LEAF MORPHOLOGICAL AND ANATOMICAL VARIATIONS OF PAPER BIRCH POPULATIONS ALONG ENVIRONMENTAL GRADIENTS ACROSS CANADA

SUBMITTED BY

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ABSTRACT

Leaf morphology and anatomy have been found to vary considerably among tree species, and leaf characteristics have widely been used for analyzing plant growth and resource use strategies because of their structural adaptation to withstand environments. Considering the changing climate projections, early-successional, broad niched species like paper birch (*Betula papyrifera* Marsh.) are expected to increase dominance due to a zonal shift of natural vegetation and/or open gaps within the current vegetation zones. Hence, it is important to understand factors such as leaf characteristics that enable these pioneer species to inhabit a wide geographic range and their increasing dominance.

Paper birch is a pioneer tree species in North America that inhabits wide climatic and geographic gradients; in addition, the species has developed different leaf morphology and anatomy that have allowed paper birch to adapt to diverse habitats. This study examines how the leaf characteristics of paper birch vary under uniform and stressed environments. The major objectives were (a) to investigate leaf characteristics variations in paper birch populations grown in uniform environmental conditions as in a greenhouse and a common garden; (b) to correlate between leaf characteristics and paper birch's environment of origins; (c) to investigate leaf characteristic variations in paper birch populations grown under different carbon dioxide concentrations [CO_2] and soil water levels to determine the relationship between leaf characteristics and individual or interacting effects of [CO_2], water levels and populations; and (d) to analyze the relationship within and between leaf morphology and anatomy of the birch populations.

The study found significant differences among paper birch populations in leaf morphological characteristics under a uniform environment at the greenhouse and the common garden. The leaf characteristic variations in the uniform environment may be related to the different genotypes of the birch inhabiting a wide environmental gradient. In paper birch populations grown in the common garden, significant differences in stomatal density, stomatal area, pore area and guard cell width were identified. As expected, the birch populations in greenhouse and common garden environments showed significant correlations of leaf characteristics, namely specific leaf area (SLA), leaf maximum width index and petiole area to latitude, longitude, elevation, temperature, precipitation and aridity index of origin. Correlation between leaf characteristics of paper birch in the greenhouse showed that populations originated in limited precipitation (during growing season) had low hair density on leaf adaxial surface, with larger leaf width and petiole area. Birch populations grown in the common garden revealed that populations originated in higher mean annual precipitation had less hair density on leaf adaxial surface with smaller leaf area and higher stomatal density. Relationships within the leaf characteristics revealed significant correlations within and between leaf morphology and anatomy as populations with larger leaf area had larger petiole area and less adaxial hair density in greenhouse. The larger petiole in larger leaf reflects the need for mechanical strengthening to support, whereas inverse relationship between leaf area and hair density possibly showed a strategy of the birch to balance water loss. In common garden, the birch populations with larger leaf area had larger specific leaf area and higher adaxial hair density but low stomatal density. All these features in paper birch populations provide a structural basis for reducing water loss through leaves and increasing water use efficiency. There was no consistency in leaf characteristics when the paper birch populations were grown in uniform environments as in the greenhouse and the common garden.

Analysis of the leaf characteristics in the birch showed significant differences due to the interaction and/or main effects of [CO_2], water levels and populations. Paper birch had decreased leaf area and increased stomatal density under elevated [CO_2] which might have reduced stomatal conductance and increased water-use efficiency. Under low soil water level, paper birch populations studied had smaller stomatal area, pore area and guard cell width. Contrasting with the expectation neither stomatal area was larger nor stomatal density increased under low water level. A trade-off between stomatal area and density in this study showed that stomatal area per unit leaf area remained the same. Hence, smaller stomatal area and guard cell width under low water level must have improved [CO_2] diffusion and decreased water loss compared to larger stomatal area and guard cell width.

The results of this study confirmed significant genotypic difference in leaf characteristics of paper birch populations irrespective of a uniform growing environment. The characteristics, namely leaf area, maximum width, SLA, stomatal density and stomatal area, appear related to the environment of origin; however, these relationships were not consistent in the birch populations grown in the greenhouse and common garden. Paper birch populations acclimated to the uniform environments; differences in leaf area, stomatal density and stomatal area in paper birch populations under different [CO_2] and soil water levels prove the birch's ability to acclimate to environmental changes. Lastly, integration of leaf morphology and anatomy enhanced paper birch's ability to balance between [CO_2] gain and water loss.

Key words: Phenotypic plasticity, genotypes, leaf area, leaf shape, petiole area, leaf hair density, stomatal density, stomatal area, paper birch, pioneer tree and climate change

Preface

Publications arising from this dissertation

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CHAPTER 1

INTRODUCTION

Trees in nature vary along environmental gradients at the intraspecies level. Ecologists have suggested that different genotypes within a species have evolved phenotypic changes resulting in intraspecies variation (Darwin 1859, Reich et al. 1998, Wright et al. 2004). These phenotypic variations have enabled species to inhabit a wide environmental gradient. Yet, within the habitat of widespread plant species, the individuals are likely to face a heterogeneous environment representing a unique set of resources; i.e., biotic and abiotic conditions. Consequently, the individuals regulate their structure and function to acclimate under environmental conditions resulting in phenotypic variations often termed as phenotypic plasticity (Coleman et al. 1994, Violle et al. 2007, Jung et al. 2010). A species' phenotypic plasticity includes genotypic differences and the adaptive mechanism of the individual to different environments (Coleman et al. 1994, Mal and Lovett 2005). Hence, the major goal of plant ecology is to understand these phenotypic changes in plants species in relation to the available resources in the growing environment (Coleman et al. 1994).

Genotypes contribute to phenotypic variations in plants, displayed in leaf morphological and anatomical characteristics. The leaf characteristics are sensitive to the inhabiting environmental conditions, and so the assessment of the leaf characteristics shows the broad spectrum of a plant's resource use strategies through leaf investment (Reich et al. 1997, Hajek et al. 2013) and their strong associations with climate and geographic position (Wright et al. 2004). The majority of previous studies compared and recognized differences in leaf characteristics between different species or within a species inhabiting different habitat range (Cordell et al. 1998, Guerin et al. 2012, Marcysiak 2012). It is often useful to determine whether leaf characteristic variations in different plant populations are observed when grown under a uniform environment, and to examine how plants modify their leaf to acclimate to the change in climate and available resources such as carbon dioxide, precipitation, temperature and humidity.

Uncertain changes in temperature, precipitation, humidity, snow cover and frequency, and severity of extreme events associated with the changing environment (Le Houerou 1996) have created extreme selection pressure on natural plant species. Climate change projections indicate there will be a shift in the climate zone over years; consequently, tree species will either adapt, migrate or become extinct due to changes in available resources (Aitken et al. 2008). In view of the rapidity of global warming, fast migratory responses are needed for species to cope with the changes; thus, species with restricted habitats may be more vulnerable to extinction (Johnston et al. 2009, Lindner et al. 2010). Several studies suggested that early-successional, broad-niched species such as birch would increase dominance or migrate due to a zonal shift of natural vegetation (Stocklin and Baumler 1996, Johnston et al. 2009, Garamvolgyi and Hufnagel 2013) and/or open gaps within the current vegetation zones (Johnston et al. 2009, Garamvolgyi and Hufnagel 2013), if predicted global warming occurs. More importantly, effects of changing climate on plants are likely to be different for different species and the response of species with similar climatic niches cannot be expected to respond consistently (Werkman and Callaghan 2002, Baselga and Araujo 2009, Johnston et al. 2009, Butof et al. 2012, Garamvolgyi and Hufnagel 2013). Plants can easily add, modify and remove parts such as leaves and branches as per the available resources (Coleman et al. 1994); therefore, analyzing the causes and consequences of leaf characteristic variations is fundamental to understanding a species' ability to adapt in a wide habitat range and changing climatic conditions.

The paper birch

The birches (*Betula* L.) are common trees of the boreal and temperate zones of the Northern hemisphere (Furlow 1990, Jong 1993). Among birches, paper birch (*Betula papyrifera* Marsh.) is one of the most widely distributed pioneer tree species in Canada (Carlson et al. 1999), found in all forested regions and north to tree line (Safford et al. 1990). The birch grows in a wide variety of soil types and is abundant on upland terrain, floodplain, open slopes, swamp margins and in bogs (Carlson et al. 1999), and includes poorly drained, well drained and extremely dry sites (Safford et al. 1990). The wide geographic distribution of the birch is due to its ability to regenerate on sites of poor quality, and to its tolerance to flood and drought (Peterson et al. 1997). This ability might have been obtained by morphological, anatomical and physiological modifications due to genetic diversity among the birch populations.

Until recently, the birch has been looked upon as a weed species, and few studies have focused on the physiological variations in paper birch populations (Li et.al. 1996, Wang et al. 1998, Benowicz et al. 2001), while no studies on leaf morphology and anatomy were found by this researcher. With its commercial potential, the birch is now recognized as a suitable reforestation species (Peterson 1997, Carlson et al. 1999). It is gaining ecological significance because of its productivity, easy regeneration, few serious damaging agents (Klinka et al. 2000), and its ability to cycle nutrients, add organic matter by the loss of leaves and increase site productivity (Parish 1996). Knowledge of intraspecific leaf variations of paper birch populations must be addressed to understand and manage the birch more effectively under potential impacts of expected changes in climate.

The leaf and stem growth in birch

The primary growth and branching processes of plants depend upon the activity of meristems located at each axis and or at leaf axils; they involve the differentiation of organ primordia from meristems, including the inception of new organs (organogenesis) and extension of these primordia into fully developed organs (Puntieri et al. 2000, Puntieri et al. 2002). The inception of new organs results from the functioning of undifferentiated cells that constitute the apical meristem located at the tip of stem. During an active phase, these meristems form small cell masses that would develop into embryonic leaves and leaves on elongated stems (Barthelemy and Caraglio 2007). In many temperate plants, primordial organs remain dormant in buds and develop into mature organs after a certain time period. They are referred to as preformed organs. On the other hand, the inception and extension of organs may proceed sequentially without an intervening dormancy period, resulting in organs termed neoformed (Pollard and Logan 1974). In cold regions and temperate zones, the major part of shoots developed by trees consists of organs that are performed in a growing season previous to that of shoot extension. A minor proportion of shoots in these species develop neoformed organs during the growing season, which may benefit them in favorable environmental conditions (Puntieri et al. 2000, Puntieri et al. 2002).

Shoots that are not fully preformed in the winter bud are long shoots that produce two types of leaves, early and late leaves. The early leaves emerge shortly after bud break and late leaves appear in the growing season, after the first leaves are well expanded (Kozlowski and Clausen 1966). Early leaves emerge on short shoots whose internodes are not elongated and which lack late leaves. In tree species such as *Betula*, *Acer*, *Eucalyptus* and *Populus,* some shoots are not fully preformed in the winter bud and exhibit neoformation by producing both early and late leaves, termed as heterophyllous leaves (Pallardy 2010).

The birch has heterophyllous leaves and there is evidence that leaves of the same shoot may have different developmental and functional attributes (Kozlowski and Clausen 1966, Pallardy 2010). It has been suggested that earlier leaves as in *Betula platyphylla* flush first to avoid damage by late frost, and utilize higher temperatures for expansion of shoots (Kozlowski and Clausen 1966, Koike 1995). These two sets of leaves produced by the birch frequently differ in leaf size, venation size, toothing, thickness, stomatal development and other leaf characteristics (Kozlowski and Clausen 1966). Such dissimilarities are justified by the fact that these two kinds of leaves extend at different ages, times and under different environmental conditions (Guedon et al. 2006). To reduce these variations within trees, previous studies on the intraspecific comparisons of leaves used samples collected at approximately the same height, location and either on the same date or after growth has stopped (Blue and Jensen 1988, Bruschi et al. 2000, 2003).

Objective of the study

The overall goals of this dissertation are (a) to analyze and understand why leaf morphology and anatomy vary in paper birch populations (originating across different habitats) raised under uniform environmental conditions; and (b) to analyze how paper birch population modifies leaf morphological and anatomical characteristics when raised under the elevated carbon dioxide concentration and limited soil water levels.

Chapter 2 investigates if leaf morphology varies among paper birch populations when grown under uniform environmental conditions as in a greenhouse; and if the resulted variations of morphological characteristics are related to climate of the population's origin. Twenty three paper birch populations, aged six months, and from different environmental origins were analyzed for leaf characteristics in this study.

Chapter 3 includes the analysis of the leaf morphological and stomatal characteristics of sixteen paper birch populations grown in a common garden in Thunder Bay. The study's objectives were to analyze differences in leaf morphological and stomatal characteristics of two-years-old seedlings of paper birch populations grown in the common garden; to explore the correlations between leaf morphological and stomatal characteristics; and to determine the relation of leaf characteristic to climate variables of origin. If paper birch populations grown in the common garden maintain leaf morphological differences at the population origin, I could assume that the differences were due to underlying genotypic differences.

The leaf characteristic variations in Chapters 2 and 3 provide a framework for Chapter 4 to study leaf morphological and anatomical characteristics of paper birch populations treated under the stress of elevated [CO_2] concentration and decreased soil water level. Chapter 4 examines the effect of these changes on leaf characteristics and explores the capacity of birch populations to adapt under the stress. I chose four paper birch populations from different geographic origins with mean annual precipitation that ranged from 279mm to 1032mm.

CHAPTER 2: Leaf morphological variation among paper birch (*Betula papyrifera* Marsh.) populations: A greenhouse experiment

ABSTRACT

Increasing dominance of broad niched, early successional species such as paper birch are expected as a result of increasing climatic variability, if the predicted climate change comes true because the species appear to have considerable genotypic and leaf morphological variations that have allowed them to inhabit wide environmental gradients. Analyzing one of the factor (leaf characteristics) that enables these species to occupy such variant habitats is of paramount importance This study examines variations in leaf morphological characteristics of 23 paper birch populations across Canada and grown in a greenhouse; furthermore, the study explores whether the variations in leaf morphological characteristics are related to the climate of the population's origin.

I found significant differences in all leaf morphological characteristics (*P*=<0.001) measured among the birch populations. Thus, I expected that the morphological variations in birch might be related to natural diversity in birch populations due to environmental differences at habitat origin. Principal component analysis (PCA) reduced thirteen leaf morphological variables to five principal components (PC), which explained 90.2% of the total variance in the original data. PCs accumulated with leaf maximum width index and aspect ratio, and specific leaf areas were significantly negatively related to mean annual precipitation at the population's origin. The correlation analysis within leaf morphological characteristics showed significant positive relation between leaf width index and petiole sizes.

Hence, the birch populations had significant genotypic variations in leaf morphology, but most of these variations were unrelated to environment of origin. Unexpected relations of SLA, the width index and aspect ratio to the habitat of origin, raised the possibility of the birch populations' ability to acclimate in the growing environment. Significant relation within leaf morphological characteristics resulted in this study showed that the leaf characteristics provide a basis for the birch to mechanically strengthen and reduce evaporation through leaf surfaces during drought.

INTRODUCTION

Plant species inhabiting environmental gradients exhibit genotypic and phenotypic differences (Via and Lande 1985, Jonas and Geber 1999). It has been suggested that as a strategy to maximize growth rate, plants respond to these environmental changes by differentially allocating biomass to capture optimum light, water, nutrients and carbon dioxide (Bloom et al. 1985). Plants develop the ability, often referred to as phenotypic plasticity, to produce different phenotypes as a response to abiotic stress (McLellan 2000). The characterization of geographical patterns of morphological variation in natural plant populations suggests possible patterns of genotypic variation and plastic responses to environmental gradients (Ohsawa and Ide 2008, Uribe-Salas et al. 2008). These plasticity responses are expressed at different levels such as plant morphology, anatomy, physiology and growth.

Leaves are the important organs for plant growth and are sensitive to the inhabiting environment (Coleman et al. 1994). Leaf morphological variations for plants growing in contrasting habitats have long been studied in numerous species such as *Azadirachta indica* (Kundu and Tigerstedt 1997), *Eucalyptus sideroxylon* (Warren et al.

2005), and *Quercus rugosa* (Uribe-Salas et al. 2008). With increasing temperature and decreasing precipitation, leaf size and specific leaf area decrease whereas the thickness of the leaf increases. Previous studies suggest that small leaves with their low boundary resistance and efficient sensible heat exchange can avoid heating much above air temperature although they cannot cool much below air temperature, whereas large and wide leaves suffer from overheating when water is limited (Gates et al. 1968, Warren et al. 2005). Smaller and narrower leaves are often associated with higher elevation habitats that have higher temperatures (Cordell et al. 1998), and where precipitation (McDonald et al. 2003) and aridity index are limited (Roderick et al. 2000). Furthermore, small leaves are characterized by a smaller specific leaf area (SLA) and less leaf hair density. It is suggested that thick (low SLA) leaves can better withstand wilting in comparison to thinner leaves in dry and hot environments (Warren et al. 2005, Milla and Reich 2007). Additionally, leaf hairs could influence leaf water relations by increasing the boundary layer resistance (Donselman and Flint 1982, Hilaire and Graves 1999) and decreasing leaf temperature by reflecting radiation (Ehleringer and Mooney 1978). Consequently, increased leaf hairs in hot and arid habitats have significant influence on reducing solar radiation, leaf temperature and transpirational losses (Ehleringer and Bjorkman 1978, Ehleringer et al. 1981, Picotte et al. 2007).

Narrower leaves are viewed as a plant’s adaptation to dry and hot environments, while wider leaves are an adjustment to wet and cold environments. It has been established that in comparison to wider leaves, narrower leaves provide structural reinforcement to withstand wilting in hot, sunny and dry environments (Werger and Ellenbroek 1978, Abrams 1990, 1994). Similarly, petiole length influences leaf arrangement, affecting light interception efficiency under different circumstances

(Niinemets et al. 2004). Previous studies have shown that petiole area increases in larger leaves along decreasing drought gradients, which probably reflects the need for mechanical strengthening to support large leaves (Niinemets et al. 2006, Poorter and Rozendaal 2008). However, within the deciduous broadleaved trees, petiole area increases in drought-prone habitats, which may be a mechanical device to promote leaf cooling (Meng et al. 2009).

The majority of studies on leaf morphological variation in response to climatic factors have included species inhabiting different environments. Results of these studies showed remarkable leaf morphological variation in relation to their inhabiting environments (Joel et al. 1994, Bruschi et al. 2003, Calagari et al. 2006, Uribe-Salas et al. 2008). For example, species of *Betula* from different habitats often show significant differences in leaf morphology such as leaf area (Dancik and Barnes 1974, Sharik and Barnes 1979, Senn et al. 1992, Aspelmeier and Leuschner 2006) and shape (Dancik and Barnes 1974, Sharik and Barnes 1979, Aspelmeier and Leuschner 2006). Most of these studies on leaf morphological response to environmental factors have either included comparative studies among multiple species (Abrams 1994) or species inhabiting different locations along an environmental gradient (Abrams 1990, 1994, Ashton et al. 1998). Therefore, it is important to determine whether leaf morphology differs in wide-ranging pioneer species like paper birch grown in a uniform environment.

Paper birch, the most widely distributed pioneer tree species in Canada (Farrar 1995), is an ecologically and economically important hardwood species. The interaction of genetic diversity and wide environmental range within its distribution may have resulted in morphological variation as it is in other species (Gurevitch 1992, Warren et al. 2005). In this study, I examined if leaf morphology varies among paper birch populations across Canada and if the variations in these leaf morphological characteristics are related to the latitude, longitude, elevation and climates of origin. Based on geographic and climatic differences in a population's origin, I hypothesized that leaf morphological characteristics would vary among paper birch populations despite of the same growing environment. The differences in leaf morphology of the birch populations are predicted to correlate with the environmental conditions at the population's origin. Larger, wider and thinner leaves (i.e. higher specific leaf area, leaf area and maximum width index) with larger petiole size but less leaf hair density were expected in the birch populations originated in areas of higher precipitation and aridity index but along decreasing temperature, longitude, latitude and elevation gradients. Thirdly, leaf morphological characteristics are hypothesized to be correlated with each other as a strategy to reduce water loss through leaves.

MATERIALS AND METHODS

Sample collection and leaf morphological data

Seeds of 23 paper birch populations were collected from Newfoundland, Prince Edward Island, New Brunswick, Quebec, Ontario, Saskatchewan, and British Columbia. The populations' origins ranged from 20 meters to 840 meters elevation, with mean annual precipitation at 279 mm to 2062 mm, and 0.9^{o}C to 8.9^{o}C annual mean temperature (Appendix I). Seeds of the 23 paper birch populations were germinated in horticultural trays (28cm x 56cm) filled with a 2:1 (volume) mixture of peat moss and vermiculite in a greenhouse at Lakehead University. Three randomly selected birch seedlings from each population were grown for six months (January to June 2010) in containers that were 21-25cm (upper circle size) and 41.5cm deep. The seedlings were well watered and fertilized once a week with a regular fertilizer (N-P-K, 20:20:20). The containers were rearranged randomly on a weekly basis to minimize the effects of environmental patchiness in the greenhouse. In July 2010, I randomly sampled five well-developed leaves from each seedling from the middle crown of the seedlings for leaf morphological measurements and analysis.

Using WinFolia software (Winfolia 2007), I measured leaf area (LS), perimeter (P), blade length (BL), petiole length (PL), petiole area (PS), maximum width (MW) of the leaf blade, position of the maximum width (PMW) of the leaf blade, horizontal width (HW) of the leaf blade, and vertical length (VL) of the leaf (Aas 1993, Bruschi et al. 2000, Kremer et al. 2002, Curtu et al. 2007, Du et al. 2007). I counted hairs on three parts of each leaf surface (0.20 cm^2) using an Academic sterezoom microscope at 30X magnification, and calculated the average number of hairs on each adaxial and abaxial leaf surface for further analysis. Subsequently, sampled leaves were dried at 70°C for 42 h, the leaf dry mass (DM) was measured, and specific leaf area (SLA) was calculated (Appendix II). In addition, some leaf characteristics were calculated as ratios, since ratios relate to shape rather than size and may thus provide additional information (Winfolia 2007).

Climate data

Mean annual and growing season temperatures and precipitation data for the population's origin were taken from Environment Canada's normalized climate data from years 1971 to 2001 (Environment Canada). I used De Martonne's equation to calculate the mean annual aridity index (De Martonne 1926, Migalina et al. 2009), and Sijors's (1974) equation to calculate the aridity index during the growing season (Appendix II).

Statistical analysis

Leaf characteristics like leaf area, specific leaf area, blade length, maximum width index, petiole length, petiole area, and petiole index (ratio between petiole length and total leaf length) were log transformed. Assumptions of normality and homogeneity were checked for all leaf morphological characteristics with Shapiro-Wilk's Test and the Levene Test, respectively. Aspect ratio, form coefficient, adaxial and abaxial hair density were square root transformed after normality testing. Analysis of variance (ANOVA) was used to analyze differences in leaf morphology of paper birch populations; the Tukey HSD test was used to analyze the morphological differences between populations.

A principal component analysis was performed as described by Johnson and Wichern (1992), Tausz et al. (1998), Warren et al. (2005) and Uribe-Salas et al. (2008). The objectives of the analysis were to identify patterns in the original data sets, reduce variables without losing much information and facilitate the extraction of accumulated variables not generally accessible for measurement. The principal component (PC) analysis was based on twelve leaf morphological variables and these variables were reduced to five principal components that represent most of the information in the original data set. An acceptable PC solution was based on the Kaiser criterion (all eigenvalues greater than 1) and visual examination of Scree plot (Tausz et al. 1998, Warren et al. 2005). PCs were determined after Varimax with Kaiser Normalization rotation to maximize the variance of loadings (Johnson and Wichern 1992). I analyzed correlation among leaf morphological characteristics and climatic variables using Pearson's correlation. In reference to the results of previous studies (Santiago et al. 2004, Warren et al. 2005, Russo et al. 2010), correlation coefficients (r) are considered for discussion if the values are ≥ 0.25 at $p<0.5$ significance level. All statistical analyses were conducted using SPSS-18 (SPSS, Chicago, IL, USA) and R-2.12.1 (R-Development Core team, 2011).

RESULTS

Variations in leaf morphological characteristics

Leaf morphology differed significantly among paper birch populations grown in Lakehead University greenhouse (Table 2.1). Leaf area, specific leaf area, petiole area, aspect ratio, form coefficient, petiole index and leaf hair density varied significantly (*P*=<0.001, Table 2.1). Populations from Amanita and Petawawa had the smallest leaf areas, which differed significantly from Timmins, Wayerton, Bush Creek and Mars Creek populations. Although Amanita Lake population had smaller leaf area, it had significantly larger petiole area and index compared to Milvale and Bells Fall populations. Additionally, Wilson Creek and Frost Lake had a significantly larger petiole index in contrast to populations from Allardville, Bells Fall, Milvale, New Brunswick and Timmins (Table 2.1).

Specific leaf area was the highest in Frost Lake and the lowest in Barnes Creek. The population from Barnes Creek significantly differed from those originating in Frost Lake, Mars Creek, and St. Mary River. Furthermore, the populations from Frost Lake, Mars Creek and St. Mary River differed significantly from the smaller specific leaf areas found in Adam Lake, Amanita Lake, Barnes Creek, Bells Fall, Little Oliver Lake, New Brunswick, Newfoundland, Timmins and Wayerton (Table 2.1).

Leaf hair density on adaxial surfaces ranged from 1.58 in Cussion Lake to 3.88 in Bells Fall; in fact, the population from Cussion Lake had the lowest density, differing significantly from Alice, Allardville, Little Oliver Lake, Mars Creek, Milvale, New Brunswick, Petawawa, Prince Albert, St. Mary River and Wayerton populations. On the other hand, leaf hair density on adaxial surfaces in the Bells Fall population did not differ significantly from Allardville, Mars Creek, St. Georges and Wayerton. Furthermore, Bells Fall had the narrowest leaf whereas Bush Creek had the widest leaf and width index. The population from Bells Fall had significantly narrower leaf size than the populations from Adam Lake, Bush Creek, Little Oliver Lake, Mars Creek, Newfoundland, Wayerton and Wilson Creek. Conversely, Bush Creek had significantly wider leaf size compared to the populations from Alice, Amanita Lake, Bells Fall, Milvale, Petawawa and St. Georges (Table 2.1).

Table 2.1 Analysis of variance for population effects on the leaf morphological characteristics in twenty three paper birch populations grown in the greenhouse. The values include mean with standard deviation in parentheses. Here, LS - leaf area in cm^2, SLA- specific leaf area (cm^2/g), MW-maximum width (cm), AR- aspect ratio, PeA- petiole area (cm^2), PeI- petiole index (ratio) and ADH- hair density on leaf adaxial surface.

Populations	**LS**	**SLA**	**MW**	**AR**	**PeA**	**PeI**	**ADH**
Newfoundland	62.51	291.31	8.59	0.71	5.38	0.44	7.03
	(22.1)	(41.6)	(1.5)	(0.1)	(3.2)	(0.1)	(4.3)
St. Georges	56.21	303.17	7.79	0.61	3.41	0.43	10.44
	(24.3)	(37.9)	(1.7)	(0.1)	(2.1)	(0.1)	(5.9)
Millvale	54.24	319.47	7.96	0.68	2.03	0.37	7.52
	(14.1)	(21.6)	(1.0)	(0.1)	(1.7)	(0.1)	(2.4)
Allardville	53.41	305.80	8.04	0.70	3.59	0.38	10.4
	(16.3)	(18.8)	(1.3)	(0.1)	(3.9)	(0.1)	(6.3)
Cap des	47.23	298.17	7.04	0.55	2.00	0.38	15.53
Rosier	(15.1)	(61.7)	(1.3)	(0.1)	(2.1)	(0.1)	(5.3)

Populations	**LS**	**SLA**	**MW**	**AR**	**PeA**	**PeI**	**ADH**
Wayerton	67.73	290.12	9.58	0.83	8.3	0.45	10.26
	(19.4)	(35.7)	(1.6)	(0.2)	(7.5)	(0.1)	(4.3)
New Brunswick	65.51	303.87	8.59	0.67	3.73	0.41	5.84
	(21.8)	(26.7)	(1.5)	(0.1)	(2.8)	(0.1)	(3.6)
Alice	53.6	335.66	7.70	0.69	3.27	0.43	8.17
	(13.4)	(29.7)	(0.9)	(0.2)	(1.7)	(0.1)	(2.4)
Petawawa	44.39	305.87	7.11	0.69	2.32	0.42	8.40
	(20.3)	(55.4)	(1.6)	(0.1)	(1.6)	(0.1)	(4.1)
Timmins	67.29	292.46	8.90	0.74	5.99	0.40	5.14
	(19.4)	(27.4)	(1.2)	(0.1)	(2.4)	(0.1)	(3.8)
Thunder Bay	55.91	334.88	8.20	0.67	3.88	0.45	4.43
	(15.8)	(43.1)	(1.3)	(0.1)	(2.6)	(0.1)	(1.8)
Prince Albert	58.42	326.82	8.46	0.67	3.28	0.40	6.86
	(21.0)	(19.5)	(1.7)	(0.1)	(2.6)	(0.1)	(3.4)
St. Mary River	59.85	346.61	8.81	0.85	7.73	0.44	5.27
	(11.8)	(32.4)	(0.8)	(0.2)	(3.6)	(0.1)	(2.4)
Wilson Ck	61.78	298.97	9.10	0.89	9.75	0.49	6.82
	(15.0)	(23.8)	(1.0)	(0.2)	(4.2)	(0.1)	(3.1)
Mars Ck	73.38	346.18	9.62	0.79	6.39	0.45	9.29
	(21.9)	(35.4)	(1.5)	(0.1)	(3.7)	(0.1)	(2.9)
Barnes Ck	56.99	275.62	8.73	0.90	8.19	0.47	5.00
	(15.0)	(21.3)	(1.1)	(0.1)	(3.1)	(0.1)	(3.2)
Bush CK	70.72	316.71	9.81	0.83	7.96	0.46	2.87
	(23.6)	(29.6)	(1.4)	(0.1)	(3.8)	(0.1)	(2.7)
Adams Lk	65.00	282.39	9.32	0.85	8.18	0.44	4.93
	(20.2)	(30.9)	(1.5)	(0.2)	(3.6)	(0.1)	(3.9)
Amanita Lake	40.29	284.8	7.12	0.88	6.78	0.48	6.98
	(8.9)	(15.8)	(0.9)	(0.2)	(2.8)	(0.1)	(2.9)
Cussion Lake	53.71	302.62	8.71	0.94	8.37	0.46	3.72
	(8.3)	(26.3)	(0.7)	(0.2)	(3.1)	(0.1)	(1.9)
Frost Lk	49.12	372.92	8.21	0.95	6.73	0.50	5.78
	(17.2)	(65.1)	(1.5)	(0.2)	(3.4)	(0.1)	(2.9)
Juniper Ck	49.02	334.44	7.91	0.81	5.41	0.44	5.53
	(16.5)	(34.3)	(1.4)	(0.2)	(4.6)	(0.1)	(2.2)
Lt. Oliver Lk	63.90	292.65	8.90	0.87	8.58	0.45	7.60
	(14.1)	(20.3)	(1.0)	(0.2)	(2.8)	(0.1)	(1.8)
F-ratio	3.5	7.8	5.2	10.6	8.6	3.8	7.7
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Ck stands for Creek and Lk stands for Lake.

Relationship between leaf morphology, and geographic coordinates and climate of a population's origin

The PCA resulted in five principal components explaining 90.20 % of the total variance in the data (Table 2.2). Communality values (a measure of how well the input variables are explained by the five PCs) were greater than 0.75 for all leaf characteristics. The eigenvectors value in PC1 was positively related to leaf dry weight, area, perimeter, and maximum width. PC2 was strongly related to petiole length, petiole area and petiole index. PC3 was related to leaf aspect ratio and maximum width index. PC4 was related to hair densities on adaxial and abaxial surfaces. Last, PC5 was related to specific leaf area (Table 2.2).

Correlation analysis revealed that the scores of PC3 were positively related to latitude (r = 0.43, P=<0.001), longitude (r = 0.53, P=<0.001) and elevation (r = 0.50, P=<0.001; Table 2.3). Along climatic variables, PC3 was significantly positively correlated to mean annual temperature (r= 0.32, P=<0.001), but was negatively related to precipitation (r= -0.43 P=<0.001; Fig 2.1) and aridity index (r= -0.48, P=<0.001; Table 2.3). There was a significant correlation of PC5 to mean annual precipitation (Fig. 2.1) and aridity index (r=-0.26 and r=-0.27, P=<0.001). Analysis of morphological variables against climate during growing season indicated that the scores of PC3 correlated strongly to precipitation and aridity index (r= -0.59 and r= -0.51, P=<0.001 respectively; Table 2.3). On the contrary, PC1, PC2 and PC4 were either weakly or insignificantly related to the environmental variables measured (Table 2.3).

Table 2.2 Principal component analysis for leaf characteristics for paper birch populations grown in a greenhouse. The data are PC loading with communalities determined after Varimax rotation. Leaf characteristics included were: SDW- square root leaf dry weight, LLS-log leaf area (cm^2), LSLA- log specific leaf area (cm^2/g), PER- perimeter (cm), MW- leaf maximum width (cm), LMWI- log maximum width index, SAR- square-root of aspect ratio (horizontal width/vertical length of leaf), LPeL- log petiole length (cm), LPeA- log petiole area (cm^2), LPeI- log petiole index (ratio) and SADH- square-root of number of hairs on leaf adaxial surface and SABH- square-root of number of hairs on leaf abaxial surface. The PC loadings >0.7 are indicated boldfaced.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	
Eigen values	3.95	2.38	1.80	1.56	1.13	
Variance %	32.95	19.83	15.03	12.96	9.43	
Cumulative %	32.95	52.78	67.81	80.77	90.20	
Leaf characteristics Eigenvectors						**Communality**
SDW	**0.95**	0.07	-0.07	-0.08	-0.27	.98
LLS	**0.98**	0.05	-0.08	-0.06	0.08	.98
LSLA	0.06	-0.08	-0.05	0.02	**0.98**	.98
PER	**0.95**	0.00	-0.08	0.03	0.12	.92
MW	**0.95**	0.13	0.18	-0.08	0.09	.97
LMWI	-0.09	0.12	**0.86**	-0.03	0.09	.77
SAR	0.01	0.28	**0.83**	0.02	-0.16	.79
LPeL	0.28	**0.95**	0.06	-0.08	-0.01	.98
LPeA	0.34	**0.72**	0.46	-0.02	-0.16	.87
LPeI	-0.27	**0.91**	0.25	-0.05	-0.03	.96
SADH	-0.16	0.00	-0.18	**0.87**	-0.01	.81
SABH	0.04	-0.11	0.16	**0.88**	0.03	.82

Table 2.3 Pearson correlations between the five principal components and the geography and climate variables of paper birch populations' origins. Here, MAT is mean annual temperature in °C, MAP is mean annual precipitation in millimeters, MAI is mean annual aridity index, GST is growing season temperature in °C, GSP is growing season precipitation in millimeters and GSA is growing season aridity index. Values are correlation coefficient (r) with p-values in parentheses.

Environment of population's origin	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Latitude	-0.05	0.12	0.43	-.07	0.15
	(0.33)	(0.01)	(<0.001)	(0.15)	(<0.001)
Longitude	0.01	0.12	0.53	-0.08	0.21
	(0.92)	(0.01)	(<0.001)	(0.07)	(<0.001)
Elevation	0.03	0.16	0.50	-0.08	0.20
	(0.47)	(<0.001)	(<0.001)	(0.08)	(<0.001)
MAT	0.04	0.14	0.32	-0.10	0.01
	(0.35)	(<0.001)	(<0.001)	(0.03)	(1.00)
MAP	0.01	0.02	-0.43	0.04	-0.26
	(0.84)	(0.63)	(<0.001)	(0.39)	(<0.001)
MAI	0.01	0.01	-0.47	0.05	-0.27
	(0.89)	(0.97)	(<0.001)	(0.34)	(<0.001)
GST	0.05	-0.07	0.16	0.10	0.18
	(0.34)	(0.16)	(<0.001)	(0.04)	(<0.001)
GSP	-0.05	-0.08	-0.53	0.07	-0.17
	(0.31)	(0.10)	(<0.001)	(0.14)	(<0.001)
GSA	-0.05	-0.05	-0.51	0.04	-0.19
	(0.32)	(0.33)	(<0.001)	(0.46)	(<0.001)

Relationships within leaf morphological characteristics of paper birch populations

Among leaf morphological characteristics, petiole area significantly increased in larger leaves with increased maximum width index and aspect ratio (Table 2.4). Increase in the maximum width index had significantly increased petiole index (Table 2.4). Although the correlation was not as strong as in maximum width index, SLA was significantly larger in elongated leaves with smaller petiole area and petiole index (Table 2.4). Leaf hair was denser on the adaxial surface of the paper birch with smaller leaf width (Table 2.4) although the correlation was not strong.

Table 2.4 Pearson correlations among leaf characteristics of paper birch populations grown in a greenhouse. Measured leaf characteristics were: LLS-log leaf area (cm^2), LSA- log specific leaf area (cm^2/g), MW- maximum width, LMWI- log maximum width index, SAR- square-root of aspect ratio, LPeA- log petiole area (cm^2), LPeI- log petiole index (ratio) and SADH and SABH- square-root of number of hairs on leaf adaxial and abaxial surfaces respectively. Values are correlation coefficient (r) with p values in parentheses.

	LLS	LSLA	MW	LMWI	SAR	LPeA	LPeI	SADH
LSLA	0.14							
	(<0.001)							
MW	0.94	0.11						
	(<0.001)	(0.02)						
LMWI	-0.13	-0.02	0.15					
	(0.01)	(0.60)	(0.01)					
SAR	-0.07	-0.17	0.13					
	(0.14)	(<0.001)	(0.01)					
LPeA	0.31	-0.18	0.47	0.34	0.66			
	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)			
LPeI	-0.24	-0.15	-0.09	0.42	0.41	0.63		
	(<0.001)	(<0.001)	(0.08)	(<0.001)	(<0.001)	(<0.001)		
SADH	-0.18	0.01	-0.22	-0.09	-0.13	-0.15	-0.06	
	(<0.001)	(0.80)	(<0.001)	(0.05)	(0.01)	(<0.001)	(0.22)	
SABH	-0.04	0.04	-0.04	0.05	0.09	-0.02	-0.09	0.54
	(0.39)	(0.36)	(0.46)	(0.29)	(0.07)	(0.65)	(0.06)	(<0.001)

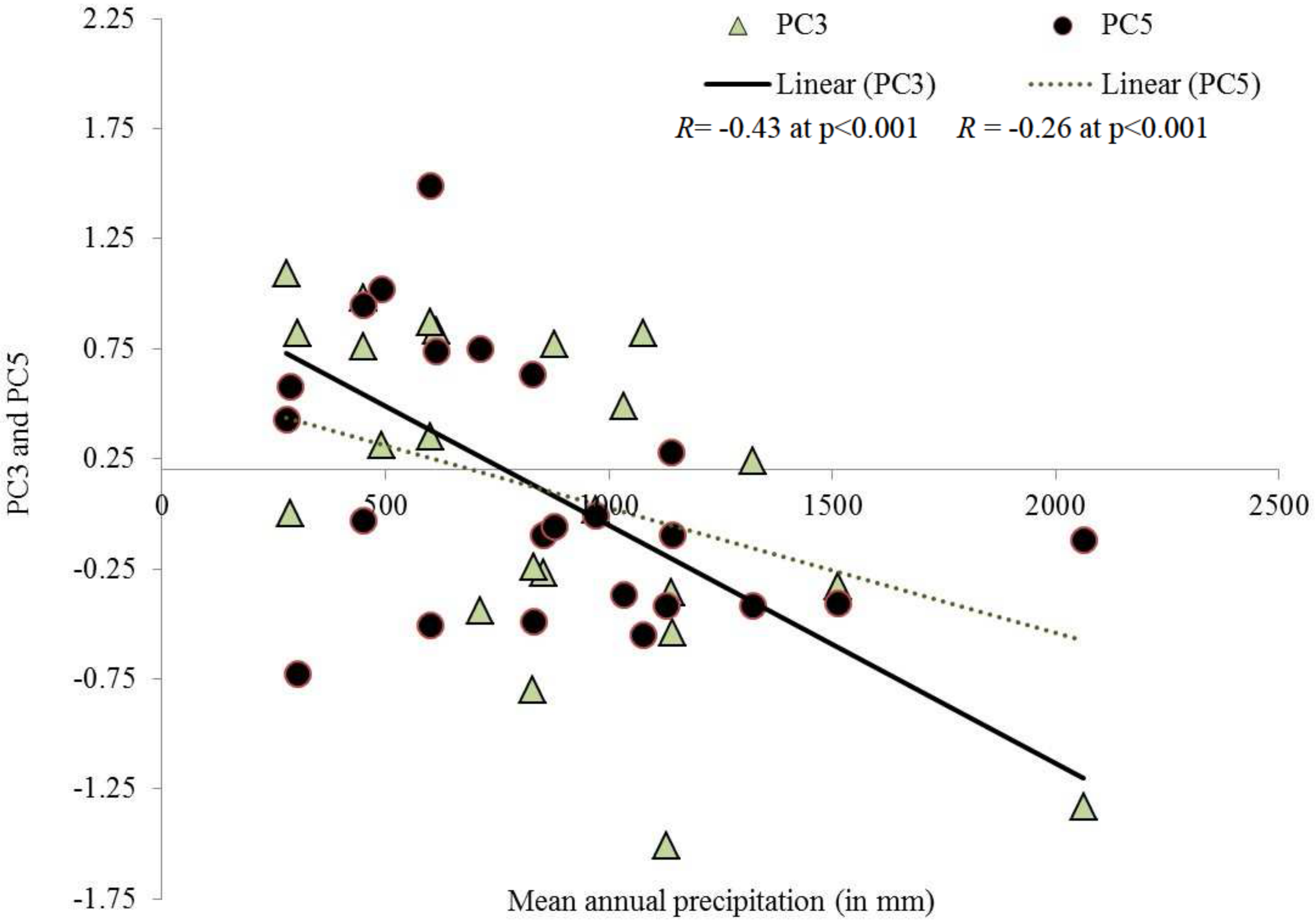

Figure 2.1
Correlation of the third principal component (PC3) and the fifth principal component (PC5) to mean annual precipitation-mm. PC3 is accumulated with leaf maximum width index and aspect ratio and PC5 is accumulated with specific leaf area (SLA)

DISCUSSION

Supporting the study's hypothesis, the results clearly demonstrate the leaf morphological diversity in paper birch populations grown under uniform conditions in a greenhouse. This coincides with the results of Kundu and Tigerstedt (1997) on *Azadirachta indica*, Teklehaimanot et al. (1998) on *Parkia biglobosa,* Bruschi et al. (2003) on *Quercus petraea*, Hovenden and Schoor (2004) on *Nothofagus cunninghamii*, Warren et al. (2005) on *Eucalyptus sideroxylon* and Possen et al. (2014) on *Betula pendula*, who found leaf morphological variations within a species. Although the populations differed in all the leaf characteristics studied, the variations among the populations were not consistent or continuous and seemingly occurred at random. Seeds of paper birch populations were from open pollination; therefore, individuals may be genetically different resulting from genotypic differences on leaf characteristics comparable to the results on *Potentilla matsumurae* (Shimono et al. 2009) and *Betula pendula* (Possen et al. 2014).

On comparing the SLA and aspect ratio, the results showed that Frost Lake population differed significantly from the majority of the birch populations in the study. The climate where Frost Lake population originated has mean annual precipitation (600 mm) that was comparatively different (either lower -mean annual precipitation >830ppm or higher -mean annual precipitation <450mm) than the birch populations studied. Mousseau and Fox (1998) reported that the maternal environmental effects on phenotypic variations cannot be entirely excluded, unless the plants are grown for more generations in a greenhouse (Shimono et al, 2009). Thus, the environmental differences at population's origin identified in this study and others studies (Kundu and Tigerstedt

1997, Warren et al. 2005, Hughes et al. 2009) likely had contributed to the SLA and aspect ratio variations.

The prevailing view is that a narrow and elongated leaf with low specific leaf area adapts better to resource poor environments where controlling water balance (Wright and Westoby 1999, Lovett and Haq 2000, Yates et al. 2010) and retaining captured resources (Wilson et al. 1999) are essential. Contrary to my expectation, both PC3 (accumulated with leaf maximum width index and aspect ratio) and PC5 (accumulated with specific leaf area) decreased with increasing annual precipitation and aridity index at a population's origin. This is consistent with previous studies on *Betula pendula* (Aspelmeier and Leuschner 2006), *Eucalyptus sideroxylon* (Warren et al. 2004) and *Fagus sylvatica* (Meier and Leuchner 2008), where SLA increased with decreasing precipitation or drought. Leaf maximum width and aspect ratio increased in warmer mean annual temperature and in regions along longitude, latitude and elevation gradients. As discussed by Warren et al. (2004) and Meier and Leuchner (2008), comparative studies on leaf morphology of interspecies along climatic gradients had showed consistent relationships, whereas within a single species such relationship are often inconsistent. It was argued that leaf life span, root structure, water use efficiency, growth stage and nutrient availability also influence SLA and leaf width (Reich et al. 1998, Warren et al. 2005, Xu and Zhou 2006), which were not analyzed in this study.

Explicitly to this study, the birch populations were originated along environmental gradients across Canada. The correlation analysis resulted that annual temperature is positively related to longitude (from East to West) and elevation gradients within the studied range, whereas annual precipitation is negatively related to these geographic gradients. Analyzing environments of Eastern (longitude 57.57 to 89 East to

West) and Western (longitude 105° to 128° East to West) Canada in different group, I found that annual temperature in Eastern Canada decreased with increasing latitude, longitude and elevation whereas annual temperature was not significantly affected by these geographical coordinates (Annex II). Previous studies on leaf characteristics had not considered as wide environmental gradient as these in this study.

Dudley (1996) and Meng et al. (2009) suggested that plants develop traits that either diminish the loss of water or reduce the need for water by increasing water use efficiency under environmental stress. The modifications in leaf areas, hair density, and petiole area such as small leaves with hairs could reduce transpiration by lowering leaf temperature (Givnish 1979, Roy et al. 1999). Thus, I assumed that correlations among leaf characteristics might have supported the birch populations to produce leaves characterized by traits other than what I hypothesized. Supporting the hypothesis, I found significant correlations among leaf characteristics: those with larger, wider and rounded leaves had larger petiole; reflecting the mechanical strength to support large leaves and promote cooling (Niinemets et al. 2004, Niinemets et al. 2006, Poorter and Rozendaal 2008). Thus, an increase in leaf petiole size with increasing leaf width and aspect ratio in this study is thought to promote leaf cooling in drier regions. In a correlation analysis, a weak negative relation found between leaf width and adaxial hair density showed a possible tradeoff between these leaf characteristics, thereby balancing the evaporation rate in wetter regions by decreasing solar radiation on leaves and lowering leaf temperature as mentioned by Ehleringer (1982) and Roy et al. (1999).

PC3 and PC5 jointly explain only about 22% of variance the rest of the variation in leaf traits remains unexplained by the presented study. A high degree of phenotypic plasticity in leaf traits may be expected and this promises some ease of adjusting to the changing climate. Part of the reason that leaf characteristics may be unexpectedly related to the geography and climate of origin is that the paper birch are considered generalist species which when grown in uniform environment would not strongly relate to the environment of seed origin (Carlson et. al. 2000). In this study, the paper birch populations in the greenhouse were watered regularly, so the populations that originated in drier and warmer habitats may have experienced more adequate soil moisture whereas those from wetter habitats may have experienced a moisture deficiency (Farley and McNeilly 2000, Pearce et al. 2006, Tomlinson et al. 2013). Thus, I concluded that paper birch as a generalist species may have acclimated to the greenhouse environment, which resulted in inconsistent leaf characteristics.

In conclusion, this study on leaf morphological variations showed significant genotypic difference in the leaf characteristics analyzed. The leaf characteristics analyzed were either more weakly or contradictorily related to environmental gradient of the birch's origin than our expectation. Two facts considered in these results are that the greenhouse had different environment than the habitat the populations would normally be exposed to. Since paper birch is a generalist species, the seedlings may have acclimated to the environment of the greenhouse, which resulted in different leaf characteristics than expected. Secondly, the correlation existed between leaf characteristics might have provided mechanical support and reduced evaporation in extreme environments. In general, the results of these analyses opened the possibility of considering leaf morphological characteristics to predict the birch's performances under changing climate.

CHAPTER 3: Leaf morphological and stomatal variations in paper birch populations across Canada: A case study from a common garden.

ABSTRACT

Variations in leaf morphology and stomatal characteristics have been extensively studied at both inter- and intraspecific levels although not explicitly in the context of paper birch populations. These populations might have developed the leaf variations that have allowed them to adapt to a wide climatic gradient. Therefore, in this study I examined variations in the leaf morphological and stomatal characteristics of sixteen paper birch populations collected across Canada and grown in a common garden. I also examined the relationship between these leaf characteristics and the climate of the population's origin. Since significant genotypic differences were found in the leaf characteristics among the birch populations, I expected that the observed leaf variations could be partly explained as natural diversity in the birch due to differences in their original environment. In fact, along with increasing mean annual precipitation and aridity index, hair density on leaf adaxial surfaces decreased whereas stomatal density increased significantly. My results showed that the populations with larger leaf area, specific leaf area and high hair density had low stomatal density. These leaf morphological and stomatal characteristics provided a structural basis in reducing water loss through leaves and increasing water use efficiency. A trade-off between stomatal area and density might be a strategy of the birch species to balance stomatal conductance in decreased precipitation.

INTRODUCTION

Plants typically express phenotypic differences in response to environmental changes (Teklehaimanot et al. 1998, Bruschi et al. 2003, Warren et al. 2005, Ivancich et al. 2012). Under different environmental conditions, plants allocate biomass in several organs to capture optimum light, water, nutrients, and carbon dioxide, and as a strategy to maximize growth rate (Bloom et al. 1985). Phenotypic plasticity also occurred to produce a range of leaf characteristics as a response to environmental effects (McLellan 2000). These differences in plants, particularly at the leaf levels, are expressed as morphological and anatomical variations.

Leaf morphological and anatomical variation in plants growing in contrasting habitats (i.e. climatic gradients) has long been studied (Teklehaimanot et al. 1998, Ivancich et al. 2012). Leaf morphological studies show that narrow and thick leaves provide structural reinforcement to withstand wilting in hot, sunny and dry environments (Werger and Ellenbroek 1978, Abrams 1990, 1994). Additionally, it is suggested that small leaves track air temperature closely, whereas large leaves suffer from over-heating when water is limited (Gates et al. 1968, Warren et al. 2005). Research on the adaptive significances of leaf hairs showed an increase in leaf reflectance, boundary thickness and prevention of stomatal obstruction by water or particulate matters (Gates 1980, Picotte et al. 2007). Consequently, increased leaf hairs in hot and arid habitats significantly reduce solar radiation at leaf surface, leaf temperature and transpirational losses (Ehleringer and Bjorkman 1978, Ehleringer et al. 1981, Picotte et al. 2007). Hence, the most commonly observed leaf morphological changes under water deficiency are reduced leaf area

(Fonseca et al. 2000), reduced specific leaf area (Cunningham et al. 1999, Fonseca et al. 2000), and increased leaf hair density (Picotte et al. 2007).

Alternatively, even during times of abundant precipitation, the cost for replenishing transpired water is high because of investment in the roots and vascular network to transport it (Pittermann 2010). Thus, the most noticeable leaf anatomical adaptation to high water transportation cost is stomatal evolution (Raven 2002, Hetherington and Woodward 2003). Stomata in plants regulate gas exchange under environmental constraints. Leaf stomata optimize between photosynthetic gain and transpirational loss to adjust during precipitation and temperature fluctuations (Hawkins et al. 2008). It has been suggested that smaller stomatal area and guard cells increase carbon dioxide diffusion per unit area of stomata and reduce water loss compared to larger stomatal area and guard cells (Abrams 1990). Fraser et al. (2009) identified that as an adaptation mechanism to water stress, stomatal density increased in *Pseudoroegneria spicata* whereas Belhadj et al. (2011) found that stomatal area decreased in *Pistacia atlantica*

Most of the studies on leaf morphological and stomatal variation in response to environmental factors have either included comparative studies among multiple species (Abrams 1994, Batos et al. 2010, Aasamaa and Sober 2011) or species inhabiting different locations along environmental gradients (Abrams 1990, 1994, Ashton and Berlyn 1994, Bacelare et al. 2004, Bayramzadeh 2011). Results of these studies showed marked genetic variation, adaptive significance and phenotypic plasticity in leaf morphology or stomata or both. However, leaf morphological and stomatal variations for individual species or multiple species inhabiting different environments do not necessary explain the variation at the intraspecific level. Therefore, it is important to determine whether leaf morphology and stomata differ in wide-ranging pioneer species like paper birch grown in a uniform environment. To my knowledge, no studies have focused on leaf morphological and stomatal variations of the birch populations grown in a uniform environment.

Paper birch adapts to a wide range of climatic and soil moisture regimes in North America (Safford et al. 1990), and the species is increasingly significant in commercial forestry (Safford et al. 1990). Thus, paper birch populations may have developed leaf morphological and stomatal variations that have allowed them to adapt to a wide climatic gradient. In this study, I addressed whether leaf morphology and stomata differ among paper birch populations that originate from different environments but are grown in the same environment, and whether differences in leaf morphology and stomata are related to the environmental factors of a population's origin. I hypothesized that genotypic differences would result in leaf morphological and stomatal variations among paper birch populations despite of the same growing environment. Leaf morphology and stomatal characteristics were expected to be correlated to the geography and climate of the populations' origin. I expected that the populations that originate from warmer regions with less precipitation have smaller leaf area and/or dense leaf hairs whereas, the population that originates from a region with higher precipitation and aridity index has lower stomata density or higher stomatal area. Lastly, I predicted that leaf morphological and stomatal characteristics are correlated to minimize water loss through the leaves.

MATERIALS AND METHODS

Sample collection, and leaf morphological and stomatal data

Seeds of sixteen paper birch populations were collected from Ontario, British Columbia, New Brunswick, Newfoundland, Nova Scotia, Prince Edward Island and Quebec. The populations' origins ranged from 10 m to 840 m in elevation, 1639 mm to 279 mm mean annual precipitation and 1.36°C to 8.88 °C mean annual temperature (Appendix I). The birch seedlings were grown for 12 weeks in Lakehead University's greenhouse. In August 2008, 30 seedlings that were uniform in height and root-collar diameter were selected from each birch population and transplanted in the common garden in Thunder Bay, Ontario (located at183.3 m above sea level, and 48°22'N and 89 °19'W). The layout of the populations followed a completely randomized design in the common garden.

The leaves of 16 two-year old paper birch populations in the common garden were randomly sampled from the middle of the crown in August 2010. The healthy and well developed leaves were selected from long branches at approximately the same height, and on the same day to minimize seedling variations (Blue and Jensen 1988, Bruschi et al. 2000, 2003). Sampling leaves on long branches in August improved the likelihood of collecting leaves that were of similar age and development. These leaves were measured and analyzed for leaf morphological and stomatal characteristics following the methods of Hovenden and Schoor (2003) and Warren et al. (2005). Leaf morphological characteristics such as leaf area and aspect ratio (horizontal width/vertical length of leaf) were measured using WinFolia software (Regent Instrument Inc. Quebec, Canada). Leaf hair densities on abaxial and adaxial leaf surfaces were counted on three parts ($0.20\mathrm{cm}^2$) of each leaf surface using an Academic sterezoom microscope at 30X magnification and average values were used for further analysis (Bruschi et al. 2000, 2003, Warren et al. 2005). Subsequently, sampled leaves were dried at 70°C for 42 h to calculate specific leaf area (leaf area per leaf dry mass).

Stomata were absent on the adaxial leaf surface; therefore stomatal replicas were assessed and analyzed for abaxial surface only. I obtained stomatal replicas from middle section of leaves by using clear nail varnish (Bacelare et al. 2004). Leaf veins were avoided as far as possible while collecting stomatal impressions. I used an electronic microscope and motic images plus 2.0 ® software (Motic Instruments Inc., Richmond, Canada) to obtain photos of stomata. I measured stomatal density (number of stomata per $1\mathrm{mm}^2$ i.e., 10^6 $\mu\mathrm{m}^2$), length, width, pore area and guard cell width per leaf for further analysis (Sun et al. 2003, Xu and Zhou 2008). The equations used for calculating stomatal area, density, epidermal cell density, pore area (Sagaram et al. 2007), stomatal shape coefficient (Batos et al. 2010) and stomatal index (Royer 2001) are listed in Appendix II.

Climate variables

Mean annual and growing season temperature and precipitation data for the population's origin were normalized climate data from 1971 to 2001 (Weather-Environment Canada). I used De Martonne's (1926) equation to calculate the mean annual aridity index (De Martonne 1926, Migalina et al. 2009), and Sijors's (1994) equation to calculate the aridity index during the growing season (Appendix II).

Data analyses

I analyzed the variations in leaf morphological and stomata characteristics using a nonparametric test because of the relatively small sample size for each population (Warren et al. 2005). Variations in leaf morphological and stomatal characteristics were analyzed using the Kruskal-Wallis test. I used Mann-Whitney U test to analyze the morphological and stomatal difference between populations. Correlation between measured leaf characteristics and climate of the paper birch population's origin were analyzed using Spearman's correlation, and I also analyzed the relationship between stomatal and morphological characteristics using Spearman's correlation. All statistical analysis was conducted using SPSS-18 (SPSS, Chicago, IL, USA) and R-2.12.1 (R-Development Core team 2011).

RESULTS

Variation in leaf morphological and stomatal characteristics

Leaf area, specific leaf area, aspect ratio and leaf hair density showed significant variation among the paper birch populations (P=<0.05; Table 3.1). Populations from Porcupine Lake, Wayerton, Pettawa and Adam Lake had significantly larger leaf area in comparison to Newfoundland, which had the smallest leaf area (Table 3.1). Populations from Wayerton and Milvale had significantly smaller specific leaf area compared to populations from Adam Lake, Porcupine Lake and Skeena River. Skimikin had lower leaf abaxial and adaxial hair densities that significantly differed from those that originated in Adam Lake, Porcupine Lake and Skeena (N=16, P=< 0.01; Table 3.2).

Apart from stomatal index and shape coefficient analyses of stomatal length, width, and area, pore length, pore width, pore area, stomatal density and guard cell width showed significant differences (N=16, *P*=<0.01) among paper birch populations. Populations from Newfoundland and Skimikin differed from the majority of populations with respect to stomatal area, pore area and stomatal density. For example, the population from Skimikin had a significantly smaller mean stomatal area and higher stomatal density (N=16, *P*=< 0.05) than those from Adam Lake and Porcupine (Fig. 3.1, Table 3.2). The population from Adam Lake had the lowest stomatal density, but the largest stomatal area, pore area and guard cell width (Fig. 3.1, Table 3.2).

Correlation between leaf characteristics and climate of population's origin

I found significant correlation between measured leaf characteristics and environmental variables of the paper birch population's origin (Table 3.3). Leaf area and aspect ratio were positively related to longitude, elevation and growing season temperature; however, they were negatively related to annual and growing season precipitation and aridity index (Table 3.3). Specific leaf area increased from east to west (*r*= 0.43, *P*=<0.001) and north to south (*r*= 0.33, *P*=<0.001), but it decreased with increasing growing season temperature (*r*= -0.29, *P*=<0.001), precipitation (*r*= -0.31, *P*=<0.001) and aridity index (r= -0.26, P=<0.001; Table 3.2). I found low hair density on the leaf's abaxial surface along increasing mean annual temperature (*r*= -0.26, *P*=<0.001). Similarly, I found less hair density on the adaxial surface with increasing mean aridity index (Table 3.3).

Longitude and growing season precipitation and aridity index of the paper birch population's origin range from 57.57-128.34, 29.55 -100.08 mm and 3.08 -13.5, respectively. All stomatal characteristics measured, except for the stomatal shape coefficient, were significantly correlated with longitude, growing season precipitation and aridity (*P*=<0.05; Table 3.3). Both stomatal area and guard cell width increased with longitude (*r*=0.35 and 0.35, respectively, *P*=<0.001) and decreased with increasing precipitation during growing season (*r*=-0.33 and -0.32, *P*=<0.001 respectively) and aridity index (*r*=-0.37 and -0.37 respectively, *P*=<0.00; Table 3.3). On the contrary, stomatal density decreased with increasing east-to-west longitude (*r*= -0.36, *P*=<0.001) decreasing mean growing-season precipitation (*r*=0.38, *P*=<0.001) and aridity index (*r*=0.39 and 0.38 respectively, *P*=<0.001; Table 3.3).

Along the temperature gradient, I found that stomatal area and guard cell width were positively correlated to mean annual temperature (*r*= 0.29 and 0.30 respectively, *P*=0.01; Table 3.3). Stomatal area and shape showed weak but significant correlations to growing season temperature. The results showed that stomatal area increased with increasing growing season temperature whereas the stomatal shape coefficient decreased with an increase in the temperature (Table 3.3). I found larger pore area in the birch populations originated in warmer temperatures (*r*= 0.30, *P*= <0.01), and lower precipitation (*r*= -0.36, *P*= <0.001) and aridity index (r= -0.38, P= <0.001) during the growing season (Table 3.3). There was no significant correlation between stomatal index and the climate of the populations' origin. Furthermore, none of the stomatal characteristics were significantly related to latitude and elevation of the origin.

Correlation between leaf morphological and stomatal characteristics

Within a leaf morphological characteristics, I found significantly higher hair density on adaxial surface in larger leaf area (*r*= 0.21, *P*=0.01) and aspect ratio (*r*= 0.26, *P*=0.001; Table 3.4). Within stomatal characteristics, leaves with higher stomatal density had smaller stomatal area (*r*= -0.72, *P*=0.001), pore area (*r*= -0.68, *P*=0.001) and guard cell width (r=- 0.56, P=<0.001; Fig. 3.2, Table 3.4). Comparing leaf morphological and stomatal characteristics, stomatal density increased in smaller leaf area (r= -0.56 P= 0.03) and specific leaf area (r= -0.51 P= 0.05) with less hair density on abaxial (r= -0.65 P= 0.01) and adaxial (r= -0.85 P=< 0.001; Fig. 3.3, Table 3.4) surfaces. Adaxial hair was denser in leaves with larger stomatal area (r= 0.64 P=0.01), pore area (r= 0.55 P= 0.03) and guard cell width (r= 0.63 P= 0.01). However these stomatal characteristics were insignificantly related to other leaf morphological characteristics (Table 3.4).

Table 3.1 Results of the Kruskal-Wallis nonparametric tests of leaf morphological and stomatal characteristics of 16 paper birch populations collected (seeds) from across Canada and grown at the common garden in Thunder Bay, Ontario. Here, LS is in cm^2, SLA is in cm^2/g, ABH is hair density on abaxial surface and ADH is hair density on adaxial surface. SL, SW, PL, PW and GCW are measured in µm. SA and PA are measured in µm^2. SD represents the number of stomata per 1mm^2.

Populations (DF=15)		
Leaf morphological characteristics	**Chi-square**	***P* value**
Leaf area (LS)	73.57	<0.001
Specific leaf area (SLA)	73.64	<0.001
Aspect ratio (AR)	74.52	<0.001
Abaxial hair (ABH)	77.94	<0.001
Adaxial hair (ADH)	83.71	<0.001
Stomatal characteristics	**Chi-square**	***P* value**
Stomatal length (SL)	49.86	<0.001
Stomatal width (SW)	48.61	<0.001
Stomatal area (SA)	51.02	<0.001
Pore length (PL)	46.43	<0.001
Pore width (PW)	24.14	.063
Pore area (PA)	39.59	<0.001
Guard cell width (GCW)	43.46	<0.001
Stomatal density (SD)	41.96	<0.001
Stomatal shape coefficient (SHC)	23.98	.0.65
Stomatal index (SI)	18.52	.236

Table 3.2 Mean values and standard deviations (in parentheses) of leaf morphological and stomatal characteristics of 16 paper birch populations originated across Canada and grown in a common garden. Here, LS-leaf area (cm^2), SLA-specific leaf area (cm^2 g^{-1}), AR-aspect ratio, ADH-leaf hair density on adaxial surface, SA-stomatal area (μm^2), PA-stomatal pore area (μm^2), GCW-guard cell width (μm), SD-stomatal density (no. of stomata/ 0.1mm^2).

Population	**LS**	**SLA**	**AR**	**ADH**	**SA**	**PA**	**GCW**	**SD**
Newfoundland	17.95	115.07	0.61	3.27	461.64	133.88	5.49	231.67
	(5.76)	(14.25)	(0.06)	(2.64)	(100.09)	(31.34)	(0.75)	(51.32)
Milvale	26.18	103.06	0.65	1.47	667.49	220.14	6.48	220.00
	(2.39)	(0.01)	(0.03)	(1.46)	(52.38)	(37.99)	(0.33)	(51.96)
Cap De Rosiers	18.74	112.92	0.63	5.33	520.00	168.31	5.41	186.67
	(8.11)	(0.01)	(0.12)	(2.04)	(49.87)	(33.22)	(0.67)	(55.08)
Allardville	23.07	118.06	0.68	11.8	710.90	216.32	6.84	134.00
	(7.05)	(11.16)	(0.05)	(7.10)	(308.45)	(70.66)	(2.74)	(28.81)
Wayerton	40.83	101.30	0.75	0.01	827.53	250.40	6.99	140.00
	(5.31)	(0.82)	(0.09)	(10.02)	(127.06)	(63.32)	(1.02)	(23.98)
Indiana Brook	22.55	116.24	0.59	6.31	871.24	260.44	7.20	190.00
	(4.42)	(0.001)	(0.04)	(2.55)	(94.66)	(58.52)	(0.82)	(17.32)
New Brunswick	26.86	111.68	0.60	1.13	898.25	294.16	7.61	141.25
	(7.49)	(6.25)	(0.07)	(4.18)	(165.55)	(87.57)	(1.25)	(33.57)
Bells Fall	32.45	100.15	0.67	6.27	625.41	193.56	6.19	176.67
	(12.44)	(0.001)	(0.04)	(5.46)	(140.23)	(52.56)	(0.17)	(30.55)
Alice	24.83	110.28	0.70	3.18	864.15	238.29	7.93	176.67
	(12.43)	(1.13)	(0.07)	(11.13)	(204.80)	(20.34)	(2.36)	(28.87)
Pettawa	37.27	117.94	0.68	2.87	697.61	209.95	7.15	173.33
	(14.04)	(0.001)	(0.08)	(2.01)	(238.71)	(76.05)	(1.34)	(77.67)
Timmins	23.65	120.50	0.65	10.47	590.86	210.39	5.86	173.33
	(6.82)	(6.12)	(0.08)	(5.51)	(142.11)	(46.13)	(1.36)	(58.31)
Porcupine Lk	42.89	123.05	0.78	13.98	1075.39	320.50	8.58	132.00
	(14.05)	(12.92)	(0.08)	(9.16)	(158.75)	(93.00)	(0.84)	(36.33)
Adam Lk	36.58	122.76	0.75	14.80	1149.00	348.14	9.25	106.67
	(5.02)	(0.001)	(0.04)	(6.12)	(121.00)	(117.54)	(1.85)	(41.63)
Skimikin	26.08	110.63	0.79	3.00	329.10	140.41	3.48	236.67
	(5.17)	(23.70)	(0.06)	(0.01)	(87.08)	(63.41)	(1.05)	(41.63)
Tabor Lk	29.00	116.93	0.69	13.93	784.87	215.71	7.83	123.33
	(8.34)	(0.01)	(0.08)	(4.50)	(93.32)	(44.28)	(1.00)	(15.28)
Skeena River	23.04	131.89	0.65	23.13	939.78	264.10	7.98	147.50
	(7.22)	(9.75)	(0.05)	(7.35)	(422.00)	(145.14)	(1.29)	(41.93)

Note: Lk stands for Lake

Table 3.3 Spearman's correlations between leaf characteristics (Chrs.) and environmental variables of paper birch populations' origin. The values are correlation coefficient with P-value in parentheses (N=16). Here, LS- leaf area (cm^2), SLA- specific leaf area (cm^2/g), AR - aspect ratio and ABH and ADH - hair density on abaxial and adaxial surfaces respectively. SA - stomatal area (μm^2), PA - stomatal pore area (μm^2), GCW -guard cell width (μm), SD- stomatal density (stomata per 1mm^2), SHC -stomatal shape coefficient and SI- stomatal index (%). For climatic data, LAT-latitude, LONG- longitude, ELE- elevation (meters), MAP -mean annual precipitation (mm), MAT - mean annual temperature (°C), MAI is mean annual aridity index, GSP - mean precipitation during growing season (mm), GST-mean temperature during growing season (°C) and GSA -mean aridity index during growing season.

Chrs.	**LAT**	**LONG**	**ELE**	**MAP**	**MAT**	**MAI**	**GSP**	**GST**	**GSA**
LS	-0.05	0.29	0.26	-0.37	0.11	-0.32	-0.33	0.33	-0.36
	(0.54)	(<0.001)	(<0.001)	(<0.001)	(0.15)	(<0.001)	(<0.001)	(<0.001)	(<0.001)
SLA	0.43	0.33	-0.11	0.06	0.18	-0.16	-0.31	-0.29	-0.26
	(<0.001)	(<0.001)	(0.14)	(0.43)	(0.02)	(0.04)	(<0.001)	(<0.001)	(<0.001)
AR	0.24	0.33	0.51	-0.53	0.08	-0.53	-0.42	0.26	-0.44
	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.33)	(<0.001)	(<0.001)	(<0.001)	(<0.001)
ABH	0.10	-0.03	0.11	-0.05	0-.26	-0.15	0.02	-0.21	0.04
	(0.19)	(0.74)	(0.17)	(0.51)	(<0.001)	(0.06)	(0.77)	(0.01)	(0.61)
ADH	0.04	0.18	0.13	-0.23	-0.16	-0.30	-0.17	0.04	-0.18
	(0.58)	(0.02)	(0.09)	(<0.001)	(0.03)	(<0.001)	(.02)	(0.59)	(0.02)
SA	-0.07	0.35	0.05	-0.12	0.29	-0.31	-0.33	0.22	-0.37
	(0.52)	(<0.001)	(0.66)	(0.27)	(0.01)	(0.01)	(<0.001)	(0.05)	(<0.001)
PA	-0.08	0.36	0.03	-0.21	0.19	-0.32	-0.36	0.30	-0.38
	(0.49)	(<0.001)	(0.76)	(0.07)	(0.09)	(<0.001)	(<0.001)	(0.01)	(<0.001)
GCW	-0.03	0.35	0.03	-0.12	0.30	-0.32	-0.32	0.14	-0.37
	(0.80)	(<0.001)	(0.81)	(0.31)	(0.01)	(<0.001)	(<0.001)	(0.22)	(<0.001)
SD	-0.14	-0.36	-0.13	0.24	-0.05	0.39	0.39	-0.18	0.38
	(0.21)	(0.001)	(0.25)	(0.03)	(0.69)	(<0.001)	(<0.001)	(0.12)	(0.001)
SHC	0.18	-0.002	-0.04	0.001	0.02	0.04	-0.05	-0.23	-0.02
	(0.11)	(0.99)	(0.72)	(0.99)	(0.83)	(0.72)	(0.64)	(0.04)	(0.88)
SI	-0.22	-0.27	-0.09	0.003	-0.07	0.11	0.26	0.16	0.22
	(0.05)	(0.02)	(0.42)	(0.98)	(0.51)	(0.34)	(0.02)	(0.15)	(0.05)

Table 3.4 Spearman's correlations coefficient (with p value in parentheses) within of leaf morphological and stomatal characteristics (Chrs.) of 16 (N) paper birch populations originated across Canada and grown at the common garden in Thunder Bay, Ontario. Here, SA is stomatal area (μm^2), PA is stomatal pore area (μm^2), GCW is guard cell width (μm), SD is stomatal density (number of stomata per $1 mm^2$), SHC is stomatal shape coefficient, SI is stomatal index (%), LS is leaf area (cm^2), SLA is specific leaf area (cm^2/g), AR is aspect ratio, ABH is hair density on abaxial surface and ADH is hair density on adaxial surface.

Chrs.	SA	PA	GCW	SD	SHC	LS	SLA	AR	ABH
PA	0.87								
	(<0.001)								
GCW	0.84	0.55							
	(<0.001)	(<0.001)							
SD	-0.72	-0.68	-0.56						
	(<0.001)	(<0.001)	(<0.001)						
SHC	-0.20	-0.24	.07	0.20					
	(0.08)	(0.04)	(0.55)	(0.07)					
LS	0.41	0.34	0.46	-0.56	-0.07				
	(0.12)	(0.20)	(0.07)	(0.03)	(0.80)				
SLA	0.42	0.35	0.48	-0.51	0.39	-0.14			
	(0.11)	(0.19)	(0.06)	(0.05)	(0.13)	(0.80)			
AR	0.18	0.11	0.27	-0.38	0.03	0.51	-0.04		
	(0.50)	(0.68)	(0.32)	(0.15)	(0.90)	(<0.001)	(0.62)		
ABH	0.41	0.35	0.43	-0.65	-0.12	-0.03	0.14	0.17	
	(0.12)	(0.18)	(0.10)	(0.01)	(0.66)	(0.67)	(0.07)	(0.03)	
ADH	0.62	0.55	0.63	-0.85	-0.17	0.21	0.14	0.26	0.80
	(0.01)	(0.03)	(0.01)	(<0.001)	(0.53)	(0.01)	(0.07)	(0.001)	(<0.001)

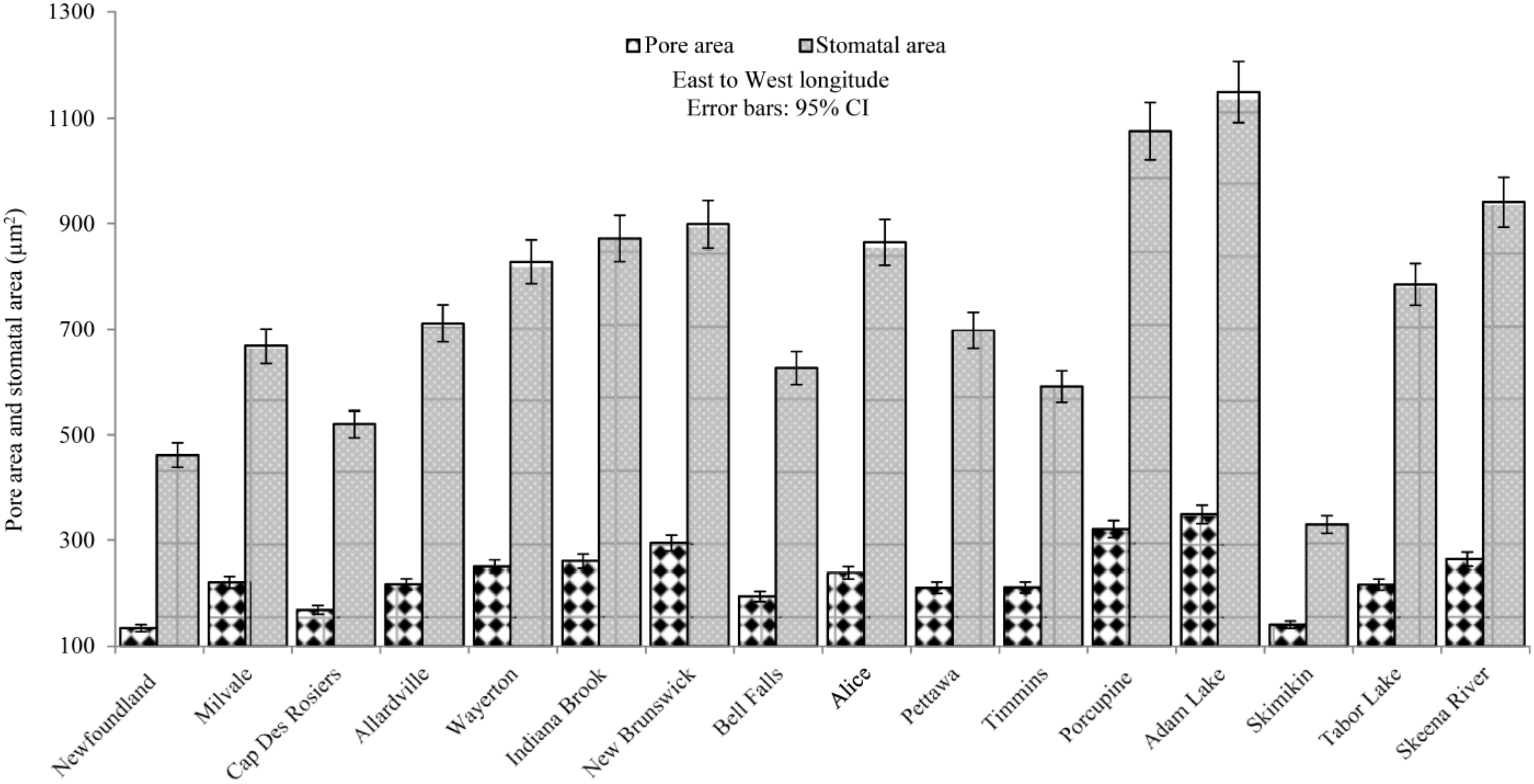

Figure 3.1 Intraspecific variation in stomatal area (μm^2) and pore area (μm^2) among paper birch populations grown in the common garden - Thunder Bay. The populations are arranged from east to west as per longitude gradient (ranging from 57.57 to 128.34). Error bars represents 95 % confidence interval (CI).

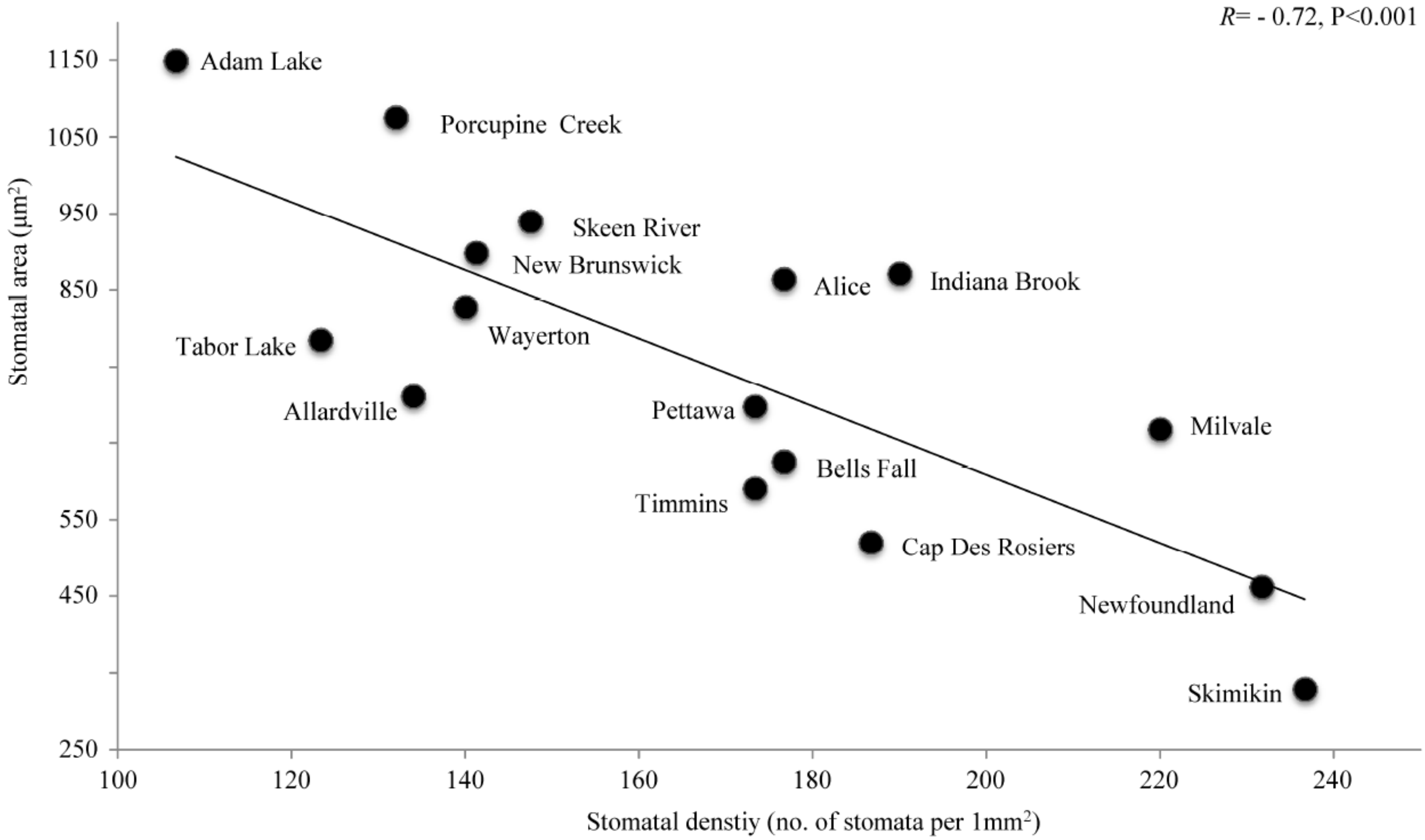

Figure 3.2 The correlation between stomatal density and area for 16 paper birch populations grown in the common garden, Thunder Bay. Spearman's correlation coefficient (r) is listed in the plot. Dots represent paper birch populations originated across Canada.

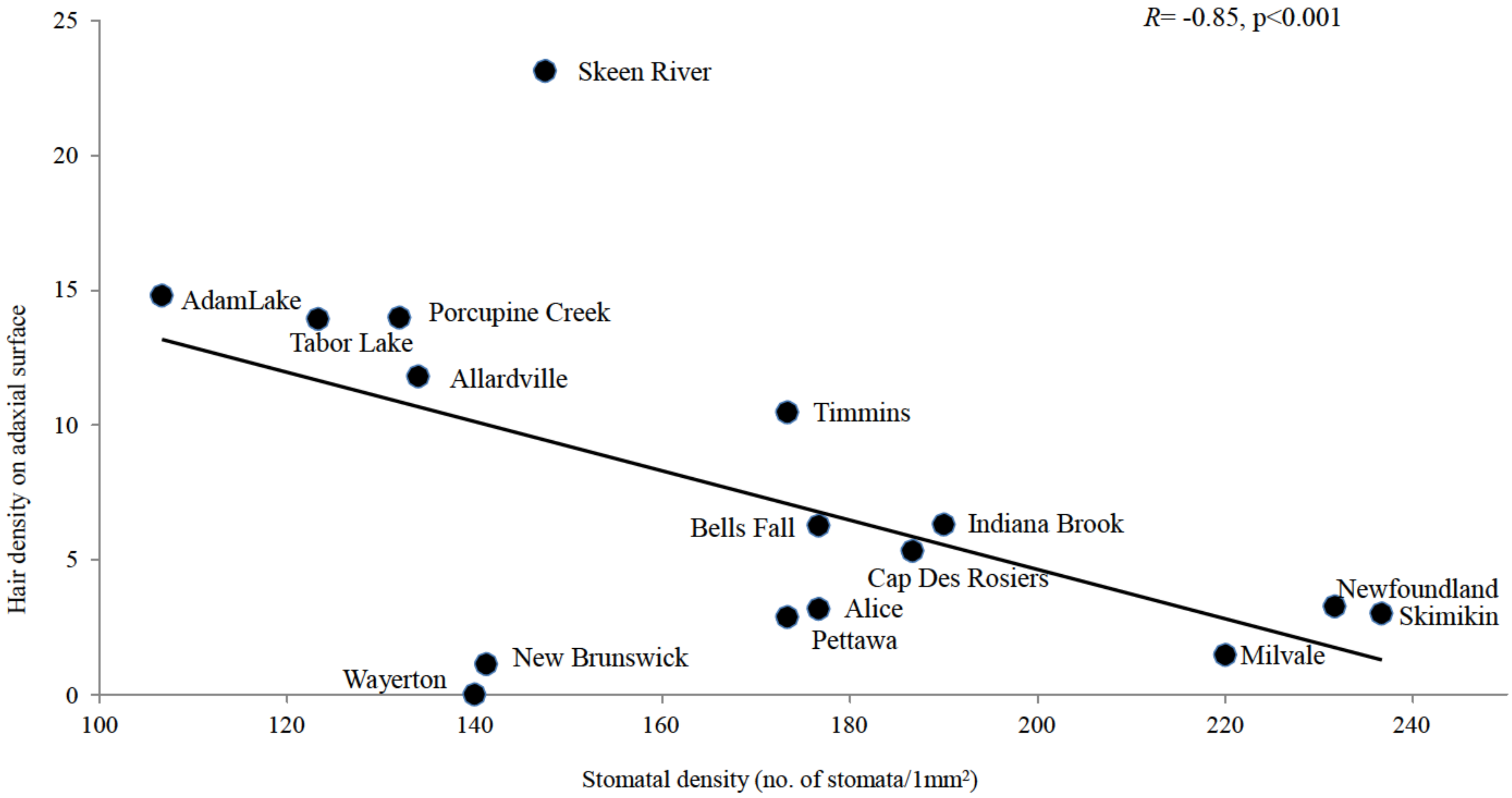

Figure 3.3 The correlation between mean stomatal density (no. of stomata/1mm^2) and leaf hair density on adaxial surface (no. of hairs/0.20cm^2) for sixteen paper birch populations grown in the common garden- Thunder Bay. Spearman's correlation coefficient (r) is listed in the plot. Dots represent paper birch populations originated across Canada.

DISCUSSION

The results of this study demonstrate significant variations in leaf morphological and stomatal characteristics of paper birch populations grown under uniform conditions in a common garden. The patterns in the variations are consistent with the results of other studies on *Quercus petraea* and *Parkia biglobosa* (Teklehaimanot et al. 1998, Bruschi et al. 2003). The among- population's variation observed in this study suggest that morphological and stomatal characteristics of birch leaves vary under a uniform environment, except for the stomatal index and stomatal shape coefficient. This supports the hypothesis that the birch populations significantly vary in leaf morphological and stomatal characteristics. These variations may be related to genotypic differences, but it is possible that environmental differences at the population's origin identified in this study and elsewhere (Warren et al. 2005, Hughes et al. 2009) contributed to leaf variation among the paper birch populations.

I tested if the differences in the leaf characteristics were related to the environment of the birch population's origin. Supporting my hypothesis, the results showed the leaf characteristics that varied in the paper birch populations were significantly related to longitude, precipitation and aridity index of the birch population's origin. Species show a range of mechanisms of adaptation to water deficiency such as reduction in leaf area, specific leaf area and aspect ratio (Kundu and Tigerstedt 1997) and increase in leaf hair density (Johnson 1975). Consistent with previous studies and supporting my hypothesis, results showed that paper birch leaves have higher adaxial hair density with decreasing annual precipitation and aridity index.

Conversely, larger and wider leaf area was noticed in elevation, longitudinal and temperature (during growing season) gradients with decreasing precipitation and aridity index. Although my results did not support the hypothesis that a smaller leaf originates in warmer regions, the strong positive correlation between hair density on the leaf adaxial surface and leaf area may explain reducing evapo-transpiration from larger leaves during water deficiency. Similar to my result, most studies on intraspecific variation show an inconsistent relationship between leaf traits and climatic variables in comparison to interspecific variation. For instance, a study on *Cistus salvifolius* found bigger leaves in a drier area (Farley and McNeilly 2000) whereas the opposite trend was found in *Cistus ladanifer* (Warren et al. 2005). Similarly, an inconsistent relationship was found between leaf morphology of *Eucalyptus siderosylon* and rainfall of different population origins (Warren et al. 2005). The study on *Cistus salvifolius* demonstrated that leaf traits of plants grown in different conditions such as a greenhouse generally differ from those in natural populations (Farley and McNeilly 2000), which might be the case in this common garden study on paper birch.

In my study, longitude and aridity index (both mean annual and growing season) appear to be major environmental variables that are significantly related to stomatal characteristics of paper birch. Stomatal area and density characterize species' resistance to drought (Balok and Hilaire 2002, Belhadj et al. 2011); a small stomatal area with higher density was noticed in *Populus trichocarpa* from xeric environments (Dunlap and Stettler 2001) and stomatal density increased in *Lolium perenne* under elevated temperature (Ferris et al. 1996). In contrast to these studies, I found that paper birch had larger stomatal and pore areas and lower density of stomata under decreasing growing-season precipitation and aridity index. Even though these results did not agree with my prediction, they are consistent with a study on paper birch populations from water-deficit sites that had larger and fewer stomata per unit leaf area (Li et al. 1996).

The tradeoff between stomatal area and density; that is, either larger stomatal area with low density or smaller stomatal area with high density, was revealed by the strong correlations in my study, and it is consistent with other studies (Hetherington and Woodward 2003, Camargo and Maremco 2011). Although stomatal area reduced with precipitation and aridity gradients in this study, stomatal area per unit leaf area remained unchanged due to an increase in stomatal density. Similar to my result, temperate species from drier habitats also had smaller stomatal area and higher stomatal densities that were associated with higher stomatal conductance (Abrams 1994). Alternatively, larger stomatal area and lower stomatal density in deciduous tree species were associated with a slow increase in stomatal conductance under unfavorable conditions, such as warmer temperatures (Aasamaa and Sober 2011). Thus, I conclude that the tradeoff between stomatal area and density and their correlation to the climate of origin of the paper birch populations might be a strategy, by the species, to balance stomatal conductance in drier habitats. Furthermore, stomatal density and index displayed a negative relationship with increasing longitude, whereas stomatal area was positively related to longitude. Hence, populations that originated on the west coast had smaller stomatal densities with larger stomatal area in comparison to populations from the east coast. In the present study, I found no significant relationship between stomatal density and elevation, which was consistent with other studies (Holland and Richardson 2009, Russo et al. 2010). Results from previous studies on either the increase or decrease of stomatal density to increased elevation were contradictory (Korner et al. 1986, Korner 1999). For example, Hetherington and Woodward (2003) suggest that small stomata can open and close more rapidly and would increase rapid stomatal conductance to maximize [CO_2] diffusion into the leaf during favorable conditions. In my study, stomatal area was positively correlated with the mean annual temperatures of origin, which was consistent with previous studies (Abrams et al. 1992, Hilaire and Graves 1999).

Under environmental stress such as water deficiency, plants develop traits that either diminish the loss of water or traits that reduce the need for water by increasing water use efficiency (Dudley 1996). Small leaves with hairs could reduce transpiration by lowering leaf temperature or by changing boundary layer conditions (Givnish 1979, Roy et al. 1999). Furthermore, if small leaves have fewer stomata, water use efficiency for a species will change. Although I did not subject the populations to any stress, increased precipitation and aridity index during the growing season at the origin positively increased stomatal density and decreased stomatal area, leaf area, specific leaf area and aspect ratio. Supporting my hypothesis, results showed significant correlations among stomatal density, area, and leaf characteristics. Correlations between leaf morphological and stomatal characteristics revealed that populations with larger leaf area, specific leaf area and higher hair density had low stomatal density. Furthermore, populations with higher hair density on the adaxial surface had larger stomatal area, pore area and guard cell width. All these features provide a structural basis in reducing water loss through leaves and water use efficiency (although I did not measure water use efficiency in this study). Nevertheless, leaf morphological and stomatal studies are valuable for identifying ecologically important traits that can be further analyzed in other experiments (Wade and Kalisz 1990, Roy et al. 2001).

My results raise the possibility that intraspecific variation in paper birch might evolve due to genotypic variation and environmentally induced variation in leaf morphological and stomatal characteristics. Contrary to my expectations, several leaf characteristics appeared unrelated to the environmental gradient of the birch's origin. Yet, I should consider the fact that the common garden was located at a climatic condition (mean annual precipitation 711mm, temperature 2.25 °C and aridity index 74.73) that was different from the environment the populations would normally be exposed to. Thus, phenotypic plasticity of the birch possibly has imposed leaf characteristics contrary to my expectations to acclimate in the common garden environment (Pearce et al. 2006). Further studies involving the use of a greenhouse experiment in plants grown under different environmental conditions are necessary to better understand how morphology and stomata vary in paper birch populations across Canada and their possible phenotypic plasticity acclimates to a changing climate.

CHAPTER 4: Interactive effects of elevated [CO_2] and soil water stress on leaf morphological and anatomical characteristic of paper birch populations

ABSTRACT

The leaf morphological and stomatal characteristics of four paper birch populations, grown at four treatment conditions of carbon dioxide [CO_2] and soil water levels were investigated to determine whether future increases in atmospheric [CO_2] and decreases in water levels affect the leaf characteristics. The populations from Cussion Lake, Little Oliver Lake, Skimikin and Wayerton were grown for 12 weeks under ambient (360ppm) and elevated (720ppm) [CO_2] at either high or low soil water levels. The birch populations significantly differed in leaf area and most of stomatal characteristics due to the interaction effects of [CO_2], soil water levels and population differences. Unlike soil water levels, the main effects of [CO_2] and populations resulted in significant differences in most leaf morphology features. Significantly, the water levels had affected leaf morphology when combined on with other factors. Elevated [CO_2] alone barely affected stomatal area of the birch populations, while elevated [CO_2] at both soil water levels stimulated measured stomatal characteristics within and between the populations. Overall, elevated [CO_2] reduced leaf area and increased stomatal density; and low soil water level resulted in smaller stomatal area, pore area and guard cell width. Unexpected effects on leaf characteristics seen in this study showed that the birch populations acclimate to [CO_2] and soil water levels either by decreasing stomatal area under low soil water level or by increasing stomatal density under elevated [CO_2]. The paper birch must have acclimated to these adverse environment by integrating its leaf morphological and stomatal characteristics to maintain balance between [CO_2] gain and water loss.

INTRODUCTION

Atmospheric carbon dioxide [CO_2] concentration has increased from pre-industrial level of 280 ppm to more than 390ppm and is predicted to increase almost two-fold, reaching 730 ppm by the end of 2100 (IPCC 2007, Sitch et al. 2008). As consequence, the rise in [CO_2] together with other greenhouse gases could increase the average global temperature by 0.6-4.0 °C and bring uncertainty in both magnitude and degree of precipitation (Houghton et al. 2001, IPCC 2007). Furthermore, elevated [CO_2], together with rising temperatures, may increase the rate and depth of evaporation, lowering soil water table (Catovsky and Bazzaz 1999, Volk et al. 2000). Atmospheric [CO_2] and soil water availability are key resources for plant growth, structure and function. Hence, it is essential to understand the effects of predicted [CO_2] and reduced soil water levels on plant structure, such as morphology and anatomy (Korner 2003).

The effects of elevated [CO_2] and soil water levels on plants have been reported in numerous studies (Pritchard et al. 1999, Ferris et al. 2001, Hetherington and Woodward 2003). These studies suggest that in elevated [CO_2] and reduced soil water levels, plants modify their leaf morphology and anatomy, often referred to as plasticity, which enables them to thrive well under environmental stress (McLellan 2000). Many studies suggest that elevated [CO_2] enhances leaf area (Kerstiens et al. 1995, Heath and Kerstiens 1997) and decreases specific leaf area (SLA) (Norby and O'Neill 1991). But, under drought conditions, leaf area decreases whereas petiole area, foliar tissue density and stomatal pore area increase, acting as mechanical support, promoting leaf cooling (Li et al. 1996, Meng et al. 2009), resistance to physical damage by desiccation

(Mediavilla et al. 2001), and inducing efficient water use and lowering evapo-transpiration (Abrams 1990, de Lillis 1991, Bruschi et al. 2000).

While there is no doubt that elevated [CO_2] and drought affect leaf area, stomatal area and stomatal density, there is no consensus among studies concerning the increase or decrease in these leaf characteristics. For example, in response to the main effects of elevated [CO_2] or decrease in soil water, some studies report increases in leaf area (Norby et al. 1995, Sims et al. 1998), stomatal area (Li et al. 1996) and stomatal density; whereas others report decreases in leaf area (Norby and O'Neill 1991, Pettersson et al. 1993), stomatal area (Woodward and Kelly 1995, Beerling et al. 1996) and stomatal density (Lin et al. 2001). But, relatively few studies have addressed the consequences of elevated [CO_2] and low soil water levels on leaf area, specific leaf area, stomatal area and stomatal density (Beerling et al. 1996, Xiao et al. 2005). These studies on the responses of leaf morphological and anatomical characteristics to environmental stresses are at multispecies-specific leaf characteristics (Beerling et al. 1996, Li et al. 1996, Paoletti et al. 1998) . Thus, integrating both leaf anatomical and morphological changes of species in elevated [CO_2] and low soil water level is needed to understand these effects at intraspecific level.

Studies on pioneer species, including paper birch that inhabits a wide climatic gradient, have shown remarkable leaf morphological and anatomical variations (Dancik and Barnes 1974, Sharik and Barnes 1979, Senn et al. 1992, Pyakurel and Wang 2013). Paper birch adapts to a wide range of climatic regimes in North America and the species is significantly gaining ecological and economic importance (Safford et al. 1990). However, less is known about how such widely distributed species respond to similar environmental stress, such as elevated [CO_2] and less soil water level, with respect to variations in leaf morphology and anatomy.

The aim of this study was to identify effects of [CO_2], water levels, and their interactions with selected paper birch populations on leaf morphology and anatomy. The major objectives of this study were to determine the individual effects of elevated [CO_2] and water levels, as well as their interaction, on the leaf characteristics and to explore the ability of the four different birch populations to adapt to predicted environmental stresses. I hypothesized that interaction and main effects of [CO_2], soil water levels and different populations would result in significant leaf morphological and anatomical variations among the selected paper birch populations. Among the birch populations, I expected that the interaction or main effects of elevated [CO_2] and high soil water level would increase leaf area, but decrease specific leaf area, petiole area and foliar tissue density. The interaction or main effect of elevated [CO_2] and low soil water level would decrease stomatal density, stomatal area, pore area and guard cell width. Secondly, I expected that significant correlation would exist among leaf characteristics (such as stomatal density, stomatal area, leaf area, foliar tissue density, petiole area and specific leaf area) to support the plants in balancing water loss from their leaves.

MATERIAL AND METHODS

Plant material

For the experiment four paper birch populations were purposely selected along annual precipitation gradient (higher ->1000 ppm and lower-<500ppm). Little Oliver (1322.00 ppm) and Wayerton (1032.60 ppm) populations were from higher mean annual precipitation; and Cussion Lake (450.00 ppm) and Skimikin (279.00 ppm) populations were from lower mean annual precipitation. The birch populations used in this study also varied in latitude, longitude and elevation at its originating habitats Wayerton (47.22 N, 65.93 W and 300 m), Skimikin (50.43 N, 120.25 W and 547 m), Cussion Lake (52.53 N, 122.24 W and 760 m) and Little Oliver Lake (54.48 N, 128.16 W and 150 m).

Seeds were initially germinated in petri dishes for 15 days in greenhouses at Lakehead University, Thunder Bay. Seeds were germinated in the same greenhouses in which the experiment was conducted to ensure that seedlings were growing in their appropriate experimental conditions from the moment of emergence. Twenty seedlings were transplanted into plastic containers of 21 - 25 cm (upper circle) diameter, 41.5cm deep and grown in a 2:1 (v/v) mixture of peat moss and vermiculite. These 20 seedlings were selected from each population and assigned to each treatment condition randomly. The experiment lasted for 12 weeks (February to April, 2012).

The experiment followed a factorial design (split-split plot), with atmospheric [CO_2] (ambient = 360ppm; elevated = 720ppm) being the main plots and two soil water levels (well watered and limited water) as sub-plots on four paper birch populations. There were four treatment combinations; ambient [CO_2]/limited water, ambient [CO_2]/well watered, elevated [CO_2]/limited water and elevated [CO_2]/well watered. Because of available greenhouses the [CO_2] treatments was not repeated. The [CO_2] was achieved using Argus [CO_2] generators and monitored by an Argus control system (Argus, Vancouver, Canada). Soil water levels were controlled experimentally by varying the frequency and quantity of watering (Tschaplinski and Norby 1991, Tschaplinski et al. 1995, Catovsky and Bazzaz 1999). In well-watered treatment, seedlings were watered every three days and all containers freely drained. But in limited water treatment, seedlings were watered every four days, with limited water in order to eliminate free draining of containers. Seedlings were fertilized once a week with 20-20-20 NPK water soluble fertilizer which was scheduled on watering days.

During the entire experiment, air temperature in the greenhouses were maintained at 20-26°C during the day and at 15-19°C overnight. The relative humidity was 50±5% for the entire experiment period. The supplemental light system was programmed between 5:00 hours and 21:00 hours on a cloudy day, defined as when light levels fell below 200 μmol m^{-2} s^{-1}. The seedlings were randomly positioned and were moved around every week throughout the experiment to remove block effect.

Sampling and data collection

In May 2012, five well developed leaves from each seedling of the five randomly selected seedlings were sampled from each treatment for leaf morphological and anatomical measurements. The samples collected were weighted for fresh mass and stored immediately in sealed plastic bags and kept at 4 °C in the dark for 24 hours. Leaf morphological data, such as leaf area (LS), perimeter (P), blade length (BL), petiole length (PL), petiole area (PA), maximum width (MW), position of maximum width (PMW), horizontal width (HW) and aspect ratio (AR) were measured using WinFolia software (Regent Instrument Inc. Quebec, Canada). Stomatal data were collected by obtaining stomatal impressions from the middle section of the leaves using clear nail varnish (Bacelar et al. 2004). While collecting stomatal impressions leaf veins were avoided as much as possible. I used electronic microscope and Motic Images Plus 2.0 software (Motic Instruments Inc., Richmond, Canada) to obtain photos of stomata. Stomatal density (number of stomata per $0.1mm^2$ i.e., 100,000 μm^2), length, width, pore area and guard cell width per leaf (Xu and Zhou 2008) were estimated on the JPEG

(Joint Photographic Experts Group) image, acquired through the Motic Images Plus. After morphological and anatomical measurements, the sample leaves were oven-dried in paper bags at 70 °C for at least 48h and leaf dry masses were weighted. The equations used for calculating the leaf characteristics are listed in Appendix II.

Data analysis

In this design, three factors can contribute to the variance observed for leaf characteristics: (1) [CO_2] levels were high or low, (2) whether soil water levels were high or low, and (3) whether the leaves were from Skimikin, Cussion Lake, Little Oliver or Wayerton population. [CO_2] levels, soil water levels and populations were designated as fixed factors and split plot design was used to analysis the variance of leaf characteristics. [CO_2] and soil water had two levels each whereas populations had four levels; therefore, the degree of freedom for [CO_2], soil water level and populations were one, one, and three, respectively. The sum of squares for each components and the total model sum of squares provide a direct measure of the effect of each model component on the variance of leaf characteristics (Annex-III, the table of expected values of mean square).The analysis was considered significant at $P \leq 0.01$. The ANOVA analysis followed the procedure explained by Doncaster and Davey (2007) and Niklas and Cobb (2010).

In addition to the analysis, Tukey’s honest significant difference test and student *t* test were used for pair-wise means comparison when ANOVA results were significant for any given characteristics. Pearson correlation was used to analyze the correlation within and between leaf morphological and anatomical variables. Assumptions of normality and homogeneity were checked for all leaf morphological characteristics with

Shapiro-Wilk's Test and Levene test, respectively. All statistical analysis was conducted using IBM SPSS Statistics-21 (SPSS, Chicago, IL, USA) and R- 2.12.1 (R Development Core team, 2011).

RESULT

Leaf morphology

Leaf area was significantly affected by the interaction effect of [CO_2], soil water levels and populations (P=<0.01; Fig. 4.1a, Table 4.1). Under both ambient and elevated [CO_2], and at both soil water levels, leaf areas of populations from Cussion Lake, Little Oliver Lake, Skimikin and Wayerton significantly differed from each other (Fig. 4.1a). The population from Wayerton had significantly larger leaf area when treated under higher [CO_2] and soil water levels whereas Skimikin had significantly smaller leaf area (Fig. 4.1a) under these treatments. Under elevated [CO_2] and lower water treatment, Little Oliver had significantly smaller leaf area that differed from ambient [CO_2] at different soil water levels (Fig. 4.1a). Two-way interactions between [CO_2] and soil water level resulted in significantly larger and smaller leaf areas (respectively) in Cussion Lake population that differed significantly from Little Oliver, Skimikin and Wayerton populations. There was no significant three-way interaction on SLA, petiole area and aspect ratio (P>0.05; Table 4.1).

On examining two way interactions, I found that interactions between elevated [CO_2] and low soil water level generally had the smallest petiole area, aspect ratio (Fig. 4.1b) and petiole index ratio in the birch populations (Table 4.1). There was no significant effect of the interaction between [CO_2] and soil water levels on SLA, foliar tissue density and succulence (Table 4.1). The interaction effect of soil water levels and populations showed that an increase in soil water level had significantly increased average leaf area in Cussion Lake and Little Oliver whereas decreased in Skimikin and Wayerton. Under high soil water levels, petiole area significantly decreased in Cussion Lake and Wayerton whereas significantly increased in Little Oliver. Although interaction effects of [CO_2], soil water levels and populations did not have significant effect on specific leaf area, and foliar tissue density, these leaf characteristics varied significantly between two different [CO_2] levels (Table 4.1). Paper birch populations under elevated [CO_2] had a significantly smaller aspect ratio (Fig. 4.1b), petiole area and specific leaf area compared to the populations that were treated under ambient [CO_2] (Table 4.2).

As a main effect of population, Tukey test showed that population from Skimikin had significantly different leaf succulence (mean value± standard error 0.05±0.002) among the four paper birch populations. Student *t* test comparison between the SLA from elevated and ambient [CO_2] and foliar tissue density from elevated and ambient [CO_2] showed that the mean values of SLA and foliar tissue density of the two [CO_2] levels differed significantly with 99% confidence interval. The mean values (with ± standard error mean) of SLA and foliar tissue density in elevated [CO_2] were 45.46±1.74 and 0.30±0.01 respectively whereas mean values (with ± standard error mean) of SLA and foliar tissue density in ambient [CO_2] were 55.68±2.25 and 0.25±0.01 respectively.

Leaf anatomy

There was significant interaction effect of [CO_2], soil water and population on stomatal characteristics such as stomatal density, area, pore area, guard cell width, stomatal intensity and stomatal shape coefficient (Fig. 4.1c and 4.1d, Table 4.1) at

P<0.01 significance level. Skimikin population treated under elevated [CO_2] and high water level had significantly more stomatal density, and smaller stomatal area compared to other paper birch populations treated under both [CO_2] and at both water levels (Fig. 4.1c and 4.1d). At the same time, Cussion Lake treated under ambient [CO_2] and high water level had significantly low stomatal density that differed significantly from Cussion Lake seedlings treated under elevated [CO_2] and low soil water level. Under the ambient and elevated [CO_2] levels, high soil water treatment had significantly increased average stomatal area, pore area and guard cell width in compare to the low water treatment in the birch populations (Fig. 4.1d, and 4.1e). For example, interaction of high water treatments to ambient and elevated [CO_2] resulted in significantly different mean stomatal areas in the populations from Cussion Lake, Little Oliver, and Wayerton compare to the birch seedlings treated under the interactions of low soil water treatment to ambient and elevated [CO_2] levels (Table 4.2). Under elevated [CO_2], high soil water treatment had significantly decreased mean stomatal area per population in Skimikin than the population at the low soil water level, whereas pore area and guard cell width were comparatively larger in Skimikin (Table 4.2). Little Oliver showed significant decrease in average stomatal pore area at decreased soil water levels under both ambient and elevated [CO_2] which differed within the birch populations. Population from Skimikin had significantly rounded stomata (i.e., larger stomatal shape coefficient) under low soil water treatments at both ambient and elevated [CO_2] with mean value 71.49% and 65.60% respectively, despite the fact that more rounded stomata were observed in Wayerton (69.10%), and Cussion Lake (69.24%) for the interactions between high soil water levels to ambient [CO_2] and elevated [CO_2] respectively (Table 4.2).

Table 4.1 Analysis of variance (ANOVA) with p-values for the main and interaction effects of carbon dioxide [CO_2], soil water level and populations on leaf area in cm^2 (LS), aspect ratio (AR), petiole area in cm^2 (PeA), petiole index in percentage (PeI%), specific leaf area in cm^{2}g^{-1} (SLA), succulence, foliar tissue density, stomatal density (SD), stomata area in μm^2 (SA), pore area in μm^2 (PA), guard cell width in μm (GCW) and stomatal shape coefficient (SHC). DF denotes degrees of freedom. The significance level is <0.01 with 99% confidence interval.

Source	**[CO_2]**	**Soil water**	**Populations**	**CO_2* Soil**	**CO_2* pop**	**Soil* Pop**	**CO_2* Soil *P**
LS	.001	.184	<0.001	.005	.001	<0.001	.001
AR	.008	.565	<0.001	.001	.007	.002	.154
PeA	.001	.167	<0.001	<0.001	.070	.003	.608
PeI%	.118	.682	.837	<0.001	.007	.001	.954
SLA	.001	.537	.628	.057	.686	.139	.647
SU	.999	.792	.010	.792	.295	.514	.569
TD	.003	.745	.113	.527	.723	.188	.444
SD	.003	.200	<0.001	.358	<0.001	<0.001	.004
SA	.972	<0.001	<0.001	.001	<0.001	.004	<0.001
PA	<0.001	<0.001	<0.001	.807	<0.001	<0.001	<0.001
GCW	<0.001	<0.001	<0.001	.025	<0.001	.075	<0.001
SHC	.002	.001	.033	<0.001	<0.001	.001	.003
DF	1	1	3	1	3	3	3

Table 4.2 Mean values of leaf characteristics for each paper birch population treated under two carbon dioxide [CO_2] levels (360and 720ppm) and two soil water levels (low and high). Leaf characteristics presented here are LS-leaf area (cm$^{2)}$, AR-leaf aspect ratio, PeA-petiole area (cm^2), SLA-specific leaf area (cm^{2}g^{-1}), SD-stomatal density, SA-stomata area (µm^2), PA-pore area (µm^2) and GCW-guard cell width (µm).

CO_2	Water levels	Populations	LS	AR	PeA	SLA	SD	SA	PA	GCW
360	Low	Skimikin	65.41	0.66	4.57	53.94	15	281.15	105.32	4.02
	High		59.32	0.68	3.37	65.62	15	384.32	141.59	4.78
720	Low		54.49	0.64	1.47	41.39	15	291.51	102.6	4.26
	High		55.89	0.66	3.53	49.77	20	286.81	103.39	4.38
360	Low	Cussion Lake	103.94	0.67	6.89	50.55	11	516.91	192.41	5.51
	High		109.91	0.60	3.19	60.10	10	562.75	193.88	5.88
720	Low		111.02	0.65	5.04	51.71	15	500.95	144.88	6.18
	High		115.54	0.66	5.84	41.36	12	656.58	230.04	7.33
360	Low	Little Oliver Lake	148.73	0.65	8.07	48.73	11	503.73	180.25	5.64
	High		146.75	0.65	7.18	56.03	12	659.69	214.09	6.92
720	Low		98.52	0.61	3.84	38.31	12	492.41	147.42	5.98
	High		149.72	0.64	7.07	41.40	11	539.04	156.93	6.56
360	Low	Wayerton	181.92	0.72	10.79	55.23	14	417.54	157.49	4.97
	High		168.43	0.71	5.71	55.29	15	510.52	176.11	5.92
720	Low		171.8	0.70	6.46	57.77	13	530.27	160.1	6.23
	High		158.18	0.69	7.31	41.97	14	537.06	160.38	6.46

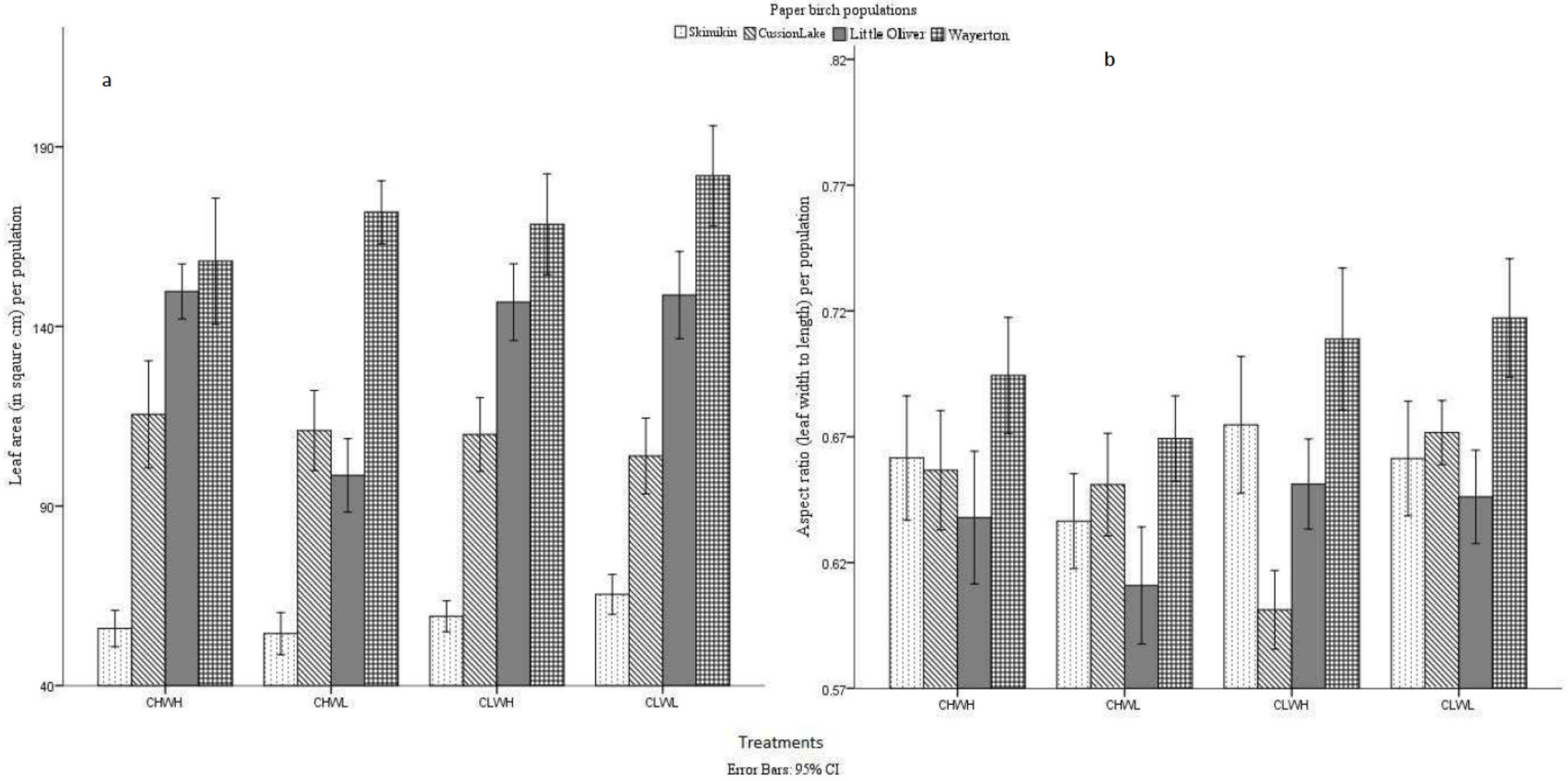

Figure 4.1 Interaction effects of carbon dioxide [CO_2] levels (360 ppm and 720 ppm), soil water levels (high and low) on leaf characteristics of four paper birch populations. Figures (a) and (b) are leaf area (cm^2) and aspect ratio (ratio of leaf width to leaf length) (per population). CHWH, CHWL, CLWH, CLWL are interactions of elevated CO_2 and high soil water level, elevated CO_2 and low soil water level, ambient CO_2 and high soil water level and ambient CO_2 and low soil water level respectively.

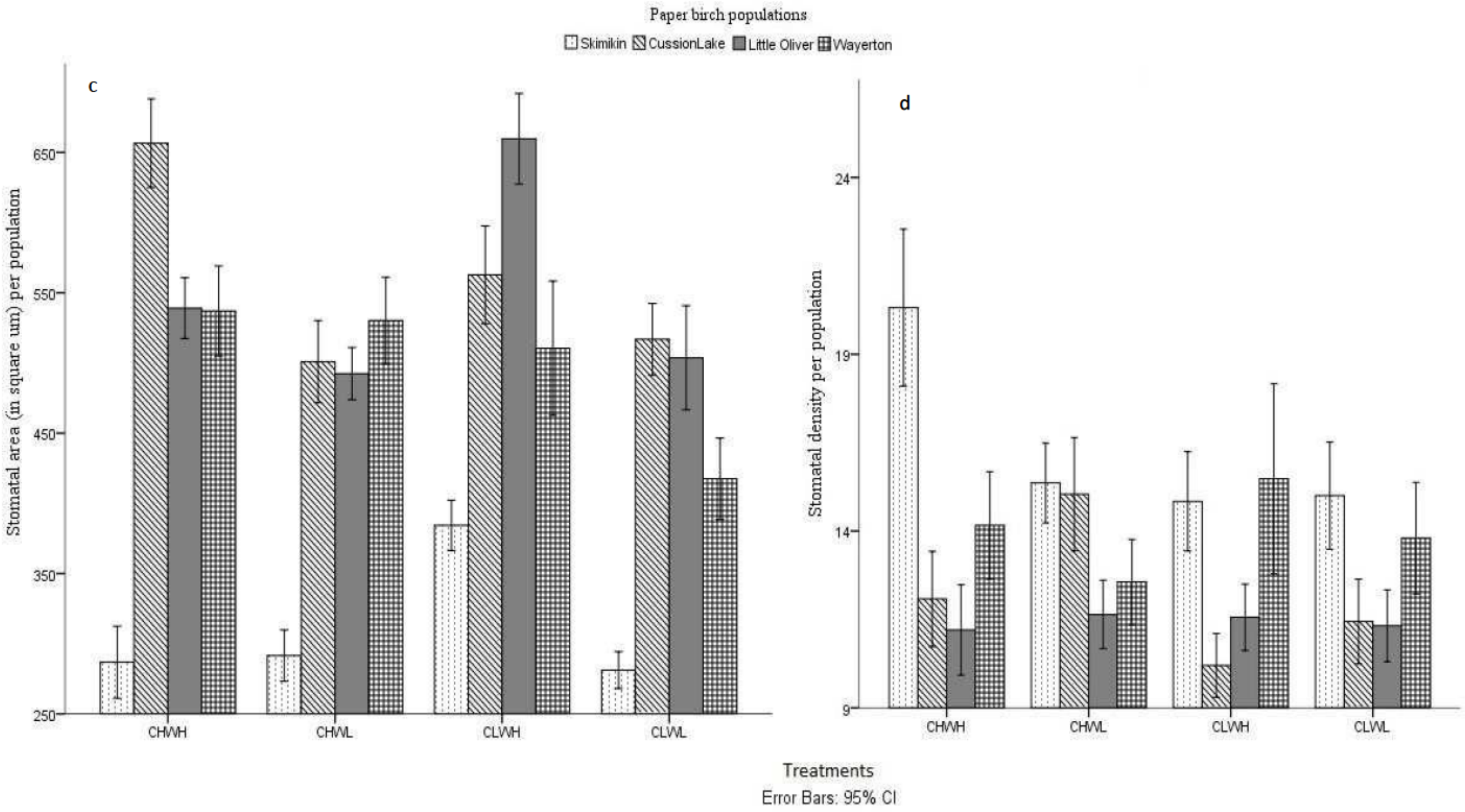

Figure 4.1 Contd...Interaction effects of carbon dioxide [CO2] levels (360 ppm and 720 ppm), soil water levels (high and low) on leaf characteristics of four paper birch populations. Figures (c) and (d) are stomatal area (μm^2) and stomatal density respectively (per population). CHWH, CHWL, CLWH, CLWL are interactions of elevated CO2 and high soil water level, elevated CO2 and low soil water level, ambient CO2 and high soil water level and ambient CO2 and low soil water level respectively

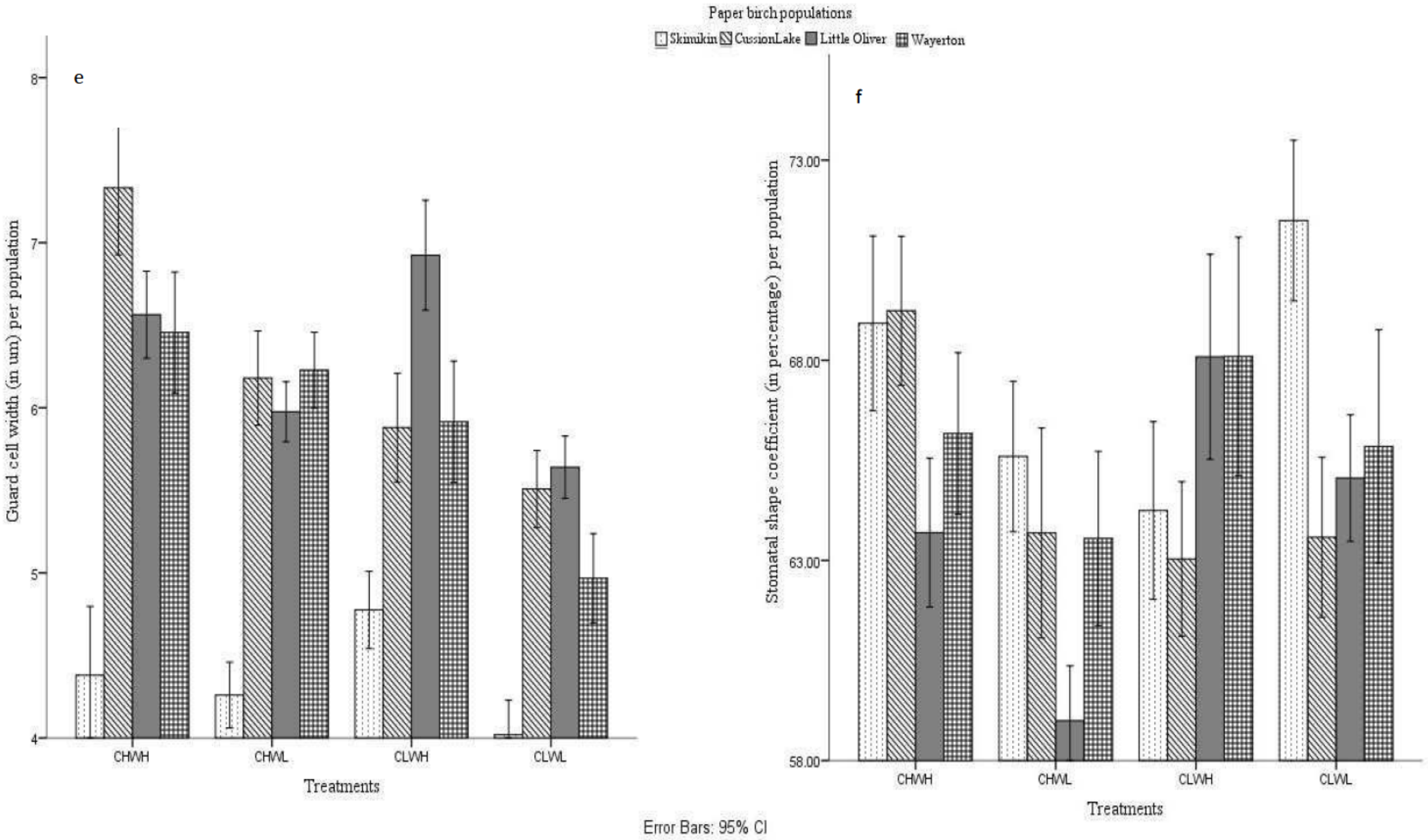

Figure 4.1 Contd...Interaction effects of carbon dioxide [CO2] levels (360 ppm and 720 ppm), soil water levels (high and low) on leaf characteristics of four paper birch populations. Figures (e) and (f) are guard cell width (µm) and stomatal shape coefficient (%) respectively (per population). CHWH, CHWL, CLWH, CLWL are interactions of elevated CO2 and high soil water level, elevated CO2 and low soil water level, ambient CO2 and high soil water level and ambient CO2 and low soil water level respectively

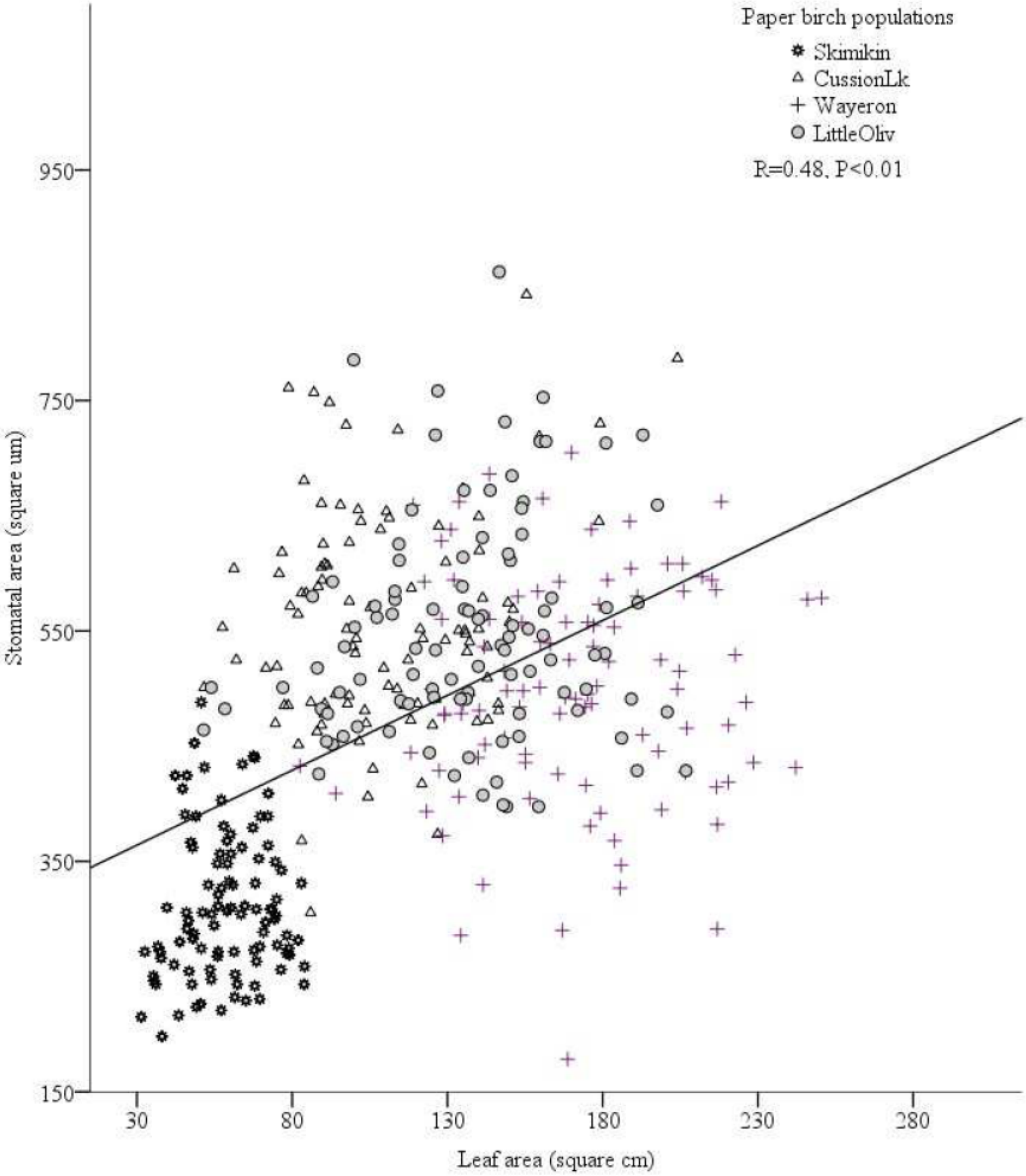

Figure 4.2 Correlation between leaf area (in cm^2) and stomatal area (μm^2) per paper birch populations. Different dots types represent origin of the paper birch populations.

Correlation between leaf morphological and stomatal characteristics

Leaf with larger stomatal area had lower stomatal density (*r*= -0.49, *P*=<0.001; Table 4.3). Within leaf morphological characteristics larger leaf area had significantly larger petiole area (*r*= 0.56, *P*=<0.001), and wider aspect ratio (*r*= 0.30, *P*=<0.001; Table 4.3); while none of these leaf characteristics were correlated with specific leaf area (thus, not included in Table 4.3). Comparing leaf morphological and stomatal characteristics, leaf area was positively correlated with stomatal area (Fig. 4.2), pore area and guard cell width (Table 4.3) but was weakly correlated with stomatal density (Table 4.3). However, stomatal density, stomatal area, pore area and guard cell width were not significantly correlated with specific leaf area and aspect ratio (Table 4.3).

Table 4.3 Pearson correlations between stomatal density (SD), stomata area in μm^2 (SA), pore area in μm^2 (PA), guard cell width in μm (GCW), leaf area in cm^2 (LS), aspect ratio (AR), petiole area in cm^2 (PeA), and succulence (S) of paper birch populations. The leaf morphological characteristics (chrs.) used in Pearson correlation are an average per seedling per population (N= 80). Values are correlation coefficient (r) with p values in parentheses.

Chrs.	**SD**	**SA**	**PA**	**GCW**	**LS**	**AR**	**PeA**
SA	-0.49 (<0.001)						
PA	-0.49 (<0.001)	0.87 (<0.001)					
GCW	-0.32 (<0.001)	0.85 (<0.001)	0.59 (<0.001)				
LS	-0.24 (<0.001)	0.48 (<0.001)	0.37 (<0.001)	0.45 (<0.001)			
AR	.09 (0.09)	-0.05 0.37	-0.03 0.58	-0.05 0.30	0.30 (<0.001)		
PeA	-.16 (0.001)	0.24 (<0.001)	0.24 (<0.001)	0.19 (<0.001)	0.56 (<0.001)	0.43 (<0.001)	
S	-0.41 (<0.001)	0.43 (<0.001)	0.38 (<0.001)	0.41 (<0.001)	0.47 (<0.001)	0.11 (0.33)	0.43 (<0.001)

DISCUSSION

Leaf morphological and anatomical characteristics are sensitive to environmental changes such as rising [CO_2] and reduced water availability for plants (Woodward and Kelly 1995, Teklehaimanot et al. 1998, Pritchard et al. 1999). Supporting my hypothesis, the results showed that leaf area and stomatal characteristics differed as a result of an interactive effect of [CO_2], soil water levels and paper birch populations. However, the interaction had no significant effect on leaf morphological characteristics such as shape (leaf aspect ratio), petiole area, specific leaf area, foliar tissue density and succulent. This indicated that stomatal characteristics are more sensitive to water stress as compared to [CO_2] levels. For different species, both an increase and a decrease in leaf area have been reported as an effect of elevated [CO_2] (Norby and O'Neill 1991, Pettersson et al. 1993, Norby et al. 1995, Sims et al. 1998, Pritchard et al. 1999). Partly rejecting my hypothesis, this study showed a reduction in the leaf area of paper birch populations from Little Oliver Lake, Skimikin and Wayerton either under elevated [CO_2] or the interaction between elevated [CO_2] and soil water levels. Comparable to the present study, leaf area was reduced in elevated [CO_2] in *Castanea sativa, Liriodendron tulipifera* seedlings, and *Betula pendula* (Mousseau and Enoch 1989, Norby and O'Neill 1991, Pettersson et al. 1993). These species had a more significant effect of elevated [CO_2] on root growth rather than shoot or leaf growth, which might be true in the case of paper birch seedlings. Cussion Lake population had larger average leaf area in elevated [CO_2] irrespective of soil water levels, similar to a study on *Phaseolus vulgaris* which also found increased leaf area by [CO_2] enrichment (Radoglou and Jarvis 1992, Pritchard et al. 1999). However, leaf area decreased in the Little Oliver Lake population treated under elevated [CO_2] and low soil moisture, and this result is consistent with a study on *P. interamericana, P. euramercana* and *P. trichocarpa* (Radoglou and Jarvis 1990) which reported smaller but more leaves per seedling under elevated [CO_2]. Although the numbers of leaves and biomass of leaf, shoot and root per seedling were not reported in this paper, I had observed a trade-off between leaf area and the number of leaves per seedling. For example, Skimikin and Cussion Lake comparatively had more branches and small, numerous leaves per seedling whereas Wayerton and Little Oliver Lake seedlings had fewer branches, with fewer but larger leaves per seedling. Thus, I concluded that an increase or decrease in leaf area in paper birch is not only related to [CO_2] levels but also population differences, as is the case of *Populus* genotypes (Gielen et al. 2001), and a trade -off between leaf morphological characteristics as in the cases of *P. interamericana, P. euramercana* and *P. trichocarpa* (Radoglou and Jarvis 1990). And these morphological characteristics together with stomatal characteristics can strongly influence water use efficiency in plant species (Woodward 1987, Mansfield et al. 1990).

The present study has confirmed that stomatal density in paper birch varied according to main effects of [CO_2] levels and population differences as well as the interaction of [CO_2], soil water levels and populations. Previously, studies had reported that stomatal characteristics are affected by [CO_2] (Knapp et al. 1994, Woodward and Kelly 1995, Woodward et al. 2002), soil water levels (Banon et al. 2004) and population differences (Pyakurel and Wang 2014). Unlike my hypothesis, stomatal density was significantly higher in elevated [CO_2] and higher water level than under ambient [CO_2] and at higher or lower water levels in paper birch populations. Furthermore, stomatal density significantly differed within Cussion Lake and Skimikin under [CO_2] treatment.

The birch populations did respond differently to the treatments and the results are consistent with previous studies of stomatal responses to [CO_2] where individual, population or species responded differently (Malone et al. 1993, Knapp et al. 1994). Nevertheless, there is considerable variation in stomatal density from large reduction, no change, to large increase under elevated [CO_2] (Woodward and Kelly 1995, Hetherington and Woodward 2003, Tricker et al. 2005). Studies suggested that stomatal density is not only relatively plastic and can potentially modify to environmental changes (Richardson et al. 2001, Lake and Woodward 2008, Sekiya and Yano 2008), but it is also genotypically differentiated (Fraser et al. 2009).

As expected, the interaction of [CO_2], soil water levels and population further demonstrated effects on stomatal area, pore area and guard cell width. More importantly, supporting my hypothesis, elevated [CO_2] with limited soil water level reduced stomatal area, pore area and guard cell width. This observation is in agreement with the conclusion that elevated [CO_2] and water stress reduce stomatal area in *Arabidopsis* (Doheny et al. 2012), *Populus trichocarpa* (Dunlap and Stettler 2001), and *Pistacia atlantica* (Belhadj et al. 2007), respectively. Consistent with paper birch, a study on *Arabidopsis* reported reduction in stomatal area (including pore area and guard cell width) under reduced water availability and explained that small stomata would support maximal stomatal conductance (Doheny et al. 2012). Thus, it has been suggested that smaller stomata area and guard cells increase carbon dioxide diffusion per unit area of stomata and reduce water loss compared to larger stomatal area and guard cells (Abrams 1990). My result is not consistent with a study on paper birch populations from water deficit sites that had larger and fewer stomata per unit area (Li et al. 1996). Although the birch populations in this study increased stomatal area under low water levels, stomatal area per unit leaf area remained relatively similar due to a decrease in stomatal density. The trade-off between stomatal area and density; that is, either larger stomatal area with low density or smaller stomatal area with high density, revealed by the strong correlations in my study, is consistent with other studies (Hetherington and Woodward 2003, Camargo and Maremco 2011, Pyakurel and Wang 2014). Alternatively, previous studies suggested that a leaf with high stomatal density and smaller stomatal area can reduce stomatal conductance and increase water-use efficiency (Poulos et al. 2007), which might be the case in this study also.

Under environmental stress such as elevated [CO_2] and water deficiency, plants modify leaf morphological and anatomical characteristics that diminish the water loss or that increase water use efficiency (Dudley 1996). Thus, small leaf area with less stomatal density would alter water use efficiency for a species. Supporting my hypothesis, the results showed significant correlations within and between leaf anatomical and morphological characteristics. All these features provided a structural basis in reducing water loss through leaves and increasing water use efficiency. Therefore, the plasticity of leaf area and stomatal characteristics played a major role in the survival of paper birch under environmental stress.

In conclusion, the results of this study confirmed the significant effects of elevated [CO_2] on paper birch populations treated at low soil water level. Although the study was conducted in four paper birch populations, the findings of this study help to understand how the birch would change leaf morphological and anatomical structure under future elevated [CO_2] and altered precipitation patterns.

CHAPTER 5

SYNTHESIS AND ECOLOGICAL IMPLICATIONS

Plants demonstrate significant leaf morphological and anatomical differences, which underlie variation in physiology, growth rates, productivity, population and community dynamics and eventually ecosystem function (Hughes et al 2009, Hajek et al. 2013). Consensus is that these leaf variations may have resulted from the high genetic diversity and different environmental conditions providing potential for leaf morphological and anatomical adaptation. With predicted climate change and shift in climatic zones, species' adaptations such as those found in leaf characteristics are required to cope with the changes.

The change in climate might also influence boreal forest structure and function, species abundance and distribution. Hence, it is expected that early successional broadly niched species like paper birch will successfully migrate and adapt to the changes (Stocklin and Baumler 1996, Garamvolgyi and Hufnagel 2013). Therefore, this study focused on understanding leaf characteristics variations of paper birch populations in natural and stressed environmental conditions, and understanding fundamentals before suggesting possible genotypic variations and the adaptive significance of specific leaf characteristics. The major conclusions of the study are as follows:

1. The birch populations showed significant genotypic differences in leaf characteristics when grown either under uniform environments in the greenhouse (Chapter 2) and common garden (Chapter 3), or under the stresses of elevated [CO_2] and low soil water levels (Chapter 4). The genotypic differences in leaf

characteristics possibly explained natural diversity due to differences in the environment of the population's origin.

2. Leaf characteristics such as specific leaf area, petiole area, leaf hair density and stomatal density were correlated to the climate of the paper birch population's origin. The result showed environmental carry-over effects on the birch populations grown under a uniform environment of the greenhouse and common garden.
3. On comparing leaf area, specific leaf area and hair density of the birch populations grown between the greenhouse and the common garden, I found that the birch in the greenhouse had larger leaf area, specific leaf area and leaf hair density. The result demonstrated that the birch populations might have acclimated to climate and biotic conditions in the common garden.
4. Under the elevated [CO_2] and low soil water levels, the increase in [CO_2] reduced leaf area and increased stomatal density whereas low soil water level resulted in smaller stomatal area, pore area and guard cell width.
5. The birch populations showed plastic response to [CO_2] and soil water levels either by decreasing stomatal area under low soil water level or by increasing stomatal density under elevated [CO_2] which might indicate an adaptive strategy in limited resources.
6. Integration between and within leaf morphological and stomatal characteristics such as smaller leaf area with less stomatal density, larger leaf area, high hair density and low stomatal density might have supported paper birch to maintain balance between [CO_2] gain and water loss.

Paper birch is an early successional species that is increasingly recognized for its role in nutrient cycling. Hence, the birch is expected to increase in importance for natural regeneration and plantation purposes. As the genotypic and phenotypic differences in the leaf characteristics of the birch populations have been resulted from adaptive differences to available resources, the information on leaf variation is beneficial in delineating seed zones and tree breeding. As suggested for *Quercus rugosa* (Uribe-Salas et al. 2008) and *Populus* spp. (Hajek et al. 2013), paper birch may achieve higher adaptability to predicated climate change over other species with less intraspecific variation in leaf characteristics. The birch may expand the range of its habitat types it currently occupies, or by moving from one type of habitat to another. Hence, the birch may be used for restoration of degraded land with limited available resources or maintaining intraspecific diversity.

Intraspecific variation in leaf characteristics can have significant effects at individual (plant), population, community and ecosystem levels. For instance, the variations and integration of leaf characteristics must have supported the birch populations to balance light energy, transpirational loss, carbon gain and other physiological activities at the plant level. The genetic diversity and adaptive function of leaf characteristics might have supported the birch populations to inhabit a wide environmental gradient and expand habitat range over other species. However, leaves grown under elevated [CO_2] showed lower plant quality for herbivores by decreasing nitrogen levels and increasing levels of starches, fiber and secondary compounds such as condensed tannins (Roth et al. 1998, Lindroth et al. 2001a, 2001b, Robinson et al. 2012). Hence, the leaf quality and quantity of the birch would affect competition, palatability, herbivore interaction and pest interaction at the community level, also affecting nutrient cycle, species fitness and species' habitat at the ecosystem level.

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APPENDICES

Appendix I: Environmental variables of paper birch populations' origins

Latitude (LAT), longitude (LONG), elevation (ELE) in meters, mean annual precipitation (MAP) in millimeters, mean annual temperature (MAT) in degrees Celsius, mean annual aridity index (MAI), precipitation during growing season (GSP) in millimeters, temperature during growing season (GST) in degrees Celsius, aridity index during growing season (GSA) of 16 paper birch populations collected (seeds) from across Canada and grown at the common garden in Thunder Bay, Ontario.

Populations	LAT	LONG	ELE	MAT	MAP	MAI	GST	GSP	GSA
Newfoundland	47.37	57.57	140.00	4.70	1513.70	102.97	12.00	100.08	13.50
St. Georges	48.50	58.16	70.00	4.42	2062.60	143.04	12.93	110.15	14.26
Millvale	46.40	63.40	70.00	5.23	1140.70	74.90	15.08	91.00	10.77
Allardville	47.36	65.25	100.00	3.91	969.90	69.73	15.80	90.90	10.46
Cap Des Rosiers	48.11	65.41	200.00	3.33	1147.20	86.06	13.33	90.78	11.55
Wayerton	47.22	65.93	300.00	3.99	1032.60	73.81	15.80	90.90	10.46
Indiana brook	46.21	66.33	13.00	6.16	1639.50	101.45	14.13	93.85	11.54
New Brunswick	45.54	66.38	20.00	5.30	1143.30	74.73	16.25	90.35	10.21
Bells Fall	46.50	75.10	330.00	3.28	1014.70	76.41	16.83	93.38	10.33
Alice	45.75	77.13	300.00	4.07	829.65	58.97	16.45	83.73	9.39
Petawawa	45.97	77.47	130.00	4.28	853.30	59.75	16.18	76.85	8.71
Timmins	48.34	81.22	295.00	1.36	831.40	73.19	14.35	83.03	10.12
Thunder Bay	48.22	89.19	199.00	2.50	711.00	56.88	14.43	82.18	9.98
Prince Albert	53.12	105.46	440.00	0.90	286.90	26.32	14.88	63.78	7.61
St. Mary River	49.38	116.03	990.00	5.70	451.00	28.73	15.55	41.45	4.81
Porcupine Lk	49.15	117.00	840.00	8.05	551.00	30.53	14.85	44.08	5.26
Wilson Ck	50.04	117.23	800.00	7.40	879.00	50.52	15.85	38.08	4.37
Mars Ck	51.22	118.18	760.00	4.60	490.70	33.61	14.88	42.03	5.01
Barnes Ck	50.34	118.50	850.00	7.70	305.00	17.23	17.10	40.65	4.45
Bush Ck	50.59	119.45	650.00	8.90	279.00	14.76	16.78	34.20	3.79
Adam Lk	51.43	119.83	400.00	5.01	1076.50	71.72	13.38	52.33	6.64
Skimikin	50.43	120.25	547.00	8.88	279.00	14.78	18.50	29.55	3.08
Amanita Lk	54.08	121.47	615.00	5.20	600.00	39.47	13.38	52.33	6.64
Tabor Lk	53.55	122.22	800.00	5.01	600.00	39.97	13.38	52.33	6.64
Cussion Lk	52.53	122.24	760.00	6.50	450.00	27.27	13.28	48.98	6.24
Frost Lk	53.47	122.38	650.00	5.20	600.00	39.47	15.58	77.53	9.00
Juniper Ck	55.08	127.43	350.00	3.90	613.00	44.10	12.88	43.23	5.61
Lt. Oliver Lk	54.48	128.16	150.00	6.30	1322.00	81.10	14.18	55.00	6.75
Skeena River	54.30	128.34	70.00	6.32	1160.00	71.08	14.18	55.00	6.75

Note: Ck stands for Creek and Lk stands for Lake

Appendix II: Equations used to calculate leaf characteristics and climatic variables

Equations used for leaf morphological and anatomical data collection on paper birch populations. Where, PeI-petiole index (ratio), PeL-petiole length (cm), LL-total leaf length (cm), MWI-maximum width index, MW-maximum leaf width (cm), FC-form coefficient, P-leaf perimeter (cm) S-leaf succulence ($\mathrm{mgH_2Ocm^{-2}}$), LFM is leaf fresh mass (gm), LDM-leaf dry mass (gm), LS-leaf area ($\mathrm{cm^2}$), SLA-specific leaf area ($\mathrm{cm^{-2}gm^{-1}}$), FTD-foliar tissue density (Bacelar et al. 2004); SA is stomatal area ($\mathrm{\mu m^2}$), SL-stomatal length (µm), SW-stomatal width (µm), SD- stomatal density, ED- epidermal cell density, PA-stomatal pore surface area ($\mathrm{\mu m^2}$), PL-pore length (µm), PW- pore width (µm), SI-stomatal intensity, SHC-stomatal shape coefficient (Sagaram et al. 2007, Batos et al. 2010). MAI-mean annual aridity index, MAP-mean annual precipitation (millimeters), MAT-mean annual temperature (°C), GSA-mean aridity index during growing season, GSP-mean precipitation during growing season (millimeters), GST-mean temperature during growing season (°C) and Nv-length of growing season (days).

Equations		
Morphological Characteristics	**Anatomical Characteristics**	**Climatic variables**

Appendix III: Correlation between environmental variables of paper birch populations' origins

Pearson correlations between the geography and climate of paper birch populations' origins. Here, Env is environmental variables, Long is longitude (West), Lat is latitude (North), Ele is elevation in meters, MAT is mean annual temperature in ^{o}C, MAP is mean annual precipitation in millimeters, MAI is mean annual aridity index, GST is growing season temperature in ^{o}C, GSP is growing season precipitation in millimeters and GSA is growing season aridity index. Longitude from 57-89 (East to West) is considered as Eastern Canada whereas Longitude from 105-128(East to West) is considered as Western Canada for the correlation analysis. Values are correlation coefficient (r) and significance level are $p<0.05$ (indicated as ** for $p\leq0.001$, * for $p\leq0.05$, and ^{ns} for >0.05 in following table).

Long.	Env.	Lat	Long	Elev	MAT	MAP	MAI	GSP	GST
57- 89W	Long	0.01^{ns}							
105- 128W		0.46^{ns}							
57-128W		0.85^{**}							
57-89W	Elev	0.22^{ns}	0.50^{ns}						
105-128W		-0.76	-0.35^{ns}						
57-128W		0.48^{*}	0.75^{**}						
57-89W	MAT	-0.64^{*}	-0.67^{*}	-0.66^{*}					
105-128W		-0.48^{ns}	0.41^{ns}	0.37^{ns}					
57-128W		0.16^{ns}	0.42^{*}	0.47^{*}					
57-89W	MAP	0.29^{ns}	-0.79^{**}	-0.49^{ns}	0.45^{ns}				
105-128W		0.29^{ns}	0.52^{ns}	-0.59*	0.06^{ns}				
57-128W		-0.39^{ns}	-0.64^{**}	-0.71^{**}	-0.13^{ns}				
57-89W	MAI	0.46^{ns}	-0.70^{*}	-0.37^{ns}	0.25^{ns}	0.98^{**}			
105-128W		0.38^{ns}	0.46^{ns}	-0.67	-0.11^{ns}	0.98			
57-128W		-0.44^{*}	-0.71^{**}	-0.76^{**}	-0.30^{ns}	0.98^{**}			
57-89W	GSP	0.37^{ns}	-0.82^{**}	-0.41^{ns}	0.40^{ns}	0.95^{**}	0.93^{**}		
105-128W		0.56^{ns}	-0.05^{ns}	-0.37^{ns}	-0.54^{ns}	0.17^{ns}	0.25^{ns}		
57-128W		-0.65^{**}	-0.91^{**}	-0.80^{**}	-0.53^{*}	0.71^{**}	0.79^{**}		
57-89W	GST	-0.71^{*}	0.38^{ns}	0.09^{ns}	0.15^{ns}	-0.63^{*}	-0.71^{*}	-0.63^{*}	
105-128W		-0.67	-0.38^{ns}	0.53^{ns}	0.48^{ns}	-0.41^{ns}	-0.51^{ns}	-0.24^{ns}	
57-128W		-0.33^{ns}	0.04^{ns}	0.24^{ns}	0.31^{ns}	-0.43^{*}	-0.46^{*}	-0.19^{ns}	
57-89W	GSA	0.53^{ns}	-0.72^{*}	-0.32^{ns}	0.23^{ns}	0.92^{**}	0.94^{**}	0.95^{**}	-0.85^{**}
105-128W		0.66	0.03^{ns}	-0.46^{ns}	-0.60	0.24^{ns}	0.34^{ns}	0.98	-0.43^{ns}
57-128W		-0.57^{**}	-0.88^{**}	-0.79^{**}	-0.53^{*}	0.76^{**}	0.84^{**}	0.98^{**}	-0.35^{ns}

Appendix IV: Expected values of mean square for a three factors split plot design

Expected values of mean squares (Ems) for a three factors split plot design. Factor carbon dioxide (C) c with levels; factor soil water (W) with w levels, factor populations (P) with p levels and s is a whole plot treatment (unreplicated). Here all three factors are designed as fixed. DF denotes degree of freedom.

Sources (factors)	**DF**	**Ems**
For whole plot	sp-1=1	-
C	(c-1) =1	$\sigma_e^2 + wp\sigma_\alpha^2 + swp\sigma_c^2$
W	(w-1) =1	$\sigma_e^2 + p\sigma_{\alpha\pi}^2 + scp\sigma_w^2$
WC	(c-1)(w-1)=2	$\sigma_e^2 + p\,\sigma_{\alpha\pi}^2 + sp\sigma_{cw}^2$
Whole plot residual	c(s-1)(w-1)=0	$\sigma_e^2 + p\,\sigma_{\alpha\pi}^2$
P	(p-1)=3	$\sigma_e^2 + \sigma_{\alpha\pi'}^2 + scw\sigma_p^2$
CP	(c-1)(p-1)=3	$\sigma_e^2 + \sigma_{\alpha\pi'}^2 + sw\sigma_{cp}^2$
WP	(w-1)(p-1)=3	$\sigma_e^2 + \sigma_{\alpha\pi'}^2 + sc\sigma_{wp}^2$
CWP	(c-1)(w-1)(p-1)=3	$\sigma_e^2 + \sigma_{\alpha\pi'}^2 + s\sigma_{cw_p}^2$
Total residual	cwp(s-1)=0	$\sigma_e^2 + \sigma_{\alpha\pi'}^2$