The K_L is a measure of the hydraulic sufficiency of the segment to supply water to leaves (Tyree & Zimmermann, 2002). Leaf area was measured with a LI-3100 area meter (Li-Cor, Lincoln, NE, USA).

2.3.4 Xylem anatomy

All xylem anatomical measurements were carried out on the same branch segments used for measuring hydraulic conductivity and cavitation resistance. Vessel diameters were measured on cross-sections of 30-35 μm thickness. Sections were prepared with a microtome (Leica SM2400) and analyzed with a Leica DM3000 microscope at 200× magnification. Images of each cross-section were captured with a Leica DFC420C camera and analyzed using image analysis software (Image-Pro Plus 6.1, Media Cybernetics, Silver Spring, MD, USA). Vessel diameters were measured in three radial sectors representing the two outermost growth rings. Mean hydraulic vessel diameters (D_V) were calculated based on the Hagen-Poiseuille equation. The vessel diameter that corresponds to the average lumen conductivity was calculated as $D_V = [(\Sigma\, d^4)/n]^{1/4}$, where n is the number of vessels measured, and d is the individual vessel lumen diameter. Wood density was measured following the methods of Hacke *et al.* (2000) and Pratt *et al.* (2007). Segments were cut into 3 cm pieces and split in half. Bark and pith were removed. Xylem density was measured by water displacement on an analytical balance (CP224S; Sartorius, Göttingen, Germany). Samples were dried in an oven at 70 $^{\circ}C$ for at least 48 h and density was measured as dry mass (g) / fresh volume (cm^3).

2.3.5 Statistical analysis

Aspen and hybrid poplar plantations were nearby separate trials established at different times. Since they were not part of the same randomized experimental design, we did not apply a formal statistical evaluation of differences between aspen and hybrid poplars. Instead, we present box plots to illustrate the differences between these two groups (*Fig. 2.1*). For statistical analyses of intra-group differences between physiological and wood anatomical traits, we calculated means of row plots summarized at the clone and provenance level, taking advantage of the blocked experimental design (*Table 2.4*, *2.5* & *2.6*). Analysis of variance was carried out with PROC MIXED of the SAS statistical software package (SAS Institute, 2008), where block and genotype within groups were specified as random factors.

2.4 Results

2.4.1 Physiological differences between hybrid poplars and aspen provenances

Many of the measured hydraulic and wood anatomical traits differed between the hybrid poplars and aspen provenances (*Fig. 2.1*). In particular, traits such as P_{50}, ψ-*Leaf*, leaf-to-sapwood area ratio (A_L:A_S), K_L, and $\delta^{13}C$ differed considerably. Compared to aspen, hybrid poplars were more vulnerable to cavitation and correspondingly exhibited higher (less negative) leaf water potentials (*Fig. 2.1*). Branches of hybrid poplars tended to show higher K_L values than aspen branches. This was mainly a result of lower A_L:A_S ratios of hybrid poplars since xylem-specific conductivities were similar in both plant groups. Native embolism (PLC_N) levels varied between 36.7 and 58.7 % and did not differ between plant groups. Wood densities were similar, but showed greater variation within hybrid poplars than within aspen provenances.

2.4.2 Xylem cavitation resistance, leaf water potentials and safety margins

Vulnerability curves for hybrid poplars and aspen provenances were similar in shape, but aspen curves were shifted toward more negative xylem pressure, i.e., greater resistance to cavitation (*Fig. 2.2*). Most hybrid poplars and all aspen provenances exhibited relatively steep sigmoidal curves with a well-defined cavitation threshold. The P_{50} varied from -1.51 to -1.97 *MPa* in hybrid poplars, and from -2.05 to -2.44 *MPa* in aspen. Hence there was no overlap in P_{50} between the two plant groups. The P_e varied between -0.72 and -1.44 *MPa* in hybrid poplars, compared with a range between -1.41 and -1.91 *MPa* in aspen. No clear relationship between cavitation resistance and growth performance was apparent in either plant group. No significant differences in P_{50} existed within hybrid poplars and aspen (*Table 2.4*). Variation in P_{50} was also not correlated with differences in D_V or d_W. Leaf water potential varied from -1.07 to -1.47 *MPa* in hybrid poplars and from -1.57 to -1.93 *MPa* in aspen (*Fig. 2.3*). Safety margins can be implied by the difference between P_e and ψ*-Leaf*. A genotype with a safety margin of zero would plot on the 1:1 line in *Fig. 2.3a*. Higher and lower safety margins would plot below and above the diagonal, respectively. Although no correlation existed within hybrid poplars and aspen provenances, there was a significant ($P < 0.02$) correlation across all data points. The slope of the regression line did not differ from the 1:1 line, indicating that there was a general agreement between leaf water potentials and cavitation threshold. Safety margins ranged from -0.78 to 0.38 *MPa* and did not differ between aspen and hybrid poplars (t-test, $P = 0.43$). It should be noted that in transpiring plants, ψ*-Leaf* is more negative than the xylem pressure. Therefore, the actual safety margins will be larger than our estimates that were based on ψ*-Leaf* values. Lower leaf water potentials in aspen trees corresponded with less negative $\delta^{13}C$ values than in hybrid poplars (*Fig. 2.3b*), suggesting aspen trees were more water-use efficient. Variation in $\delta^{13}C$ was larger in hybrid poplars than in aspen provenances, and was not related to performance within groups (*Table 2.5*) or provenances (*Table 2.6*).

2.4.3 Height growth and links with other parameters

Of all parameters measured, only D_V showed strong correlations with height (and *DBH*) in both aspen and hybrid poplars (*Fig. 2.4*). Surprisingly, greater height growth corresponded with narrower vessel diameters. Tree height varied between 5.6 and 11.3 m in the aspen provenances and between 7.1 and 14.7 m in the hybrid poplars. In other words, the best performers in each group exhibited about twice the height compared with the slowest growing genotypes. The absolute height values cannot be compared between the aspen and hybrid poplars since they were confounded by the microenvironment at the test site and by the age of the trees. Nevertheless, the fastest and slowest growing aspen genotypes had comparable growth rates to the fastest and slowest growing hybrid poplar clones with an approximate adjustment for age. Within the aspen as much as 87.4 % of the variance in height (and 82.4 % of the variance in *DBH*) could be explained by region (*Table 2.6*). Like height, vessel diameters exhibited large variation within each plant group. Within hybrid poplars 50.4 % of the variance in D_V could be explained by performance groups (*Table 2.5*), and the means between performance groups showed significantly smaller vessel diameters of Walker vs. poor, and high vs. poor performers (*Table 2.4*). Similarly, within the aspen 55.5 % of the variance in D_V could be explained by region (*Table 2.6*), and the means showed significantly smaller vessel diameters for Minnesota vs. British Columbia source (*Table 2.4*).

2.5 Discussion

Differences in cavitation resistance between plant groups

Our results show that hybrid poplars and aspen differed greatly in some key hydraulic parameters, including cavitation resistance and leaf water potentials. Hybrid poplars were more vulnerable to cavitation than aspen, and correspondingly, maintained less negative leaf water potentials. The fact that most data points fell on or near the 1:1 line of the P_e versus ψ-*Leaf* relationship (*Fig. 2.3a*) indicates that predicted safety margins from hydraulic failure were similar in both plant groups. The data shown in *Fig. 2.3a* also suggests that leaf water potentials were constrained by the cavitation threshold. This was an expected finding given the fact that all vulnerability curves showed a steep slope after the onset of cavitation (*Fig. 2.2*, see also Fichot *et al.* (2010)). The fact that hybrid poplars were found to be highly vulnerable to cavitation agrees with previous work on *Populus* species (Blake *et al.*, 1996, Hacke & Sauter, 1996, Pockman & Sperry, 2000, Rood *et al.*, 2000). Many of the hybrid poplars analyzed in this study were derived from cottonwoods (sensu

Rood *et al.* (2003), Cooke & Rood (2007)). Riparian cottonwoods are dependent on shallow groundwater which is often linked to stream water. Given that there is access to such relatively stable water sources, phreatophytic cottonwoods can persist even in semi-arid regions (Rood *et al.*, 2003). Trembling aspen, by contrast, has ecophysiological adaptations to nonriparian zones and is widespread on upland sites (Lieffers *et al.*, 2001, Rood *et al.*, 2007). Differences in cavitation resistance between the two plant groups agree with these ecological characteristics. Correlations between cavitation resistance and other traits, aside from ψ-*Leaf*, were weak or absent, as observed previously in a study on eight hybrid poplar genotypes (Fichot *et al.*, 2010). Our failure to identify such correlations may have been due, at least in part, to the fact that variation in P_{50} remained relatively small. Moreover, if cavitation resistance in poplar is determined by differences in pit membrane ultrastructure (Jansen *et al.*, 2009), then variation in P_{50} will not necessarily be linked with traits such as D_V and d_W. If a direct causal link between cavitation resistance and other vessel traits does not exist, then it may be possible to breed poplar genotypes that show improved transport safety while maintaining high transport efficiency.

δ^{13} and leaf water potentials

Our results show that hybrid poplar and aspen also differed distinctively in their $\delta^{13}C$ and ψ-*Leaf* values (*Fig. 2.3*), suggesting that aspen regulated its stomata more conservatively in order to avoid xylem cavitation and excessive water loss. Previous work has shown that stomata in aspen operate in a way that maintains ψ-*Leaf* above a critical threshold value between -2 and -2.5 *MPa* (Hogg & Hurdle, 1997, Hogg *et al.*, 2000). Considering that aspen clones can be quite large, tree water use is likely to exert a strong feedback on the future availability of soil moisture in the area occupied by the clone. This may have led to more selection pressure for increased water use efficiency in the aspen (Ted Hogg, personal communication). We conclude that aspen appears to be more water-use efficient than hybrid poplars at a boreal planting site.

Growth performance and vessel diameters

Height was negatively correlated with d_W in hybrid poplars (r = -0.82, $P < 0.02$; data not shown). In aspen, variation in d_W was much smaller than in hybrid poplars, and there was no clear relationship with height or *DBH*. Again, it should be noted that d_W was measured in branch segments. Stronger correlations between height and d_W may have been found if d_W had been measured in the trunk. The only other parameter that scaled with height in both hybrid poplars and aspen was D_V. The fact that strong negative correlations between tree height and D_V existed in both plant groups was unexpected. Another interesting finding was that differences in both height and D_V were highly conserved in trees from different aspen provenances. Trees from the two Minnesota provenances showed very similar values of height growth and D_V, as did trees from the two Alberta and the two British Columbia provenances (*Fig. 2.4*). The negative correlations between height and D_V seen in these mature trees contrast with observations on hybrid poplar saplings growing in a controlled environment without being subjected to abiotic stress. In such saplings, faster growth was correlated with wider vessels (Hacke *et al.*, 2010). Why was height at our boreal planting site associated with narrower vessels at the expense of potentially lower transport efficiency? At our study site, long-distance water transport in the xylem is not only constrained by drought-induced cavitation, but also by freezing. Wider vessels are more vulnerable to freezing-induced embolism than narrow vessels (Davis *et al.*, 1999, Stuart *et al.*, 2007). Relatively small differences in D_V can lead to large differences in vulnerability. Although we did not measure native embolism during winter, it seems reasonable to assume that trees with narrow vessels exhibited lower embolism levels in the winter than trees with wider vessels. Unlike other species such as birch, poplars do not reverse winter embolism by developing root pressure (Sperry *et al.*, 1994). The amount of winter embolism and differences in D_V may be significant in the context of this study because, despite some variation, a functional linkage exists between the embolism level in late winter and the timing of spring bud break across ring- and diffuse-porous angiosperms and conifers (Wang *et al.*, 1992, Tyree & Zimmermann, 2002). Lower embolism levels may allow for a relatively early bud break in spring and an adequate water supply to the developing foliage in Minnesota trees. Available records for this common garden trial from 2008 indicate that Minnesota provenances did in fact leaf out approximately one week earlier than sources from central Alberta (Li *et al.*, 2010), an observation opposite to normal latitudinal trends in budbreak, where sources from cooler environments break bud relatively earlier to take advantage of a shorter available growing season (Leinonen & Hänninen, 2002). This departure from normal trends was explained as an adaptation of Minnesota sources to take advantage of favourable early season growing conditions in Minnesota (Li *et al.*, 2010). Minnesota receives one and a half times more precipitation throughout the year (700 *mm* versus 463 *mm* for central Alberta and 449 for north-eastern BC) (*Table 2.7*), and when temperatures reach growing season conditions (5 °C) in spring, precipitation is two and a half times higher in Minnesota (50 *mm* / month) than typically recorded in Alberta and north-eastern British Columbia (very dry with only 20 *mm* / month) (*Fig. 2.5*). Our hydraulic data provides additional information that might help us to understand how Minnesota sources are adapted to their local climatic conditions, and why they grow exceptionally well in central Alberta, exceeding locally adapted sources by 30-40 % in height and diameter growth. For a given spring temperature, Minnesota sources start growing early and are therefore more likely to be exposed to freeze-thaw events in spring. The small vessel diameters that we observed in this study for Minnesota sources may provide effective protection against embolism caused by freeze-thaw events in spring. On the other hand wider vessels found in the British Columbia sources may have evolved to ensure high water supply to the leaves in environments with extremely short growing seasons. Such an adaptation however, would come at the cost of minimizing cavitation resistance (xylem safety) for the benefit of maximizing water transport efficiency and growth.

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In hybrid poplars, differences in xylem anatomy were due to differential genetic backgrounds rather than natural selection. Nevertheless, narrower vessels appear advantageous for growth within the hybrid poplar group as well: Walker exhibited the greatest height growth and also had the narrowest vessel diameters, followed with increasingly larger vessel diameters by the high, average, and poorly performing groups. A complicating factor in the analysis of D_V in trees of different height is the well-known fact that vessel diameters in the trunk vary with tree height (Tyree & Zimmermann, 2002, McCulloh & Sperry, 2005, Petit *et al.*, 2010). When D_V is measured at the same height in trees of different sizes, as was done in our study, D_V may be expected to be wider in larger trees than in smaller ones (Weitz *et al.*, 2006). We observed the opposite, suggesting that the trend in D_V was not just the consequence of a size effect. While these explanations are speculative, they provide a framework to guide future research aimed at linking xylem traits, winter embolism, plant growth and climatic characteristics. Such work could be useful to identify genotypes that are well adapted to drought conditions as well as freeze-thaw cycles which could become more frequent under a warmer and more variable future climate. In conclusion, large differences in hydraulic traits existed between hybrid poplar clones and aspen provenances. Hybrid poplars exhibited less negative water potentials and were more vulnerable to drought-induced cavitation than aspen genotypes. Within groups, traits like wood density and $\delta^{13}C$ showed wide variation within hybrid poplars but not within the aspen provenances. By contrast, vessel diameter and height growth varied substantially in both plant groups, and much of this variation in aspen was related to geographic seed source. In both plant groups, height growth was negatively correlated with vessel diameters. Vulnerability to freezing-induced embolism is closely related to vessel diameter, and genetically determined differences in vessel diameter could play an important role in explaining differences in tree performance.

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2.7 Tables

Table 2.1 – *Geographic origin of aspen seed sources and height and diameter at breast height (DBH) measured after 11 growing seasons in the field in a provenance field trial in central Alberta. The standard error of the mean is given in brackets.*

Region	**Provenance #**	**Latitude**	**Longitude**	**Elevation (m)**	**Height11[1] (m)**	**DBH11[2] (cm)**
British Columbia	9	58° 12′ N	123° 20′ W	1177	5.6 (0.2)	7.0 (0.5)
British Columbia	10	58° 36′ N	122° 20′ W	335	6.0 (0.5)	8.0 (0.5)
Alberta	25	55° 36′ N	113° 25′ W	762	8.8 (0.3)	9.5 (0.6)
Alberta	26	54° 56′ N	112° 44′ W	545	7.7 (0.3)	8.8 (0.5)
Minnesota	39	47° 12′ N	93° 48′ W	405	11.3 (0.2)	13.5 (0.6)
Minnesota	41	47° 30′ N	93° 36′ W	433	11.0 (0.2)	13.9 (0.5)

[1] Height after 11 growing seasons
[2] Diameter at breast height after 11 growing seasons

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Table 2.2 – *Seven selected hybrid poplar clones with code, parental background, performance grouping, height and DBH data measured in 2008 as well as percent survival for the time period 1993-2008. Standard error of the mean is given in brackets.*

Performance group	**Code/clone name**	**Genus**	**Femal parent[1] (species/hybrid)**	**Male parent[1] (species/hybrid)**	**Height16[2] (m)**	**DBH16[3] (cm)**
High	P38P38	*Populus*	*balsamifera*	*simonii*	13.9 (0.5)	16.7 (1.3)
High	Brooks #1	*Populus*	*deltoides*	× *petrowskyana*[4]	14.4 (0.4)	20.4 (1.7)
Average	4435	*Populus*	*balsamifera*	× *euramericana*[5]	11.6 (0.7)	9.9 (1.6)
Average	TACN 1	*Populus*	*laurifolia*	*nigra*	13.0 (0.1)	15.8 (1.0)
Poor	DTAC 24	*Populus*	*angulata*	*trichocarpa*	7.1 (0.5)	5.4 (0.3)
Poor	DTAC 22	*Populus*	*angulata*	*trichocarpa*	7.9 (0.6)	7.3 (0.9)
Reference	FNS 44-52/Walker	*Populus*	*deltoides*	× *petrowskyana*[4]	14.7 (0.5)	15.4 (1.1)

[1] Hybrids are designated by an × in front of the parent.
[2] Height after 16 growing seasons.
[3] Diameter at breast height after 16 growing seasons.
[4] *P.* ×*petrowskyana* is a hybrid of *P. laurifolia* and *P. nigra*.
[5] *P.* ×*euramericana* is a hybrid of *P. deltoides* and *P. nigra*.

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Table 2.3 – *List of all physiological traits measured in chapter 2 with symbols and units.*

Symbol	**Definition**	**Unit**
PLC_N	Percent loss hydraulic conductivity / Native embolism	%
P_{50}	Pressure causing 50 % loss of hydraulic conductivity	MPa
P_e	Air entry pressure	MPa
ψ-Leaf	Leaf water potential	MPa
V_D	Vessel diameter	µm
K_S	Xylem-specific hydraulic conductivity	kg m^{-1} MPa^{-1} s^{-1}
K_L	Leaf-specific hydraulic conductivity	10^{-4} kg m^{-1} MPa^{-1} s^{-1}
A_L:A_S	Leaf-to-sapwood area ratio	m^2 cm^{-2}
δ^{13}C	Leaf carbon isotope compostition	‰
d_W	Wood density	g cm^{-3}
Height16	Hybrid poplar height after 16 growing seasons	m
DBH16	Hybrid poplar diameter at breast height after 16 growing seasons	cm
Height11	Aspen height after 11 growing seasons	m
DBH11	Aspen diameter at breast height after 11 growing seasons	cm
MAT	Mean annual temperature	°C
MGP	Mean growing season precipitation (May-September)	mm
MAP	Mean annual precipitation	mm

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Table 2.4 – *Group means of physiological parameters and growth traits for hybrid poplar and regional means for aspen provenances. Significant differences among performance groups or regions are indicated by different letters (α = 0.05). Standard error of the mean is given in brackets. We did not test for significant differences between aspen and hybrid poplar because traits were confounded by test site and age of trees.*

	Hybrid Poplar				**Aspen**		
Physiological parameter	*High*	*Average*	*Poor*	*Walker*	*MN*	*AB*	*BC*
PLC_N (%)	40.3 (5.5)^{A}	40.5 (5.4)^{A}	54.6 (3.3)^{A}	36.7 (6.6)^{A}	41.0 (3.7)^{A}	58.7 (4.9)^{A}	48.3 (5.6)^{A}
P_{50} (MPa)	-1.81 (0.07)^{A}	-1.68 (0.07)^{A}	-1.64 (0.08)^{A}	-1.98 (0.09)^{A}	-2.37 (0.07)^{A}	-2.15 (0.07)^{A}	-2.28 (0.07)^{A}
ψ-Leaf (MPa)	-1.18 (0.05)^{AB}	-1.51 (0.05)^{B}	-1.12 (0.05)^{A}	-1.24 (0.07)^{AB}	-1.68 (0.05)^{A}	-1.90 (0.05)^{A}	-1.72 (0.06)^{A}
V_D (μm)	27.15 (0.42)^{B}	28.5 (0.41)^{AB}	30.66 (0.72)^{A}	25.41 (0.57)^{B}	24.82 (0.41)^{B}	27.01 (0.50)^{AB}	29.37 (0.57)^{A}
K_S	1.73 (0.12)^{A}	1.37 (0.16)^{A}	1.77 (0.23)^{A}	1.32 (0.10)^{A}	1.10 (0.09)^{B}	1.66 (0.15)^{A}	1.60 (0.13)^{A}
K_L ($\times 10^{-4}$)	3.79 (0.30)^{A}	2.67 (0.31)^{A}	3.09 (0.27)^{A}	3.25 (0.35)^{A}	1.72 (0.18)^{A}	2.33 (0.29)^{A}	3.16 (0.34)^{B}
A_L:A_S	0.52 (2.1)^{A}	0.54 (0.04)^{A}	0.61 (0.05)^{A}	0.42 (0.03)^{A}	0.74 (0.04)^{A}	0.80 (0.04)^{A}	0.59 (0.05)^{A}
δ^{13}C (‰)	-29.6 (0.4)^{A}	-29.8 (0.1)^{A}	-28.7 (0.2)^{A}	-30.0 (0.3)^{A}	-27.5 (0.3)^{A}	-27.8 (0.2)^{A}	-28.0 (0.3)^{A}
d_W (g/cm^3)	0.39 (0.00)^{A}	0.40 (0.01)^{A}	0.46 (0.01)^{A}	0.42 (0.01)^{A}	0.41 (0.01)^{A}	0.40 (0.01)^{A}	0.39 (0.01)^{A}
16 & 11-Year *Height* (m)	14.4 (0.3)^{A}	12.8 (0.5)^{A}	7.6 (0.4)^{B}	14.5 (0.5)^{A}	11.2 (0.1)^{A}	8.2 (0.3)^{B}	5.8 (0.3)^{C}
16 & 11-Year *DBH* (cm)	18.8 (1.2)^{A}	14.0 (1.5)^{AB}	6.5 (0.6)^{B}	15.3 (1.1)^{B}	13.7 (0.4)^{A}	9.2 (0.4)^{B}	7.5 (0.4)^{B}

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Table 2.5 – *Analysis of Variance for physiological parameters and growth traits measured in hybrid poplar clones. We report variance components due to performance groups (high, average, poor), clones within performance groups, as well as block effects of the experimental design. Significant effects are indicated in bold ($\alpha = 0.05$).*

	Variance component (%)				Prob (>F-value)		
Physiological parameter	*Block*	*Group*	*Clone(Grp)*	*Error*	*Block*	*Group*	*Clone(Grp)*
PLC_N (%)	0	9.5	0	90.5	0.605	0.164	0.596
P_{50} (MPa)	3.8	0	24.5	71.7	0.135	0.567	**0.007**
ψ-Leaf (MPa)	0	58.1	4.9	37	0.907	0.085	0.369
V_D (µm)	0.5	50.4	0	49.1	0.493	**0.014**	0.592
K_S	4.4	0	19.3	76.3	0.149	0.675	**0.011**
K_L ($\times 10^{-4}$)	3.9	2.3	18	75.8	0.139	0.54	**0.016**
A_L:A_S	2.6	0	41.7	55.7	0.168	0.788	**<0.001**
δ^{13}C (‰)	3.5	0	65.9	30.6	0.105	0.748	**<0.001**
d_W (g/cm^3)	3.2	36	33.8	27	0.078	0.239	**<0.001**
16 & 11-Year *Height* (m)	9.1	80.4	2.2	8.3	**<0.001**	**0.006**	0.064
16 & 11-Year *DBH* (cm)	9.7	59.1	12.2	19	**0.003**	**0.048**	**0.008**

Table 2.6 – *Analysis of Variance for physiological parameters and growth traits measured in aspen provenances. We report variance components due to regions (MN, AB, BC), provenances within regions, as well as block effects of the experimental design. Significant effects are indicated in bold ($\alpha = 0.05$).*

	Variance component (%)				Prob (>F-value)		
Physiological parameter	*Block*	*Group*	*Clone(Grp)*	*Error*	*Block*	*Group*	*Clone(Grp)*
PLC_N (%)	0	10.5	6.3	83.2	0.539	0.19	0.293
P_{50} (MPa)	0	0	19.8	80.2	0.667	0.584	**0.037**
ψ-Leaf (MPa)	13.6	27.2	32.1	27.1	0.086	0.202	**0.042**
V_D (μm)	1.7	55.5	0.2	42.6	0.278	**0.013**	0.49
K_S	1.4	22.7	0	75.9	0.44	**0.012**	0.909
K_L ($\times 10^{-4}$)	14.2	26.3	0.8	58.7	**0.035**	0.066	0.396
A_L:A_S	0	17.9	11.9	70.2	0.536	0.242	**0.078**
δ^{13}C (‰)	13.8	1.3	0.8	84.1	0.088	0.392	0.425
d_W (g/cm^3)	0	0	0	100	0.94	0.151	0.872
16 & 11-Year *Height* (m)	1.7	87.4	2.4	8.5	**0.041**	**0.005**	**0.032**
16 & 11-Year *DBH* (cm)	1	82.4	0	16.6	0.197	**0.003**	0.405

Table 2.7 – *Mean annual temperature (MAT), mean growing season precipitation (MGP), and mean annual precipitation (MAP) for the planting site as well as the aspen provenance locations. Climate variables were calculated using a time period from 1993-2007 for the planting site and the 1961-1990 climate normals for the aspen provenance locations.*

		Provenances		
	Planting site	*Minnesota*	*Alberta*	*British Columbia*
MAT	2.2	3.7	0.8	-1.1
MPG	347	456	326	302
MAP	489	700	463	449

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2.8 Figures

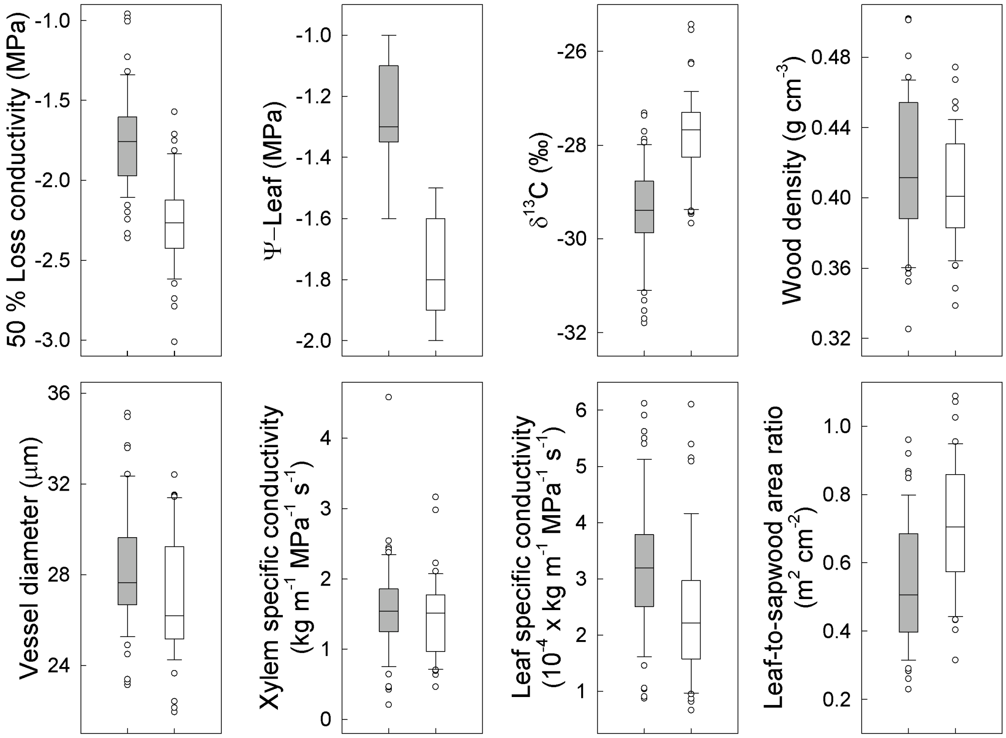

Figure 2.1 – *Box plots of hydraulic and wood anatomical properties contrasting poplar clones (grey) with aspen provenances (white). The central box in each box plot represents the 25th and 75th percentile with the median (50th percentile).Whiskers indicate the 10th and 90th percentile. Every outlier is shown as a circle.*

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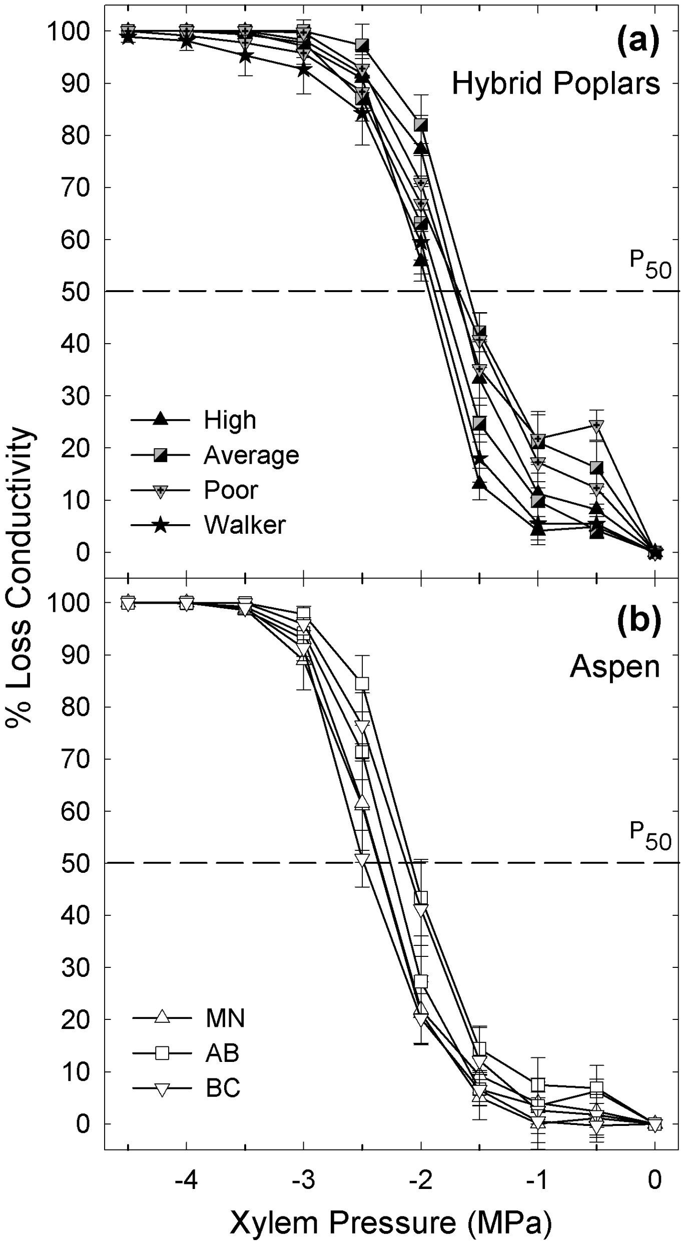

Figure 2.2 – *Vulnerability curves of all hybrid poplar clones (a) and aspen provenances (b). The dashed lines indicate 50 % loss of hydraulic conductivity. Closed symbols represent hybrid poplar clones; open symbols represent aspen provenances. Error bars are representing the standard error of the mean.*

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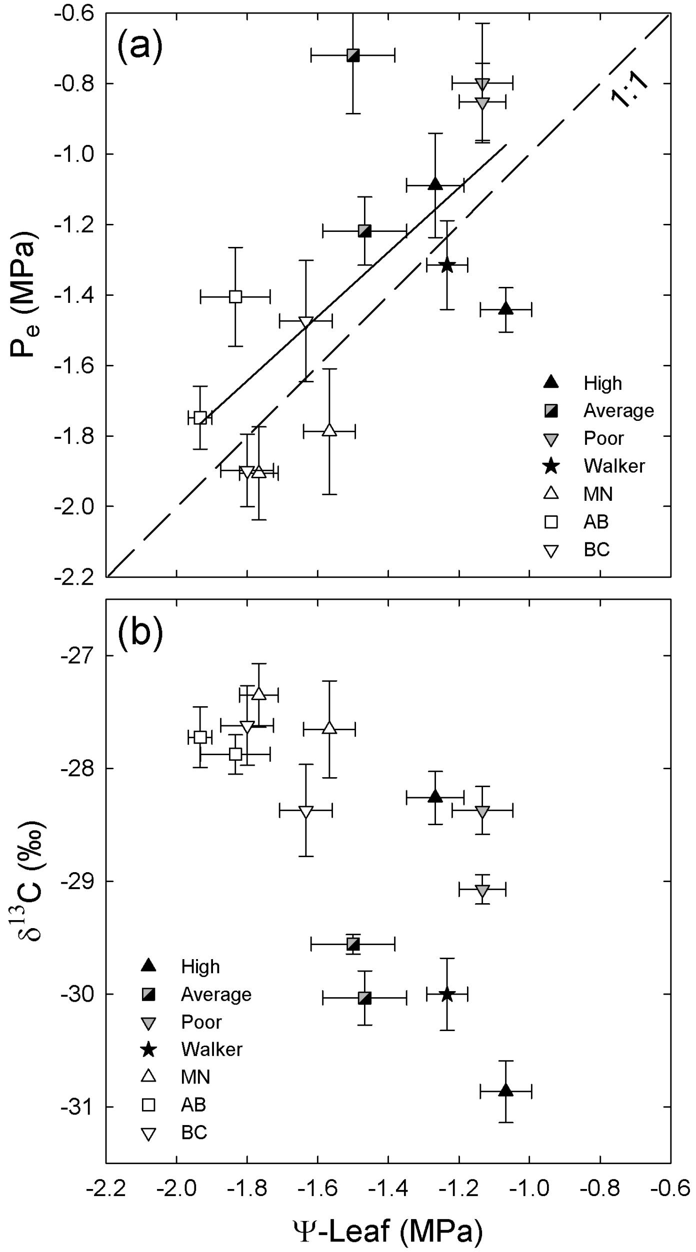

Figure 2.3 – *Relationship between leaf water potential (ψ-Leaf) and the air entry pressure (P_e) at which loss of hydraulic conductivity begins to increase rapidly (a) as well as leaf carbon isotope composition (b). Closed symbols represent hybrid poplar clones; open symbols represent aspen provenances. The dashed line in a) represents the 1:1 line separating the plot in a lower and upper area indicating larger and smaller safety margins, respectively. Error bars are representing the standard error of the mean.*

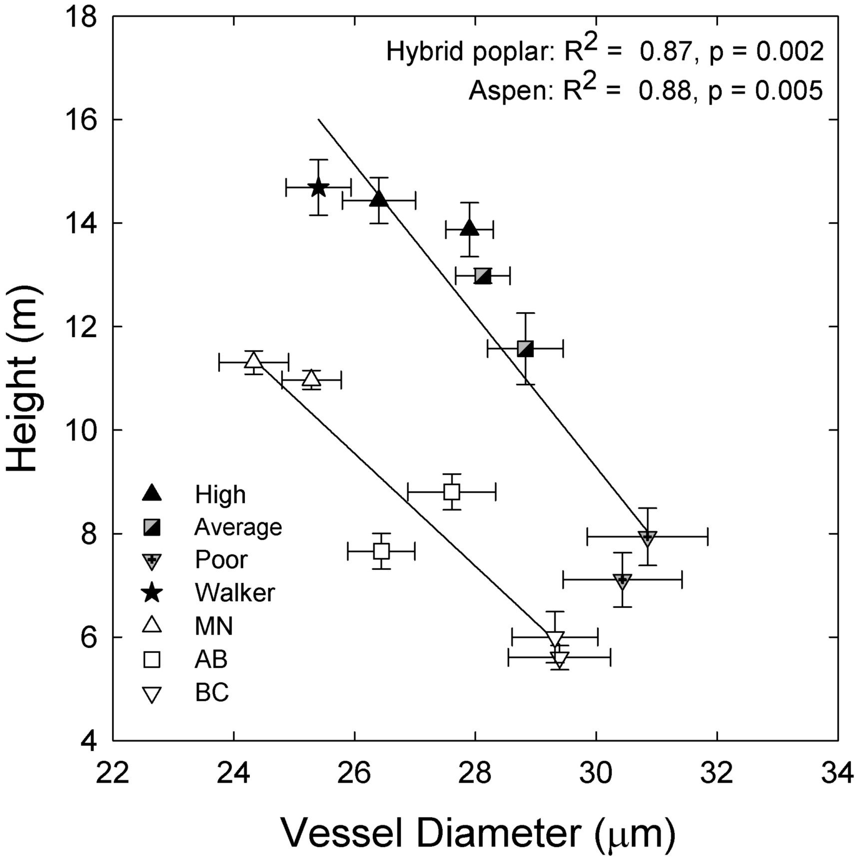

Figure 2.4 – *Correlation between tree height and vessel diameter. Closed symbols represent hybrid poplar clones; open symbols represent aspen provenances. Error bars are representing the standard error of the mean.*

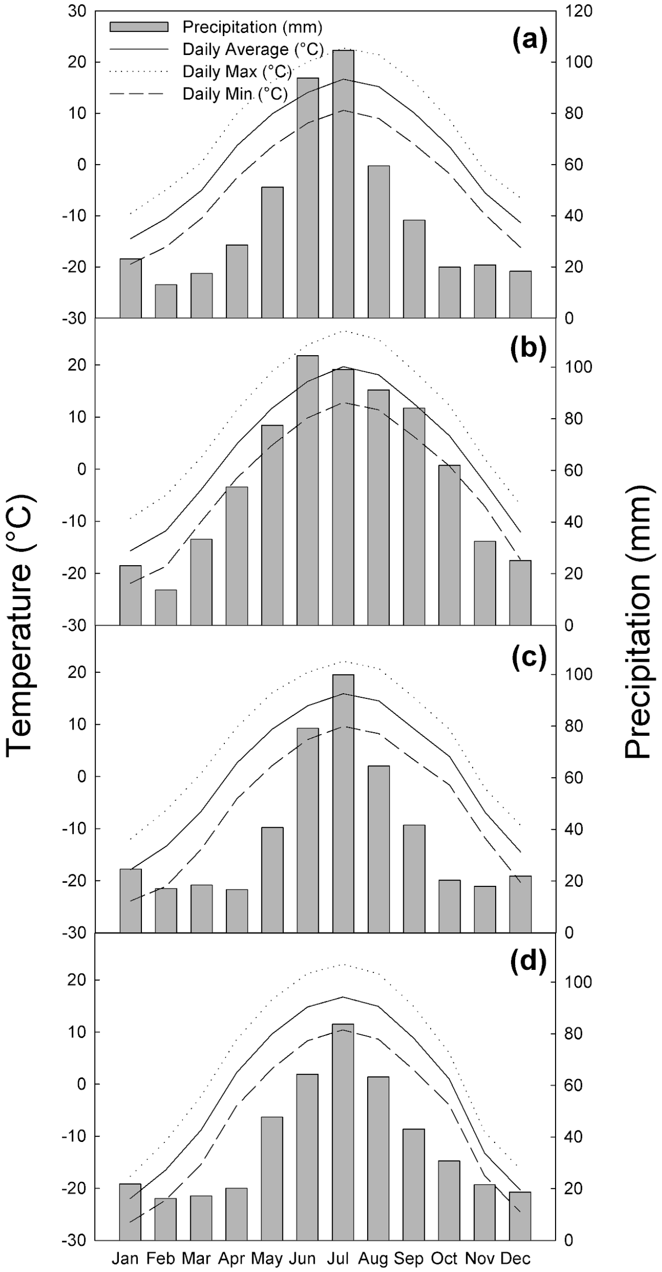

Figure 2.5 – *Characterization of local climate conditions for the planting site (a) and for the three aspen provenances locations Minnesota (b), Alberta (c) and British Columbia (d). Climate variables were calculated using a time period from 1993-2007 (a) and 1961-1990 climate normals for the aspen provenance locations (b,c,d).*

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CHAPTER 3

Sixteen years of winter stress: an assessment of cold hardiness, growth performance and survival of hybrid poplar clones at a boreal planting site[1]

Contents

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[1] A version of this chapter has been accepted for publication. Schreiber, S.G., Hamann, A., Hacke, U.G., & Thomas, B.R. 2012. *Sixteen years of winter stress: an assessment of cold hardiness, growth performance and survival of hybrid poplar clones at a boreal planting site*. Plant, Cell & Environment. DOI: 10.1111/j.1365-3040.2012.02583.x

3.1 Summary

In recent years, thousands of hectares of hybrid poplar plantations have been established in Canada for the purpose of carbon sequestration and wood production. However, boreal planting environments pose special challenges that may compromise the long-term survival and productivity of such plantations. In this study, we evaluated the effect of winter stress, i.e. frequent freeze-thaw and extreme cold events, on growth and survival of 47 hybrid poplar clones in a long-term field experiment. We further assessed physiological and structural traits that are potentially important for cold tolerance for a selected set of seven clones. We found that trees with narrow xylem vessels showed reduced freezing-induced embolism and showed superior productivity after 16 growing seasons. With respect to cold hardiness of living tissues, we only observed small differences among clones in early autumn, which were nonetheless significantly correlated to growth. Maximum winter cold hardiness and the timing of leaf senescence and budbreak were not related to growth or survival. In conclusion, our data suggests that reduction of freezing-induced embolism due to small vessel diameters is an essential adaptive trait to ensure long-term productivity of hybrid poplar plantations in boreal planting environments.

3.2 Introduction

Intensive plantation forestry with fast-growing hybrid poplars has been advocated for its CO_2 sequestration potential, and in Canada thousands of hectares of hybrid poplar plantations have been established under the federal *Forest 2020* afforestation initiative to help meet greenhouse gas reduction targets (Dominy *et al.*, 2010). The boreal and sub-boreal planting environments throughout Canada pose special challenges however, and planting stock needs to be well adapted to harsh winter conditions. While drought tolerance and productivity of hybrid poplars has been relatively well researched (Monclus *et al.*, 2006, Hogg *et al.*, 2008, Silim *et al.*, 2009, Soolanayakanahally *et al.*, 2009, Schreiber *et al.*, 2011), studies that investigate winter biological traits of hybrid poplars are limited, particularly linking these traits with growth performance (cf. McCamant & Black, 2000, Tsarouhas *et al.*, 2001, Friedman *et al.*, 2008). Specifically, extreme minimum temperatures and frequent freeze-thaw events may play an important role in survival and long-term productivity of hybrid poplar plantations in boreal planting environments.

Plants exposed to frequent freeze-thaw events must develop mechanisms to overcome the stress applied to their organs to remain functional. A recent study by Schreiber *et al.* (2011) showed a strong negative relationship between height and branch vessel diameters of hybrid poplars (*Populus* spp.) as well as trembling aspen (*Populus tremuloides* Michx.) at a boreal planting site. This planting site is characterized by frequent freeze-thaw events in spring, and winter minimum temperatures often as low as -40 °C. A possible mechanism explaining the unexpected negative relationship between growth and vessel diameter may be the occurrence of frost-induced embolisms. It has been frequently shown that wider xylem conduits are more likely to embolize when exposed to frequent freeze-thaw events (Davis *et al.*, 1999, Mayr *et al.*, 2003a, Pittermann & Sperry, 2003, Cobb *et al.*, 2007, Choat *et al.*, 2011) and thus trees with wider vessels would experience impaired water conduction after budbreak in spring, decreased photosynthetic rates and eventually reduced growth (Wang *et al.*, 1992, Castro-Diez *et al.*, 1998, Cavender-Bares *et al.*, 2005). This may be particularly pronounced in poplars since they do not seem to refill winter embolism by developing root pressure, a behaviour that for example is seen in birch, or alder species (Sperry *et al.*, 1994, Hacke & Sauter, 1996).

Freeze-thaw events, besides inducing embolism, may also significantly contribute to frost injury and shoot dieback over winter. Living tissues are mostly affected when water transitions from the liquid into the crystalline state causing plasma membrane destruction and eventually cell death (Sakai & Larcher, 1987). Injury to tissues can also occur by means of extracellular freezing, resulting in considerable desiccation stress on the protoplasm as a result of water removal from cells (Burke *et al.*, 1976). Multiple freeze-thaw events during winter may amplify the extent of frost injury due to mechanical wear of cell components, caused by volume changes of water from the liquid into the crystalline state and vice versa. Frost injury damage often results in cankers, dieback, frost cracked stems and distortion of developing organs (Cayford *et al.*, 1959, Zalasky, 1976). Late spring frosts can also severely harm a tree in which growth is already initiated and early tissues are not lignified, particularly due to warmer winters and earlier dormancy release (Beaubien & Hamann, 2011).

In this present study we analyzed a long-term, repeatedly measured field experiment with 47 hybrid poplar clones in Alberta, Canada. Height and winter survival were evaluated in autumn and spring for the first four years after planting. Subsequently, height, diameter at breast height, and survival were measured at the end of an additional 12 growing seasons. Further, we evaluated physiological and structural traits that are considered potentially important for cold adaptation. The timing of leaf senescence and budbreak was quantified for all clones, and in a selected subset of seven clones we measured the amount of native xylem embolism in autumn, winter and spring of 2010 and 2011, as well as frost hardiness of living tissue several times throughout autumn of 2011. The objective of this paper is to identify traits relevant for cold adaptation, ensuring survival and long-term productivity of hybrid poplars in a boreal planting environment. Specifically, we hypothesized that the degree of freezing-induced embolism may play an important role in cold adaptation and growth performance of hybrid poplars based on previous research (Schreiber *et al.*, 2011) in which the authors found a strong negative relationship between vessel diameter and tree height at a boreal planting site. We also evaluated repeated field measurements in conjunction with historical climate data to understand genetic differences in growth and survival among clones as a function of environmental stressors. This research may help to guide selection of appropriate hybrid poplar clones and species for future afforestation efforts in boreal environments by identifying key elements in adaptation to winter stress.

3.3 Materials and Methods

3.3.1 Plant material

We evaluated a hybrid poplar field trial established by Alberta-Pacific Forest Industries Inc. near Boyle, Alberta, Canada (54° 49′N, 113° 3′W, 570 *m a.s.l.*). The trial was established with 47 clones in June 1993 using over-winter dormant stock and planted in a randomized complete block design with five blocks and five-tree row plots of each clone in each block (*Table 3.1*). Trees were planted on a 2 *m* within row by 3 *m* between row spacing. The trial was managed intensively for the first three years using cultivation and hand weeding, after which time pulp mat sheets were placed around each tree to control competition. *Height* and *DBH* (Diameter at breast height) measurements for all clones were taken annually in autumn until 2008, except for the year 1998, and additional height measurements in spring were recorded for the years 1994-1997 to capture winter dieback during the first four years after planting. Since height and *DBH* in 2008 were highly correlated ($r = 0.94$, $P < 0.05$), we only discuss one trait, but the complete set of measurements is provided in *Table 3.1*. Other measurements taken on all individuals of the experiment were the timing of leaf senescence and budbreak (see details below).

For the evaluation of physiological and wood structural traits, we sampled a total of seven clones with contrasting growth performances for subsequent testing. These clones were labelled as *High*, *Average* and *Poor* performing genotypes based on height in 2008 and survival (*Fig. 3.1*, *Table 3.2*). The chosen subset included two clones of each performance group plus the *Walker* clone as a reference, since it is widely used in plantations and shelterbelts in western Canada. Cold hardiness, xylem vessel diameter and the amount of native embolism were measured on these seven clones with eight replicates per clone on multiple dates (see sections below). All samples were taken from 2-3 year-old branches from approximately 6 *m* using a 4 *m* telescope pruner and were processed within the next seven days. The material was packed in plastic bags and stored at 4 °*C* in a walk-in refrigerator at the University of Alberta.

3.3.2 Climate data

Daily minimum and maximum temperatures (*TMIN*, *TMAX*) for the period from 1980 to 2009 were obtained from the National Climate Data and Information Archive (`http://www.climate.weatheroffice.gc.ca`) for the climate station Athabasca 2 (Station ID 3060321). Furthermore, a 30-year daily average temperature (*TAV*) was calculated to identify seasonal climate anomalies. Winter dieback, measured in metres, was calculated as the difference in height between the spring measurement of a given year and the preceding height measurement in autumn. Freeze-thaw events throughout the winter season were calculated as the difference between *TMAX* and *TMIN* for only those days on which *TMAX* was equal to or greater than 5 °*C* and *TMIN* equal to or less than -5 °*C* (*Fig. 3.2* & *Fig. 3.5*).

3.3.3 Spring and autumn phenology

Timing of leaf senescence and budbreak were measured in autumn 2010 and spring 2011, respectively, on an eight-level senescence scale based on Fracheboud *et al.* (2009) and a five-level bud development scale (*Table 3.3*). Leaf senescence was measured on 21-Sep, 23-Sep, 25-Sep, 28-Sep and 02-Oct, and budbreak on 08-May, 11-May, 13-May, 15-May and 17-May. The average day of year at which score 4 (more yellow than green leaves, representing timing of senescence) and score 2 (leaves extended but unfolded, representing the timing of budbreak) were calculated for each individual tree. If the required score was recorded multiple times, the date of the phenological event was calculated as an average. If the required score was not directly recorded, the date of the phenology event was inferred by means of linear regression from the bracketing scores.

3.3.4 Cold hardiness

Cold hardiness of living tissue was measured using the electrolyte leakage method (Zhang & Willison, 1987, Morin *et al.*, 2007), which quantifies the amount of frost damage in living tissue by measuring the electrolyte leakage from the symplast into the apoplast due to damaged plasma membranes. Plant material was collected in 2011 on 22-Aug, 12-Sep and 10-Oct. Current year branches were cut into 5 cm pieces and placed in 30 ml high-density polyethylene bottles (Fisherbrand, Fisher Scientific). To induce ice formation, 5 ml of deionized water was added to the sample before freezing treatments were applied. The freezing treatments were 8 $^{\circ}C$ (control), -5 $^{\circ}C$, -10 $^{\circ}C$, -20 $^{\circ}C$, -40 $^{\circ}C$ on 22-Aug; 8 $^{\circ}C$ (control), -10 $^{\circ}C$, -20 $^{\circ}C$, -40 $^{\circ}C$, -60 $^{\circ}C$ on 12-Sep; and 8 $^{\circ}C$ (control), -10 $^{\circ}C$, -20 $^{\circ}C$, -40 $^{\circ}C$, -80 $^{\circ}C$ on 10-Oct. A programmable freezer (Model 85-3.1A, Scientemp Corp., Adrian, MI, USA) was used to cool samples at a rate of 7 $^{\circ}C$ per hour, holding the target temperature for one hour, before re-warming to 8 $^{\circ}C$. Subsequently, each segment was cut into 5 mm pieces, 20 ml deionized water was added, and samples were stored for 15-18 hours at 8 $^{\circ}C$, and manually shaken three times during storage. After storage, the relative amount of electrolyte leakage (%REL) was measured at room temperature using a conductivity meter (Oakton Acorn CON 6 Meter, Oakton Instruments, Vernon Hills, IL, USA) and conductivity readings were taken before (c1) and after (c2) all samples were boiled at 100 $^{\circ}C$ for 30 min. REL was calculated as (c1/c2)×100 and used to determine the amount of cell lysis (L) in percent, where $\overline{REL_C}$ is the mean value of the control samples: $L = \frac{REL-\overline{REL_C}}{100-\overline{REL_C}} \times 100$

3.3.5 Native embolism

Percent native embolism (PLC_N) was measured using the flushing method (Sperry *et al.*, 1988). Long branches were cut from trees in 2010 and 2011 on 02-Oct, 08-Apr and 23-May. In the laboratory, two 14 cm-long segments were cut from these branches under water and hydraulic conductivity (K_h) was measured as described previously (Hacke & Jansen, 2009). Briefly, K_h was calculated as the quotient of flow rate through a segment and the pressure gradient. The tubing apparatus consisted of an elevated water reservoir connected to an electronic balance (CP225D; Sartorius, Göttingen, Germany) via Tygon tubing. The balance was interfaced with a computer using Collect 6 software (Labtronics, Guelph, Canada) and logged K_h every 10 seconds. An initial measurement represented the native conductivity of the segment. The segment was then flushed with filtered (0.2 mm) measuring solution (20 $mM\ KCl$ + 1 $mM\ CaCl_2$) at 40 kPa for 15 min and the maximum conductivity was determined. PLC_N was calculated as the percentage loss of conductivity relative to the maximum conductivity.

3.3.6 Vessel diameter

Xylem vessel diameters (D_V) were taken from a previous study (Schreiber *et al.*, 2011) for analysis in a new context. All other traits in this study were measured on the same individual trees as xylem vessel diameters in Schreiber *et al.* (2011). Briefly, mean hydraulic vessel diameters were calculated based on the Hagen-Poiseuille equation. The vessel diameter that corresponds to the average lumen conductivity was calculated as $D_V = [(\Sigma\, d^4)/n]^{1/4}$, where n is the number of vessels measured, and d is the individual vessel lumen diameter.

3.3.7 Statistical analysis

Statistical analyses were carried out using the R programming environment (R Development Core Team, 2011). Data exploration and plotting were carried out using the R packages *plyr* (Wickham, 2011) and *ggplot2* (Wickham, 2009). To take advantage of the blocked experimental design, the data were analyzed using a mixed effects model available through the R package lme4 (Bates *et al.*, 2011) with the *lmer()* function. The dependent variables were native embolism (PLC_N) and cell lysis (L) and the fixed effects in this model were *group* (*Walker*, *High*, *Average* and

Poor) and *sample date*. The random terms were *block*, *clone* and each clone's unique *ID* to remove temporal pseudoreplication for multiple measurements on the same clone at different dates. In cases where inferences were based on multiple statistics, experiment-wise P-values were reported using the Holmes adjustment according to Peres-Neto (1999) for multiple correlations, or using the Tukey adjustment for multiple mean comparisons after ANOVA.

3.4 Results

3.4.1 Growth and survival in the field

Survival and height after 16 growing seasons was highly variable among the 47 clones tested in the field experiment (*Fig. 3.1*, *Table 3.1*). Overall, height and survival were significantly correlated (r = 0.46, P = 0.009, df = 42 due to three clones that had no surviving individuals by the final measurement). The high performing clones as well as the *Walker* clone showed high survival (84 %, 87.5 % and 96 %) and were among the tallest trees after 16 growing season (14.3 m, 13.7 m and 14 m). The average performing clones showed low survival (35.3 % and 40 %) but were among the tallest trees (12.3 m and 11.9 m). The poor performing trees had intermediate survival rates (60 % and 65 %) but were the shortest of the seven clones (6.2 m and 8.3 m).

Putative frost damage, measured as the height of each tree in autumn minus the height of the leader after budbreak in spring is shown in *Fig. 3.2a*. Of the first four years, the 1995/96 winter appears the most extreme with a variety of potential stress conditions (*Fig. 3.2b*, see *Fig. 3.3* for data from all winter seasons). The 1995/96 winter season shows very low extreme cold events exceeding -40 °C, which are rare events that were only observed at four other dates between 1980 and 2009 (data not shown). In addition, winter temperatures in 1996 were highly fluctuating with unusual warm periods in January and the first half of February followed by large temperature drops of 35 to 50 °C. Furthermore, a late spring frost in early

May 1996 was recorded with minimum temperatures of -7 °C. The 1995/96 winter season coincided with a dieback of ∼1.35 m of the two clones 32 and 52, selected from the poor performance group (*Fig. 3.2a*). Other clones from the same performance group suffered an average dieback of ∼30 *cm* during the 1995/96 winter season. No significant dieback was observed in the other performance groups.

3.4.2 Cold hardiness

The onset of cold hardiness of living tissue, evaluated as percent cell lysis (%*L*), occurred relatively early, between mid-August and mid-September on all hybrid poplars tested (*Fig. 3.4a*). Late summer measurements showed significant differences between performance groups at -10 °C and -20 °C on 22-August (*Table 3.4*). It should be noted that frost events of -10 °C or -20 °C in mid-August are extremely unlikely, and were not observed over the course of this field experiment (data not shown). Nevertheless, we note that cell lysis at -10 °C on 22-August significantly correlated with height (*Fig. 3.4b*). For both, cell lysis at -10 °C and -20 °C in late summer, significant differences were found between the poor and high performing groups (*Table 3.4*). In contrast, by mid-September and especially mid-October, all clones appear to be well hardened. By early October, cell lysis was low even when tested under extreme artificial freezing conditions of -40 °C and -80 °C (*Table 3.4*). While we did observe significant differences between average and poor performing groups in October, these differences were not correlated with height as in August and remained below 33 % (*Table 3.4* & *Table 3.5*).

3.4.3 Native Embolism

The degree of native embolism was measured three times (*Fig. 3.5*). The first measurement was taken at the end of the growing season in 2010 and prior to any major frost event in order to act as a baseline or control; the second measurement was taken during the peak period of freeze-thaw events in early April 2011, when plants were still dormant; and finally, the third measurement was taken well into the subsequent growing season at the end of May 2011. Performance groups differed significantly at the 02-Oct and 23-May measurements, but not at the 08-Apr measurement (*Fig. 3.5a*, *Table 3.4*). Within groups and across dates only the average and poor performing groups differed significantly in the amount of embolism when measured on different dates (*Table 3.4*). No significant differences in native embolism were found for *Walker* and the high performing group across different dates. Notably, native embolism in May was positively correlated with vessel diameter (*Fig. 3.6a*) and negatively correlated with tree height (*Fig. 3.6b*).

3.4.4 Timing of budbreak and leaf senescence

The onset of leaf senescence in autumn, and the timing of budbreak in spring appeared to be remarkably uniform among performance groups, which showed virtually identical timing (*Table 3.4*). In addition, variance of clones within performance groups was minimal, with all clones breaking bud or showing leaf senescence within a week (*Fig. 3.7*). No significant correlations of leaf senescence and budbreak with native embolism, vessel diameter or height were found.

3.5 Discussion

Our results allow us to investigate and discuss several alternative mechanisms that may play a role in cold adaptation and potentially impacting growth performance of boreal forest trees. The synchrony of budbreak and leaf senescence with the available growing season, the timely onset of frost hardiness and absolute winter hardiness, and the structural xylem properties are all potentially important traits for cold adaptation. The most notable result appears to be a strong differentiation of performance groups in measurements of native embolism (*Fig. 3.5*), a strong positive correlation of native embolism with vessel diameter (*Fig. 3.6a*), and a strongly negative correlation of native embolism with height (*Fig. 3.6b*). Notably, these correlations are not just a result of a size effect, which has been previously discussed in Schreiber *et al.* (2011). In our study, branches were similar in age and were taken from the same height, regardless of the size of trees. In this situation, vessel diameters may be expected to be wider in larger trees than in smaller ones (Weitz *et al.*, 2006). We observed the opposite, suggesting that the trend in vessel diameter was not a consequence of a size effect.

These results suggest that narrow vessel diameters minimize the extent of freezing induced embolism. Embolized xylem tissue would result in decreased hydraulic conductivity, which in turn limits photosynthetic rates and eventually growth (Wang *et al.*, 1992, Castro-Diez *et al.*, 1998, Tyree, 2003, Cavender-Bares *et al.*, 2005). Similarly to height, survival was negatively correlated with vessel diameter (r = -0.56, P = 0.192), cell lysis (r = -0.39, P = 0.394) and native embolism (r = -0.70, P = 0.107). While none of these correlations were significant, the trends do indicate that under boreal planting environments, there appear to be no fundamental differences in trade-offs with respect to height versus survival. Adaptive traits that increase the probability of survival (e.g. small vessel diameters) also result in larger trees after multiple growing seasons. Hence, narrower vessels would explain the observed greater height for trees in an environment that is characterized by frequent freeze-thaw events. A negative correlation between vessel diameter and tree height is likely restricted to boreal or high elevation environments (e.g. Fisher *et al.*, 2007, Schreiber *et al.*, 2011), while an opposite correlation has been observed in tropical environments (e.g Zach *et al.*, 2010, Poorter *et al.*, 2010, Fan *et al.*, 2012).

Further, our data showed that the amount of native embolism (PLC_N) decreases over winter and increased again right after budbreak at the start of the growing season (*Fig. 3.5*). A decrease in PLC_N from autumn to winter was not expected and may be due to recovery of embolized vessels. Mayr *et al.* (2003b) observed similar trends for conifers at the alpine timber line and proposed the existence of refilling mechanisms that enable species to recover from embolism in late winter. In diffuse-porous beech trees (*Fagus sylvatica*) Cochard *et al.* (2001) observed similar results and postulated restoration of branch hydraulic conductivity due to a combination of active refilling of embolized vessels through root pressure (although rather weak in beech), and through the formation of new functional xylem after cambial activity was initiated. In the current study however, we observed a recovery mechanism that must have occurred before growth was initiated (*Fig. 3.5*). Since poplars are not known for developing root pressure, an alternate hypothesis may be that in late autumn when soils were still unfrozen and leaves were shed, near atmospheric xylem pressures led to bubble dissolution.

Figure 3.5a,b, as well as *Table 3.4* also show a significant increase in PLC_N for the average and poor performing groups when measured on 08-Apr and 23-May of 2011, indicating a possible threshold vessel diameter at which freezing induced embolism increased significantly. The reference clone *Walker*, as well as the high performing group, did not show significant differences between the three dates at which PLC_N was measured (*Table 3.4*) suggesting an optimal mean vessel diameter of < 28 μm (*Fig. 3.5a*) given the local climate conditions. Previous studies (Davis *et al.*, 1999, Pittermann & Sperry, 2003) demonstrated that plants with a mean conduit diameter below 30 μm experienced little embolism following a single freeze-thaw event at a xylem pressure of -0.5 MPa while species with conduit diameters greater than 30 μm exhibited considerable embolism. Fisher *et al.* (2007) also observed mean vessel diameters of 27.5 μm for high elevation populations of *Metrosideros polymorpha* experiencing occasional freezing, compared to 35.5 and 32.9 μm for populations found at middle and low elevation experiencing no freezing. The critical conduit diameter likely depends on several factors including xylem pressure, the number of freeze-thaw cycles, the minimum freezing temperature, and length of the freezing period (Cavender-Bares & Holbrook, 2001, Mayr *et al.*, 2003b, Pratt *et al.*, 2005, Choat *et al.*, 2011). Taken together, these results underpin the adaptive significance of vessel diameter in influencing tree height and performance in an environment characterized by frequent freeze-thaw events, and that mean vessel diameter may be an important trait to consider for poplar breeding programs in the Canadian prairies.

As a possible alternative trait that could be important for cold tolerance, we tested the effect of frost injury, measured as percent cell lysis (%*L*), on tree height (*Fig. 3.4*). These results only show clear differences in clonal performance for %*L* at -10 °C and only for trees sampled on 22-Aug, implying a very early date for the onset of frost hardiness. *High* performing trees appear to be hardier than average and poor performing trees in August and were hardy enough to sustain moderate sub-zero temperatures without major damage. By the end of August all groups were hardy enough to sustain moderate sub-zero temperatures without major damage. By October, all clones could withstand -40 °C frost events that were extremely rare in the field, even in mid-winter. Once hardy, these clones even withstood extreme experimental treatments of -80 °C in October, which agrees with previous research showing that poplar cells can, once hardy, survive extreme freezing through vitrification (Hirsh *et al.*, 1985). We therefore conclude that damage of living tissue due to lack of cold hardiness in hybrid poplar clones cannot serve as a defendable explanation for the observed differences in performance. Although we observed a negative relationship of cell lysis in August with performance, the observed correlation cannot explain differences in growth since August temperatures did not fall to -10 °C at our study site over the entire 16-year period.

During the winter of 1995/96, we observed severe dieback of poor performing clones (*Fig. 3.2a*). *Fig. 3.2b* demonstrates that this winter was characterized by highly fluctuating daily temperatures. Temperatures in January varied between +10 °C and -42 °C, in February between +8 °C and -41 °C, and in March between +14 °C and -32 °C. April appeared to be normal relative to the 30-year temperature average. Early May was characterized by a distinctive temperature drop which can be considered a late spring frost. We neither observed an unusually low amount of snow, which could have increased the total degree of frost damage, nor was there an unusual drought event preceding the winter of 1995/96, which could have weakened the trees (data not shown). Hence, we hypothesize that the observed dieback may be a cumulative effect of a high number of freeze-thaw events in winter as well as a late spring frost in May (*Fig. 3.2b*). Parent type (*Table 3.2*) may also play into the vulnerability of these different groups since *Walker* as well as the high and average performing trees share many of the same parent species, e.g. *P. balsamifera*, *P. deltoides* and *P. nigra*, of which *P. balsamifera* is the northernmost North American hardwood; *P. deltoides* is native to the continental and eastern United States, and *P. nigra* to Europe and central Asia. Hybrids of these three species often share characteristics for superior growth performance and survival in boreal planting environments. *P. trichocarpa* on the other hand, a common parent in the poor performing group, is a species of moist and bottomland sites of the Pacific Northwest which may have contributed to the poor performance of its hybrids in the cold and dry Canadian prairies.

Finally, synchronization of budbreak and the onset of leaf senescence with the available growing season could not serve as a plausible explanation for differences among clones in growth and survival. The timing of budbreak in spring appears to be remarkably uniform among performance groups and among tested clones, even though a wide variety of hybrids from diverse genetic backgrounds were included in this field trial (*Table 3.1*).

By excluding several alternate hypotheses, we conclude that the degree of native embolism restricts hydraulic conductivity during the growing season, and ultimately limits tree height and performance in boreal planting environments. Vessel diameter appears to be a key trait responsible for variation in native embolism in environments that experience frequent freeze-thaw events. Interestingly, we did not find significant differences in native embolism over time in the high performing group. This suggests that small vessel diameters minimize freezing induced embolism throughout the year, which in turn maximizes xylem conductivity. We should provide a cautionary note however, since we arrive at our conclusions by exclusion of alternate explanations, and obviously we cannot exhaustively test all conceivable traits that are potentially responsible for cold adaptation, growth and survival. Additional systematic studies in controlled environments and field conditions should strengthen or challenge our conclusions regarding key traits for predicting growth performance of hybrid poplars in boreal environments.

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3.7 Tables

Table 3.1 – *Hybrid poplar clones (Populus spec) with code, parental background, height and DBH data measured in 2008, percent survival for the time period 1993-2008 as well as timing of leaf senescence and budbreak. Clones are ordered based on height in 2008. Standard error is given in parentheses. DoY= Day of Year. Missing values are indicated as n/a*

Clone	Code	Genus	Female parent (species/hybrid)	Male parent (species/hybrid)	Height 2008 (m)	DBH 2008 (cm)	Survival 1993-2008 (%)	Leaf senescence (Score 4; DoY)	Budbreak (Score 2; DoY)
33	P38P38	*P.*	*balsamifera*	*simonii*	14.3 (0.6)	19.8 (0.6)	84	267 (0.2)	134 (0.1)
24	Walker	*P.*	*deltoides*	×*petrowskyana*^{2}	14.0 (0.6)	14.7 (0.6)	96	266 (0.3)	135 (0.1)
8	1014	*P.*	*balsamifera*	*deltoides*	14.0 (1.3)	19.1 (1.3)	50	n/a	133 (0.3)
3	410	*P.*	*balsamifera*	*deltoides*	13.9 (0.9)	17.8 (0.9)	90	n/a	133 (0.2)
42	TACN 1	*P.*	*laurifolia*	*nigra*	13.8 (0.7)	17.4 (0.7)	56	267 (0.3)	134 (0.1)
36	Brooks #1	*P.*	*deltoides*	×*petrowskyana*^{2}	13.7 (0.6)	17.5 (0.6)	87.5	267 (0.3)	135 (0.1)
10	1017	*P.*	*balsamifera*	*deltoides*	13.6 (1.3)	17.3 (1.3)	26.7	n/a	133 (0.3)
9	1016	*P.*	*balsamifera*	*deltoides*	13.4 (1.3)	19.2 (1.3)	80	n/a	133 (0.3)
22	4753	*P.*	*balsamifera*	*deltoides*	13.1 (0.6)	15.2 (0.6)	84.2	265 (0.3)	133 (0.2)
25	Assiniboine	*P.*	*deltoides* ×(×*petrowskyana*^{2})	Unknown	13.1 (0.6)	12.6 (0.6)	80	n/a	134 (0.1)
11	1062	*P.*	*balsamifera*	*deltoides*	12.9 (0.6)	19.1 (0.6)	77.8	n/a	135 (0.1)
47	Brooks #1	*P.*	*deltoides*	×*petrowskyana*^{2}	12.7 (0.6)	16.1 (0.6)	82.6	267 (0.3)	135 (0.1)
26	Manitou	*P.*	*deltoides* ×(×*petrowskyana*^{2})	Unknown	12.6 (0.6)	14.1 (0.6)	84	n/a	134 (0.1)
37	Brooks #1	*P.*	*deltoides*	×*petrowskyana*^{2}	12.6 (0.6)	15.4 (0.6)	52	267 (0.4)	135 (0.1)
27	Northwest	*P.*	*balsamifera*	*deltoides*	12.4 (0.6)	14.5 (0.6)	91.7	n/a	137 (0.2)
18	4435	*P.*	*balsamifera*	×*euramericana*^{3}	12.3 (0.9)	11 (0.9)	35.3	266 (0)	136 (0.2)
23	4914	*P.*	*balsamifera*	*nigra*	12.0 (0.6)	12.7 (0.6)	80	265 (0.1)	132 (0.2)

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Table 3.1 continued

Clone	Code	Genus	Female parent[1] (species/hybrid)	Male parent[1] (species/hybrid)	Height 2008 (m)	DBH 2008 (cm)	Survival 1993-2008 (%)	Leaf senescence (Score 4; DoY)	Budbreak (Score 2; DoY)
12	1080	*P.*	*balsamifera*	*deltoides*	12.0 (0.9)	14.2 (0.9)	90	n/a	135 (0.2)
5	638	*P.*	*balsamifera*	*nigra*	11.9 (0.6)	13.6 (0.6)	88.9	266 (0.5)	134 (0.1)
51	Dunlop	*P.*	*laurifolia*	*nigra*	11.9 (0.6)	13.2 (0.6)	79.2	265 (0.4)	136 (0.1)
38	DN 91	*P.*	*deltoides*	Unknown	11.9 (0.6)	15.2 (0.6)	76	268 (0.6)	136 (0.1)
48	TACN 1	*P.*	*laurifolia*	*nigra*	11.9 (0.7)	13.3 (0.7)	40	267 (0.3)	134 (0.1)
50	Tristis #1	*P.*	*tristis*	*balsamifera*	11.8 (0.6)	12.3 (0.6)	94.7	265 (0.2)	133 (0.1)
21	4714	*P.*	*deltoides* × *trichocarpa*	*balsamifera*	11.8 (0.6)	11.2 (0.6)	76.5	n/a	132 (0.3)
16	4346	*P.*	*balsamifera*	*nigra*	11.8 (0.6)	13.2 (0.6)	52.9	n/a	133 (0.1)
31	DTAC 8	*P.*	*deltoides*	*trichocarpa*	11.5 (1.3)	11.3 (1.3)	12	n/a	n/a
14	4293	*P.*	*nigra* × *trichocarpa*	*balsamifera*	11.1 (0.7)	11.8 (0.7)	75	266 (0.2)	131 (0.1)
4	636	*P.*	*balsamifera*	*nigra*	11.1 (0.7)	11.1 (0.7)	73.7	n/a	132 (0.2)
6	639	*P.*	*balsamifera*	*nigra*	10.9 (0.6)	14.4 (0.6)	75	267 (0.4)	134 (0.1)
15	4299	*P.*	*nigra* × *trichocarpa*	*balsamifera*	10.2 (0.9)	9.9 (0.9)	75	n/a	131 (0)
39	D208	*P.*	*angulata*	*trichocarpa*	10.1 (0.6)	10.9 (0.6)	80	268 (0.5)	134 (0.1)
28	DN 5	*P.*	×*euramericana*[3]	Unknown	9.8 (0.7)	12.3 (0.7)	100	269 (0.8)	136 (0.1)
30	DTAC 19	*P.*	*angulata*	*trichocarpa*	9.5 (0.7)	9.6 (0.7)	65	268 (0.6)	133 (0.3)
20	4563	*P.*	×*jackii*[4]	*balsamifera*	9.2 (0.6)	10.2 (0.6)	52.6	267 (0.1)	134 (0.2)
45	NM 2	*P.*	*nigra*	*maximowiczii*	9.1 (0.6)	10.8 (0.6)	92	272 (0.8)	134 (0.1)
17	4418	*P.*	*balsamifera*	×*jackii*[4] × *grandidentata*	8.9 (0.9)	7.4 (0.9)	90	n/a	135 (0.2)
49	ANS 7	*P.*	*angulata*	*simonii*	8.9 (0.9)	8.1 (0.9)	60	268 (0.6)	136 (0.2)
52	DTAC 24	*P.*	*angulata*	*trichocarpa*	8.3 (0.6)	8.3 (0.6)	65	268 (0.9)	135 (0.2)
46	TXM Mix	*P.*	*trichocarpa*	*maximowiczii*	7.7 (0.6)	5.7 (0.6)	25	269 (0.6)	131 (0.2)

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Table 3.1 continued

Clone	Code	Genus	Female parent[1] (species/hybrid)	Male parent[1] (species/hybrid)	Height 2008 (m)	DBH 2008 (cm)	Survival 1993-2008 (%)	Leaf senescence (Score 4; DoY)	Budbreak (Score 2; DoY)
41	DN 184	*P.*	*deltoides*	*nigra*	6.7 (0.7)	5.3 (0.7)	60	270 (0.4)	134 (0.3)
32	DTAC 22	*P.*	*angulata*	*trichocarpa*	6.2 (0.6)	4.5 (0.6)	60	268 (0.5)	134 (0.1)
19	4523	*P.*	*balsamifera*	*nigra*	5.1 (0.6)	4 (0.6)	70	n/a	134 (0)
35	50-182	*P.*	*trichocarpa*	*deltoides*	2.9 (1.3)	2.1 (1.3)	4	n/a	n/a
34	44-137	*P.*	*trichocarpa*	*deltoides*	2.4 (1.3)	1.3 (1.3)	4	n/a	n/a
40	60-290	*P.*	*deltoides*	*trichocarpa*	n/a	n/a	0	n/a	n/a
43	44-132	*P.*	*deltoides*	*trichocarpa*	n/a	n/a	0	n/a	n/a
44	52-229	*P.*	*deltoides*	*trichocarpa*	n/a	n/a	0	n/a	n/a

[1] Hybrids are designated by an × in front of the parent.

[2] *P.* ×*petrowskyana* is a hybrid of *P. laurifolia* and *P. nigra*.

[3] *P.* ×*euramericana* is a hybrid of *P. deltoides* and *P. nigra*.

[4] *P.* ×*jackii* is a hybrid of *P. balsamifera* and *P. deltoides*.

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Table 3.2 – *Seven selected hybrid poplar clones with code, parental background, performance grouping, height and DBH data measured in 2008 as well as percent survival for the time period 1993-2008. Standard error of the mean is given in parentheses*

Clone ID	**Code/name**	**Genus**	**Female parent[1] (species/hybrid)**	**Male parent[1] (species/hybrid)**	**Performance group**	**Height 2008 (m)**	**DBH 2008 (cm)**	**Survival 1993-2008 (%)**
24	Walker	*Populus*	*deltoides*	× *petrowskyana*[2]	Walker	14.0 (0.6)	14.7 (1.1)	96.0
33	P38P38	*Populus*	*balsamifera*	*simonii*	High	14.3 (0.6)	19.8 (1.1)	84.0
36	Brooks #1	*Populus*	*deltoides*	× *petrowskyana*[2]	High	13.7 (0.6)	17.5 (1.1)	87.5
18	4435	*Populus*	*balsamifera*	× *euramericana*[3]	Average	12.3 (0.9)	11.0 (1.7)	35.3
48	TACN 1	*Populus*	*laurifolia*	*nigra*	Average	11.9 (0.7)	13.3 (1.4)	40.0
52	DTAC 24	*Populus*	*angulata*	*trichocarpa*	Poor	8.3 (0.6)	8.3 (1.2)	65.0
32	DTAC 22	*Populus*	*angulata*	*trichocarpa*	Poor	6.2 (0.6)	4.5 (1.1)	60.0

[1] Hybrids are designated by an × in front of the parent.
[2] *P.* ×*petrowskyana* is a hybrid of *P. laurifolia* and *P. nigra*.
[3] *P.* ×*euramericana* is a hybrid of *P. deltoides* and *P. nigra*.

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Table 3.3 – *Phenology score key to assess timing of leaf senescence and budbreak. Boldface indicates scores and codes used for further analyses in this study.*

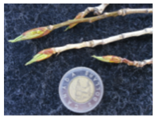
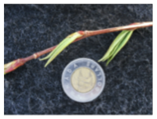
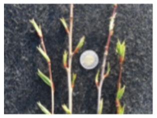
Score	Leaf senescence (Fracheboud *et al.*, 2009)	Budbreak description	Budbreak image
0	leaves uniformly green	buds dormant	
1	more dark green than pale green leaves	buds break	
2	more pale green than dark green leaves	**leaves extending but unfolded**	
3	more green than yellow leaves	leaves extending and partly unfolded	
4	**more yellow than green leaves**	leaves fully unfolded	
5	only yellow leaves		
6	mainly gold or brown leaves		
7	more than 90 % leaf abscission		

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Table 3.4 – *Means (Cell lysis, PLC$_N$) and medians (Day of year), for 7-clone subset and all clones) of physiological and phenological parameters for each performance group. The standard error of the mean is given in parentheses. Different upper case letters indicate significant mean differences after Tukey adjustment for multiple mean comparisons between performance groups for each date (to be read horizontally). Different lower case letters indicate significant differences within performance group for different dates (to be read vertically, only for PLC$_N$). No letters indicate that no statistical significance testing was carried out.*

	Performance group			
Physiological/ phenological parameter	*Walker*	*High*	*Average*	*Poor*
Cell lysis (%)				
22-Aug, -10 °C	34.6 (4.2)^{AB}	25.2 (1.5)^{A}	35.9 (2.3)^{B}	48.4 (2.3)^{C}
22-Aug, -20 °C	73.8 (2.0)^{AB}	70.3 (1.5)^{A}	69.5 (1.9)^{A}	79.1 (1.5)^{B}
11-Oct, -40 °C	4.9 (0.7)^{AB}	14.3 (5)^{AB}	1.4 (0.5)^{B}	18.8 (2.2)^{AC}
11-Oct, -80 °C	17 (2.1)^{AB}	24.6 (4.4)^{AB}	12.7 (2.2)^{B}	32.6 (1.6)^{AC}
PLC$_N$ (%)				
02-Oct	30.0 (4.1)^{AB,a}	21.7 (2.4)^{A,a}	38.3 (3.5)^{BC,b}	47.8 (3.6)^{C,b}
08-Apr	19.8 (3.3)^{A,a}	19.8 (2.6)^{A,a}	19.8 (1.8)^{A,a}	28.0 (3.0)^{A,a}
23-May	26.8 (3.7)^{A,a}	31.7 (3.0)^{AB,a}	45.7 (5.1)^{BC,b}	57.0 (3.5)^{C,b}
Phenology (day of year)				
Leaf abscission, score 4	267 (24-Sep)	267 (24-Sep)	267 (24-Sep)	266.5 (23-Sep)
Budbreak, score 2	135 (15-May)	135 (15-May)	135 (15-May)	134 (14-May)

Table 3.5 – *Mean cell lysis in % for seven selected clones by performance groups for all sampling dates and temperatures.*

Performance group	Temperature	Sampling date	Cell lysis (%)	Standard error
Walker	-5	11-08-22	1.6	0.6
High	-5	11-08-22	2.3	0.7
Average	-5	11-08-22	1.4	0.4
Poor	-5	11-08-22	4.6	1.5
Walker	-10	11-08-22	34.6	4.2
High	-10	11-08-22	25.2	1.5
Average	-10	11-08-22	35.9	2.3
Poor	-10	11-08-22	48.4	2.3
Walker	-20	11-08-22	73.8	2
High	-20	11-08-22	70.3	1.5
Average	-20	11-08-22	69.5	1.9
Poor	-20	11-08-22	79.1	1.5
Walker	-40	11-08-22	73.3	2.5
High	-40	11-08-22	73.9	2
Average	-40	11-08-22	74.3	2
Poor	-40	11-08-22	80.3	1.2
Walker	-10	11-09-12	8.2	2.6
High	-10	11-09-12	5.7	2
Average	-10	11-09-12	6	2.2
Poor	-10	11-09-12	6.9	3.9
Walker	-20	11-09-12	68.1	2.4
High	-20	11-09-12	66.4	1.7
Average	-20	11-09-12	67.3	1.1
Poor	-20	11-09-12	73	0.8
Walker	-40	11-09-12	71.2	2.5
High	-40	11-09-12	69.8	1.5
Average	-40	11-09-12	66.5	1.6
Poor	-40	11-09-12	74.2	1.3
Walker	-60	11-09-12	75.6	1.1
High	-60	11-09-12	72.2	1.9
Average	-60	11-09-12	70.6	1.1
Poor	-60	11-09-12	75.5	1

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Table 3.5 continued

Performance group	Temperature	Sampling date	Cell lysis (%)	Standard error
Walker	-10	11-10-11	0.3	0.2
High	-10	11-10-11	4.8	2.4
Average	-10	11-10-11	1.2	0.5
Poor	-10	11-10-11	2.9	0.7
Walker	-20	11-10-11	6.1	1.3
High	-20	11-10-11	12.9	5
Average	-20	11-10-11	2.9	1.1
Poor	-20	11-10-11	14	2.3
Walker	-40	11-10-11	4.9	0.7
High	-40	11-10-11	14.3	5
Average	-40	11-10-11	1.4	0.5
Poor	-40	11-10-11	18.8	2.2
Walker	-80	11-10-11	17	2.1
High	-80	11-10-11	24.6	4.4
Average	-80	11-10-11	12.7	2.2
Poor	-80	11-10-11	32.6	1.6

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3.8 Figures

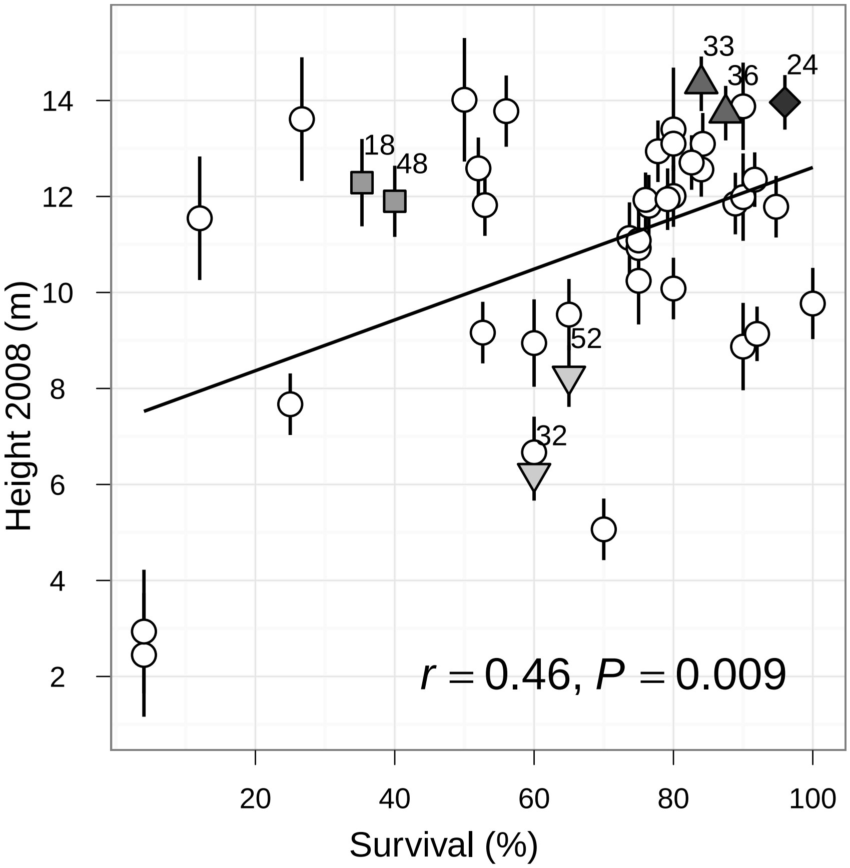

Figure 3.1 – *Relationship of average height and survival of each clone in 2008 after 16 growing seasons. Survival, expressed in percent, was calculated as individual tree count per clone at the end of 2008. Symbols represent performance groups: Triangles: High performer; Squares: Average performer; upside down triangles: Poor performer; Diamond: Reference clone Walker. A grey fill indicates clones that were selected for physiological measurements in this study.*

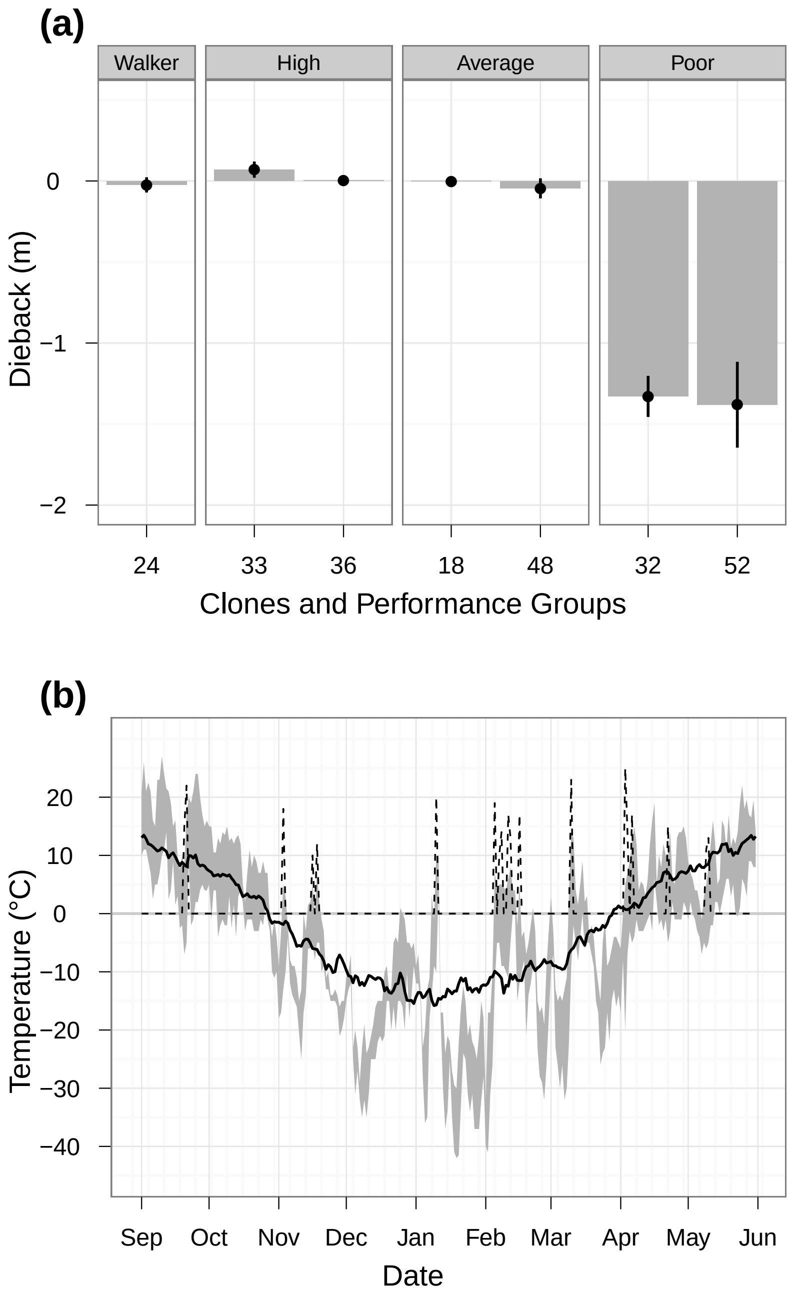

Figure 3.2 – *(a) Mean winter dieback for the season 1995/96 in metres for seven selected clones grouped by performance (Walker, High, Average, Poor). Error bars represent the standard error of the mean. (b) Daily minimum and maximum temperature for the winter season 1995/96 (grey-shaded ribbon) and the 30-year daily average temperature (solid black line) for the time period 1980-2009. The dashed grey line represents freeze-thaw events for days when TMIN was equal or less than -5 °C and TMAX equal or greater than +5 °C (see text for details).*

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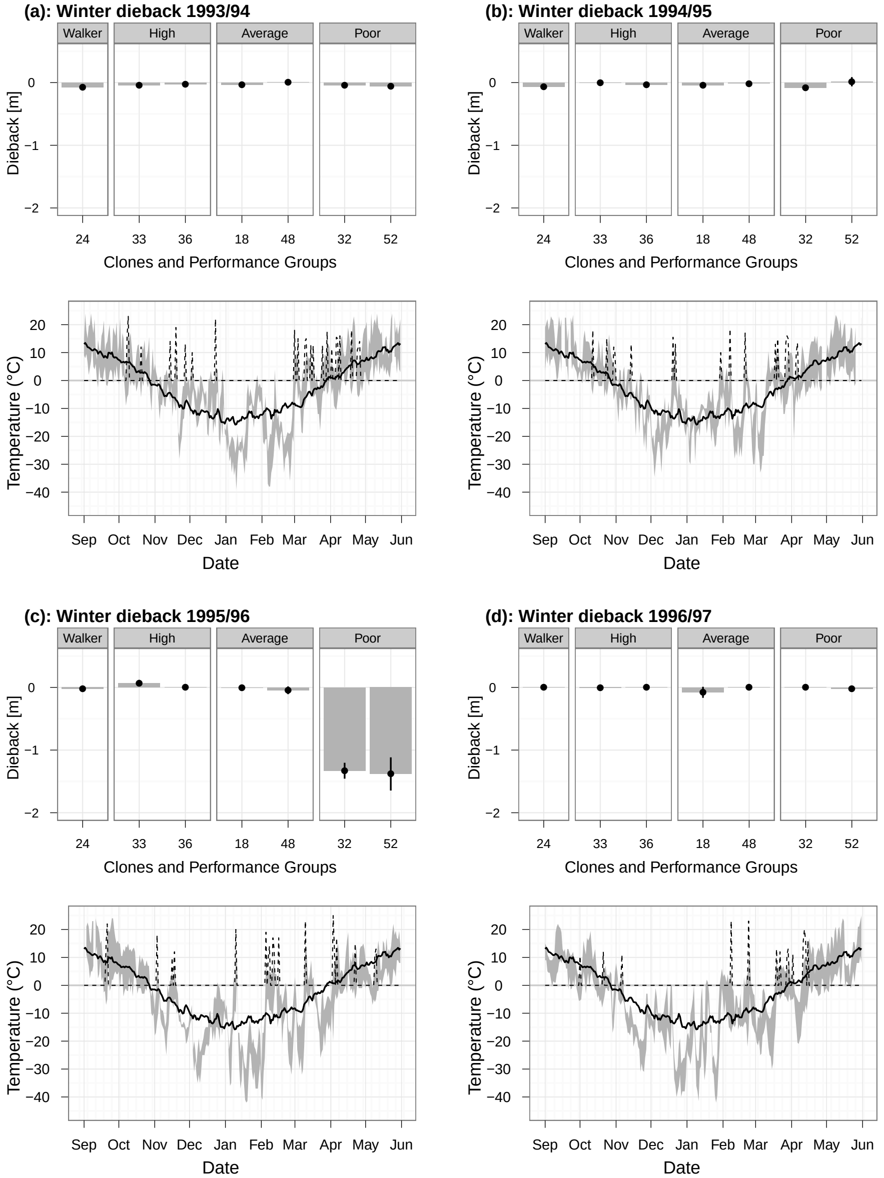
(a): Winter dieback 1993/94

(b): Winter dieback 1994/95

(c): Winter dieback 1995/96

(d): Winter dieback 1996/97

Figure 3.3 – *Mean winter dieback and the respective climate for the years 1993/94, 1994/95, 1995/96 and 1996/97. Dieback is given in metres for seven selected clones grouped by performance (Walker, High, Average, Poor). Error bars represent the standard error of the mean. Daily minimum and maximum temperatures are shown as a grey-shaded ribbon and the 30-year average daily temperature for the period 1980-2009 as a solid black line. The dashed grey line represents freeze-thaw events for days when TMIN was equal or less than -5 °C and TMAX equal or greater than +5 °C (see text for details).*

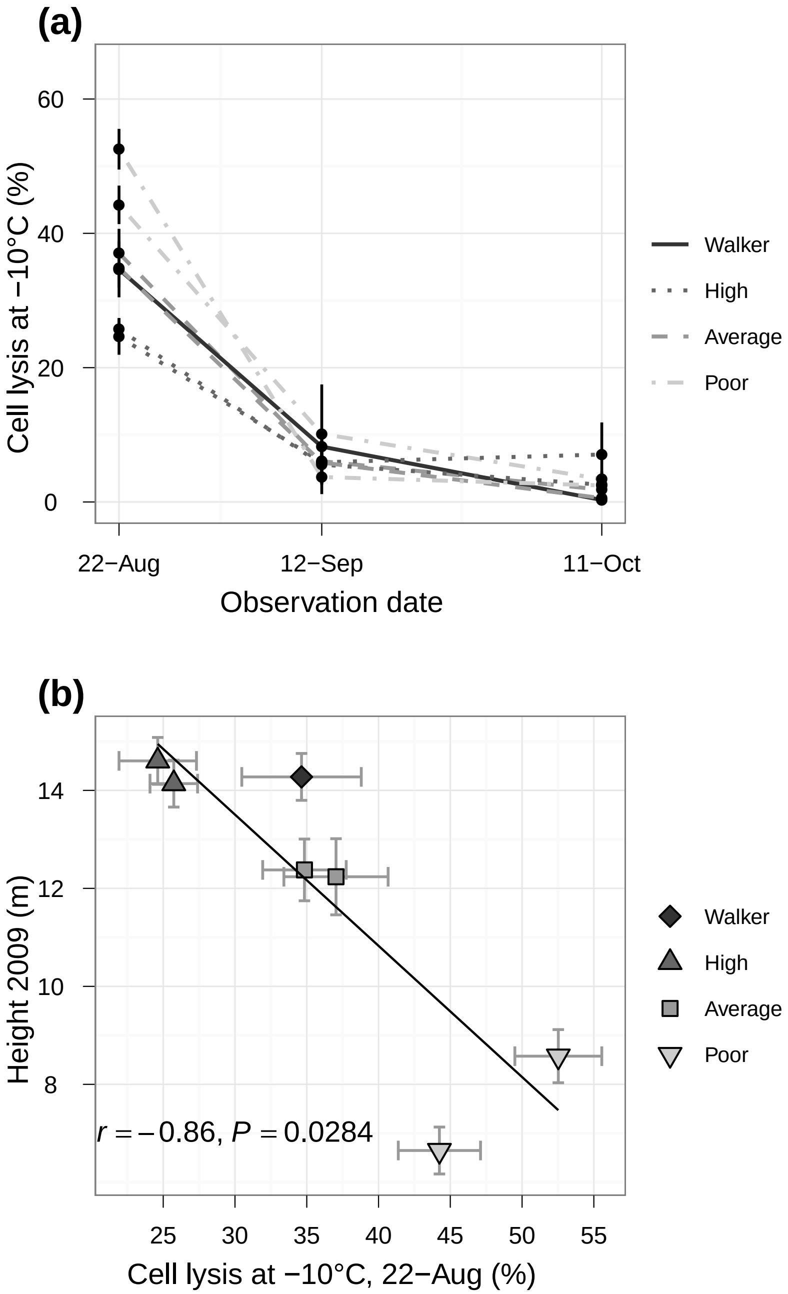

Figure 3.4 – *(a) Mean cell lysis at -10 °C measured three times in autumn 2011 (22-Aug, 12-Sep and 11-Oct). A solid line signifies the Walker clone, a dotted line individuals of the high performing group, a dashed line the average performing group and a dot-dashed line the poor performing group. (b) Correlation between tree height and average cell lysis at -10 °C for 22-Aug. Error bars represent the standard error of the mean.*

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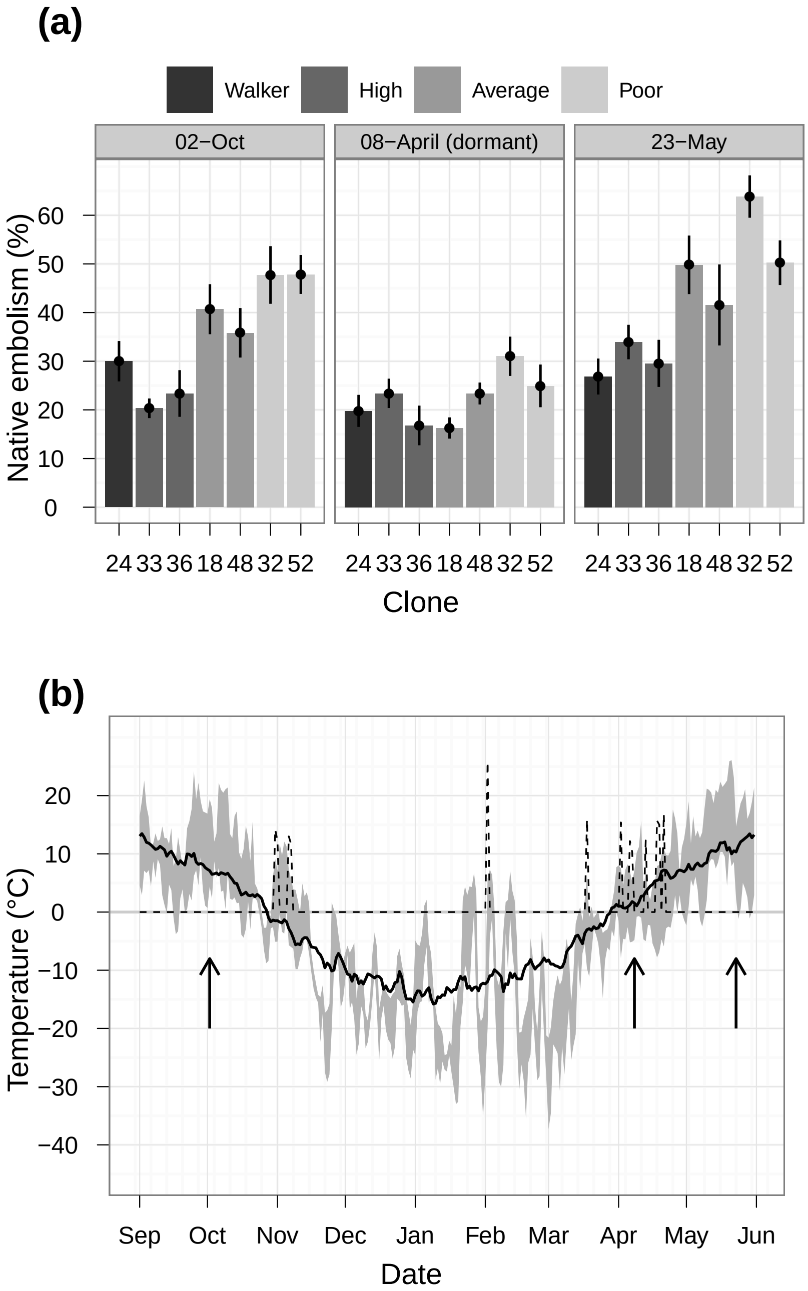

Figure 3.5 – *(a) Mean native embolism in percent measured over three dates in 2010/11 (02-Oct, 08-Apr, 23-May) is given for the seven selected clones grouped by performance (Walker, High, Average and Poor). Error bars represent the standard error of the mean. (b) Daily minimum and maximum temperature for the winter season 2010/2011 (grey-shaded ribbon) and the 30-year daily average temperature (solid black line) for the time period 1980-2009. The dashed grey line represents freeze-thaw events for days when TMIN was equal or less than -5 °C and TMAX equal or greater than +5 °C (see text for details). The arrows indicate the sample dates.*

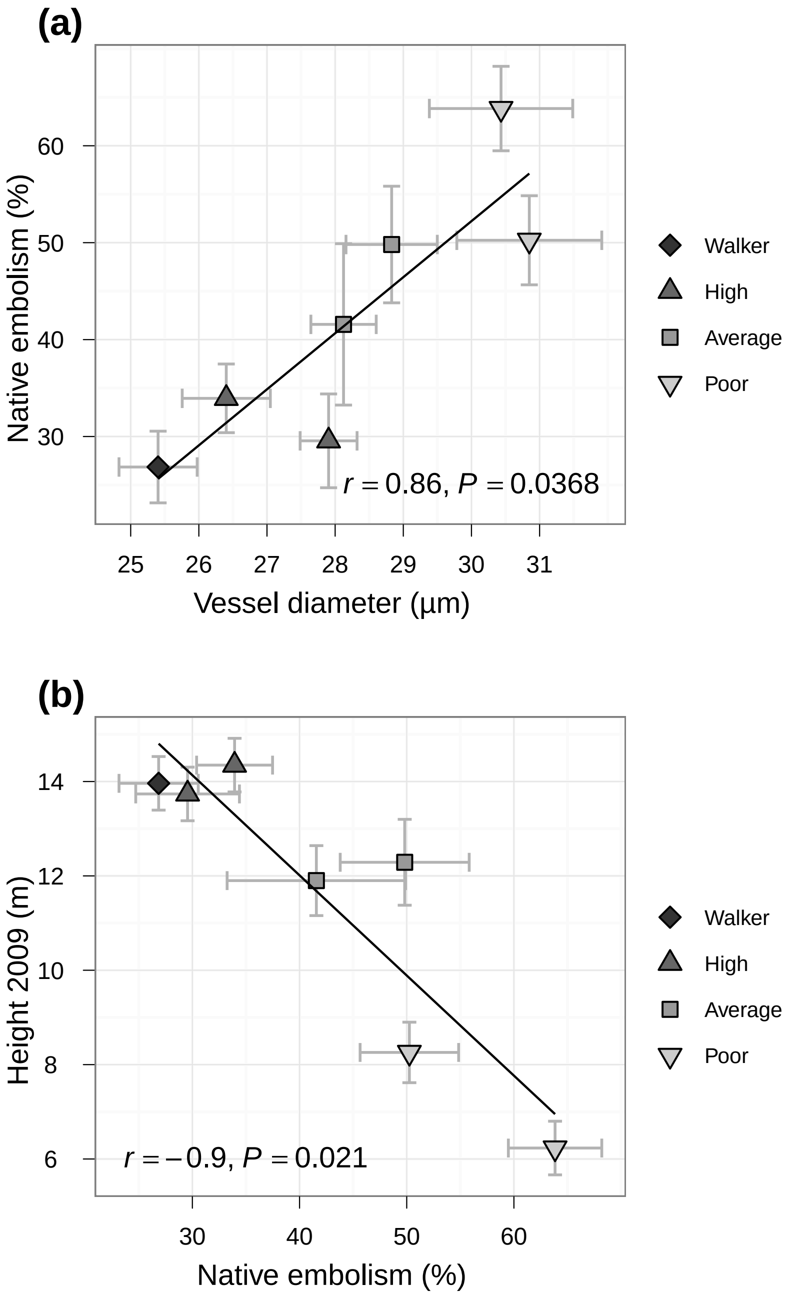

Figure 3.6 – *Correlations between mean native embolism measured in May of 2011 and vessel diameter (a) as well as tree height (b) for seven selected clones grouped by performance. Error bars represent the standard error of the mean.*

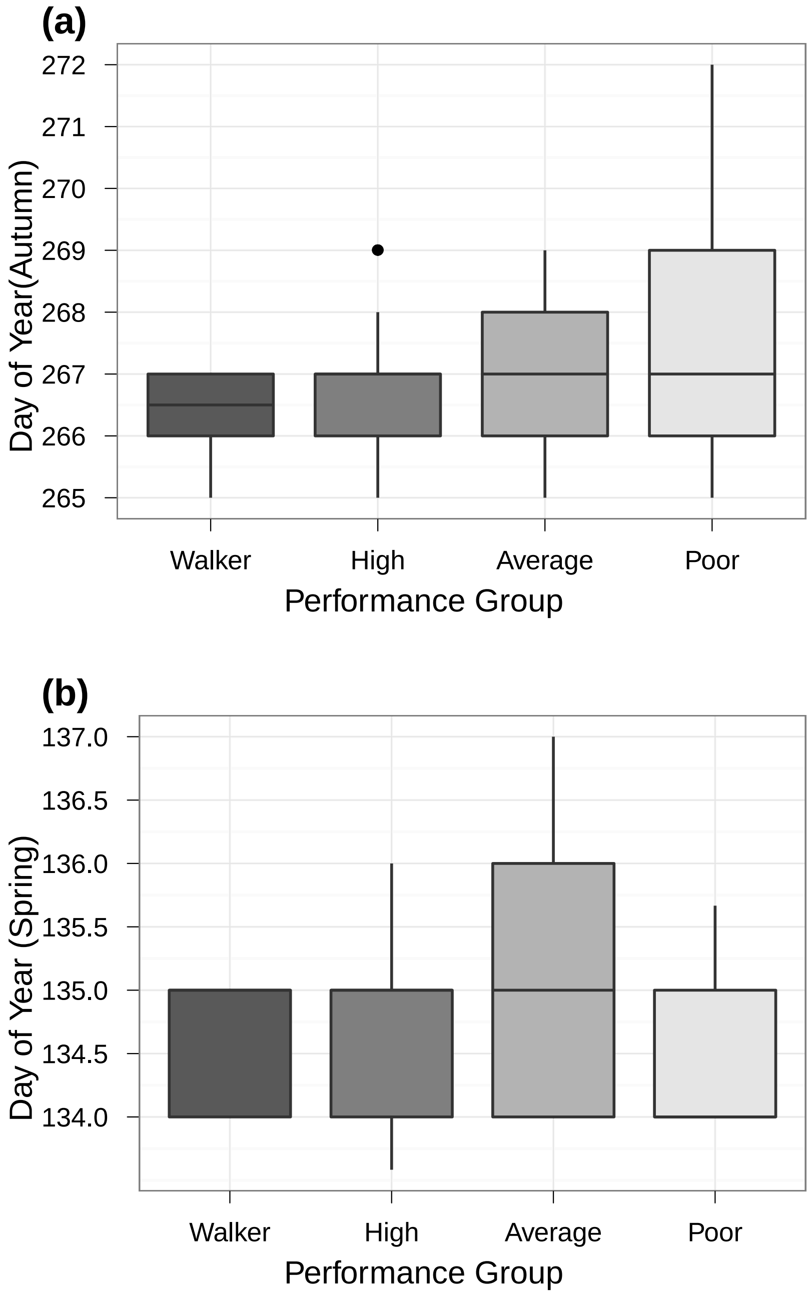

Figure 3.7 – *Boxplots representing the day of year at which leaf senescence score 4 (a) and budbreak score 2 (b) was reached grouped by performance. Leaf senescence score 4 = more yellow than green leaves. Budbreak score 2 = leaves extending but unfolded. Incomplete boxplots are due to lack of spread in data.*

CHAPTER 4

Frost hardiness versus growth performance in trembling aspen: results of a large-scale reciprocal transplant experiment[1]

Contents

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[1] A version of this chapter has been submitted for publication. Schreiber, S.G., Ding, C., Hamann, A., Hacke, U.G., Thomas, B.R. & Brouard, J.S. 2012. *Frost hardiness versus growth performance in trembling aspen: results of a large-scale reciprocal transplant experiment*. This chapter represents a joint thesis chapter between S.G. Schreiber and C. Ding. CD lead the field and lab work with SGS's assistance. SGS wrote the paper with CD's assistance. The data analysis was a 50 % shared effort.

4.1 Summary

According to the range limit hypothesis, the distribution of many temperate species is restricted by a trade-off between their adaptive capacity to survive winter extremes in the north (or high elevation), and their ability to compete with better-adapted species in the south (or low elevation range limits). This trade-off has important implications in forestry, particularly in the context of managed seed movement under climate change. In this study, we aim to quantify trade-offs among growth, frost hardiness, and timing of leaf senescence and budbreak in populations of trembling aspen (*Populus tremuloides* Michx.), which were observed in a series of reciprocal transplant experiments with provenances ranging from northeast British Columbia to central Minnesota. After 10 years, we found pronounced increases in productivity as a result of long-distance transfers in northwest direction. For example, provenances moved 1,600 km northwest from Minnesota to central Alberta (a shift of 7° latitude to the north) had produced almost twice the biomass of local sources. Similarly, provenances moved 800 km from central Alberta to northeast British Columbia (4° latitude north) also produced twice the biomass of local sources. We further found that increased growth was not associated with lower survival rates in this study. Budbreak in provenances transferred northwest generally occurred slightly later than in local sources, suggesting decreased risk of spring frost injury. Leaf abscission was later in provenances transferred in northwest direction, but they appeared to be extremely frost hardy, well ahead of very rare early autumn frost events. Based on the results of this study and a review of other research, we conclude that potential benefits appear to outweigh potential risks associated with northward movement of aspen populations in forestry operations, especially in the context of climate change.

4.2 Introduction

Trembling aspen (*Populus tremuloides* Michx.) is the most widespread North American tree species and is particularly abundant in the western boreal forest. It is the leading species in the forests of northeast British Columbia, northern and central Alberta and central Saskatchewan, covering an area of approximately 60 million hectares of boreal mixed wood forest (Canadian Forest Service, 2011). Aspen is also an important commercial forest resource in this region, accounting for approximately half of the annual forest harvest and is primarily processed into oriented strand board (*OSB*) for construction purposes. However, aspen wood is also processed into pulp and paper production, and more recently also used to generate biofuels and other potential biomaterials (Balatinecz *et al.*, 2001, Sannigrahi *et al.*, 2010).

Given current and predicted climate change for western Canada (IPCC, 2007, Mbogga *et al.*, 2009), this important renewable forest resource is under considerable threat. Over the last two decades, loss of forest productivity as well as heat and drought induced dieback of aspen and other tree species has been severe along the southern fringe of the boreal forest (Hogg *et al.*, 2008, Allen *et al.*, 2010, Peng *et al.*, 2011). Michaelian *et al.* (2011) conducted a detailed survey covering an area of 11.5 million hectares in western Canada to assess the impact of drought induced aspen dieback. They report 45 megatonnes (*Mt*), of dead aboveground biomass, which represented 20 % of the total aboveground biomass (226 *Mt*) in the surveyed area.

One way to address these issues is to afforest the affected areas with different species, or differently adapted planting stock to better match current and anticipated climate conditions. In a study with lodgepole pine, (Rehfeldt *et al.*, 2001) suggests that adapting to global climate change requires a major redistribution of forest tree species and genotypes across the landscape. They report, for example, that genotypes which are best suited to future climates in northeast British Columbia (latitude 60°) are currently located as much as 9° latitude farther to the south. Similar work for aspen indicates that relocating aspen planting material northwards by 1-2° latitude is required just to account for climate change observed over the last two decades (Gray *et al.*, 2011).

Any movement of planting stock, however, could increase the risk of freezing injury if migrated genotypes are not properly synchronized with the available growing season (Aitken & Hannerz, 2001). Frost hardiness and de-hardening coincides with leaf senescence in autumn and budbreak in spring. Early spring growth is particularly susceptible to late spring frosts, since tissues are actively growing and not lignified. Budbreak is a direct response to temperature and is initiated after a certain heat sum is acquired (Li *et al.*, 2010, Hunter & Lechowicz, 1992). In contrast, autumn leaf senescence in most species, including aspen, is triggered by photoperiod (Horvath *et al.*, 2003, Keskitalo *et al.*, 2005, Fracheboud *et al.*, 2009). Notably, the timing of leaf abscission and onset of frost-hardiness in autumn is decoupled from the actual selective environmental factor (temperature), which poses a special concern when moving seed.

The distribution of many temperate tree species is thought to be determined by their adaptive capacity to survive winter extremes in the north or at high elevation, and their ability to compete with better adapted species in the south or at low elevation range limits (MacArthur, 1984, Woodward, 1987). This is a consequence of trade-offs between maximizing growth by fully utilizing the available growing season, and avoiding injury or mortality due to late spring or early autumn frosts(Loehle, 1998, Aitken & Hannerz, 2001, Leinonen & Hänninen, 2002, Koehler *et al.*, 2012). According to Loehle (1998) frost protection requires significant plant resources, being achieved by structural investments (e.g. increased lignification, thicker leaves and cell walls), physiologic responses (e.g. accumulation of lipids, sugars or membrane proteins), and conservative growth strategies (e.g. early autumn leaf senescence and late budbreak).

In order to successfully move planting stock, such trade-offs should be considered. Hence, the objectives of the present study are: (1) to assess the impact of moving aspen seed sources throughout western Canada on growth and survival in a large-scale reciprocal transplant experiment; (2) to investigate geographic patterns of genetic variation in adaptive traits, including the timing of budbreak and leaf senescence, and the onset and degree of frost hardiness; (3) to quantify frost risk environments in early autumn and late spring to which local and transferred aspen populations need to be adapted; and (4) to assess risks and potential benefits of seed movement throughout the western boreal forest. This information could in principle be used to develop sophisticated climate change adaptation strategies that account for uncertainty in future climate projections. For example, we could potentially lower risks (e.g. due to variable future climate), by sacrificing some growth potential (e.g. through a more conservative growth strategy). However, to keep the scope of the study manageable, we do not model performance and trade-offs under uncertain future environments. Instead, this papers aims at management recommendations that enhance health and productivity of planted aspen forests under current climate conditions.

4.3 Materials and Methods

4.3.1 Plant material and experimental design

The effects of moving planting stock to new growing environments were tested with a reciprocal transplant experiment, established by the industrial members of the Western Boreal Aspen Cooperative in 1998. The planting and collection locations were chosen to represent broad geographic regions, corresponding to forest management areas of the participating Canadian forestry companies: Saskatchewan (*SK*), Alberta foothills (*ABf*), central Alberta (*cAB*), northern Alberta (*nAB*) and northeast British Columbia (*BC*), and also included five seed lots from Minnesota (*MN*) (*Fig. 4.1*, *Table 4.1*). In total, 43 half-sib families were tested at five test sites. In the subsequent text, we refer to half-sib families from this trial also as provenances or collection locations. All trials were laid out as randomized complete blocks de-

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signs with six replications per seed source, planted in five-tree row plots. In order to minimize error caused by environmental effects, trials were surrounded by two rows of border trees. The trials were established with over-winter dormant stock in spring of 1998 and height and diameter measurements were taken after 9 growing seasons in autumn of 2006. An additional height measurement was carried out for the central Alberta site in 2008 after 11 growing seasons. Total above ground biomass for trembling aspen was calculated according to the Canadian national biomass equations in *kg* dry weight (Ung *et al.*, 2008).

4.3.2 Climate data and frost risk assessment

In order to assess current frost risk environments to which plant populations are putatively adapted, we used daily weather station data for the 1961-1990 normal period from the National Climate Data and Information Archive for Canada (Environment Canada; `http://www.climate.weatheroffice.gc.ca`) and the Minnesota Climatology Working Group for historical climate data for Minnesota, USA (University of Minnesota; `http://climate.umn.edu/doc/historical.htm`) (*Table 4.1*).

Frost risk for each provenance location was derived by first calculating means and standard deviations for daily minimum temperatures for each day of the year. The probability of a frost event was then estimated based on a normal distribution characterized by daily means and standard deviations. Frost thresholds of interest (-5, -10, -20, -30 and -40 °C) were first converted to z-scores, by subtracting the mean daily minimum temperatures and subsequently dividing by the daily standard deviations. The probability of a frost event equal or exceeding a given frost threshold was then calculated for each day as an integral under the normal distribution, using the function *pnorm()* of the R programming environment (R Development Core Team, 2011). The resulting time series were subsequently smoothed with a 7-day moving average, since day-to-day variation simply reflects variability in daily climate data and not true day-to-day variation in frost risk.

4.3.3 Phenology measurements

Leaf senescence was determined based on an eight-level senescence scale according to Fracheboud *et al.* (2009). Scoring was carried out at the central Alberta planting site in autumn of 2010 at seven dates: 14-Sep, 18-Sep, 21-Sep, 23-Sep, 25-Sep, 28-Sep and 02-Oct. The average day of year at which senescence score 5 (all leaves yellow) was reached was recorded for each individual tree. If the target score was recorded multiple times, the date of the phenological event was calculated as an average. If the required score was not directly recorded, the date of the phenology event was inferred by means of linear regression from the bracketing dates and scores. The corresponding day length (*DL*) for the day of year when score 5 was reached was calculated as a function of latitude (*LAT*) and day of year (*DoY*) according to Forsythe *et al.* (1995):

$$DL = 24 - \frac{24}{\pi}\cos^{-1}[\tan\frac{LAT\pi}{180}\tan\phi]$$

where ϕ is the declination angle of the sun, calculated as :

$$\phi = \sin^{-1}[0.3979\cos\theta]$$

and where θ is the revolution angle, calculated as:

$$\theta = 0.2163 + 2\tan^{-1}[0.9671\tan[0.0086 \times (DoY - 186)]]$$

Assuming that leaf senescence is primarily controlled by day length, which is well documented for temperate tree species including poplars (Horvath *et al.*, 2003, Keskitalo *et al.*, 2005, Fracheboud *et al.*, 2009), we inferred differences in the day of leaf senescence for provenances based on the latitude of the other four planting sites. These estimates are meant to broadly characterize the average date of leaf senescence. We note that there may be temperature-modulated year-to-year variations in the date of leaf abscission, but for the purpose of interpreting geographic patterns of adaptive genetic variation, these can be ignored.

Budbreak scores, similar to the leaf senescence data described above, were obtained from a previous study using the identical plant material (Li *et al.*, 2010). Here, we re-analyzed this data in a different research context, inferring the average day of budbreak (score 3: buds broken and leaves extending) for the seven regions of our study design, using daily weather station data for the 1961-1990 normal period. The expected date of budbreak for each individual tree was calculated according to a model optimized for aspen in the boreal forest (Beaubien & Hamann, 2011). Required heat sums for budbreak were determined as the daily sum of average temperatures with a start date set as January 1st and a threshold value set as 0 °C. This summation continues up to the day at which a budbreak score 3 was reached, yielding a required heat sum statistic for the observed event. Based on the well supported assumption that budbreak is determined by a genetically controlled heat sum requirement (Hunter & Lechowicz, 1992), an expected date of budbreak could then be estimated for all provenances at all test sites.

4.3.4 Cold hardiness measurements

Cold hardiness was measured using the electrolyte leakage method (Zhang & Willison, 1987, Morin *et al.*, 2007), which quantifies frost damage by measuring the leakage of cell sap into the extracellular space due to ruptured plasma membranes. The plant material was collected in autumn 2011 on 22-Aug, 12-Sep and 10-Oct at the central Alberta test site. Current year branches were cut into 5 *cm* pieces and placed in 30 *ml* high-density polyethylene bottles (Fisherbrand, Fisher Scientific). Adding 5 *ml* of deionized water to the samples before freezing treatments was applied to ensure ice formation. The freezing treatments were 8 °C (control), -5 °C, -10 °C, -20 °C, -30 °C on 22-Aug; 8 °C (control), -10 °C, -30 °C, -50 °C, -60 °C on 12-Sep; and 8 °C (control), -30 °C, -60 °C, -70 °C, -80 °C on 10-Oct. A programmable freezer (Model 85-3.1A, Scientemp Corp., Adrian, MI, USA) cooled the samples at a rate of 5 °C per hour, holding the target temperature for one hour, before re-warming to 8 °C. Each segment was subsequently cut into 5 *mm* pieces, topped up with 20 *ml* deionized water, stored for 20-24 hours at 8 °C, and manually shaken three times during storage. The amount of electrolyte leakage was measured at room temperature (approximately 20 °C) using a conductivity meter (Oakton Acorn CON 6 Meter, Oakton Instruments, Vernon Hills, IL, USA). Conductivity readings were taken before (c1) and after (c2) all samples were boiled at 100 °C for 50 *min*. Cell lysis (L) was calculated as:

$$L = \frac{REL - \overline{REL}_C}{100 - \overline{REL}_C} \times 100,$$

where REL is the relative amount of electrolyte leakage of sample undergoing freezing treatments calculated as (c1/c2)×100, and $\overline{REL}_C$ is the mean value of the control samples.

4.3.5 Statistical analysis

Statistical analyses were performed using the R programming environment (R Development Core Team, 2011), and graphics were prepared with the R package *ggplot2* (Wickham, 2009). Statistical null hypothesis testing was carried out for the variable cell lysis (L). To take advantage of the blocked experimental design, the data were analyzed using a mixed effects model implemented with the *lmer()* function available through the R package *lme4* (Bates *et al.*, 2011). The fixed effects in this model were the selected regions *MN*, *cAB*, *BC*, the random terms were block and provenance. Experiment-wise *P*-values were calculated using Tukey's adjustment for multiple mean comparisons.

4.4 Results

4.4.1 Growth data

At the age of 10, or after 9 growing seasons in the field, we found pronounced increases in productivity as a result of long-distance seed transfers in a northwest direction. For example, provenances moved 1,600 *km* northwest (and 7° latitude north) from Minnesota to central Alberta were 34 % taller and had 84 % more biomass than local sources (*Table 4.2* & *Table 4.3*). Similarly, provenances moved approximately 800 *km* northwest (and 4° latitude north) from central Alberta to northeast British Columbia produced twice the biomass compared to local sources and were 15 % taller. The farthest seed transfer tested in our design, which was from Minnesota to northeast British Columbia (2,300 *km* northwest and 11° latitude north), still outperformed local sources by 17 % in height and had more than twice the biomass. Increased performance as a result of northwest transfers was not associated with reduced survival. Minnesota provenances had survival rates similar to local sources at all sites. The next most southern group, the Alberta Foothills provenances, had better survival rates at all northern test sites relative to other sources than at its own local planting site, where it ranked second-lowest. Similar to Minnesota provenances, survival rates of the Saskatchewan and central Alberta provenances were comparable to local sources when transferred to the northern Alberta or northeast British Columbia test sites (*Table 4.2*).

The northeast British Columbia and the northern Alberta provenances always ranked as the lowest and second lowest group of provenances at more southern planting sites (but there was no or only small reductions in survival). For example, the northeast British Columbia provenances were 16 %, 28 %, and 50 % smaller in height than the local sources at the northern Alberta, central Alberta, and the Alberta Foothills test site, respectively. Northern Alberta provenances had only somewhat reduced height of 5 %, and 8 % at the central Alberta and Foothills test site, respectively.

4.4.2 Spring and autumn phenology

At the central Alberta test site, where phenology was recorded, the sequence of leaf senescence started with the most northern provenances (BC and nAB), followed by mid-latitude provenances (cAB, ABf and SK), and ended with Minnesota provenances turning yellow 10 days later than the first provenances from the north (*Table 4.4*). For the inferred day of leaf senescence at other planting sites we found no discernible differences in leaf senescence among sites.

Budbreak occurred latest for the central Alberta and Saskatchewan provenances, while the northeast British Columbia provenances consistently broke bud earliest with the Minnesota provenances having an intermediate date of budbreak (*Table 4.4*). Inferred budbreak dates for 1961-1990 normal climate differed only by a few days, with provenances flushing first at the central Alberta site, followed by the Saskatchewan, Alberta Foothills, and northern Alberta sites, and last at the northeast British Columbia site.

The above observations are also reflected in a relatively strong correlation between height and leaf senescence at the central Alberta (*cAB*) test site, where phenology measurements were carried out ($R^2 = 0.36$, $P < 0.0001$). This correlation appears to be driven by early leaf senescence of the northern British Columbia sources, and late leaf senescence of the Minnesota sources, when transferred to a central Alberta common garden (*Fig. 4.2a*). For budbreak, a strong latitudinal differentiation was not apparent (*Fig. 4.2b*). Sources from northeast British Columbia had the lowest heat sum requirements and broke bud first, but otherwise there was more within- than among-regional variation in the date of budbreak, which could not explain variation in height ($R^2 = 0.07$). There was also no correlation between height and the utilized growing season, calculated as the day of leaf senescence minus the day of budbreak ($R^2 = 0.002$, $P = 0.78$, data not shown).

4.4.3 Cold hardiness

The amount of freezing injury, expressed in % cell lysis (%L), revealed a general trend in which trees from Minnesota appear to be more vulnerable than trees from central Alberta and trees from northeast British Columbia, where the onset of frost hardiness occurs first (*Fig. 4.3a*). Our cell lysis data suggests clear regional differences with very little variation of frost hardiness within regions (*Table 4.5*, *Fig. 4.4*). At the August sample date, the -10 and -20 °C treatments resulted in significantly higher vulnerability of Minnesota sources. Regional differences were most pronounced at all freezing treatments in September, with a sequence of increasing vulnerability from British Columbia to Alberta to Minnesota. At the October sample date, all sources showed generally low amounts of cell lysis, even under -80 °C freezing treatments. However trees from Minnesota were still the most vulnerable (*Table 4.5*, *Fig. 4.3a*).

The onset of frost hardiness measured as cell lysis at -30 °C in September also showed a strong correlation with leaf senescence (*Fig. 4.3b*). Trees from Minnesota were the least hardy and senesced the latest. On the other hand, the BC provenances showed a high degree of hardiness and also being the first to turn uniformly yellow. The central Alberta provenances ranked in between, however showing a larger spread of approximately 12 % in cell lysis.

4.4.4 Phenology, hardiness and frost risks

A joint representation of phenology and regional frost risks is shown in *Fig. 4.5*. Generally, the probability of frost curves indicates a progression from relatively mild winters in Minnesota, to more severe winters in Alberta and British Columbia. For example the British Columbia planting site has a 30-40 % chance of a -30 °C or colder frost events at any given day in January, whereas the corresponding probability in Minnesota is about 10-15 %. Nevertheless, the time where mild frost events of -5 °C or colder can be expected at the three planting sites is remarkably similar, although the probability increases much faster in autumn and decreases more rapidly in spring at the northern test sites.

The phenology of local provenances further appears to be remarkably well attuned to the frost risks of their local environments. The central Alberta provenances appear to perfectly avoid any frost risk without sacrificing the available growing season at their local central Alberta test site. The British Columbia provenances utilize the available growing season in spring more aggressively, but also avoid spring frost risks well in their local environment and in central Alberta. The Minnesota provenances on the other hand utilize the available growing season more aggressively in autumn. However, by mid-September, when -5 °C frost risks start to appear at all planting sites, they are already quite well hardened against -10 °C frosts (*Table 4.5*). By mid-October, when -10 °C frost risks start to appear with very low probabilities, Minnesota provenances are similarly hardened against -30 °C to -80 °C freeze events. That said, overall cell lysis values indicate that British Columbia and Alberta provenances were always more hardened at any given time than Minnesota sources. For example, British Columbia provenances sustained the same level of damage at -50 °C that Minnesota sources sustained at -10 °C in mid-September (around 20 %).

4.5 Discussion

Survival versus capacity adaptation are normally expected to be important drivers in trade-offs for temperate tree species (Leinonen & Hänninen, 2002). Reproductive success of trees from high-latitude ecosystems should be strongly influenced by their ability to withstand harsh frost, whereas trees from milder climates should be favoured by natural selection based on higher growth rates and competitive fitness (Loehle, 1998). By moving trees north out of their local habitat one would generally expect an increasing risk of frost damage in autumn due to delayed growth cessation (Howe *et al.*, 1995). Interestingly, that is not what we predominantly found in this study with boreal aspen provenances.

Spring phenology was quite similar across all provenances observed in a common garden in central Alberta, except perhaps for the northern Alberta provenances. It is not uncommon that provenances from very high latitudes or very high elevation are adapted to make the most out of a short period of favorable temperatures and extended photoperiods, and tend to more aggressively utilize the available growing season (cf. Beuker, 1994, Aitken & Hannerz, 2001). In our case, this means that northward movement of more southern provenances would typically lead to similar or slightly delayed onset of growth of introduced genotypes relative to local provenances, and therefore northward transfers would not pose additional risks.

Another interesting observation is that the inferred dates of budbreak and leaf senescence (for the regions *BC*, *nAB*, *ABf*, *SK*, *MN*), were not drastically different from the common garden site at which they were all observed (*cAB*), and this may have two explanations: While the severity of winters increases from southeast to northwest, there are virtually no differences in the frost free period from Minnesota to northeast British Columbia (*Fig. 4.5*). Secondly, the date of leaf senescence of aspen populations coincides exactly with the inflection point of the day length curve (*Fig. 4.6*). This means, that although the day length trigger is temperature-decoupled, it will nevertheless work more or less appropriately under latitudinal transfers, because the day length does not vary with latitude around the date of the autumnal equinox (September 22), which happens to be when we observed leaf senescence in aspen provenances selected for our study. The true critical daylength that initiates senescence must be somewhat earlier than the date where we observe leaf senescence, so there may be small shifts in the timing of senescence under long distance transfers. However, we find these time shifts quite small in absolute terms. For example, Minnesota provenance senesced six days later than the local sources when moved over 7° of latitude to the central Alberta planting site (*Table 4*).

Perhaps the most striking result of this experiment is that moving aspen as far as 2,300 km northwest from Minnesota to northeast British Columbia did not result in higher mortality rates or inferior growth. In fact, trees from Minnesota outperformed all local sources at Saskatchewan, Alberta and northeast British Columbia test sites. That said, we should acknowledge that there are clearly discernible differences in frost hardiness from southeast to northwest, suggesting a typical trade-off between investments in growth (Minnesota sources) versus investments in cold resistance (northeast British Columbia sources). However, when looking at the corresponding risk environments, investments in cold resistance appear non-optimal for current climate conditions, i.e. too conservative (*Fig. 4.3b*). All provenances appear to be sufficiently hardy early enough to withstand extremely unlikely cold events, for example -30 °C in mid-September.

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At this point we have to mention that more pronounced differences in the timing of growth cessation and initiation of frost hardiness may be found when assessing bud set instead of timing of leaf senescence. These differences could have shed more light on the question whether the observed differences in height are in fact a result of early versus late growth cessation in northern versus southern aspen provenances. However, this would not alter our statement that northwest transfer of aspen in our study is associated with increased productivity and low risk of frost damage.

While the assumption of optimality of local adaptation is an important foundation in forest management, it is widely known that local sources do not always represent the most optimal genotypes. This may have several reasons that could well apply to aspen (e.g. Namkoong, 1969, Leinonen & Hänninen, 2002). There may be founder-effects and persistence of genotypes, which could very well apply to aspen, which predominantly regenerates clonally via root suckers. Another reason for naturally occurring non-optimality is gene flow that may overcome local selection pressures, typically observed along elevational gradients. A third, and perhaps most plausible cause is environmental change that exceeds the speed of evolutionary change, referred to as adaptational lag. Last but not least, apparent non-optimality in growth observed over a short period of time may not indicate non-optimality in terms of evolutionary fitness. Non-optimality is therefore not a surprising finding in itself. It is the magnitude of seed transfers with beneficial effects on growth that we find remarkable in this experiment.

Conclusions

Should we make long-distance transfers of aspen provenances in a north or northwest direction a general management recommendation, based on the results of this study? We could argue that there may be other important trade-offs, where more northern sources sacrifice growth and instead invest in resistance mechanisms to biotic or abiotic risk factors that we have not considered. One possible risk factor, drought, was excluded by a related study (Schreiber *et al.*, 2011) that showed that the Minnesota provenances tested at the central Alberta site also had the smallest xylem vessel diameters, which conferred the greatest drought resistance across all genotypes tested in this experiment. Adaptations to biotic factors such as pests and diseases by northern provenances that are absent in southern sources also appear unlikely. Sources from warmer environments and milder winters would generally be expected to be more exposed, and therefore better adapted to pest and disease factors. We therefore conclude that potential benefits appear to outweigh potential risks associated with a northward movement of aspen populations in forestry operations. We are confident in recommending that seed transfer guidelines in western Canada allow a moderate movement of planting material 2-3 degrees of latitude northward in response to observed and predicted climate warming, as suggested by Gray *et al.* (2011). As for true long-distance transfers, notably the use of Minnesota sources in western Canada, we encourage forest companies and government agencies to pursue this option first on a relatively small operational scale. General recommendations of long-distance transfers should await the outcome of the present test series near rotation age, and concurrent experience gained from small-scale operational plantations.

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4.7 Tables

Table 4.1 – *Regional representation of seed collections, geographic information of test sites, source of weather station data, and average temperature values for the 1961-1990 climate normal period.*

		Test site coordinates				**Temperature (°C)[2]**			
Region	*Number of provenances*	*Latitude*	*Longitude*	*Elevation*	*Weather stations[1]*	*EMT*	*TMIN01*	*TMAX07*	*TAV*
Minnesota (MN)	5	-	-	-	216612	-42.2	-20.2	27.2	4.9
Saskatchewan (SK)	8	53°20′N	105°36′W	480	4056240	-49.4	-26.1	24.2	0.6
Alberta Foothills (ABf)	11	52°44′N	114°47′W	970	3015520-3	-44.1	-18.2	21.8	2.6
Central Alberta (cAB)	11	54°53′N	113°18′W	570	3060321	-46.1	-20.6	22.3	1.8
Northern Alberta (nAB)	5	56°46′N	117°28′W	525	3075040	-48.9	-22.9	22.4	0.8
Northeast British Columbia (BC)	3	58°32′N	122°20′W	335	1192940	-47.2	-26.3	23	-1

[1] Weather station IDs according to `http://www.climate.weatheroffice.gc.ca` for Canada and according to `http://www.noaa.gov/` for Minnesota.

[2] All temperature values based on the 1961-1990 climate normal period.*EMT*, 30-year extreme minimum temperature; *TMIN*01, mean monthly minimum temperature for January; *TMAX*07, mean monthly maximum temperature for July; *MAT*, mean annual temperature. All climate variable were calculated for the 1960-1990 normal period.

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Table 4.2 – *Height (m) and survival (%) of provenances grown in the reciprocal transplant experiment at age nine. Test sites are ordered along northwest gradient. Local sources are marked in bold, and standard errors are given in parenthesis.*

	Test site				
Origin of seed source	*SK*	*ABf*	*cAB*	*nAB*	*BC*
Height (m) at age nine					
Minnesota (MN)	4.0 (0.06)	4.0 (0.23)	6.9 (0.23)	5.0 (0.11)	3.0 (0.08)
Saskatchewan (SK)	**3.3 (0.14)**	3.5 (0.09)	5.6 (0.08)	5.0 (0.16)	2.9 (0.07)
Alberta Foothills (ABf)	3.1 (0.12)	**3.1 (0.11)**	5.2 (0.15)	5.1 (0.16)	2.8 (0.14)
Central Alberta (cAB)	3.4 (0.11)	3.4 (0.11)	**5.2 (0.15)**	5.1 (0.11)	2.9 (0.09)
Northern Alberta (nAB)	3.1 (0.15)	2.9 (0.16)	5.0 (0.09)	**5.3 (0.06)**	3.6 (0.11)
Northeast British Columbia (BC)	2.5 (0.15)	1.6 (0.24)	3.7 (0.11)	4.4 (0.12)	**2.5 (0.11)**
Survival (%) at age nine					
Minnesota (MN)	61.6 (6.01)	76.4 (6.96)	91.8 (1.53)	92.8 (1.36)	87.8 (2.24)
Saskatchewan (SK)	**66.0 (4.72)**	89.0 (2.00)	94.5 (1.43)	95.9 (1.43)	92.4 (2.07)
Alberta Foothills (ABf)	67.3 (4.54)	**74.2 (3.95)**	88.9 (3.91)	90.7 (3.11)	80.9 (2.90)
Central Alberta (cAB)	72.4 (2.19)	79.5 (5.18)	**94.8 (1.68)**	95.4 (1.61)	87.5 (2.29)
Northern Alberta (nAB)	73.6 (2.40)	78.8 (1.62)	92.0 (3.13)	**92.2 (2.63)**	90.0 (1.34)
Northeast British Columbia (BC)	65.3 (3.53)	44.7 (4.41)	84.7 (2.33)	97.7 (2.33)	**77.7 (5.21)**

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Table 4.3 – *DBH (cm) and total dry mass (kg) of provenances grown in the reciprocal transplant experiment at age nine. Test sites are ordered along northwest gradient. Local sources are marked in bold, and standard errors are given in parenthesis.*

	Test site				
Origin of seed source	*SK*	*ABf*	*cAB*	*nAB*	*BC*
DBH (cm) at age nine					
Minnesota (MN)	3.14 (0.17)	3.05 (0.2)	7.97 (0.28)	3.21 (0.17)	2.44 (0.13)
Saskatchewan (SK)	**2.39 (0.32)**	2.73 (0.1)	6.72 (0.12)	3.42 (0.16)	2.26 (0.11)
Alberta Foothills (ABf)	2.42 (0.14)	**2.45 (0.12)**	6.93 (0.22)	3.69 (0.15)	2.09 (0.18)
Central Alberta (cAB)	2.68 (0.19)	2.64 (0.13)	**6.65 (0.19)**	3.55 (0.11)	2.25 (0.12)
Northern Alberta (nAB)	2.43 (0.14)	2.09 (0.18)	6.34 (0.11)	**3.65 (0.12)**	2.79 (0.08)
Northeast British Columbia (BC)	2.25 (0.16)	0.99 (0.13)	4.84 (0.11)	2.69 (0.14)	**1.65 (0.17)**
Total dry mass (kg) at age nine					
Minnesota (MN)	1.09 (0.12)	1.05 (0.17)	11.45 (1.13)	1.39 (0.17)	0.52 (0.07)
Saskatchewan (SK)	**0.62 (0.14)**	0.73 (0.06)	6.66 (0.31)	1.6 (0.19)	0.43 (0.05)
Alberta Foothills (ABf)	0.54 (0.07)	**0.56 (0.08)**	6.81 (0.57)	1.92 (0.17)	0.4 (0.08)
Central Alberta (cAB)	0.72 (0.11)	0.69 (0.08)	**6.22 (0.47)**	1.73 (0.14)	0.44 (0.05)
Northern Alberta (nAB)	0.53 (0.08)	0.38 (0.07)	5.33 (0.24)	**1.87 (0.13)**	0.79 (0.06)
Northeast British Columbia (BC)	0.38 (0.07)	0.05 (0.02)	2.43 (0.15)	0.87 (0.11)	**0.21 (0.05)**

Table 4.4 – *The inferred average date of leaf senescence for four test sites based on a day length trigger measured at the cAB planting site in autumn 2011, and the average date of budbreak for the 1961-1990 climate normal conditions inferred from heat sum requirements observed at the cAB planting site in spring of 2009. Test sites are ordered along northwest gradient. The response in the native environment are marked in bold, and standard errors are given in parenthesis.*

	Performance group				
Origin of seed source	*SK*	*ABf*	*cAB*	*nAB*	*BC*
Leaf senescence (day of year)					
Minnesota (MN)	269 (0.3)	269 (0.3)	270 (0.3)	269 (0.2)	269 (0.2)
Saskatchewan (SK)	**262 (0.2)**	262 (0.2)	263 (0.2)	263 (0.2)	263 (0.2)
Alberta Foothills (ABf)	262 (0.3)	**262 (0.3)**	263 (0.3)	262 (0.2)	263 (0.2)
Central Alberta (cAB)	263 (0.2)	262 (0.2)	**264 (0.2)**	263 (0.2)	263 (0.2)
Northern Alberta (nAB)	259 (0.4)	259 (0.4)	260 (0.4)	**260 (0.3)**	261 (0.3)
Northeast British Columbia (BC)	259 (0.8)	258 (1.0)	260 (0.8)	260 (0.7)	**260 (0.8)**
Budbreak (day of year)					
Minnesota (MN)	134 (0.7)	134 (0.7)	131 (0.7)	133 (0.7)	136 (0.7)
Saskatchewan (SK)	**140 (0.9)**	141 (1.1)	137 (1.0)	140 (1.0)	142 (1.0)
Alberta Foothills (ABf)	137 (0.8)	**137 (0.9)**	134 (0.9)	137 (0.9)	139 (0.9)
Central Alberta (cAB)	139 (1.0)	140 (1.2)	**137 (1.1)**	140 (1.1)	142 (1.1)
Northern Alberta (nAB)	134 (0.9)	135 (1.0)	132 (1.0)	**135 (1.0)**	137 (1.0)
Northeast British Columbia (BC)	129 (0.7)	128 (0.3)	126 (0.3)	128 (0.3)	**131 (0.3)**

Table 4.5 – *Differences in frost hardiness measured as percent cell lysis among the regions Minnesota (MN), central Alberta (cAB) and northeast British Columbia (BC). Different letters in rows indicate significant differences at* $P < 0.05$.

	Cell lysis by region of origin (%)		
Freezing treatment	*MN*	*cAB*	*BC*
August			
-5 °C	1.4 (0.6)^{A}	1.5 (0.5)^{A}	0.4 (0.2)^{A}
-10 °C	49.6 (3.8)^{A}	31.2 (3.6)^{B}	22.7 (3.3)^{B}
-20 °C	52.7 (4.1)^{A}	31.7 (3.9)^{B}	23.0 (4.1)^{B}
-30 °C	62.4 (2.1)^{A}	56.2 (1.8)^{A}	55.3 (1.9)^{A}
September			
-10 °C	21.2 (1.8)^{A}	10.9 (1.7)^{AB}	8.0 (3.8)^{B}
-30 °C	58.7 (1.2)^{A}	44.2 (2.7)^{B}	29.1 (2.6)^{C}
-50 °C	58.8 (1.2)^{A}	40.2 (4.4)^{B}	21.0 (2.5)^{C}
-60 °C	66.0 (1.2)^{A}	44.9 (2.9)^{B}	32.1 (3.7)^{C}
October			
-30 °C	18.0 (2.4)^{A}	8.1 (0.8)^{B}	5.8 (1.1)^{B}
-60 °C	28.2 (3.5)^{A}	13.3 (0.9)^{B}	13.0 (1.6)^{B}
-70 °C	18.0 (1.7)^{A}	10.4 (1.2)^{AB}	9.9 (1.2)^{B}
-80 °C	18.8 (1.5)^{A}	11.5 (0.8)^{A}	12.1 (1.9)^{A}

4.8 Figures

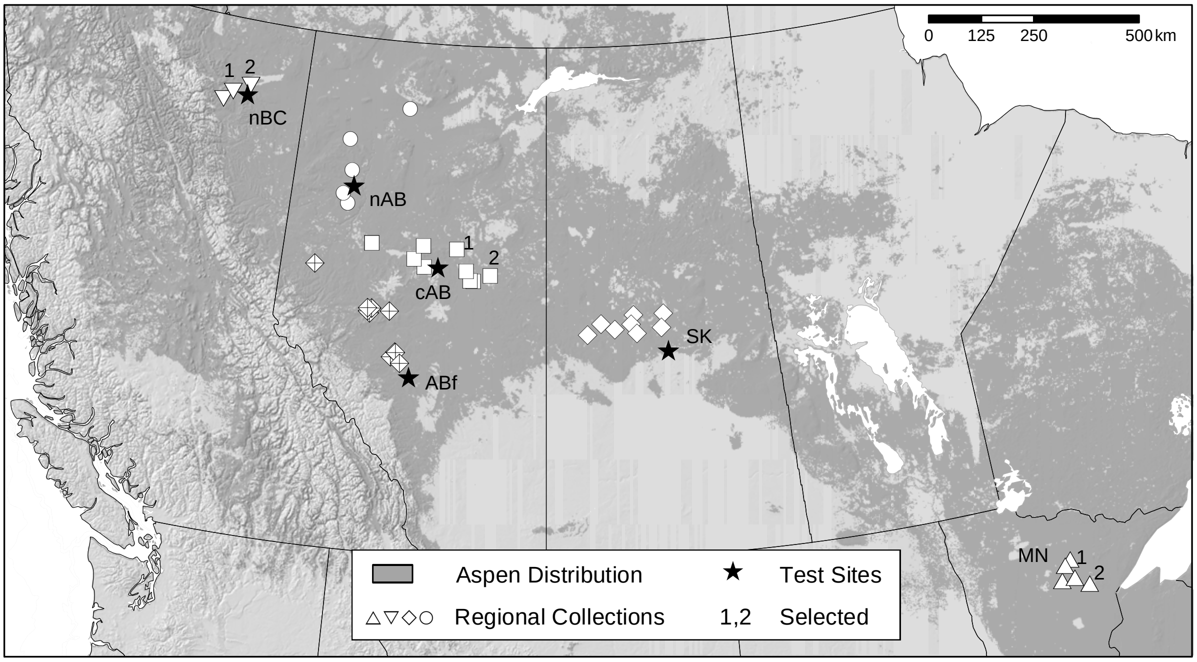

Figure 4.1 – *Collection locations, test sites of the provenance trial series. Genotypes selected for the physiological study are indicated by numbers and are, for example, referred to as MN1 or MN2 in subsequent figures.*

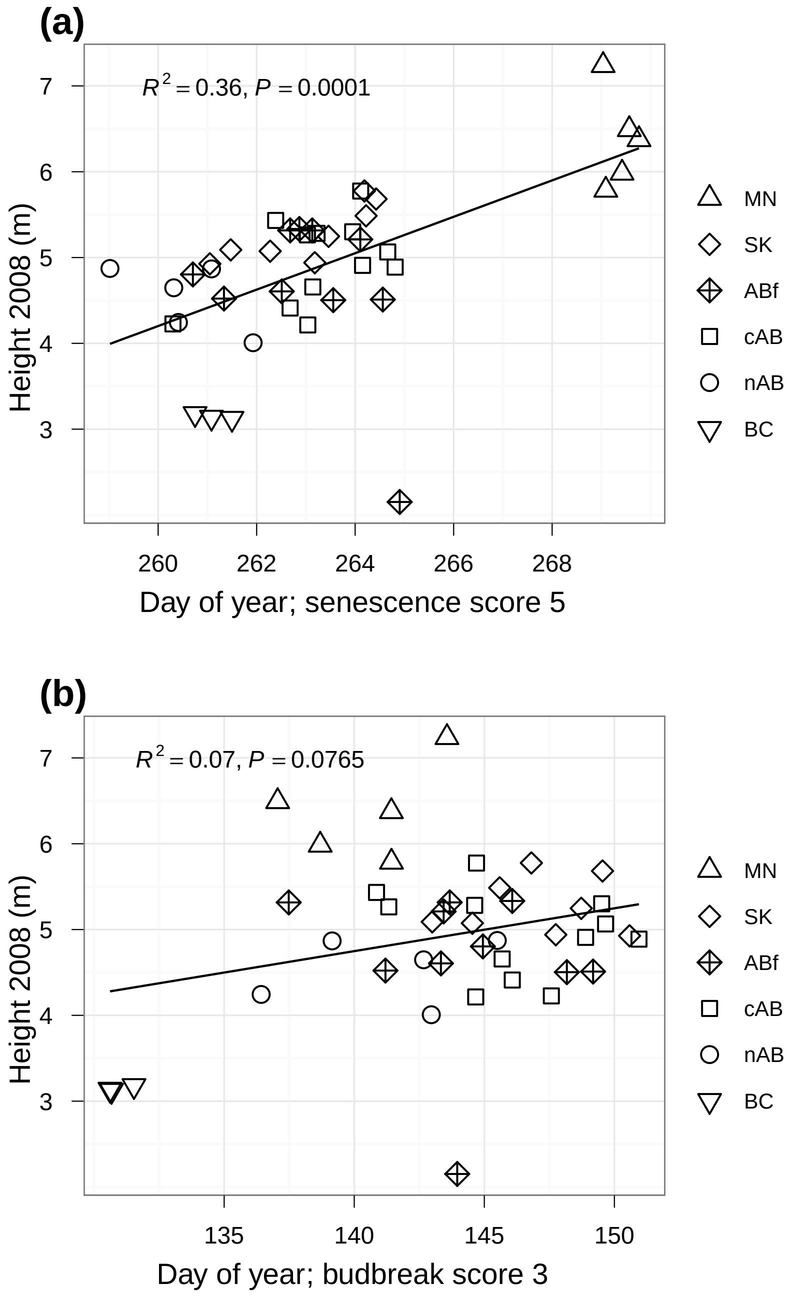

Figure 4.2 – *Correlation of 11-year height and timing of leaf senescence (a) as well as budbreak (b) in trembling aspen. Shapes represent regions ordered along northwest gradient at the central Alberta test site.*

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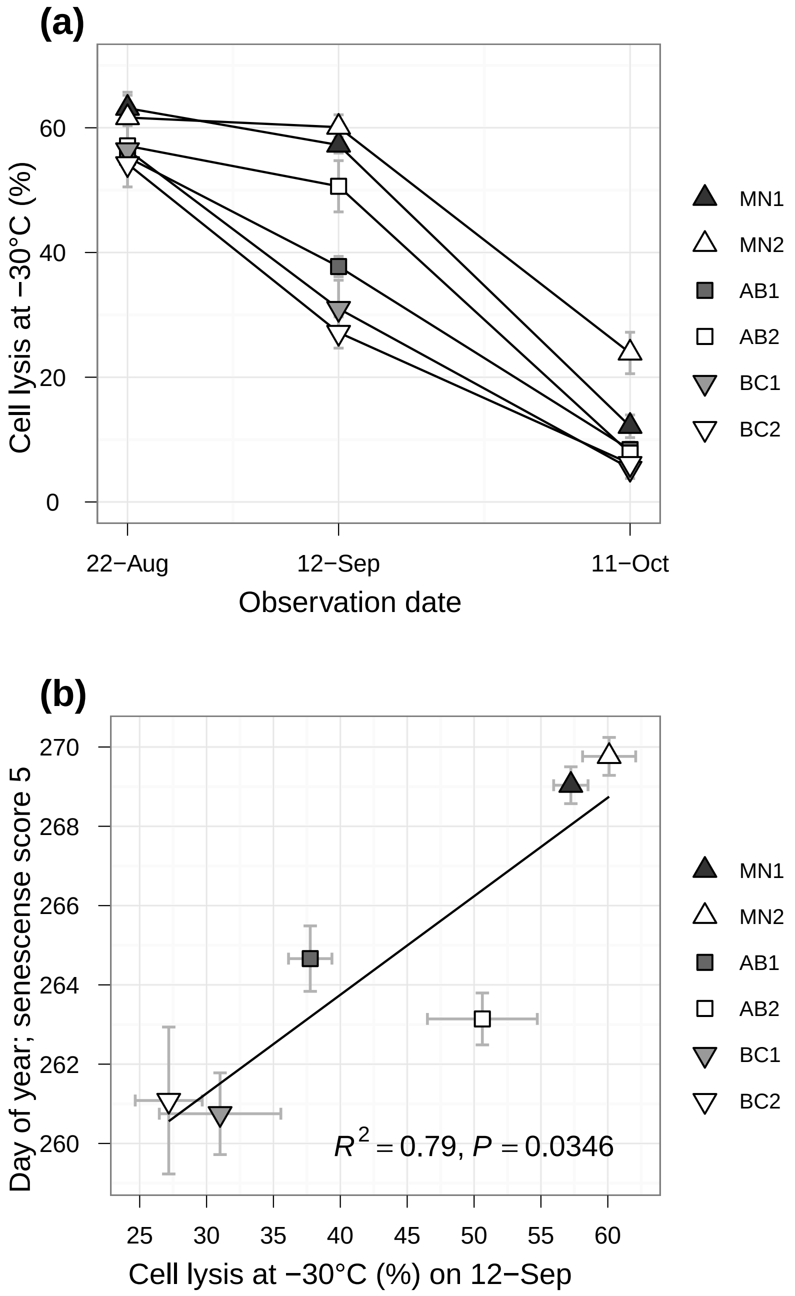

Figure 4.3 – *Cell lysis at -30 °C for six different aspen provenances measured on three dates in late summer and autumn in 2011 (a). Correlation of cell lysis at -30 °C for September 12 and timing of leaf senescence (b). Symbols and shading represents regions and genotype within region ordered along northwest gradient.*

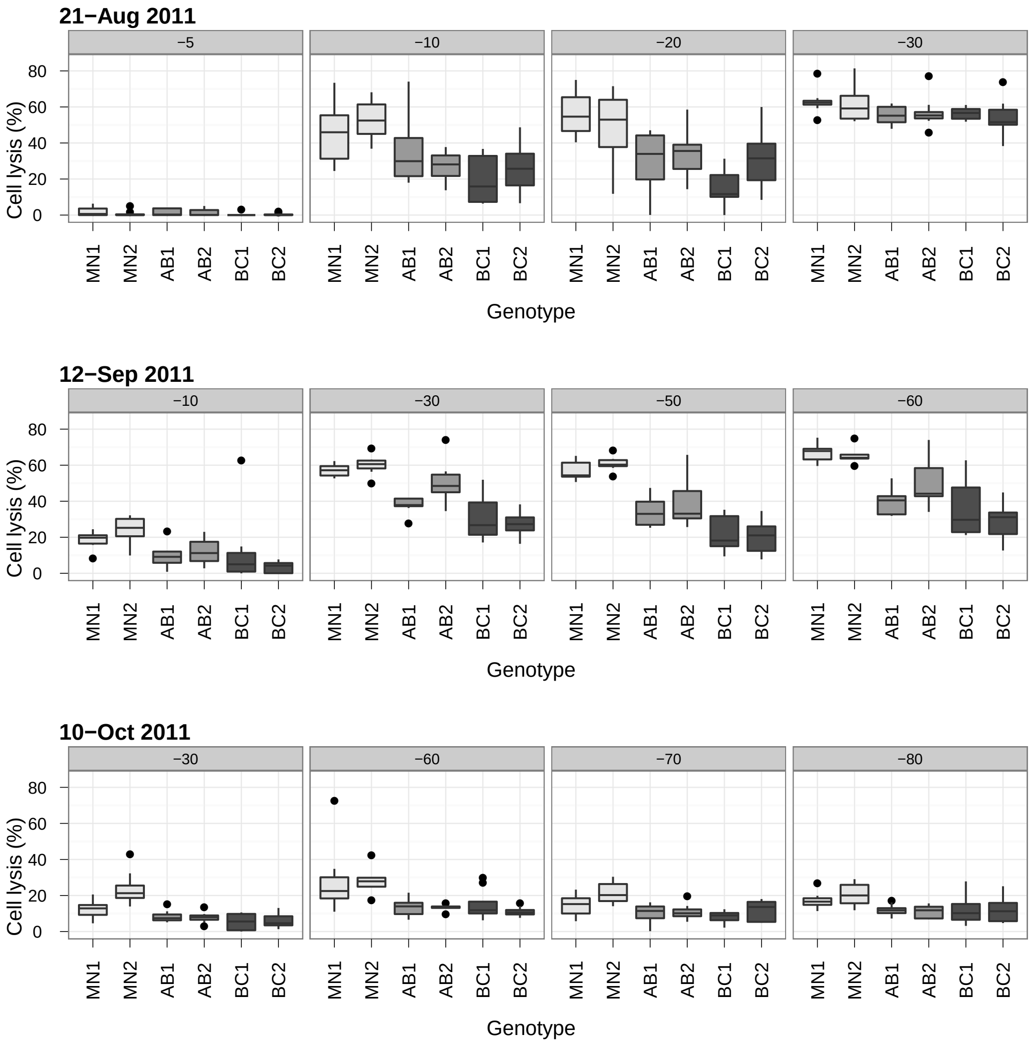

Figure 4.4 – *Cell lysis for six aspen provenances measured on 21-August (top), 12-September (middle) and 10-October (bottom) in response to different artificial freezing treatments.*

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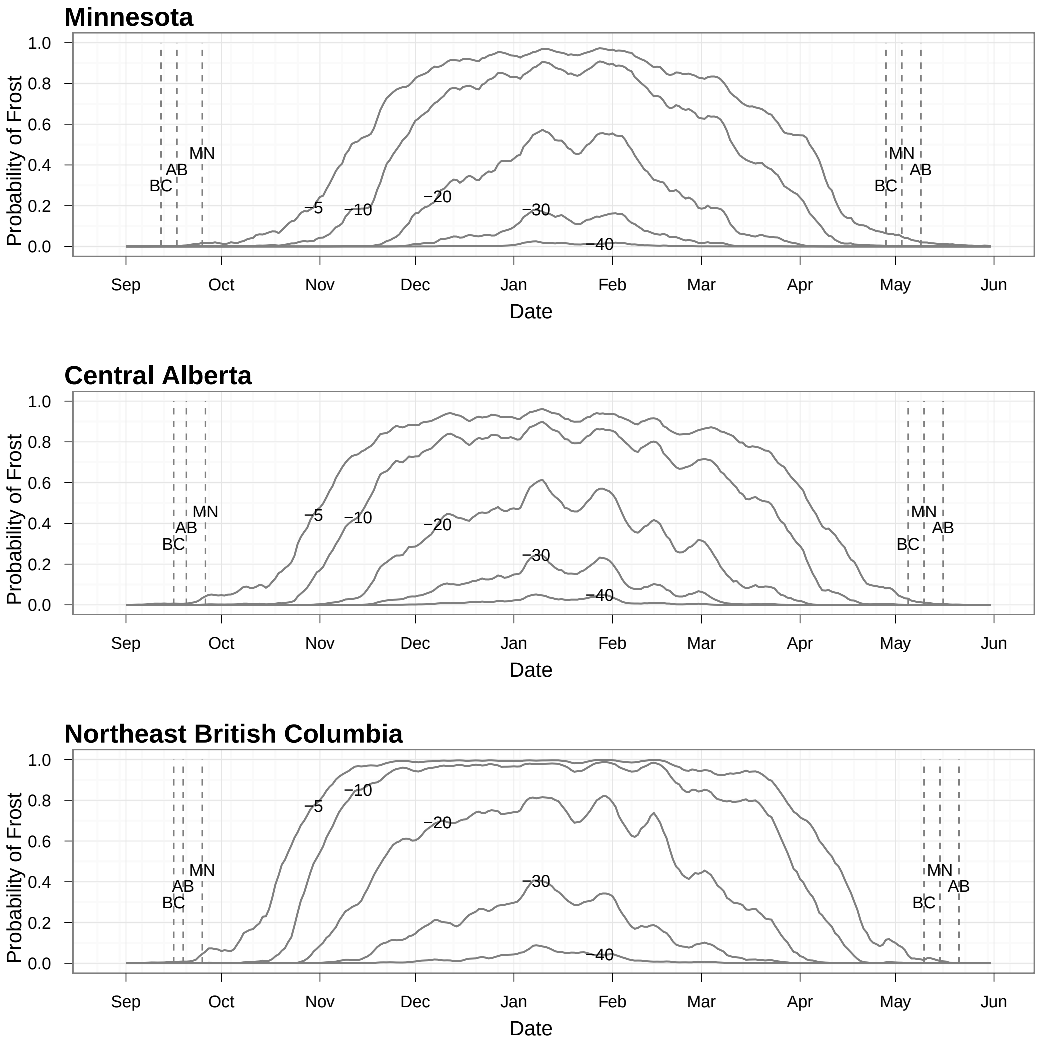

Figure 4.5 – *Probability of a frost event being equal or exceeding a certain threshold value for any given day between September 1st and May 31st at the Minnesota (top) central Alberta (middle), and northeast British Columbia (bottom) planting sites. The expected day of budbreak calculated for 1961-1990 normal climate, and the expected day of leaf senescence for the latitude of planting sites is indicated by vertical lines for provenances from central Alberta, northeast British Columbia, and Minnesota.*

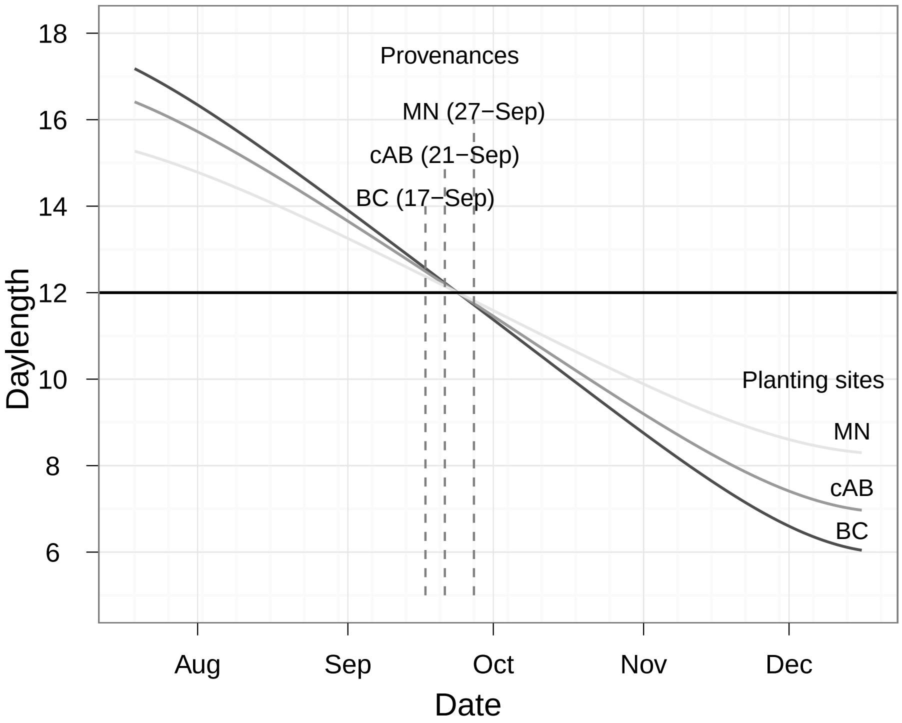

Figure 4.6 – *Changes of daylength for the latitudes of the regions Minnesota (MN), central Alberta (cAB) and northeast British Columbia (BC). The dates of leaf senescence for the corresponding provenances, observed in a common garden at the central Albert test site, are indicated by vertical lines.*

CHAPTER 5

General Discussion and Conclusions

Contents

5.1 Conclusions

The scope of my thesis was to identify physiological traits that relate to productivity, growth performance and survival of hybrid poplars and trembling aspen in western Canada. Furthermore, I also assessed the potential of long-distance seed transfer of trembling aspen as a tool for forest resource management strategies, particularly under the uncertainty of future climate change. All experiments were done on plant material that was grown in the field and was exposed to the local climate for more than ten years.

In the following section, I will conclude the key results of the present thesis, briefly describe limitations of the work, and outline how future research may follow-up in order to further increase our understanding of the ecophysiology of poplars in the boreal forest ecosystem.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

In my *first research chapter*, I assessed 56 hybrid poplar clones as well as 48 aspen genotypes and measured 10 physiological traits with the goal to:

(a) determine which plant group is better adapted to drought,

(b) identify intra- and interspecific differences in the measured traits, and

(c) identify key physiological and anatomical parameters, which can be linked to growth performance.

This chapter was supposed to build the foundation of the present thesis from which subsequent research questions were drawn. The study was designed to get an overview of physiological responses to drought stress across hybrid poplars and trembling aspen that differed in growth performance.

The results revealed large difference in traits that are related to drought resistance. Hybrid poplars were more vulnerable to drought-induced cavitation as well as less water-use efficient when compared with aspen genotypes. These interspecific differences were mainly found in measurements of xylem vulnerability to cavitation (P_{50}), leaf water potentials (ψ*-Leaf*) and leaf carbon isotope compositions ($\delta^{13}C$). Within-group comparisons showed less differences among traits, except a surprisingly strong relationship of tree height and xylem vessel diameter (D_V), which was found independently in both plant groups. On the other hand, cavitation resistance did not show a significant relationship with tree height. This suggests that xylem vessel diameters appear to be shaped by a different environmental factor other than drought, at least for the tree material sampled in this study. An alternate explanation for this relationship of tree height versus vessel diameter may be an adaption to minimize the degree of freezing-induced embolisms. Since smaller vessels are less likely to embolize, they may provide effective protection against cavitation due to frequent freeze-thaw events. A highly embolized water transport system in spring may delay the onset of growth and could explain the observed differences in height after multiple growing seasons.

The *second research chapter* was a direct follow-up on the key results of the first research chapter, in which I found that tree height and branch vessel diameter in hybrid poplars and in aspen were strongly correlated with height (*Fig. 2.4*). Specifically, I wanted to test whether

(d) smaller vessel diameters also show an increased risk of freezing-induced embolism and how this may explain observed differences in tree height, and

(e) to determine differences in cold hardiness between seven hybrid poplar clones with contrasting growth performances.

The results showed a strong correlation between the relative amount of native embolism PLC_N and vessel diameter (D_V) as well as tree height. PLC_N was measured in autumn, winter and spring right after budbreak, and did not differ significantly over time for the tallest trees in the study. This suggests that small vessel diameters minimize freezing-induced embolisms throughout the year, which in turn maximizes xylem conductivity. I further observed that maximum winter cold hardiness and the timing of leaf senescence and budbreak were not related to growth and survival. In conclusion these results suggests that reduction of freezing-induced embolism due to small vessel diameters may be an essential adaptive trait in boreal planting environments.

My first two research chapters showed that growth performance in boreal planting environments appears to be linked with small vessel diameters. My data also showed that aspen genotypes from Minnesota had the smallest vessel diameter and were also the tallest trees on a planting test site in central Alberta. This led to the question of how would aspen genotypes from Minnesota perform on other test sites in western Canada?

To answer this question, I took advantage of a large-scale reciprocal transplant experiment with aspen provenances from Saskatchewan, Alberta foothills, central Alberta, northern Alberta and northeast British Columbia, and also included five seed lots from Minnesota. The objectives of my *third research chapter* were to:

(f) quantify trade-offs among growth performance, frost hardiness, and timing of leaf senescence and budbreak for aspen provenances (particularly from Minnesota), and

(g) discuss the potential of long-distance seed transfer for forestry operations in the context of climate change.

The results of my last data chapter showed that moving aspen provenances as far as 1,600 *km* northwest from Minnesota to central Alberta, which represents a shift of 7° latitude to the north, produced almost twice the biomass of local sources. Similarly, provenances moved 800 *km* from central Alberta to northeast British Columbia (4° latitude north) also produced twice the biomass of local sources. This increased productivity was not associated with lower survival rates due to extreme minimum temperatures or higher risks of later spring frosts. Timing of leaf senescence from southern provenances was later when compared with provenances from higher latitudes, resulting in a longer utilization of the growing season without compromising a sufficient degree of cold hardiness when early autumn frosts arrive. Budbreak occurred slightly later in provenances moved northwest, which minimizes the vulnerability to late spring frosts. Taken together these results suggest that long-distance seed transfer of trembling aspen from Minnesota to central Alberta may be an attractive option for forestry operations to compensate for reduced productivity of local trees due to current and predicted climate change scenarios.

5.2 Outlook

My PhD thesis represents a first approach to identify physiological traits that may explain observed difference in growth performance and survival of hybrid poplar and aspen trees under boreal field conditions. To determine physiological and functional trade-offs, I took advantage of large-scale common garden experiments in western Canada. My analyses focused on key hydraulic and wood anatomical traits of the above-ground plant biomass. These results have the potential to improve future poplar breeding programs and may help to optimize afforestation efforts under the assumption of current and predicted climate change.

For instance, xylem vessel diameter is an easily measurable trait and appears to be a very good predictor of hybrid poplar and aspen growth performance. It may be used as a screening tool to identify superior clones at an early age. However, there are still questions that need to be answered before vessel diameter may be used to predict growth performance reliably. My thesis has shown that smaller vessel diameters are correlated with taller trees. However, it is still not clear what are the selective forces that shape vessel diameter under boreal climate conditions. Is it an adaptation to drought in order to minimize the risk of xylem cavitation? Or is it an adaptation to minimize the degree of freezing-induced embolism? If yes, then small vessel diameters would be favoured by natural selection in regions in which drought or freeze-thaw events are likely to occur. However, it may also plausible to assume that larger vessel diameters are favoured in regions with very short growing season in order to maximize water transport to the leaves. In the end, it can also be a combination of multiple scenarios. Furthermore, the questions to which extent genotype × environment effects are responsible for the vessel diameters observed in my thesis, and to which extent vessel diameters represent a heritable trait still need to be answered.

Lastly, long-distance seed transfer of aspen genotypes from Minnesota into central Alberta, resulted in almost twice the biomass when compared with the local sources, while keeping survival rates on a similar level. This finding may be of particular interest to forest companies and government agencies when planning future afforestation efforts.

However, further research is needed to strengthen, challenge or extend beyond the observed results. Particularly, work aiming to identify causal relationships between the size of xylem vessels under various climate conditions in the boreal ecosystem, as well as studies and questions related to carbon allocation, root biology and forest genetics are vital to explain the observed differences in phenotypes.