

**LATITUDINAL COMPENSATION: FOLIAR PHYSIOLOGY  
OF NARROWLEAF COTTONWOODS  
FROM THE FULL LATITUDINAL RANGE**

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## Abstract

For deciduous trees, the growth season becomes progressively shorter with increasing latitude or elevation. To overcome the challenge of sufficient growth and development within the limited interval, deciduous trees may have adapted with increased physiological capacity. To test this ‘latitudinal compensation’ theory, I investigated the growth, foliar morphology, and some foliar biochemical and gas exchange characteristics of narrowleaf cottonwoods originating from the full range of its distribution. This study was carried out in a common garden at the University of Lethbridge, Alberta, Canada, near the northern limit of the species’ distribution. Across 167 genotypes from 9 populations representing the full 16° latitudinal range, heights and stem diameters were associated negatively ( $p < 0.1$ ) with the latitude of origin, while foliar morphological characteristics of blade width and leaf mass tended to be positively associated with latitude ( $p < 0.1$ ), and leaf mass per area (LMA) was positively associated ( $p < 0.05$ ). Foliar chlorophyll (chl) and nitrogen (N) content were also significantly associated with latitude, and carbon isotope composition ( $\delta^{13}\text{C}$ ) was positively correlated with chl and N. With a more intensive analysis for a subset of three populations from near the southern middle, and northern limits of the distribution, these same patterns were confirmed and additionally, stomatal ratio was higher in the leaves for the northern population. Subsequently, the light-saturated photosynthetic capacity ( $A_{max}$ ) was higher in genotypes from the northern population. I conclude that increased foliar morphological and biochemical characteristics of narrowleaf cottonwoods contribute to increased photosynthetic capacity for northern populations, providing evidence for latitudinal compensation in a deciduous tree from the northern hemisphere.

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The responsibility is entirely my own for any errors or inadequacies that may remain in this work.

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## Abbreviations

AB	- Alberta, Canada
ABSD	- Abaxial stomatal density
ADSD	- Adaxial stomatal density
AZ	- Arizona, USA
CCI	- Chlorophyll Content Index
Chl	- Chlorophyll
CnGV	- Counter Gradient Variation
DMSO	- Dimethyl sulphoxide
GT	- Genotypes
LMA	- Leaf Mass per Area
N	- Nitrogen
Rubisco	- Ribulose-1,5-biphosphate carboxylase/oxygenase
UT	- Utah, USA
WUE	- Water Use Efficiency
WY	- Wyoming, USA
$\delta^{13}\text{C}$	- Carbon isotope composition
$\Delta^{13}\text{C}$	- Carbon isotope discrimination

## Chapter 1. Introduction

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### 1.1 Native cottonwoods

Cottonwood trees belonging to the genus *Populus* can be found along many rivers as gallery forests throughout the Northern Hemisphere. They are the ecological pioneers that are specifically adapted to riparian conditions and provide vital woodland habitat for wildlife (Finch & Ruggiero, 1993; Cooke & Rood, 2007). Canadian landscapes are rich with seven species of naturally occurring poplars (Cooke & Rood, 2007). The only native trees in parts of southern Alberta are poplars and the riparian forests in this area are an assemblage of four species of poplars that hybridise (Brayshaw, 1965; Floate, 2004).

Out of the six sections in the genus *Populus*, the native riparian cottonwoods in North America belong to two sections, *Aigeiros* and *Tacamahaca* (Eckenwalder, 1996).

*Populus deltoides* and *P. fremontii* have been classified under the section *Aigeiros* and they are primarily limited to lower elevations and latitudes. *P. angustifolia*, *P.*

*balsamifera*, and *P. trichocarpa* are within the section *Tacamahaca*, and commonly found at higher elevations and latitudes (Braatne, Rood & Heilman, 1996). While *P.*

*balsamifera* is widely distributed in North America, *P. trichocarpa* largely occurs in the western parts. *P. angustifolia* (narrowleaf cottonwood) is widely distributed in the Rocky Mountains and plains from southern Canada to northern Mexico (Dickmann, 2001).

Throughout its range, *P. angustifolia* is found at elevations higher than *P. deltoides* or *P. fremontii*, but lower than *P. trichocarpa* or *P. balsamifera* (Braatne et al., 1996).

This intermediate distribution reflects relatively narrow environmental requirements; consequently, *P. angustifolia* occurs in disjunct woodlands (Cooke & Rood, 2007).

## **1.2 Common gardens**

Common garden studies of various *Populus* species have been carried out and they have involved comparisons of different traits for trees collected from different environments and brought together and grown under the same environment at one site (Rood, Braatne & Hughes, 2003). The pioneer common garden study by Pauley & Perry (1954) has shown patterns of height growth cessation with the latitude of origin for *P. trichocarpa* and *P. deltoides*. Similar studies carried out for *Populus tremula* genotypes grown in a common garden in Sweden by Hall et al. (2007) and Luquez et al. (2008) have shown strong latitudinal patterns of growth cessation and similar results have been found by Friedman, Roelle & Cade (2011) for *P. deltoides*.

Gornall & Guy (2007) have investigated *P. trichocarpa* in two range-wide common garden experiments in British Columbia, Canada. They have shown strong latitudinal gradients with growth characters, foliar morphological characters and ecophysiological characters. Another common garden study by Soolanayakanahally et al. (2009) has also found latitudinal and elevational gradients in growth and physiological traits from Canada-wide collection of *P. balsamifera* populations representing 21 provenances. Most of these previous studies have shown higher photosynthetic capacity for plants from higher latitudes and elevations.

### **1.3 Latitudinal compensation**

Distributional ranges of plants are dependent upon the seasonal climatic patterns especially for northern ecoregions. The summer growth seasons progressively become shorter with increasing latitude or elevation. In order to overcome the challenge of growth and reproduction within the limited interval with sufficient temperature, deciduous trees at the northern latitudes may have adapted with increased physiological capacity. This concept has been observed for many animals and some plants species as countergradient variation (CnGV). It occurs when the genetic influence on traits opposes environmental influences (Levins, 1968; Conover & Schultz, 1995). This will reduce the phenotypic variance across the environments within which the genotype is distributed. The gradient in question could be temperature, altitude, latitude, soil nutrient levels, etc. In this study I have investigated latitude, because latitudinal gradients in growth season length produce CnVG (Conover & Present, 1990) or “latitudinal compensation”.

Knowledge of CnGV is important in resource management and conservation as genotypes are being transplanted across environments. Phenotypes, as they appear in nature may mislead and may cause problems in transplanting at different environments. Therefore, common environment experiments or reciprocal transplanting methods are used by researchers to measure the environmental and genetic components of phenotypic differences across habitats (Conover & Schultz, 1995) .

In the common garden, representative genotypes from different populations are grown in the same environment. The common garden of narrowleaf cottonwoods used for this study was at the University of Lethbridge, AB.

In the first part of this study (Chapter 2), I have investigated associations between growth, foliar morphological and some biochemical characters, and the latitude and elevation of origin for genotypes representing the full range of distribution of narrowleaf cottonwoods.

In the second part of the study (Chapter 3), I have further investigated some morphological and gas exchange characters of a sub-sample and compared those traits for three populations representing the northern, middle and southern limits of the species distribution.

## **Chapter 2. Latitudinal compensation in trees: growth and foliar characteristics of narrowleaf cottonwoods from the full latitudinal range**

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### **2.1 Introduction**

With stationary positions and multiple-decade life-cycles, trees and shrubs must be well-adapted to their local environments. Climatic patterns of temperature and precipitation provide dominant physical factors that characterize these local environments, and subsequently define the distributional ranges of particular tree or shrub species (Woodward & Williams, 1987; Rood et al., 2003). For most global ecoregions these climatic factors vary seasonally, and in the temperate and boreal regions there are annual alternations of warm summers and cool winters. The deciduous growth habit, thus evolved with the growth and physiological activity being almost solely undertaken during the summer growth season.

At higher latitudes and elevations, progressively shorter growth seasons could impose challenges for the growth and reproduction of the deciduous trees since there would be a limited interval with sufficient temperatures. Temperature and photoperiod are the primary environmental cues that determine the growth season and there is a direct association between photoperiod and latitude. In most deciduous trees, including *Populus* species, growth cessation and associated bud set and subsequent leaf senescence are largely induced by decreasing photoperiod and temperature (Fracheboud et al., 2009; Rohde, Bastien & Boerjan, 2011). Winter chilling is the main factor required for

dormancy release (Heide, 1993) and subsequent warming temperatures enable bud flush to initiate the new growth season. To compensate for the shorter growth season at higher latitude or elevation, there could be increased physiological capacities to increase productivity to compensate for the shorter growth season (Gornall & Guy, 2007). In particular, foliar traits such as leaf size, chlorophyll content and foliar N could be elevated, thereby increases photosynthetic capacity. Also the foliar carbon isotope compositions ( $\delta^{13}\text{C}$ ) give life-time scale responses and interactions to their abiotic and biotic environments and reflects the photosynthetic capacities (Dawson et al., 2002).  $\delta^{13}\text{C}$  significantly associates with the leaf N content (Sparks & Ehleringer, 1997) and connects instantaneous measurements of water use efficiency (WUE) to more long-term, integrated measures of WUE (Farquhar, O'Leary & Berry, 1982; Condon, Farquhar & Richards, 1990; Rood et al., 2010).

As a compound measure, the leaf mass per area (LMA) is an important foliar morphological trait that relates to photosynthetic capacity through increased N content representing the Rubisco, the important enzyme in the process of photosynthesis (Meziane & Shipley, 2001). Leaf chlorophyll content is another important parameter reflecting the chloroplast density and photosynthetic pigments (Lambers, Chapin & Pons, 2008). Higher chlorophyll content has been observed in leaves of black cottonwoods and balsam poplars from northern latitudes and indicates the higher photosynthetic capacity (Gornall & Guy, 2007; Soolanayakanahally et al., 2009). Foliar N content is also positively associated with photosynthetic capacity (Reich et al., 1995; Meziane & Shipley, 2001).

To understand these ecophysiological patterns, studies have been carried out along latitudinal gradients or across elevations especially in the northern hemisphere, for both coniferous and deciduous trees. These patterns can be studied in common gardens, which involve comparisons of ecophysiological characteristics of trees collected from different environments and brought together and grown under the same environment at one site. For poplars (*Populus sp*), a pioneering common garden study was undertaken by Pauley & Perry (1954). They found associations between height growth cessation and the latitude of origin for *Populus trichocarpa* and *P. deltoides* genotypes and they concluded that photoperiod was the major factor controlling the seasonality of growth. Subsequent poplar studies have been carried out for *P. tremula* genotypes grown in a common garden in Sweden by Hall et al. (2007) and Luquez et al. (2008) and have shown strong latitudinal patterns for growth cessation. Similar results were also found by Friedman et al. (2011) for *P. deltoides*.

Gornall & Guy (2007) investigated *P. trichocarpa* in two range-wide common garden experiments in British Columbia, Canada. They found strong negative latitudinal gradients with height. They also found latitudinal variation for LMA as an integrating foliar morphological character, and ecophysiological characteristics including photosynthesis (A), stomatal conductance ( $g_s$ ), and water use efficiency (WUE). Elevation of origin was positively associated with A,  $g_s$ , stomatal density and ratio between the abaxial and adaxial stomatal densities (Q ratio) but found negatively associated with LMA and height. Soolanayakanahally et al. (2009) also found latitudinal and elevational gradients in growth and ecophysiological traits from Canada-wide collection of *P. balsamifera* populations representing 21 provenances. The traits included



LMA, chlorophyll content, foliar N,  $\delta^{13}\text{C}$  and ecophysiological characters, such as A,  $g_s$ , and WUE. Thus a few previous studies have shown higher photosynthetic capacity in plants from higher latitudes and in some cases higher elevations. Thus leads to the concept of 'latitudinal compensation' as a part of the CnGV, an evolutionary strategy (Levinton, 1983; Conover & Schultz, 1995), with increased physiological capacity of the trees adapted to the higher latitudes to compensate for the short growth seasons.

Most previous studies have involved genotypes from a wide range of distribution but none of them had representatives from the complete latitudinal range of a species. In this study we considered *P. angustifolia* (narrowleaf cottonwood) genotypes collected from across the full latitudinal range, from Alberta, Canada, south to Arizona, USA. The genotypes were grown in a common garden at the northern limit of the range.

My study species *P. angustifolia* is one of the principal native riparian trees in the semiarid regions of western North America and belongs to the family, Salicaceae. *P. angustifolia* is relatively flood-tolerant (Rood, Kalischuk & Mahoney, 1998; Amlin & Rood, 2001; Nielsen et al., 2010; Rood et al., 2010). It hybridizes with several other species of *Populus* that belonging to sections Tacamahaca and Aigeiros throughout its range (Eckenwalder, 1996; Floate, 2004). *P. angustifolia* is found at elevations higher than *P. deltoides* or *P. fremontii*, but lower than *P. trichocarpa* or *P. balsamifera* (Braatne et al., 1996). This intermediate distribution reflects relatively narrow environmental requirements and consequently, *P. angustifolia* occurs in disjunct woodlands (Cooke & Rood, 2007) along the 1800 km latitudinal gradient (Figure 2.1).

Therefore this study investigated prospective latitudinal and elevational patterns in growth, foliar morphological characters and foliar biochemical components of narrowleaf cottonwoods. The following hypotheses were investigated.

$H_0$  (null) – There is no association of growth, foliar morphological and biochemical characters with the latitude of origin, or with the elevation of origin. Thus, other factors would determine these physiological characteristics.

$H_1$  – There is a close association of growth, foliar morphological and biochemical characters with the latitude of origin. This was our primary hypothesis and would enable vigorous growth of the trees at the higher latitudes that have shorter growth season.

$H_2$  – There is a close association of growth, foliar morphological and biochemical characters with the elevation of origin. This was my secondary hypothesis and we expected reduced influence since temperature but not photoperiod would vary with elevation.

## **2.2 Materials and Methods**

### *Genotypes and Site Description*

Vegetative cuttings from nine sites were collected along the north-south distribution of narrowleaf cottonwood (Figure 2.1). Cuttings were propagated at the Northern Arizona University Research Greenhouse Facility in Flagstaff, AZ. In 2009, a common garden

was established at the University of Lethbridge, AB (49° N 113° W) along with two other common gardens in Enterprise, Utah (40° N 113° W) and Springerville, Arizona (33° N 109° W) in the United States (Evans et al., 2013). At the time of establishment, there were 363 unique genotypes from 9 populations in the common garden at Lethbridge, AB, but winter dieback and mortality reduced the number of genotypes considered in this study. A subset of 167 genotypes was selected to represent all 9 populations and Table 2.1 provides information on the number of genotypes with the geographical information for each population. At the time of establishment, all twigs were planted with 1.5 x 3m spacing in a completely randomized design. The trees that died were replanted, the garden was irrigated and weeds were mowed.

#### *Growth, foliar morphology and foliar biochemistry*

The plant height and basal diameter were used as indices for overall growth. The total height of the trees was measured using a meter stick and the basal diameter was taken using an electronic caliper (Mastercraft, Canada) with the accuracy of 0.02mm. Averages were taken when there were more than one stem at the base. Height and diameter were measured in mid-September and October, 2012 respectively. Other data were collected in second week of July and first week of August, 2012.

The 8<sup>th</sup> – 10<sup>th</sup> leaf from the base of the tallest stem was selected from all the genotypes as the sample. Selected leaves were then harvested and placed in a cooler for transport.

These were scanned with an Epson Perfection V700 scanner (Seiko Epson Corporation, Japan) at 300 dpi with 16-bit grey scale resolution, and then analyzed with the

WinFOLIA software system (Regent Instruments Inc., Canada). For each leaf, the blade length (cm), width (cm), and area (cm<sup>2</sup>) were measured. Leaves were then oven dried at 80°C for 48 h and weighed. The leaf mass per area (LMA, g·cm<sup>-2</sup>) was then calculated.

The chlorophyll content index (CCI) was measured on the selected fresh leaf using an Opti-Sciences CCM-200 meter (Hudson, NH, USA). It uses calibrated light emitting diodes (LEDs) and sensors to calculate the CCI as the ratio of percent transmission through the leaf sample at 655nm and 940 nm. A calibration curve was developed for the CCI and for this, the chlorophyll was extracted with dimethyl sulphoxide (DMSO) (Hiscox & Israelstam, 1979). Leaf disks were placed at 65°C in the dark for 45-60min, until they were completely colorless and the DMSO was green. Absorbance of the extracts was then measured at 649 nm and 665nm, relative to a DMSO blank, using a Spectronic 2000 / 33 35 05 UV-VIS Spectrophotometer (Bausch & Lomb, Rochester, NY, USA). Chlorophyll *a* (Chl<sub>a</sub>) and chlorophyll *b* (Chl<sub>b</sub>) contents were calculated as described by Wellburn (1994) to obtain the total chlorophyll content (Chl).

Leaves from 110 genotypes representing all nine populations were considered for foliar elemental analysis. Dried leaf samples were ground to a powder using a ball-mill grinder, and homogenized subsamples of ~4.5 mg were sent to the University of California at Davis Stable Isotope Facility for combustion and analysis. Carbon and nitrogen (N) contents were analyzed by an online continuous flow dual analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, Cheshire, England). The stable carbon isotope composition of leaf tissue ( $\delta^{13}\text{C}$ ) was calculated as:

$$\delta^{13}\text{C} (\text{‰}) = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}}-1) \times 1000$$

where  $\text{R}_{\text{sample}}$  and  $\text{R}_{\text{standard}}$  are the molar ratios of  $^{13}\text{C}/^{12}\text{C}$  in the sample and the standard (V-PDB, Vienna Pee Dee Belemnite) respectively (Lambers et al., 2008). Leaf N contents were calculated as per unit mass ( $\text{mg}\cdot\text{g}^{-1}$ ).

The Utah, Weber River population (UTWR,  $n=29$ ) was considered specifically to investigate the influence of elevation. This population had been sampled over the 950 m range in the elevation of native occurrence with limited variation in latitude.

Subsequently all nine populations were considered for the analyses of variation of growth and leaf characters versus latitude.

Climate data for genotype collection sites in United States were obtained from the National Oceanic and Atmospheric Administration (NOAA), USA (<http://www.noaa.gov/>) and the data for Oldman River were obtained from the Weather Canada ([http://weather.gc.ca/canada\\_e.html](http://weather.gc.ca/canada_e.html)). Temperature data were adjusted to the exact genotype collection site elevation from the weather station elevation using the previously constructed relationship (Temperature =  $0.0068 \times \text{elevation} + 18.908$ ). The river discharge data for US sites were obtained from the United States Geological Survey (USGS, <http://waterdata.usgs.gov/nwis/rt>) and the Oldman River, AB discharge data was obtained from the Water office, Environment Canada ([http://www.wateroffice.ec.gc.ca/index\\_e.html](http://www.wateroffice.ec.gc.ca/index_e.html)).

### *Statistics*

SPSS (PASW Statistics 18; IBM Corp., Somers, NY) was used for all statistical analyses. We investigated bivariate correlations for each of the growth and foliar morphological characters and foliar biochemical characters with the latitude of origin, the elevation of origin (UTWR population), and with all of the other characters. Pearson product correlations ( $r$ ) and coefficients of determination ( $r^2$ ) are reported along with corresponding probabilities. The terms ‘association, close association and strong association’ were used to describe if;  $r^2$  was 10% - 40%, 41% - 70% and  $< 71\%$  respectively. Some foliar measures were made in July and August to consider possible differences across these harvests, and analyses of covariance (ANCOVAs) were undertaken with latitude of origin as the covariate and month as a fixed factor. For the bivariate comparisons, the July, August and mean of the July and August values were investigated. For presentation, we include correlation tables as well as plots of particular measures versus the latitude of origin. For these plots, linear regressions were undertaken and solid lines are plotted for statistically significant associations ( $p < 0.05$ ), and dashed lines indicate statistical trends ( $p < 0.1$ ).

## **2.3 Results**

### *Climatic Conditions*

The day-length difference between the southernmost genotype collection site in Arizona, USA and the northern most collection site, Lethbridge, AB is 1.9 hours (Figure 2.2). Therefore the plants exposure to the light is considerably different. The average

temperature normal for the period 1971-2000 does not show a significant difference between genotype collection sites. The frost free days also do not show a significant difference between sites (Figure 2.2). River discharge from close to the genotype collection sites and precipitation normals for the period 1971-2000 are also shown in the Figure 2.3 because, the ground water availability is equally important as the rainfall to the survival and growth of riparian trees.

### *Elevation of Origin*

My analysis commenced with elevation, and I investigated this influence without the complication of latitude, by analyzing the 29 genotypes from the Weber River, Utah population (UTWR). This did not reveal any significant association between any growth or leaf characters versus the elevation of origin (Table 2.2). There were hints of positive associations of plant height and basal diameter with the elevation of origin, as evidenced by correlation probabilities that approached a statistical trend of  $P < 0.1$ . There was little evidence for variation in the foliar characteristics that was substantially associated with the elevation of origin.

### *Latitude of Origin*

Since there were no significant associations with elevation, subsequent analyses considered the latitude of origin. This removal of elevation was partly based on the close (81%) correspondence between the elevation and latitude of origin for the nine sampled populations (Figure 2.4). For most locations the elevational range was limited and

consequently there was an overall pattern in which the narrowleaf cottonwoods occurred only at lower elevations in the northerly locations of Alberta and only at higher elevations in the southerly locations of Arizona. There were broader elevational ranges for the intermediate latitudes, and especially for the Utah populations, but the analysis of the most variable UTWR population revealed little influence from elevation (Table 2.2).

For overall sapling growth, significant negative associations were shown for plant height and for basal diameter versus the latitude of origin (Figure 2.5). Both height and diameter showed a similar pattern with considerable variation for the southern populations and tighter distributions for the northern population (Figure 2.5). Of these two measures, we regarded height as somewhat confounded since many of the saplings had multiple stems and this was partly due to browsing of the apical buds by mule deer (*Odocoileus hemionus*). Conversely, the data distributions were quite similar for height and diameter, suggesting similar suitability for the overall growth analysis.

For the foliar morphological characteristics, when considered separately for the July and August samples, there was no association of leaf length, width, area or mass with the latitude of origin (Figure 2.6). The best-fit lines were however very close and with the averaging of the July and August values for each genotype, leaf mass showed a significant, but slight positive association ( $r^2=0.037$ ), and leaf width showed a statistical trend ( $r^2=0.018$ ; Table 2.3). The LMA provides a combined measure that can relate to leaf thickness and density. For leaves harvested in July and in August, the LMA was significantly associated with the latitude of origin (Figure 2.6). The regression plots were parallel and there was an apparent upward shift in August and this was confirmed by the



ANCOVA that showed a highly significant difference between months ( $F_{(1, 312)} = 64.3$ ;  $P = 0.000$ ). The average LMA across months was significantly associated with the latitude of origin (Table 2.3), thus indicating thicker or denser leaves in the northern genotypes.

The chlorophyll content index (CCI) was closely associated with the extracted total chlorophyll content (66% correspondence; Figure 2.7). This indicated that the Opti-Sciences CCM-200 meter did provide a reliable non-destructive estimate of Chl content of the leaves. The CCI by month is shown in Figure 2.8 and this provided the strongest correspondence with latitude of the measures assessed (Table 2.3). The pattern of increasing chlorophyll was significantly associated with the latitude of origin for July and for August, with an apparent increase in the regression slope (Figure 2.8). Consistent with this, there was a significant effect across the months with the ANCOVA with latitude as the covariate ( $F_{(1, 308)} = 11.1$ ,  $P = 0.000$ ). The magnitude of difference is reflected with the higher mean value for August (CCI = 21.5) versus July (CCI = 19.2). We considered the CCI distribution among genotypes for geographically isolated populations of AB, WY, UT and AZ (Figure 2.10). Chl content of AB genotypes were distributed over a wide range from low to high levels. Conversely, CCI for genotypes from AZ were clustered together at lower levels. Genotypes from WY and UT showed a Chl content distribution in between that of AB and AZ.

Foliar nitrogen (N) content was highly variable and increased with increasing latitude of origin (Figure 2.8). From the same leaf samples, there was also considerable variation in the  $\delta^{13}\text{C}$  values, especially for the mid-latitude Utah samples (Figure 2.8). There was no

significant pattern but a slight increase with latitude was observed and this approached a statistical trend ( $P=0.14$ ; Table 2.3).

### *Correspondences Between Growth and Foliar Characteristics*

The prior results relate to latitude and there is another aspect to the study – the correspondences across the genotypes. We can cluster the traits as growth, foliar morphology and foliar biochemistry. Growth traits, both height and diameter, were associated significantly with leaf morphological characters leaf blade length, width, size and mass but not with LMA (Table 2.3). Chl content and foliar N content increased with increased height or diameter. The associations were stronger for N content than Chl content.

Leaf length, width, area and mass associated significantly with both chlorophyll content and foliar N content. Further, these leaf characters were significantly associated with  $\delta^{13}\text{C}$ , except for leaf width, which approached a statistical trend (Table 2.3). N content increased with the average Chl content across months and with the  $\delta^{13}\text{C}$  (Figure 2.9). The association between N content and the Chl content was stronger than the association between N content and the  $\delta^{13}\text{C}$ . Further there was a less strong association between Chl content and the  $\delta^{13}\text{C}$ .

## 2.4 Discussion

My study involved a large number of narrowleaf cottonwood genotypes (167) that had been collected from the full latitudinal range and were grown in a common garden near the northern limit of that range. In comparing the growth, foliar morphology, and foliar biological characteristics, we observed substantial variation across the genotypes and this variation was partly associated with the geographic origin. This result rejects the null hypothesis ( $H_0$ ) that there would not be variation associated with the location from which the genotypes had been collected, and toward were presumably adapted.

We investigated the two primary physical features that characterized the geographic locations, elevation and latitude (south to north). The longitudinal extent is limited for this obligate riparian tree which occurs in a relatively limited corridor extending north to south, along rivers and creeks in valleys along the Rocky Mountains from Alberta, Canada, south to Arizona, USA. The west-to-east distribution is relatively narrow at the northern and southern range limits and wider in the middle, but we did not sample genotypes from the eastern zones of Colorado, USA (Figure 2.1). Thus, our study investigated two of the three spatial dimensions, and the narrowleaf cottonwood genotypes were primarily sampled to investigate latitude and elevation, the two spatial or geographic coordinates that primarily influence the duration of the growth season. This sampling was thus intended to investigate the prospect of latitudinal and elevational compensation.

## *Elevation*

Of the two geographic coordinates, we predicted less strong influence from elevation than latitude, since only latitude would influence both photoperiod and the temperature regime, and this provided the basis for our primary hypothesis,  $H_1$ . Elevation would only influence temperature while photoperiod would be almost constant along an elevational transect provided by a river corridor. We thus predicted less influence associated with elevation and this provided our secondary hypothesis,  $H_2$ . Opposing this hypothesis, our investigation of the 29 narrowleaf cottonwood genotypes from along the Weber River corridor in Utah, did not show any significant association between growth or leaf characteristics with the elevation of origin (Table 2.2). The hypothesis  $H_2$  was built based on the principle that the growth season for deciduous trees becomes shorter as elevation increases. We had thus predicted that, in accordance with the latitudinal compensation model, genotypes from higher elevations would compensate for the shorter growth season by displaying more rapid growth and physiology. Conversely, while the temperature and growth season would generally decrease with increasing elevation, there could be opposing environmental patterns that could promote growth. In particular, precipitation generally increases with elevation, while the evaporation demand decreases due to the decreasing temperature.

Consequently, especially within drier eco-regions, there would be benefits of increasing elevation that would provide a more favorable moisture regime and this could counter-balance the cooler temperature and shorter growth season. As well, the limited natural distribution of narrowleaf cottonwoods along the easterly flowing rivers (Halliday &

Brown, 1943), suggests that high temperatures of the prairie regions may and limit their growth and distribution and consequently, thermal stress from high temperatures could oppose the advantage from the longer growth season. These considerations of the moisture benefit at higher elevations and thermal stress at lower elevations could contribute to the lack of differentiation in the apparent physiological adaptations of the genotypes from different elevations at Utah.

In contrast to our finding of the lack of differentiation associated with the elevation of origin, prior studies have found correspondences for genotypes of the other two species of the Section Tacamacha poplars. Gornall & Guy (2007) have found significant associations between growth and physiological characters for *P. trichocarpa* with the elevation of origin in two range wide common garden experiments in British Columbia, Canada. Photosynthesis, stomatal conductance, stomatal density, stomatal ratio, and leaf N increased with the increased elevation of origin. Conversely, height and LMA decreased with the increasing elevation of origin. Soolanayakanahally et al. (2009) investigated *P. balsamifera* genotypes collected from populations across Canada, in controlled conditions at Vancouver, BC. They observed an increase in leaf N and stomatal density with increased elevation of origin. Both these studies have considered all genotypes regardless of the distribution and there could be an effect of the latitude on elevation gradients observed. Conversely our study considered genotypes distributed continuously along an elevation gradient (Figure 2.4) with minimum latitudinal variation.

### *Latitude*

We predicted stronger influence from latitude than elevation since both temperature and photoperiod varies with latitude, consequently influencing the growth season and providing the basis for our primary hypothesis ( $H_1$ ). Our investigations have shown significant associations of growth, foliar morphology and foliar biochemistry with the latitude of origin. Therefore we accept our primary hypothesis ( $H_1$ ), that there is a close association of growth, foliar morphological and biochemical characters with the latitude of origin.

We suggest that the early growth cessation of northern populations resulted in lower heights than the southern populations. Northern populations set their buds and ceased height growth earlier than the southern populations when they were grown in the northern common garden at Lethbridge, AB (unpublished data). Conversely, the active leaves of southern genotypes were available for growth for a greater part of the growth season allowing them to achieve a greater plant height. The slow growth rate of northern populations of *P. angustifolia* may have also contributed to lesser height achievement (Campbell, Mahoney & Rood, 1993; Kalischuk, Rood & Mahoney, 2001). Although, height growth cessation occurs with the bud set for northern genotypes, photosynthesis continues with a major shift in carbon allocation toward the root system (Pregitzer et al., 1990). The similar patterns of height and diameter distributions observed (Figure 2.5) in this study suggests the carbon allocation may occur towards the root system, not to the stem after the growth cessation.

The latitudinal clines in height growth as a response to the variation in photoperiod and temperature were well documented over the years for poplars such as *P. trichocarpa* (Pauley & Perry, 1954; Howe et al., 1996), *P. deltoides* (Pauley & Perry, 1954; Friedman et al., 2008; Friedman et al., 2011), *P. tremula* (Hall et al., 2007; Luquez et al., 2008) and several poplar hybrids (Kalcsits, Silim & Tanino, 2009; Rohde et al., 2011). The study carried out by Soolanayakanahally et al. (2013) for *P. balsamifera* genotypes in Vancouver, BC and Indian Head, SK common gardens has also shown less height achievement for northern populations.

Our observations indicate that there are limited latitudinal patterns associated with leaf morphology. But LMA increased with latitude of origin. This suggests a higher investment by leaves of northern genotypes in increased physiological capacity to compensate the shorter growth season. LMA depends on the leaf density and leaf blade thickness (Witkowski & Lamont, 1991) and northern genotypes may have denser and/or thicker leaves than southern genotypes. Therefore one or more layers of mesophyll may be present in these leaves. However, the components of the LMA (thickness and density) were not investigated in this study and thus there could be differences in these two components. Lower LMA gives the short term advantages of lower leaf construction per unit area for genotypes originated at southern latitudes, which may be associated with the lower rates of photosynthetic carbon gain. These results agree with the results obtained for *P. balsamifera* (Soolanayakanahally et al., 2009).

Findings of this study have shown the CCM-200 provides a good estimate of chlorophyll content in the narrowleaf cottonwoods (Figure 2.6). Similar close associations have been

observed by many other researchers who have used the CCM-200 meter to estimate the chlorophyll content in leaves (Richardson, Duigan & Berlyn, 2002; Soolanayakanahally et al., 2009). Using a meter provided an alternative method to measure the relative leaf chlorophyll level to overcome the disadvantages of chlorophyll extractions involving destruction of the leaf used for the measurement.

The higher chlorophyll content found in northern genotypes suggests higher photosynthetic capacity. Chl content has been shown to be higher in northern plants of several other poplar species, including *P. trichocarpa* (Gornall & Guy, 2007), *P. balsamifera* (Soolanayakanahally et al., 2009), as well as other woody plants (Slayback et al., 2003) across a range of latitudes. Chl content distribution among genotypes in a population also suggests variation in photosynthetic capacity within a population. Geographically isolated populations were clustered together and are genetically identified sub populations of the *P. angustifolia* (Evans et al., 2013). The wide range of distributions of Chl content among genotypes in AB suggests that there could be a wide variation in photosynthetic capacity for northern genotypes. Conversely, lower Chl content levels observed for genotypes from AZ suggest lower narrow variation in photosynthetic capacity for southern genotypes.

There was a lack of association between  $\delta^{13}\text{C}$  and latitude of origin. The slight positive trend observed suggests possible higher photosynthetic capacity, since  $\delta^{13}\text{C}$  is an integrative measure of plant photosynthetic activity over long periods (Körner, Farquhar & Wong, 1991). Agreeing with our results, Gornall & Guy (2007) did not find any significant association of  $\delta^{13}\text{C}$  with latitude for black cottonwoods. In contrast,



Soolanayakanahally et al. (2009) have shown significant associations for balsam poplar leaf  $\delta^{13}\text{C}$  and wood  $\delta^{13}\text{C}$  with the latitude of origin.

#### *Associations between growth and foliar characteristics*

Observed associations between growth, foliar morphology and foliar biochemical characteristics provide more evidence for higher productivity of northern genotypes.

Although the association between LMA and foliar N was not significant it was negative, similar to reports of other woody plants (Dijkstra, 1990; Cornelissen et al., 1997). In contrast, a positive and significant association between LMA and leaf N was observed in balsam poplars (Soolanayakanahally et al., 2009). LMA is influenced by species, genotype and environmental variables, as well as the interactions between these variables. In addition LMA depends on trade-offs between leaf functions such as photosynthesis, protection against herbivores, competition, storage and structural requirements and, therefore, it is difficult to find the same result with every species (Dijkstra, 1990).

The photosynthetic capacity of leaves is related to the foliar N content primarily because of the enzymes of the Calvin cycle and thylakoids, especially ribulose-1, 5-bisphosphate carboxylase oxygenase (Rubisco), the key enzyme in  $\text{C}_3$  photosynthesis (Evans, 1989).

Therefore, the northern genotypes with higher N content could have higher photosynthetic capacity. Previous studies have shown that there is a positive and strong association between Chl content, foliar N content and photosynthesis for the other poplar species, including *P. trichocarpa* (Gornall & Guy, 2007), *P. balsamifera*

(Soolanayakanahally et al., 2009) and *P. angustifolia* (Letts et al., 2008; Lojewski et al., 2009; Rood et al., 2010).

The findings from this study indicate ‘latitudinal compensation’ of northern latitude genotypes, with higher productivity. We propose that this higher productivity compensates for the shorter growing season. The most northern genotypes have formed leaves with higher LMA, higher Chl content and higher N content, possibly increasing the photosynthetic capacity by increasing light absorbing pigments and photosynthetic enzymes.

Table 2.1 Source locations (north to south) of 167 narrowleaf cottonwood genotypes that were studied in a common garden at Lethbridge, Alberta.

Population number, name and (abbreviation)	Mean Latitude of origin (°N)	Mean Longitude of origin (°W)	Elevation range of origin (m)	Genotypes
1. Oldman River, AB (ABOR)	49.68	-113.021	822-1136	39
2. Snake River, WY (WYSR)	43.3	-110.808	1721-1875	11
3. Weber River, UT (UTWR)	40.99	-111.487	1392-2350	26
4. Oak Creek, UT (UTOC)	39.35	-112.262	1786-1873	14
5. Corn Creek, UT (UTCC)	38.76	-112.374	1639-1701	17
6. Beaver River, UT (UTBR)	38.26	-112.505	1429-2326	15
7. Indian Creek, UT (UTIC)	37.98	-109.517	1877-1900	17
8. Pumphouse Wash, AZ (AZPW)	35.06	-111.725	1655-1986	5
9. Blue River, AZ (AZBR)	33.74	-109.064	1940-2065	23

Table 2.2 Pearson product correlations ( $r$ ) between growth and leaf characteristics, and the elevation (m) of origin for 29 narrowleaf cottonwood genotypes from Weber River, Utah (UTWR) grown at Lethbridge, AB. There were no statistically significant correlations, but height and diameter approached a statistical trend (i.e.  $P < 0.1$ ).

Variables	$r$
Plant Height (cm)	0.326 ( $P = 0.104$ )
Diameter (mm)	0.288 ( $P = 0.129$ )
Leaf Length (cm)	0.157
Leaf Width (cm)	-0.058
Leaf Area (cm <sup>2</sup> )	0.061
Leaf Mass (g)	0.098
Leaf mass per area (LMA, g·m <sup>-2</sup> )	0.144
Chlorophyll content (CCI)	-0.265
N content (mg·g <sup>-1</sup> )	0.233
Carbon isotope composition ( $\delta^{13}\text{C}$ , ‰)	0.004

Table 2.3 The matrix with Pearson product correlations ( $r$ ) relating the latitude of origin and growth and leaf characteristics of narrowleaf cottonwood genotypes grown in the common garden. Foliar morphology and chlorophyll content index are means of July and August measurements.  $t$  (trend) =  $P < 0.1$ , \* =  $P < 0.05$ ; and \*\* =  $P < 0.01$ .

	Growth		Foliar morphology					Foliar biochemistry		
	Height	Basal diameter	Leaf length	Leaf width	Leaf area	Leaf mass	LMA	Chlorophyll content index	N content	$\delta^{13}\text{C}$ (‰)
	(n=167)		(n=165)					n=166	n=110	
Latitude of origin (°N)	-0.231**	-0.201**	0.067	0.133 $t$	0.100	0.192*	0.294**	0.434**	0.219*	0.140
Height (cm)		0.864**	0.456**	0.464**	0.524**	0.542**	0.063	0.172**	0.364**	0.074
Basal diameter (mm)			0.385**	0.462**	0.498**	0.516**	0.061	0.243**	0.392**	0.058
Leaf length (cm)				0.704**	0.879**	0.817**	-0.138 $t$	0.297**	0.378**	0.222**
Leaf width (cm)					0.927**	0.866**	-0.143 $t$	0.332**	0.426**	0.179 $t$
Leaf area (cm <sup>2</sup> )						0.928**	-0.169*	0.369**	0.473**	0.196*
Leaf mass (g)							0.175*	0.405**	0.449**	0.217*
LMA (g·cm <sup>-2</sup> )								0.124	-0.099	0.067
Chlorophyll content index									0.595**	0.311**
N content (mg·g <sup>-1</sup> )										0.480**

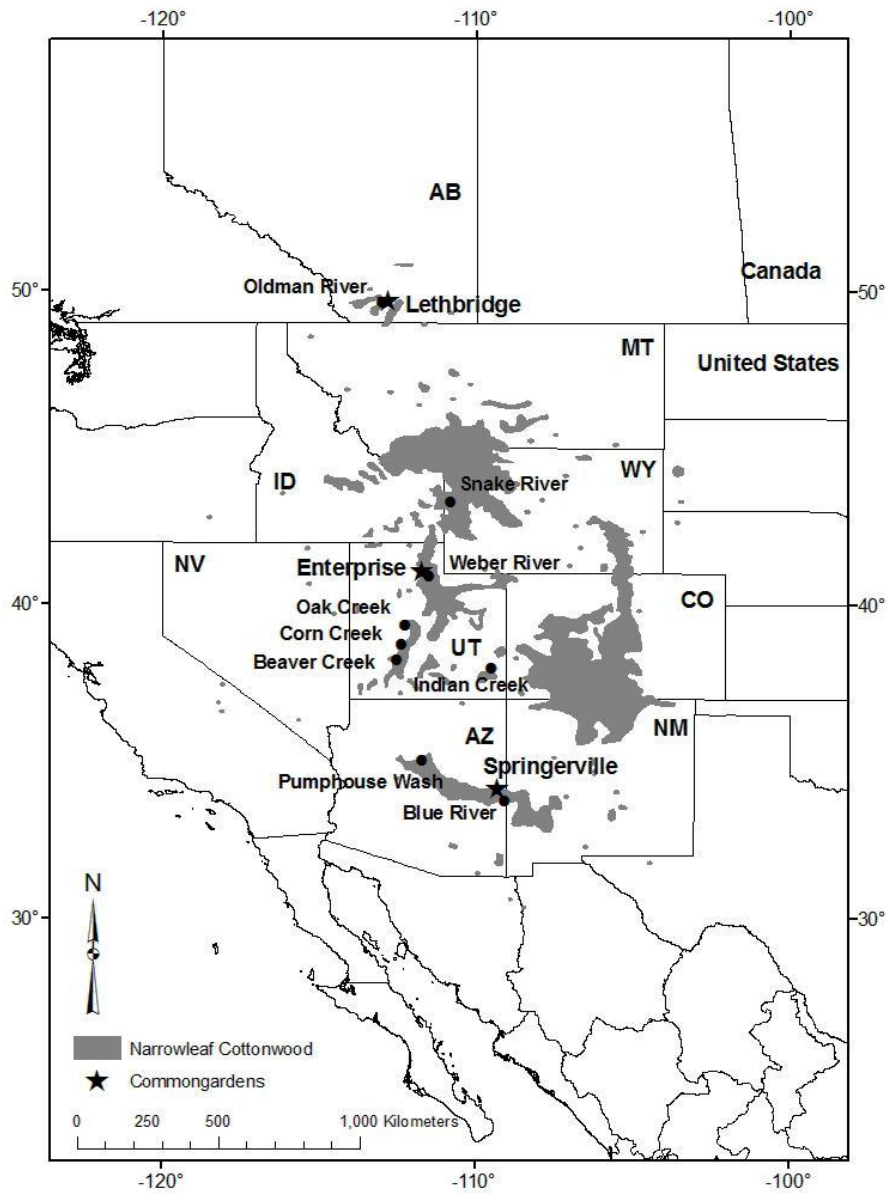


Figure 2.1 The native ranges of narrowleaf cottonwoods (shaded), and the locations of the nine population collection sites. Common gardens were established at three locations indicated by stars and this study considered the Lethbridge common garden.

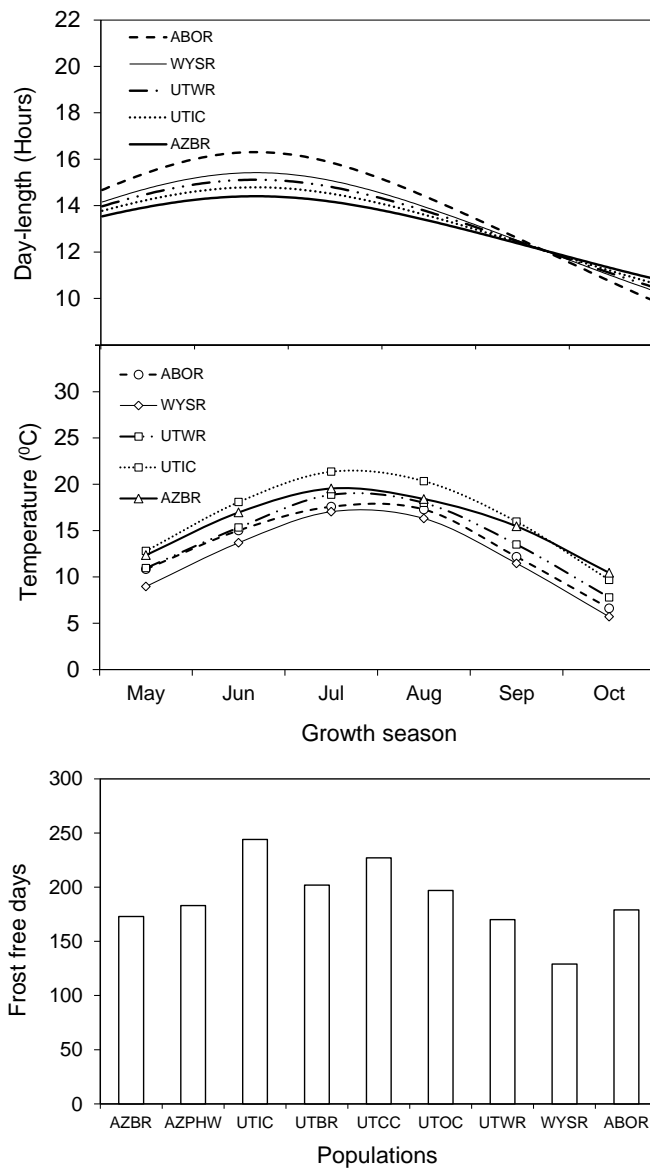


Figure 2. 2 Day-length, monthly normal (1971-2000 means) adjusted average temperature and frost free days of genotype collection sites. ABOR-Oldman River, Alberta; WYSR-Snake River, Wyoming; UTWR-Weber River, Utah; UTBR-Beaver River, Utah; UTIC-Indian Creek, Utah; AZBR-Blue River, Arizona.

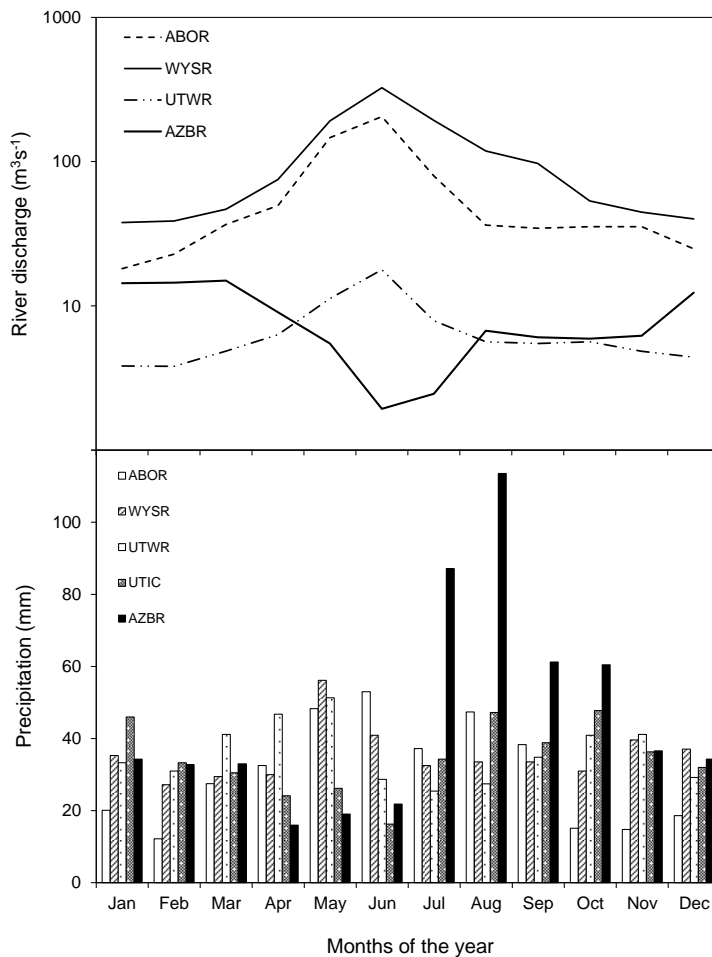


Figure 2.3 (a) River discharge data for Oldman River, near Lethbridge, AB (ABOR); Snake River near Kackson, WY (WYSR); Weber River near Coalville, UT (UTWR) and Gila River near Virden, NM (AZBR) representing genotype collection sites. (b) Monthly normal precipitation (1971-2000) for genotype collection sites. ABOR-Oldman River, Alberta; WYSR-Snake River, Wyoming; UTWR-Weber River, Utah; UTBR-Beaver River, Utah; UTIC-Indian Creek, Utah; AZBR-Blue River, Arizona.



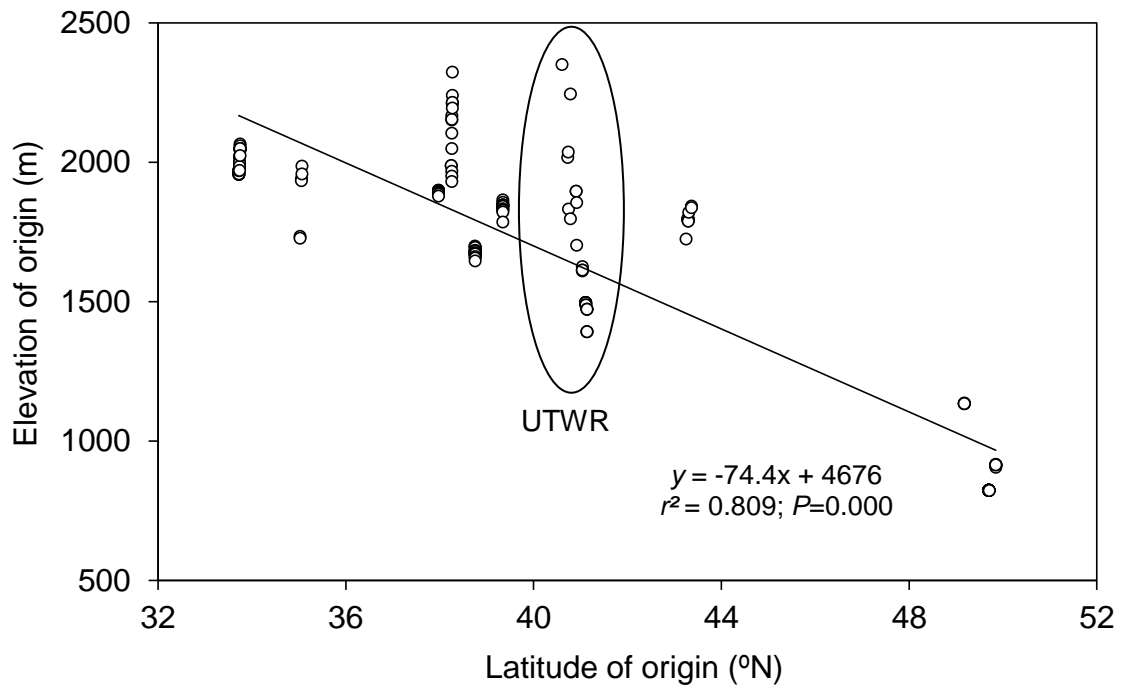


Figure 2.4 Elevation of origin versus latitude of origin for narrowleaf cottonwood genotypes (n= 167) grown in the common garden at Lethbridge, AB. For this and subsequent figures, linear regression lines are plotted for statistically significant associations ( $p < 0.05$ ). The Weber River, UT (UTWR) population is encircled in an oval and was considered for elevational associations.

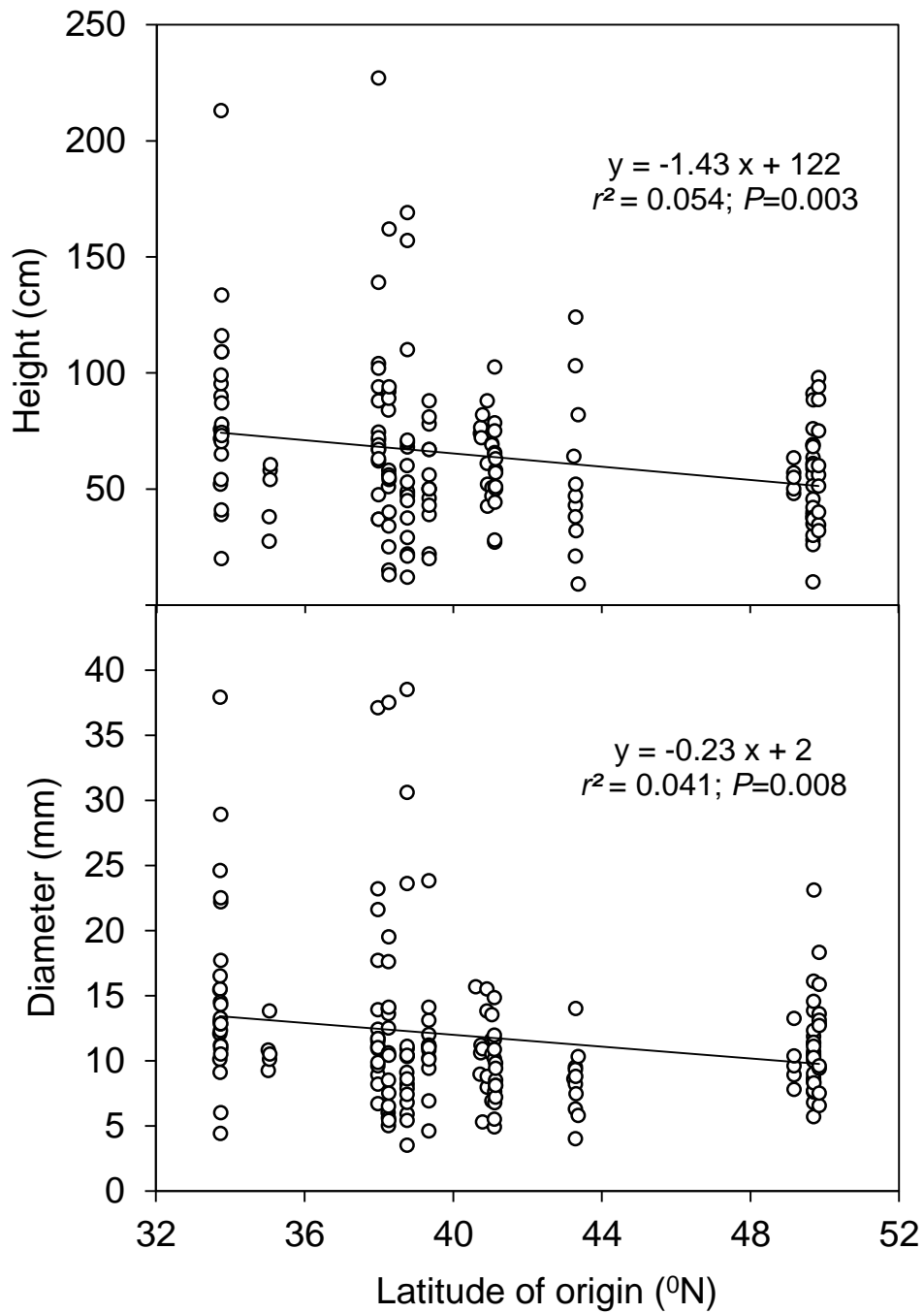


Figure 2.5 Height (top) or basal diameter versus their Latitude of origin for 167 narrowleaf cottonwood genotypes grown at Lethbridge, AB

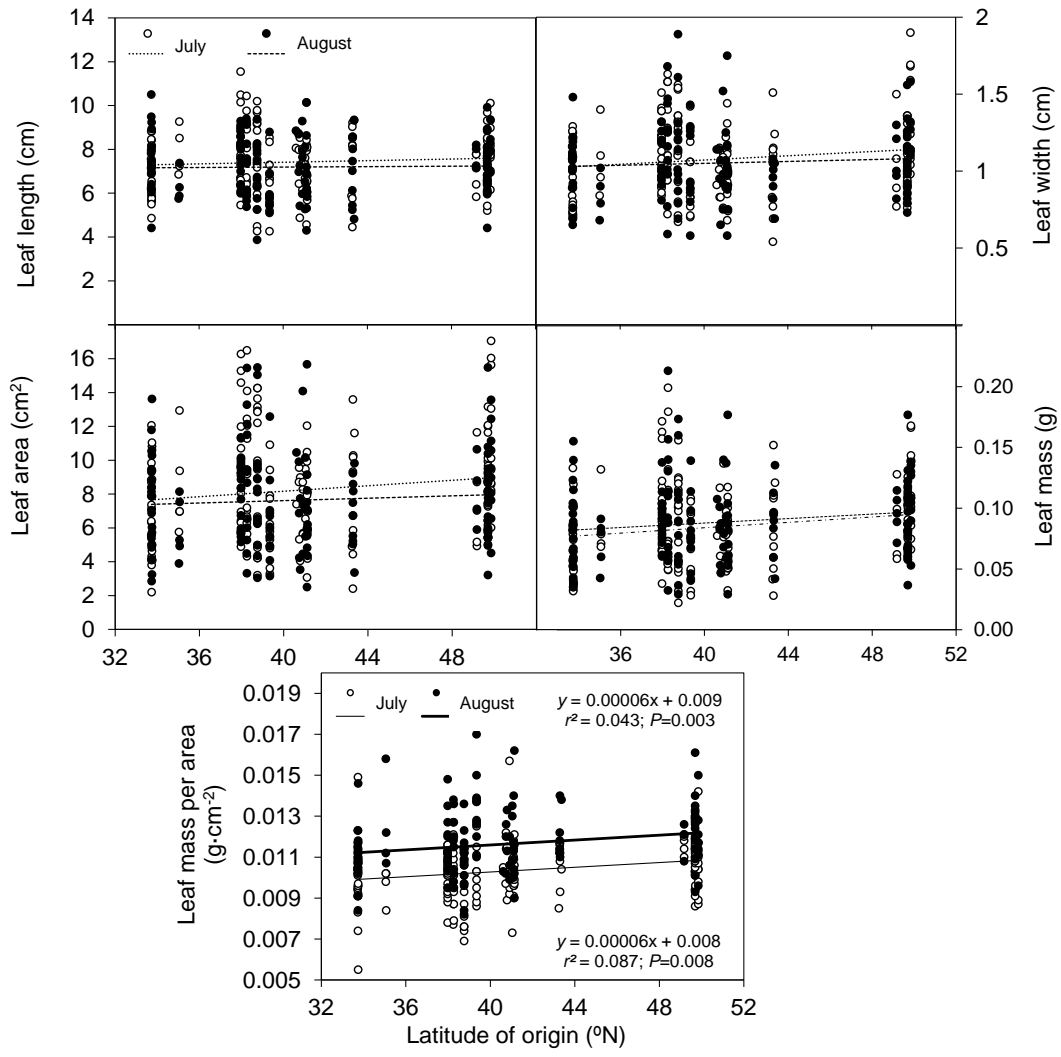


Figure 2.6 Leaf length, width, area, mass and leaf mass to area ratio (LMA) versus the latitude of origin for 167 narrowleaf cottonwood genotypes grown at Lethbridge AB. For the upper four figures, lines of best-fit are dashed since they are not statistically significant for individual months but some averages across months are (Table 2.3).

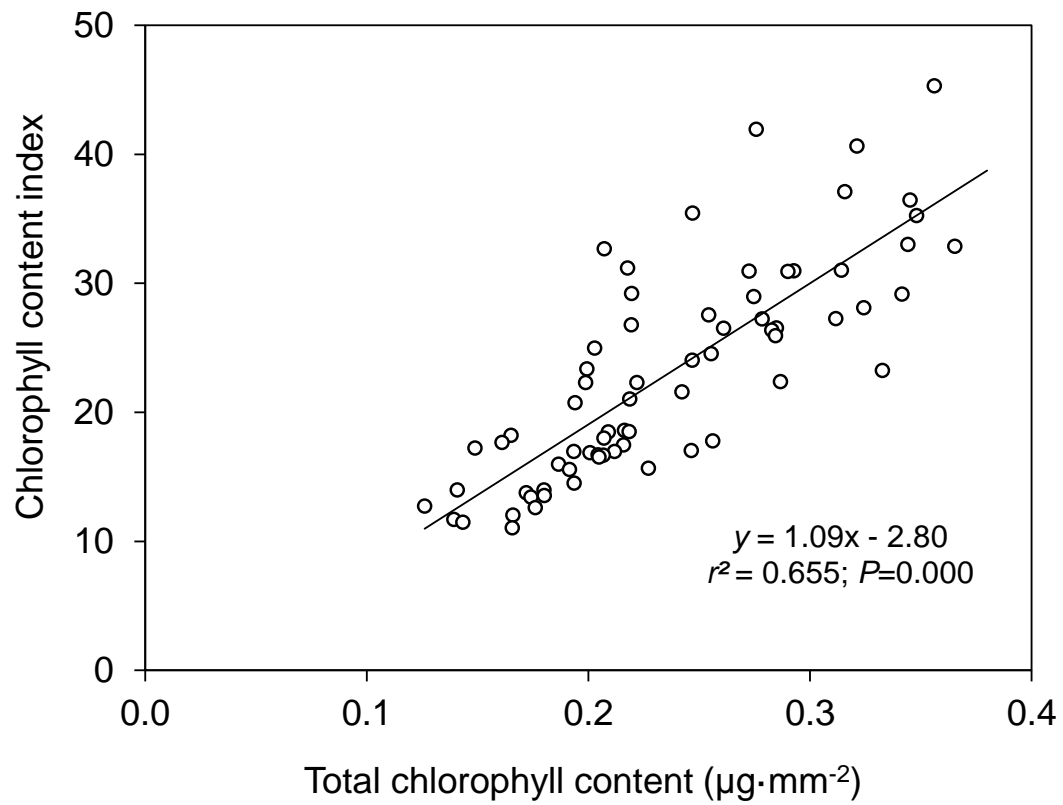


Figure 2.7 Linear regression of chlorophyll content index (CCI) versus total extractable chlorophyll content ( $\mu\text{g}\cdot\text{mm}^{-2}$ ) of narrowleaf cottonwood leaves ( $n=60$ ) grown at Lethbridge, AB.

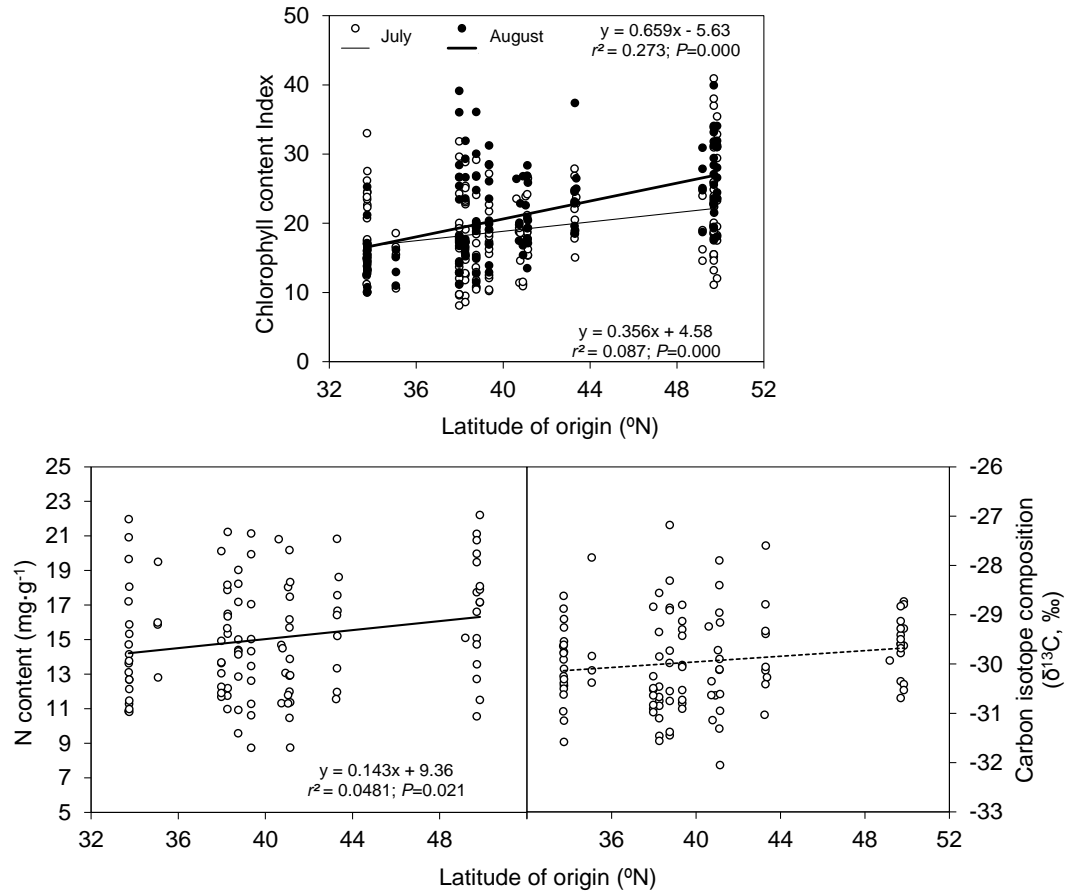


Figure 2.8 Chlorophyll content index (n=167), N content ( $\text{mg}\cdot\text{g}^{-1}$ ), and carbon isotope composition ( $\delta^{13}\text{C}$ , ‰; n = 110) of leaves versus the latitude of origin for narrowleaf cottonwood genotypes grown at Lethbridge, AB. Solid lines indicate significant ( $P < 0.05$ ) associations, while the dashed line for  $\delta^{13}\text{C}$  was not statistically significant.

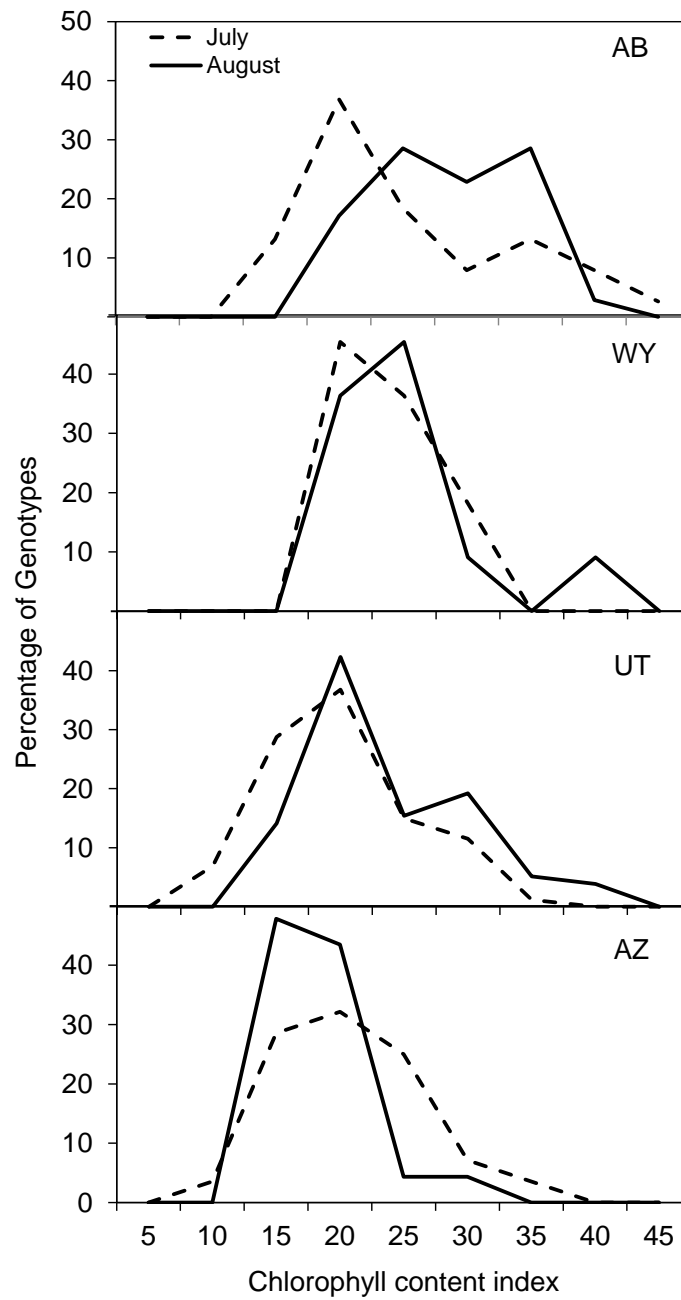


Figure 2.9 Chlorophyll content index distributions within clustered populations of narrowleaf cottonwood genotypes from Alberta (AB; n=40), Wyoming (WY; n=11), Utah (UT; n=88) and Arizona (AZ; n=28).

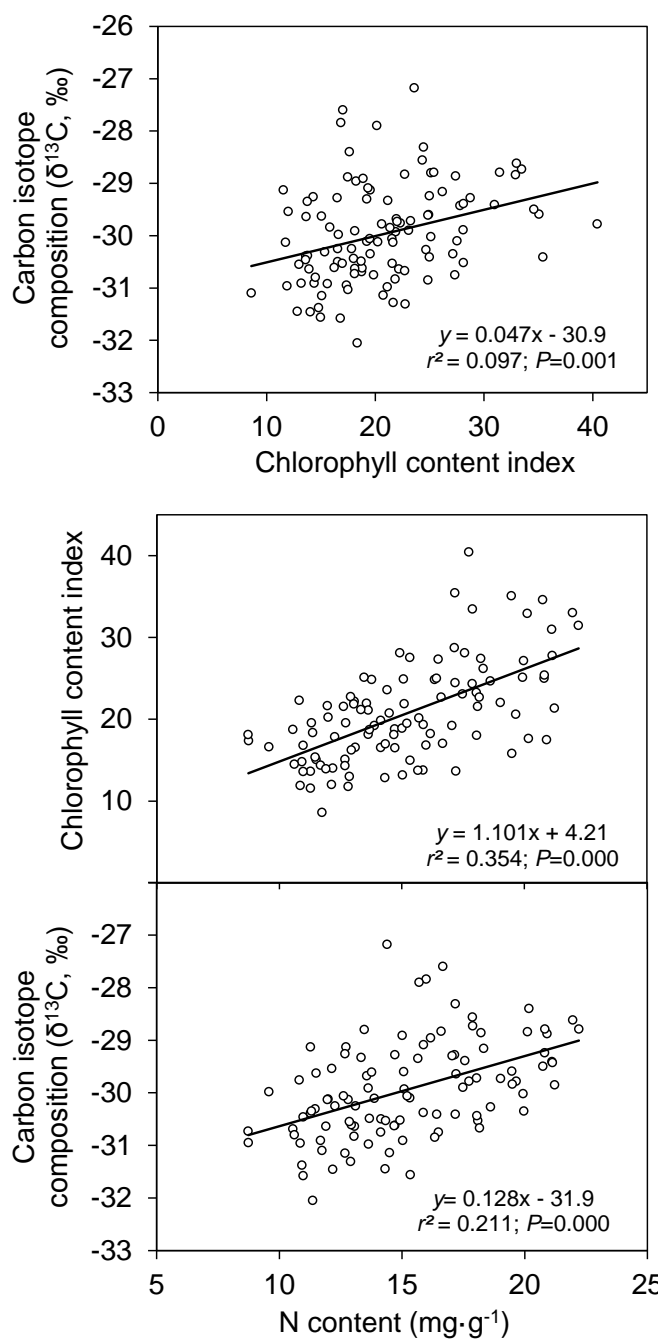


Figure 2.10 Correspondence between chlorophyll content index, foliar N content and carbon isotope composition for leaves from 110 narrowleaf cottonwood genotypes grown at Lethbridge, AB.

## **Chapter 3. Higher photosynthetic capacity from higher latitude: Foliar morphology and gas exchange of narrowleaf cottonwoods from southern, middle and northern populations.**

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### **3.1 Introduction**

Temperate and boreal ecoregions of northern latitudes are exposed to annual alterations of warm summers, that enable plant growth, and cool winters when plant growth ceases.

Temperature and photoperiod are the primary environmental cues that determine the growth season and there are direct associations between latitude and photoperiod, which provides a consistent seasonal cue (Menzel & Fabian, 1999; Friedman et al., 2008). In deciduous trees, including poplars or *Populus* species, growth cessation and associated bud set, and subsequent leaf senescence and abscission are induced by decreasing photoperiod and declining temperature (Fracheboud et al., 2009; Rohde et al., 2011).

Conversely, winter chilling is the main factor required for dormancy release (Heide, 1993) and only after this, warming temperatures enable bud flush to initiate the new growth season.

Deciduous plants at northern latitudes are likely to face a metabolic problem with the foliar phenology which is driven by climatic cues. Plant growth and development within the shorter growth season must be sufficient to allow winter survival, spring regrowth and progressive continuation of the trees life cycle. Consequently, deciduous trees and shrubs from northern latitudes could display increased physiological and foliar morphological capacities. They may follow the concept of “latitudinal compensation”, which we



(Chapter 2) and others have observed in poplars (Gornall & Guy, 2007; Soolanayakanahally et al., 2009). Thus, to compensate for the shorter growth season, there could be increased photosynthetic rates following increased plant investments in characteristics including thicker or denser leaves with increased leaf mass per area (LMA), increased stomatal densities to facilitate gas exchange, increased chlorophyll content to capture extra light, and increased foliar nitrogen, reflecting higher levels of ribulose 1, 5 bisphosphate carboxylase oxygenase and other photosynthetic enzymes.

To test this prospect, ecophysiological studies of various deciduous woody plants have been carried out along latitudinal gradients or across elevations. Investigations of plant functions with in-situ measurements of the trees and shrubs along a gradient has provided one approach (Sparks & Ehleringer, 1997). However, different environments and/or the different genotypes could underlie the observed differences, confounding interpretation. An alternate approach has been applied, when genotypes originating from different latitudes and/or elevations are collected, propagated and grown in a common garden. The genotypes thus experience very similar environmental conditions (Conover & Schultz, 1995; Rood et al., 2003).

Applying the common garden approach, Gornall & Guy (2007) investigated black cottonwoods (*Populus trichocarpa*) in two range-wide common garden experiments in British Columbia, Canada. They found strong latitudinal gradients in LMA and other foliar and physiological characteristics, including net photosynthesis ( $A$ ), stomatal conductance ( $g_s$ ) and water use efficiency (WUE). They proposed that the northern provenances have developed inherently higher photosynthetic capacity that compensates

for the shorter growth seasons, consistent with the concept of latitudinal compensation (Levinton, 1983). In a subsequent study, Soolanayakanahally et al. (2009) also found latitudinal gradients in growth and physiological traits from a Canada-wide collection of balsam poplar (*Populus balsamifera*), with populations representing 21 provenances. Their traits included LMA, chlorophyll content, foliar N,  $\delta^{13}\text{C}$  and gas exchange characteristics, including A,  $g_s$ , and WUE. Thus, these previous studies have shown higher photosynthetic capacities in poplar genotypes originating from northern latitudes. These studies did involve populations from a wide distribution range, but not the full latitudinal range of a species.

Our study involved narrowleaf cottonwood, *Populus angustifolia*, one of the principal native riparian trees in the semiarid regions of western North America. It belongs to *Populus*, section Tacamahaca, along with *P. balsamifera* and *P. trichocarpa*, and is physiologically closer to the section Aigeiros poplars than the other section Tacamahaca species (Rood et al., 2003). *P. angustifolia* resembles willows, with *Salix* being the other genus in the family Salicaceae, and is probably a more flood-tolerant poplar (Rood et al., 1998; Amlin & Rood, 2001; Nielsen et al., 2010; Rood et al., 2010). It occurs in riparian habitats along the Rocky Mountains, from southern Arizona northward to southern Alberta, Canada (Figure 3.1). In zones of natural overlap, it hybridizes with several other species of *Populus* of section Tacamahaca and of section Aigeiros (Floate, 2004). Throughout its range *P. angustifolia* is found at higher elevations than the section Aigeiros *P. deltoides* or *P. fremontii*, but lower than *P. trichocarpa* or *P. balsamifera* (Braatne et al., 1996). This intermediate distribution reflects relatively narrow environmental requirements; consequently, *P. angustifolia* occurs in disjunct woodlands

(Cooke & Rood, 2007) along its latitudinal range (Figure 3.1). These populations may have adapted to their different environments and may have differentiated from each other. Surveys of genetic polymorphisms in our narrowleaf cottonwoods from across the latitudinal range have indicated that geographical barriers result in regionally differentiated genetic subpopulations and suggest strong latitudinal clines (Evans et al., 2013).

Applying a common garden study approach, in a larger comparison, we established 363 narrowleaf cottonwood genotypes from nine populations that represented the full 1800 km latitudinal range. These were grown in three common gardens, near the southern and northern limits of the range of this tree, as well as in the middle of the latitudinal range (Evans et al., 2013). In the present study, we utilized the northern common garden and this study approach extended a strategy undertaken by other researchers. We investigated the differences in foliar and gas exchange characteristics of three narrowleaf cottonwood subpopulations representing the northern, middle and southern zones. This study considered the following, opposing hypotheses:-

$H_0$  – The null hypothesis was that there would be no significant difference in foliar or gas exchange characteristics for cottonwood populations that originated from the southern, middle or northern zones of the latitudinal range of distribution.

$H_1$  – Conversely, the primary hypothesis was that there would be a differentiation in the foliar and gas exchange characteristics, across the three populations. We anticipated increases in the traits that would particularly contribute to higher photosynthetic capacity

in genotypes that originated from northern latitudes. In testing this hypothesis, the study considered the patterns of foliar traits in narrowleaf cottonwoods relative to patterns that had been observed for other deciduous trees, and especially for other poplars.

### **3.2 Materials and Methods**

#### *Genotypes and Site Description*

This study involved three of the nine populations of narrowleaf cottonwoods that were described in Chapter 2 and in Evans et al. (2013). The three study populations represented the southern, middle and northern zones of the narrowleaf cottonwood distribution (Figure 3.1). The southern population was from the Blue River, AZ; another population was from near the middle of the latitudinal range, along the Weber River, UT; and the northern most population was from the Oldman River, AB. Cuttings of genotypes were collected from each of the three populations. Followed by rooting in a greenhouse in Flagstaff, AZ, then six saplings from each genotype were transplanted in the spring of 2009 into a common garden at the University of Lethbridge in Alberta (49° N 113° W). Table 3.1 provides the geographical details of the genotype collection sites. Some saplings were replaced with others from the same genotype in the spring of 2011 following mortality due to winter kill and/or browsing of the apical buds by mule deer (*Odocoileus hemionus*). These new saplings, used to replace the dead saplings were propagated and maintained in a nearby, sheltered garden plot. The common garden was irrigated and weeds were controlled by mowing. Initial foliar and gas exchange measurements were made during the 2011 growth season and established protocols and investigated diurnal patterns in gas exchange, and possible acropetal patterns among the

column of leaves up the stems. Subsequently, measurements for this study were made during the 2012 growth season.

#### *Selecting the sample leaf*

In each of the three key populations, twenty genotypes were represented by six individual trees. A single from one stem one individual tree of each genotype was selected for most measurements. The variation among the leaves along the stem was investigated prior to measurements (Figure 3.4). Most measurements were taken from A leaf between the 8<sup>th</sup> and 10<sup>th</sup> leaves from the base of the tallest stem was selected as the sample. The same leaf was used for gas exchange measurements followed by leaf morphological measurements, and chlorophyll traits. Samples for stomatal traits were taken, followed by drying the same leaves to obtain leaf mass. Finally, the leaves were ground up for leaf tissue analysis. Foliar characteristics, chlorophyll traits and gas exchange measurements were taken once in 6<sup>th</sup> - 13<sup>th</sup> July 2012 and again in 9<sup>th</sup> – 15<sup>th</sup> August 2012.

#### *Foliar characteristics*

Selected leaves were harvested and placed in a cooler for transport. These were scanned with an Epson Perfection V700 scanner (Seiko Epson Corporation, Japan) at 300 dpi with 16-bit grey scale resolution, and then analyzed with the WinFOLIA software system (Regent Instruments Inc., Canada). For each leaf, length (cm), width (cm), and area (cm<sup>2</sup>) were measured. Leaves were oven dried at 80°C for 48 h to a constant mass was obtained. The leaves were weighed and leaf mass per area (LMA, g·cm<sup>-2</sup>) was calculated, with adjustments for leaf disks removals done for chlorophyll extractions and stomatal traits.

Dried leaf disks were obtained from the same leaf samples, for stomatal traits. The adaxial and abaxial (bottom) surfaces were observed under a scanning electron microscope (SEM, Hitachi High-Technologies Europe, Germany) at 300 × magnification in a field of 3 mm. SEM images were taken at three random locations of both surfaces. Images were analyzed using Image J 1.20 (National Institute of Health, NIH; <http://rsb.info.nih.gov/ij>). The numbers of stomata were counted, and stomatal densities and the ratios of adaxial to abaxial (bottom) stomatal densities (Q ratio) were calculated.

The chlorophyll content was estimated as the chlorophyll content index (CCI), with an Opti-Sciences CCM-200 meter (Hudson, NH, USA), while leaves were still attached to the tree. The CCM-200 uses calibrated light emitting diodes (LEDs) and sensors to calculate the CCI, which is defined as the ratio of percent transmission at 655nm to 940 nm through a leaf sample. Leaf pigment content was also extracted with dimethyl sulphoxide (DMSO) (Hiscox & Israelstam, 1979). A fresh leaf disk from each sample leaf was used and it was incubated at 65°C in the dark for 45-60min, until it was completely colorless and the DMSO was green. Absorbance of the extracts was then measured at 649 nm, 665 nm and 480 nm relative to a DMSO blank, using a Spectronic 2000 / 33 35 05 UV-VIS Spectrophotometer (Bausch & Lomb, Rochester, NY, USA). Chlorophyll *a* ( $\text{Chl}_a$ ,  $\mu\text{g}\cdot\text{mL}^{-1}$ ) chlorophyll *b* ( $\text{Chl}_b$ ,  $\mu\text{g}\cdot\text{mL}^{-1}$ ) and total carotenoid ( $\text{Chl}_{x+c}$ ,  $\mu\text{g}\cdot\text{mL}^{-1}$ ) contents were then calculated as follows:-

$$\text{Chl}_a = 12.19A_{665} - 3.45A_{649}$$

$$\text{Chl}_b = 21.99A_{649} - 5.32A_{665}$$

$$\text{Chl}_{x+c} = (1000A_{480} - 2.14\text{Chl}_a - 70.16\text{Chl}_b) / 220$$

where,  $A_{665}$ ,  $A_{649}$  and  $A_{480}$  are absorbance measured at 665 nm, 649 nm and 480 nm respectively (Wellburn, 1994).

Completely dried leaves were ground to a powder using a ball-mill grinder, and homogenized subsamples of ~4.5 mg were sent to the University of California at Davis Stable Isotope Facility for combustion and analysis. Carbon and nitrogen (N) contents were analyzed by an online continuous flow analyzer coupled to an isotope ratio mass spectrometer (Europa Scientific Integra, Cheshire, England). The stable carbon isotope composition of leaf tissue ( $\delta^{13}\text{C}$ ) was calculated as:

$$\delta^{13}\text{C} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the molar ratios of  $^{13}\text{C}/^{12}\text{C}$  in the sample and the standard (V-PDB, Vienna Pee Dee Belemnite), respectively (Lambers et al., 2008). Leaf N concentrations were on a per unit mass basis (Leaf N,  $\text{mg}\cdot\text{g}^{-1}$ ).

#### *Gas exchange measurements*

A portable LI-6400XT gas exchange system (LI-COR Instruments, Lincoln, NE, USA) was used to take measurements twice during the active growing season. Measurements were made on the selected single leaf between 0930 h and 1330 h and the  $\text{CO}_2$  concentration of the inlet air was set to  $400 \mu\text{L}\cdot\text{L}^{-1}$ . Other conditions were set as follows; leaf temperature  $26\pm 1$  °C, vapor pressure deficit  $2.0\pm 0.1$  kPa, and a  $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  photosynthetic photon flux density (PPFD) was provided to establish a steady state condition for about 10-15 min prior to each measurement. This PPFD was supplied by a mixed red/blue light emitting diode (LED) unit mounted on top of the cuvette. Light-

saturated photosynthetic assimilation ( $A_{max}$ ,  $\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ,  $\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ), and transpiration ( $E$ ,  $\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) were the main measurements recorded.

Sub-samples of 5 genotypes from each of the 3 populations was selected to construct light response curves. Rates of net photosynthesis were measured on the same leaf selected for other measurements, using the portable LI6400XT gas exchange system, at a range of light intensities starting at high light, 1800, 1500, 1200, 1000, 800, 600, 400, 300, 200, 100, 50, and 0  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ . At each light level change, PPFD was kept constant until equilibration. The conditions were set as inlet  $\text{CO}_2$  at  $390 \mu\text{L} \cdot \text{L}^{-1}$ , the leaf temperature at  $26 \pm 1$  °C and the vapor pressure deficit at  $2.0 \pm 0.1$  kPa. The flow rate was kept constant at  $500 \mu\text{mol s}^{-1}$ .

Light response curves of net photosynthesis were constructed for each individual tree (Figure 3.8) and the maximum  $\text{CO}_2$  assimilation point ( $A_{max}$ ), the dark respiration rate ( $Rd$ ), curvature of the light response curve ( $C$ ) and the apparent quantum yield ( $QY$ ) were obtained for each individual genotype by fitting the collected data to the nonrectangular hyperbola model (Lambers et al., 2008) using the SYSTAT version 10.2 (SYSTAT Software Inc., USA). The data were fitted to the model by assuming following start, minimum and maximum values for parameters,

$$A_{max} - 25, 1, 60$$

$$Rd - 2, 0.1, 6$$

$$C - 0.8, 0.1, 0.99$$

$$QY - 0.05, 0.001, 0.07$$



The instantaneous water use efficiency ( $WUE_e$ ), was calculated as:

$$WUE_e = \frac{A_{\max}}{E}$$

where,  $A_{\max}$  is the light saturated photosynthesis and E is the transpiration rate. The intrinsic water use efficiency correlates with the carbon isotopic composition (Farquhar & Richards, 1984; Farquhar, Ehleringer & Hubick, 1989; Ponton et al., 2006).

Climate data for genotype collection sites in United States were obtained from the National Oceanic and Atmospheric Administration (NOAA), USA (<http://www.noaa.gov/>) and the data for Oldman River were obtained from the Weather Canada ([http://weather.gc.ca/canada\\_e.html](http://weather.gc.ca/canada_e.html)). Temperature data were adjusted to the exact genotype collection site elevation from the weather station elevation using the previously constructed relationship (Temperature = 0.0068×elevation + 18.908). The river discharge data for US sites were obtained from the United States Geological Survey (USGS, <http://waterdata.usgs.gov/nwis/rt>) and the Oldman River, AB discharge data was obtained from the Water office, Environment Canada ([http://www.wateroffice.ec.gc.ca/index\\_e.html](http://www.wateroffice.ec.gc.ca/index_e.html)).

### *Statistics*

SPSS (PASW Statistics 18; IBM Corp., Somers, NY) was used for all statistical analyses. Some foliar and gas exchange measures were made both in July and August. T-tests were

undertaken to compare the differences across the two months for each population separately. Subsequently, univariate analyses of variances (ANOVA) were carried out to compare the foliar morphological, biochemical and gas exchange traits among the three populations. For presentation, I include graphs with both July and August values for each study population. The significant differences ( $P < 0.05$ ) and trends ( $P < 0.1$ ) are indicated by different letters and the significant differences between months are also indicated. Subsequently, monthly values for traits were averaged and the bivariate Pearson product correlations ( $r$ ) were calculated to show associations among paired foliar morphological, biochemical and gas exchange traits. The terms ‘association, close association and strong association’ were used to describe if;  $r^2$  was 10% - 40%, 41% - 70% and  $< 71\%$  respectively.

### **3.3 Results**

#### *Climatic Conditions*

The day-length difference between the northern collection site and the southern genotype collection site is 1.9 hours (Figure 3.2). The adjusted average temperature normals for the period 1971-2000 does not show a significant difference between genotype collection sites. The frost free days for northern, middle and southern did not show a significant difference between sites (Figure 3.2). River discharge from close to the genotype collection sites and precipitation normals for the period 1971-2000 are also shown in the Figure 2.3 because, the ground water availability is equally important as the rainfall to the survival and growth of riparian trees.

### *Foliar morphological characters*

Leaf morphological characteristics, length, width, area and LMA were significantly higher for the northern population (northern P; Oldman River, AB) than the southern population (southern P; Blue River, AZ) for all leaf morphological traits (Figure 3.5). Leaf width and areas for the middle population (middle P; Weber River, UT) were significantly different from northern P while leaf length and LMA were not (Table 3.3). No significant differences in leaf morphological traits were prominent between months, except for LMA, which showed significant differences between July and August. LMA was significantly different across months for all three populations and it gives an indication of seasonal difference in leaf thickness or density or both for narrowleaf cottonwoods grown in a common garden at Lethbridge, AB.

### *Stomatal traits*

In all three populations, the majority of the stomata were observed on the abaxial (bottom) surfaces (Figure 3.6). Abaxial stomatal density (*ABSD*) of 200 per  $\text{mm}^2$  (mean<sub>all populations</sub>) was observed and it was significantly different from the adaxial stomatal density (*ADSD*) of 68 per  $\text{mm}^2$  (mean<sub>all populations</sub>). The highest *ADSD* was for the northern population and lowest for the southern P and they were significantly different from each other. Conversely, abaxial (bottom) stomatal density (*ABSD*) was highest for southern P and the difference was highly significant for both middle P and northern P. Total stomatal density was higher in leaves from the southern P (297 per  $\text{mm}^2$ ) population with greater contribution from *ABSD*. The middle P (237 per  $\text{mm}^2$ ) showed the lowest *ABSD* and was significantly different from the southern P. Total stomatal density of genotypes from the

northern P showed no difference from southern P or middle P. The Q-ratio ( $ADSD/ABSD$ ) was highest for middle P and lowest for southern P. Middle and northern populations were significantly different from southern P (Figure 3.6).

#### *Foliar biochemical characteristics*

Of the photosynthesis pigments, chlorophyll *a* ( $Chl_a$ ) was higher (mean *all populations*  $\sim 18 \mu\text{g}\cdot\text{cm}^2$ ) than chlorophyll *b* ( $Chl_b$ , mean *all populations*  $\sim 5.2 \mu\text{g}\cdot\text{cm}^2$ ) and carotenoids (mean *all populations*  $\sim 5.0 \mu\text{g}\cdot\text{cm}^2$ ) for all three populations of narrowleaf cottonwoods (Figure 3.7).

$Chl_a$  content was highest for the northern P and it was significantly different from the southern and middle populations but the middle P and the southern P did not show a significant difference between them (Table 3.3). There were no significant differences among populations for  $Chl_b$  content. Northern P showed the highest carotenoids content and it was significantly different from middle and southern populations (Figure 3.7).

The total chlorophyll content, measured as the chlorophyll content index was highest for northern P and lowest for southern P (Figure 3.8). Northern P was significantly different from middle and southern populations in both July and August. Further, the total chl contents were significantly different across months for middle and southern populations (Table 3.3). N content was higher for northern P of the three populations and it was significantly different from middle P. Southern P did not show a difference with middle or northern populations (Figure 3.8). Foliar  $\delta^{13}\text{C}$  showed no difference among the three populations (Figure 3.11).

### *Gas exchange characteristics*

Leaves were exposed to the higher PAR ( $1500 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) to obtain the light saturated photosynthesis. This will enable the leaf readily activate RUBISCO to complete the photosynthesis without any limitations. Variation of net photosynthesis among leaves of the tallest stem was investigated prior to the measurement of the gas exchange characteristics. Figure 3.4 (a) shows the limited variability among candidate leaves of individual leaves from each population. Figure 3.4 (b) shows the variation of maximum photosynthesis with the time of the day. Therefore the measurements were taken between 9.30 – 13.30 hours, when the variation of photosynthesis was limited.

Similar to the higher chlorophyll and N contents, northern P showed the highest light-saturated photosynthetic assimilation ( $A_{\text{max}}$ , mean *(July, August)*  $\sim 22 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and it was significantly different from southern P in August but showed only a trend in July. There was no significant difference or trend with the middle P (Figure 3.6). No significant differences were observed between months for any population (Table 3.3).

Individual light response curves constructed by fitting the collected data to the model are shown in the Figure 3.9. Only five individual trees from each of the population were randomly selected to construct light response curves. Individual trees from the northern populations show higher maximum net photosynthesis when compared with the southern genotypes.

Derived components from light response curves, the maximum photosynthesis, dark respiration and the apparent quantum yield showed no significant difference among populations. The maximum photosynthesis obtained from extrapolating the light response curve showed a trend in difference for northern and southern populations. No significant differences between populations were observed for transpiration (E, Figure 3.11). E measured in July was higher than in August and was significantly different for each population between the months. Instantaneous water use efficiencies ( $WUE_e$ ) calculated as  $A_{max}/E$  also showed no significant differences between populations (Figure 3.11), but was significantly higher in all population for August.

#### *Correspondence between foliar and gas exchange characteristics*

There were close associations between some foliar morphological, biochemical and gas exchange characteristics across genotypes (Table 3.2). Average values observed for July and August for all three populations were considered to show these correspondences. Leaf size showed the most associations with foliar biochemical and gas exchange characteristics (Table 3.2). There negative association between leaf size and the SD was 20%. Leaf size also showed 30% association with chl, 26% with N content and 12% association with  $\delta^{13}C$ . A stronger 35% association was observed for leaf size and  $A_{max}$  (Figure 3.12). Leaf size also associated with E and  $WUE_e$ . Although LMA showed limited association with foliar biochemical characters, it significantly associated with  $A_{max}$  and E. SD showed negative trends in associations with Chl, N content and  $A_{max}$  (Table 3.2).

Chl showed a 34% association with N content and a trend in association with  $\delta^{13}\text{C}$ . However the association between N content and  $\delta^{13}\text{C}$  was stronger (30%). Chl and N contents showed close associations with  $A_{\text{max}}$  (Figure 3.12).

$A_{\text{max}}$  highly associates with leaf size than with LMA, which provides a combined measure that relates leaf thickness and density (Figure 3.12). Further,  $A_{\text{max}}$  closely associated with Chl and N content. Northern genotypes were more towards the upper end of the regression lines in Figure 3.9, while southern genotypes were clustered towards to the lower end. N content showed a 34% association with the Chl content and 26% association with the leaf size (Table 3.2). Chl content also showed a close association (30%) with the leaf size but no such association was observed with LMA. Total SD showed a negative association with the leaf size and showed negative trends with  $A_{\text{max}}$ , Chl and N.

The  $\delta^{13}\text{C}$  showed a 30% association with N content and showed a trend in association with chlorophyll content. Although Chl content showed a trend of association with  $\text{WUE}_e$ , N content was closely associated with  $\text{WUE}_e$  (12%). We observed that  $\text{WUE}_e$  was influenced mostly by E than by  $A_{\text{max}}$  (Table 3.2).

### **3.4 Discussion**

This study involved three populations of narrowleaf cottonwoods from near the northern, middle and the southern limits of the distribution, grown in a common garden near the northern limit of the tree species. In this study I have compared traits of foliar

morphology and gas exchange for the three populations. I found significant differences among three populations for majority of the traits. This result rejects the null hypothesis ( $H_0$ ) that there would not be a significant difference between foliar and gas exchange characteristics for populations originating from the latitudinal gradient.

We observed increased leaf blade length, width, and size for northern P (Figure 3.5), and their leaves may have higher capacities to capture light. There is also a visually observable leaf size difference between northern originated and southern originated trees grown in the northern common garden at Lethbridge, AB. Increased LMA for northern P also indicates higher mass per unit area and they could therefore have thicker leaves due in part to more layers of palisade or longer palisade cells (Hanson, 1917; Castro-Díez, Puyravaud & Cornelissen, 2000) or the leaves may be denser (Witkowski & Lamont, 1991). This could increase the number of chloroplasts and the amount of photosynthetic enzymes and thus enhance the photosynthetic capacity per unit leaf area. It has also been shown that the LMA is usually high in cases where carbon availability is high as a result of the faster rate of photosynthesis at high supplies of light and CO<sub>2</sub> (Poorter et al., 2009).

Increased leaf size in the northern P may increase the light capturing capacity and subsequent activation of the Rubisco (Ribulose-1, 5-biphosphate carboxylase oxygenase) activase enzyme to initiate the photosynthesis (Lambers et al., 2008). Therefore higher LMA, leaf length, and width may indicate possible higher rates of photosynthesis in plants from northern P (Figure 3.12). Agreeing with the results we have observed, Soolanayakanahally et al. (2009) have also observed higher LMA for *P. balsamifera* genotypes originated at higher latitudes in a recent study carried out under controlled



conditions in Vancouver, BC. In contrast Gornall & Guy (2007) have not observed significant differences in LMA for northern populations of *P. trichocarpa* grown in a common garden at Surrey, BC.

The CO<sub>2</sub> essential for photosynthesis is supplied through the stomata in the leaves. However, stomatal control is a compromise between maximization of photosynthesis and minimization of transpiration. Therefore, higher stomatal density on the abaxial (bottom) surfaces of all three populations partly could be an adaptation that avoids water loss by transpiration (Lambers et al., 2008). Further, stomata respond to changes in the environment such as light, temperature, ambient humidity and soil water availability. Since guard cells of the stomata possess blue light photoreceptors, adaxial stomata could be kept open when exposed to the bright sunlight (Assmann & Shimazaki, 1999).

Riparian cottonwoods (*Populus spp.*) are very susceptible to xylem cavitation (Tyree et al., 1994; Pockman, Sperry & O'Leary, 1995). Therefore, these narrowleaf cottonwoods may control water loss partly by controlling the stomatal density and partly by controlling the stomatal aperture size, which we did not investigate in this study. Pearce et al. (2006) have reported higher total stomatal densities for *P. angustifolia* than *P. balsamifera* and *P. trichocarpa* with several more hybrids grown in a common garden at Lethbridge, AB. However the association between stomatal density and the latitude of origin was limited in this study. In another study by Soolanayakanahally et al. (2009) have also observed higher total stomatal densities in southern genotypes of *P. balsamifera* grown in controlled conditions in Vancouver, BC. However, Gornall & Guy (2007) have observed

a strong positive association between stomatal density and latitude for *P. trichocarpa* grown in a common garden in Surrey, BC.

Higher chlorophyll contents observed for the northern P support the higher photosynthetic capacity for northern originated populations with more chlorophyll present in their leaves. Even though xanthophyll and carotene contents were not analyzed separately in this study, significantly higher total carotenoids in the northern P indicate greater ability to protect their leaves from excessive light absorption, possibly supporting the maintenance of higher photosynthetic capacity.

With increased LMA, Chl and foliar N contents, the northern P showed higher mean  $A_{\max}$  (across months) of  $22 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ , which was higher than the  $A_{\max}$  observed in a previous study by Letts et al. (2008). They have observed a maximum  $A_{\max}$  of  $16 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in early summer for naturally grown mature narrowleaf cottonwoods in Lethbridge, AB. The slight difference in  $A_{\max}$  may be partly due to the different environmental and/age conditions. Plants originated from the northern P in the Lethbridge common garden displayed an early growth cessation and bud set, exposing them to a shorter growth season than other two populations (unpublished data). Therefore I suggest the increased  $A_{\max}$  observed for northern P in this study to compensate for the shorter growth season. Agreeing with the results we have observed, Soolanayakanahally et al. (2009) have also observed higher  $A_{\max}$  in *P. balsamifera* populations originated from high latitudes in controlled conditions and *P. balsamifera* originated from northern latitudes have also shown shorter growth season in a study involved two common gardens in Vancouver, BC and Indian Head, SK (Soolanayakanahally et al., 2013). Furthermore

Gornall & Guy (2007) have shown higher  $A_{\max}$  for populations of *P. trichocarpa* from higher latitudes grown in a common garden in Surrey, BC. Although we did not observe a significant seasonal difference in  $A_{\max}$  between July and August, McKown et al. (2013) observed a 25% alteration in mean value of most leaf traits including photosynthesis, particularly following height growth cessation and bud set. Their study was carried out in a common garden of *P. trichocarpa* at the University of British Columbia, BC.

Limited associations of the properties observed by the light response curves with latitude maybe partly due the insufficient sample size. There was a trend in difference among the three populations for maximum photosynthesis. Lower curvatures were expected for northern P but there was no significant difference among the three populations.

The mean instantaneous ( $WUE_e$ ) across months observed in this study were slightly higher for northern P than  $WUE_e$  observed by Letts et al. (2008) for leaves from a mature natural population of narrowleaf cottonwoods in the Oldman River valley at Lethbridge AB. The ratio of assimilation to transpiration is expected to change seasonally (Cowan, 1988) thus, intrinsic values better show the differences in water use efficiency than does instantaneous, because they integrate over time and are less susceptible to short term environmental variations. However, variations in WUE occur when there is a shift in balance between photosynthesis and transpiration. WUE will increase if the transpiration decreases or by an increase in intrinsic photosynthetic capacity.

In the present study much of the variation in WUE between genotypes could be related to variation in transpiration, because the association between  $WUE_e$  and E is strong (Table 3.2). Therefore, the lack of significant difference in E may have caused the lack of difference in  $WUE_e$  between populations. A high WUE is an adaptation to water-limited conditions because more carbon is assimilated per unit water utilized over time, allowing greater growth (Dudley, 1996). However, during the time of gas exchange measurements, the garden was well irrigated and a plant need not have high WUE when water is not limiting therefore the limited variation could be partly due to this factor. Vapour pressure deficit was kept almost constant ( $2.0 \pm 0.1$  kPa) during the gas exchange measurements. Therefore, limited variations in transpiration and water use efficiency may be also due this.

Strong, positive correlations between  $\delta^{13}\text{C}$  and WUE, at both individual leaf and whole plant levels, have been shown in numerous species including poplars (Leffler & Evans, 2001; Bonhomme et al., 2008; Letts et al., 2008; Rasheed et al., 2013). The associations observed between WUE and  $\delta^{13}\text{C}$  (Table 3.2) in this study also explains the limited variation of  $\delta^{13}\text{C}$  (Figure 3.11). Poplars are among the fastest growing trees and their high productivity is associated with large water requirements, but it has been shown that WUE and the photosynthetic characters collectively enables the plant to grow vigorously throughout the growing season despite the changes in the water availability for *P. angustifolia* (Letts et al., 2008). However, Rood et al. (2013) have carried out a study with long term river flow data for *P. angustifolia* that demonstrated  $\Delta^{13}\text{C}$  decreased with decreased river levels, and therefore the cottonwoods are vulnerable to drought.  $A_{\text{max}}$  was

also closely correlated with the conductance ( $g_s$ ) within months July ( $r^2=0.489$ ;  $P=0.00$ ) and August ( $r^2=0.792$ ;  $P=0.00$ ).

*Associations between foliar and physiological traits.*

I found close associations between the traits leaf area, LMA, SD, chl, foliar N and  $A_{max}$  (Table 3.2). N content on an area basis has shown by many researchers to present associations with photosynthesis (Evans, 1989; Poorter et al., 2009). We have showed the close association between  $A_{max}$  and the N content in Figure 3.9 and showed the increased photosynthesis for the northern P. This could be partly due to the increased investments in the photosynthetic apparatus. There is a 10 fold variation in the  $A_{max}$  with N content when considered as per unit area. A large portion of foliar N is found in Rubisco, which is an essential enzyme for photosynthesis. However, it has been suggested that high investments of nitrogen in the photosynthetic apparatus have positive returns only if abundant site resources allow plants to maximize assimilation and growth (Chapin, Schulze & Mooney, 1990; Reich, Walters & Ellsworth, 1992). Plants may respond to N and water availability by controlling LMA and it associates negatively with N availability (Chapin et al., 1987). Therefore plants' capacity to control the leaf area and mass could play a significant role in photosynthetic acclimation (Evans & Poorter, 2001; Benomar, DesRochers & Larocque, 2011).

My observations of strong positive associations of  $A_{max}$  with N content have been observed by other researchers for several other poplar species. For example, Gornall &

Guy (2007) have observed similar results for *P. trichocarpa* grown in Surrey, BC and Soolanayakanahally et al. (2009) have also reported a strong positive association between assimilation rate ( $A$ ), and leaf N. In addition, they found a negative correlation between mesophyll conductance and frost free days (FFD) resulting in increased photosynthesis as well as an enhancement of WUE. Their results suggest that increased photosynthesis and N, could be due to the shorter growing season for *P. balsamifera* grown under controlled conditions. Agreeing with their results, narrowleaf cottonwoods from northern P grown at Lethbridge common garden also displayed a shorter growth season when compared with other two populations (unpublished data). Ripullone et al. (2003) have also observed similar strong associations between  $A_{\max}$  and foliar N for hybrid poplars. Positive correlations between foliar N and rates of photosynthesis have been observed across many other species (Evans, 1989; Reich et al., 1994).

My results include that light saturated photosynthetic rate increases with increased latitude for narrowleaf cottonwood trees. This may be a generalized pattern among deciduous trees of the northwest of North America. We propose that northern populations may have inherently higher  $A_{\max}$  with higher LMA, higher chlorophyll content, and higher leaf nitrogen, and this may help to compensate for the shorter growth seasons, consistent with the latitudinal compensation.

Table 3.1 Source locations of three sampled narrowleaf cottonwood populations that were studied in a common garden at Lethbridge, AB.

Population	Latitude of origin (°N)	Longitude of origin (°W)	Elevation range (m)
1. Southern (Blue River, AZ)	33.74	-109.064	1940- 2065
2. Middle (Weber River, UT)	40.99	-111.487	1392-2350
3. Northern (Oldman River, AB)	49.68	-113.021	822-1136

Table 3.2 The matrix with Pearson product correlations (r) among trait means for genotypes from three populations (3×20; n=60, averaged measurements for July and August) of narrowleaf cottonwoods grown in a common garden at Lethbridge, AB.  
*t* (trend) =  $P < 0.1$ , \* =  $P < 0.05$ ; and \*\* =  $P < 0.01$

	Foliar morphology		Foliar biochemistry			Gas exchange characteristics		
	LMA	SD	Chl	N	$\delta^{13}\text{C}$	$A_{\text{max}}$	$g_s$	Instantaneous Water Use Efficiency ( $\text{WUE}_e$ )
<i>Foliar morphology</i>								
Leaf area ( $\text{cm}^2$ )	- 0.082	- 0.447**	0.549**	0.507**	0.350**	0.589**	0.376**	0.054
Leaf mass per area (LMA, $\text{g}\cdot\text{m}^{-2}$ )		0.148	0.138	- 0.109	- 0.038	0.276*	0.297*	- 0.069
Stomatal density (SD, # $\text{cm}^{-2}$ )			- 0.261 <i>t</i>	- 0.229 <i>t</i>	- 0.089	- 0.248 <i>t</i>	0.024	- 0.180
<i>Foliar biochemistry</i>								
Chlorophyll content index				0.582**	0.242 <i>t</i>	0.481**	0.191	0.250 <i>t</i>
N content ( $\text{mg}\cdot\text{g}^{-1}$ )					0.547**	0.443**	0.081	0.351**
Carbon isotope composition ( $\delta^{13}\text{C}$ , ‰)						0.148	- 0.015	0.282*
<i>Gas exchange characteristics</i>								
Light saturated photosynthesis ( $A_{\text{max}}$ , $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )							0.802**	- 0.261*
Stomatal conductance ( $g_s$ , $\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )								- 0.637**



Table 3.3 Probability values of the 2-way analysis of variation for foliar morphological, foliar biochemical and physiological characteristics of southern, middle and northern populations (factor 1) made in July and August (factor 2).

P < 0.1 – Italics, P < 0.05 – Bold, P < 0.01 – Italic bold.

Traits	P - value		
	Population	Month	Population × Month
<i>Foliar morphological characteristics</i>			
Leaf length	0.178	0.349	0.026
Leaf width	<b>0.010</b>	0.392	0.517
Leaf area	<b>0.010</b>	0.862	0.403
Leaf mass	<i><b>0.001</b></i>	<b>0.015</b>	0.701
Leaf mass per area (LMA)	<i>0.053</i>	<b>0.000</b>	0.556
<i>Foliar biochemical characteristics</i>			
Chlorophyll content (CCI)	<i><b>0.000</b></i>	0.964	<b>0.034</b>
<i>Physiological characteristics</i>			
Light saturated photosynthesis	<i><b>0.007</b></i>	0.138	0.591
Transpiration	<b>0.020</b>	<b>0.000</b>	0.580
Instantaneous water use efficiency	0.733	<b>0.000</b>	0.241

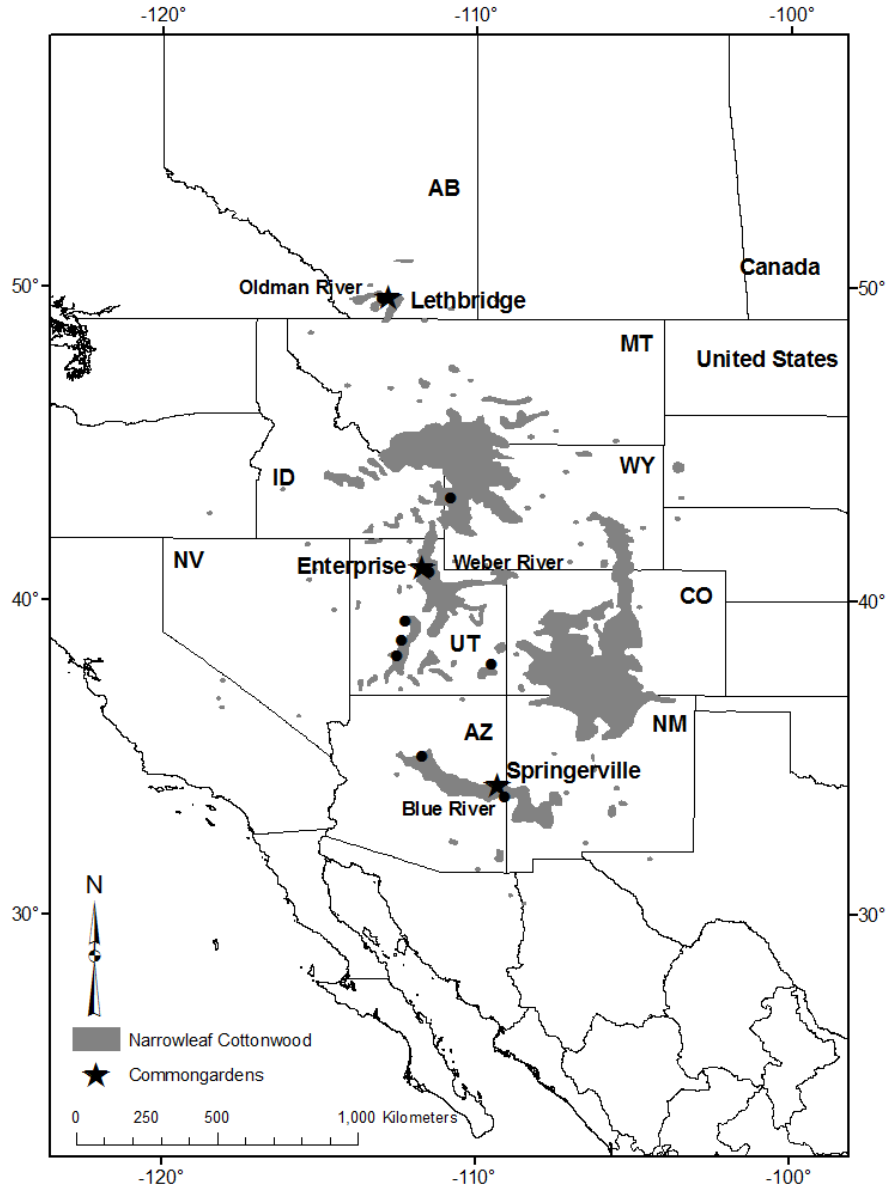


Figure 3.1 Native distributional range of narrowleaf cottonwoods and the locations of the population collection sites northern (Oldman River, AB), middle (Weber River, UT) and southern (Blue River, AZ). Common garden at Lethbridge is indicated by a star and this common garden comprises with genotypes collected from other sites (•) and common gardens (★) by Evans et al. (2013).

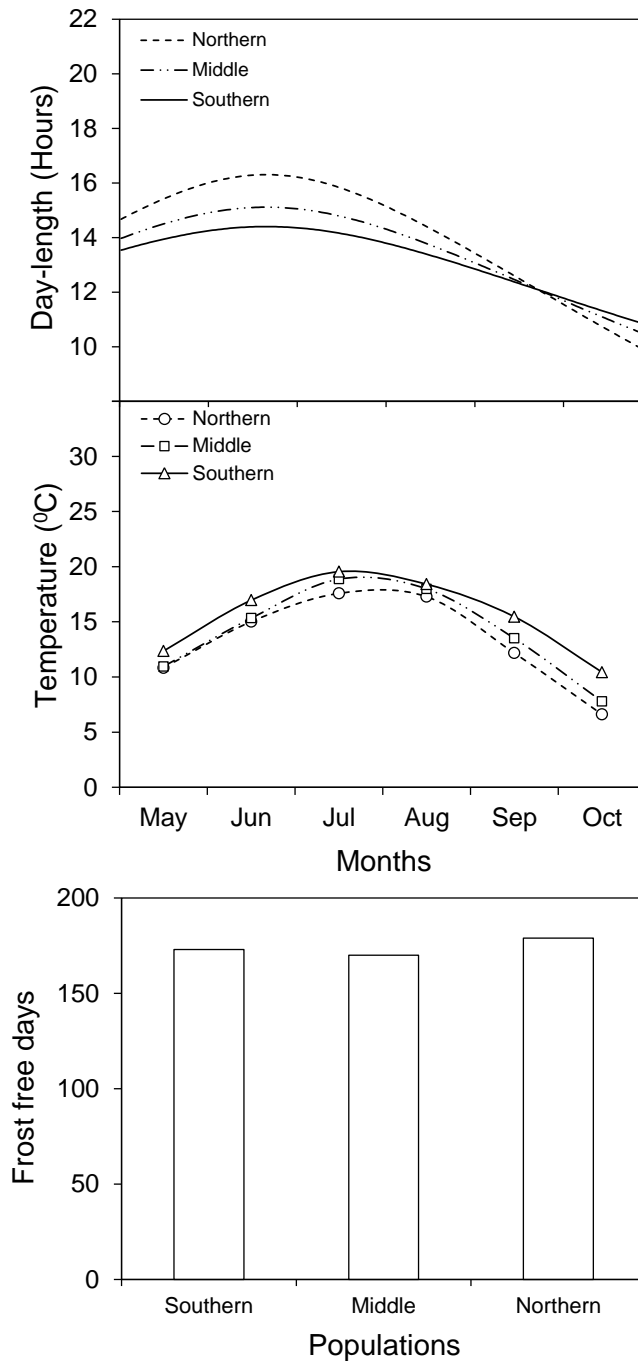


Figure 3.2 Day-length, monthly normal (1971-2000 means) adjusted average temperature, and frost free days for the genotype collection sites of northern, middle and southern population for the growth season (May – October).

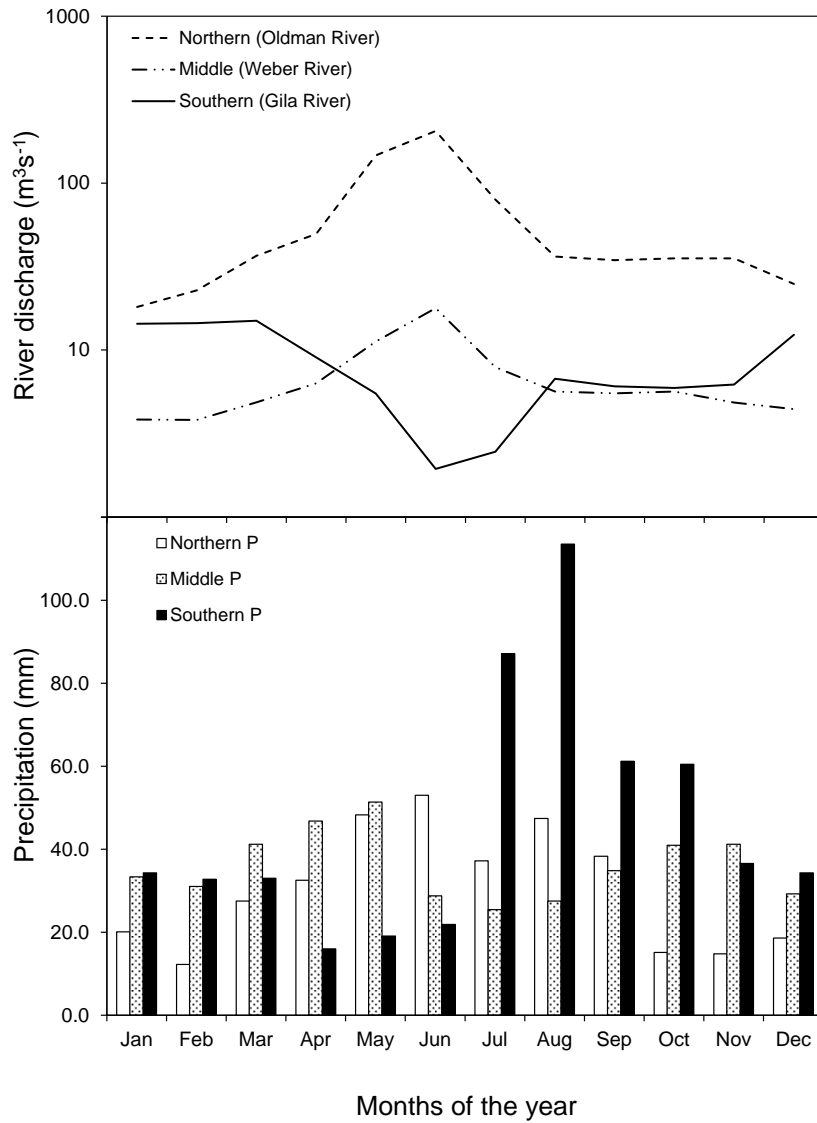


Figure 3.3 River discharge data for Oldman River, near Lethbridge, AB; Weber River near Coalville, UT; and Gila River, near Virden, NM representing northern, middle and southern population collection sites. (b) Monthly normal precipitation (1971-2000) for genotype collection sites, the northern (Oldman River, Alberta), middle (Weber River, Utah) and southern (Blue River, Arizona).

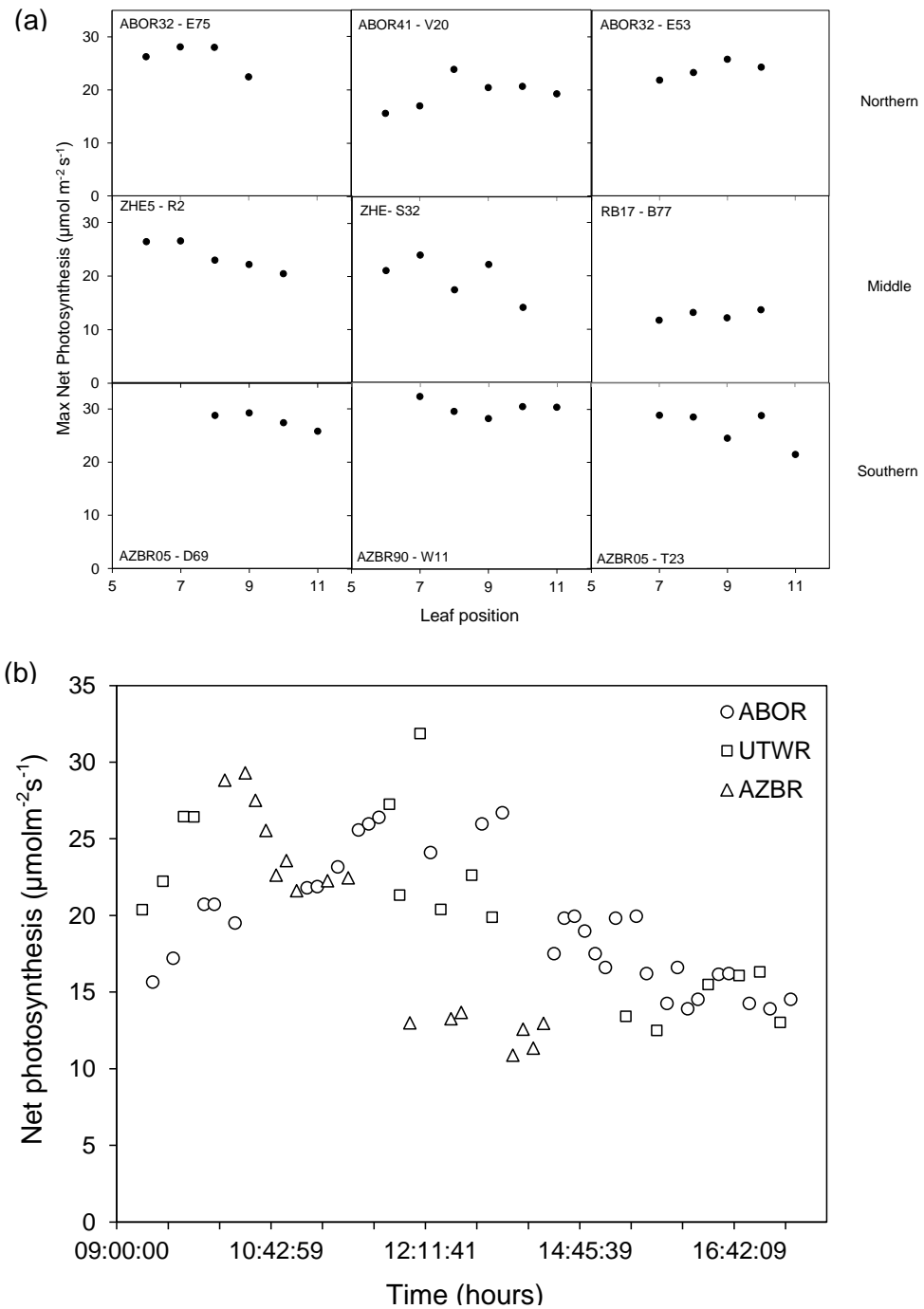


Figure 3.4 (a) Variation of net photosynthesis for leaves from the base along the tallest stem of individual trees for the northern, middle and southern populations. (b) Variation of net photosynthesis during the day.

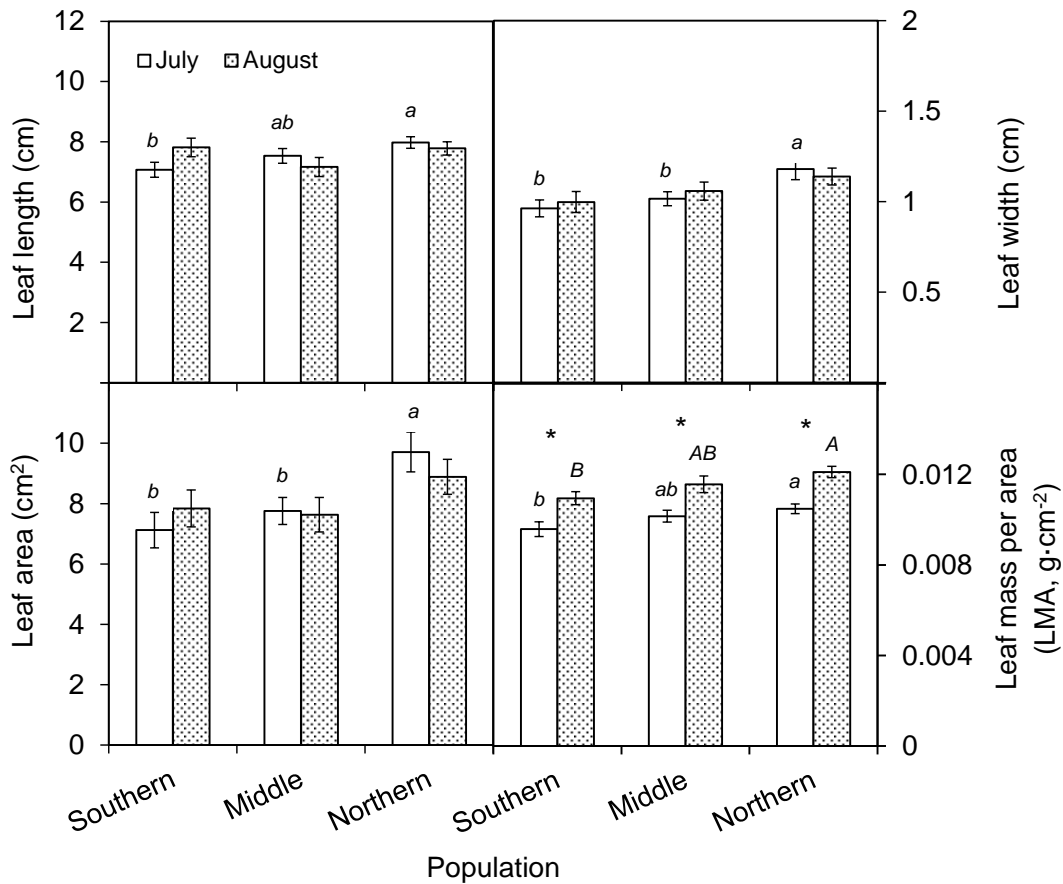


Figure 3.5 Leaf length, width, area and leaf mass per area ratio (LMA) of narrowleaf cottonwoods from southern, middle and northern populations grown in a common garden at Lethbridge, AB (n=20 from each population). Different letters indicate significant differences ( $P < 0.05$ ) for individual traits within months. Lower case letters are for July and upper case letters are for August. Asterisks (\*) indicate July versus August is significantly different ( $P < 0.05$ ).

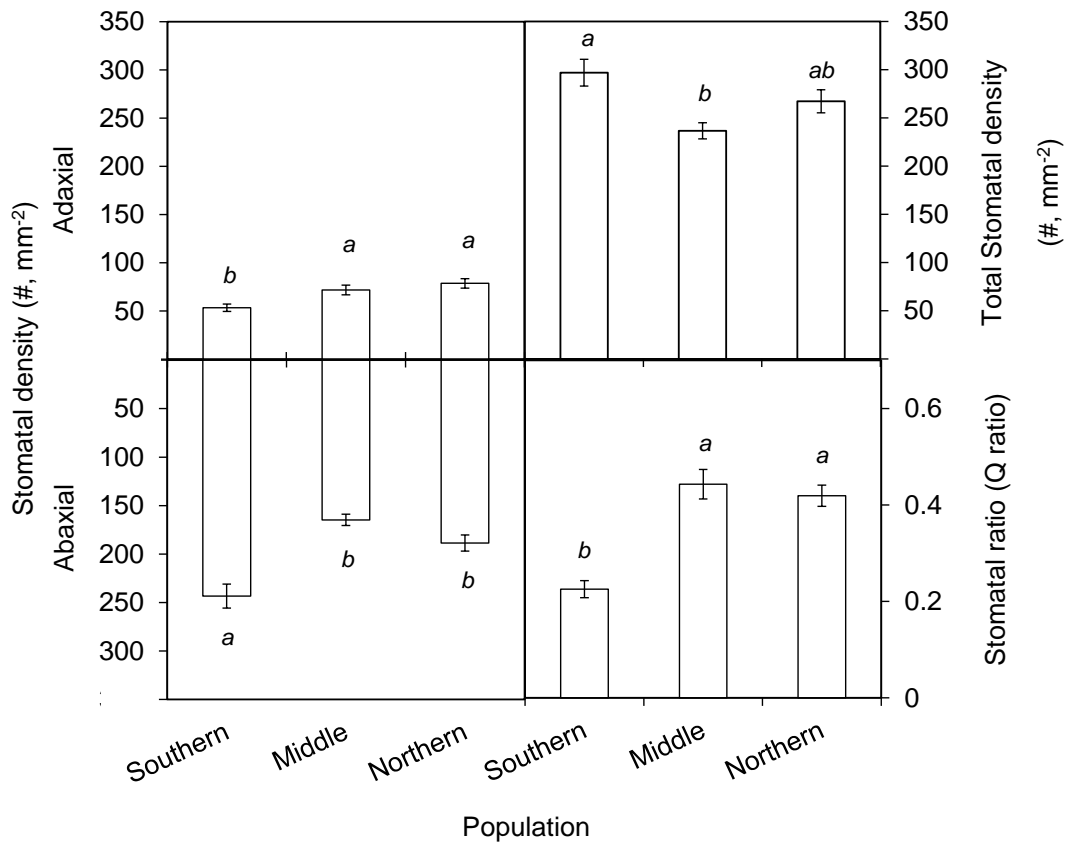


Figure 3.6 Adaxial (AD), abaxial (AB), total stomatal density and Q ratio (AD/AB ratio) of narrowleaf cottonwoods from southern, middle and northern populations grown in common garden at Lethbridge, AB (n=20 from each population). Different letters indicate significant differences ( $P < 0.05$ ) for individual traits.

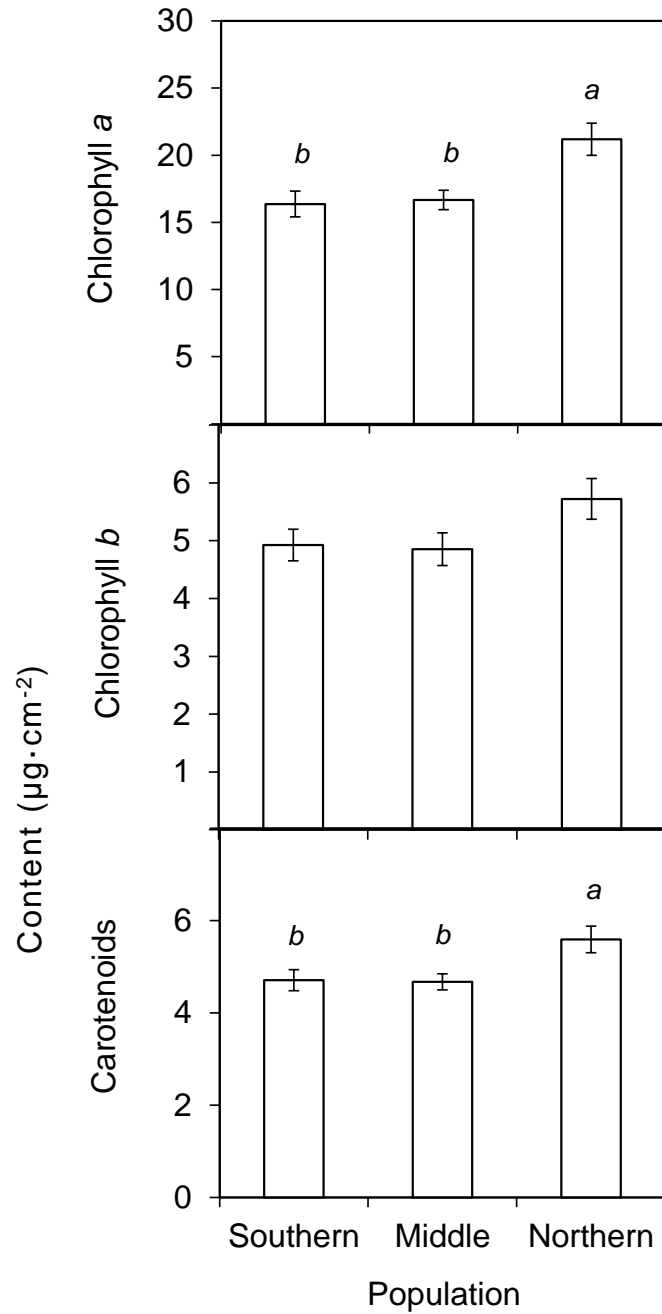


Figure 3.7 Chlorophyll *a*, chlorophyll *b* and carotenoid contents of narrowleaf cottonwoods from southern, middle and northern populations grown in a common garden at Lethbridge, AB (n=20 from each population). Different letters indicate significant differences ( $P<0.05$ ) for individual traits.



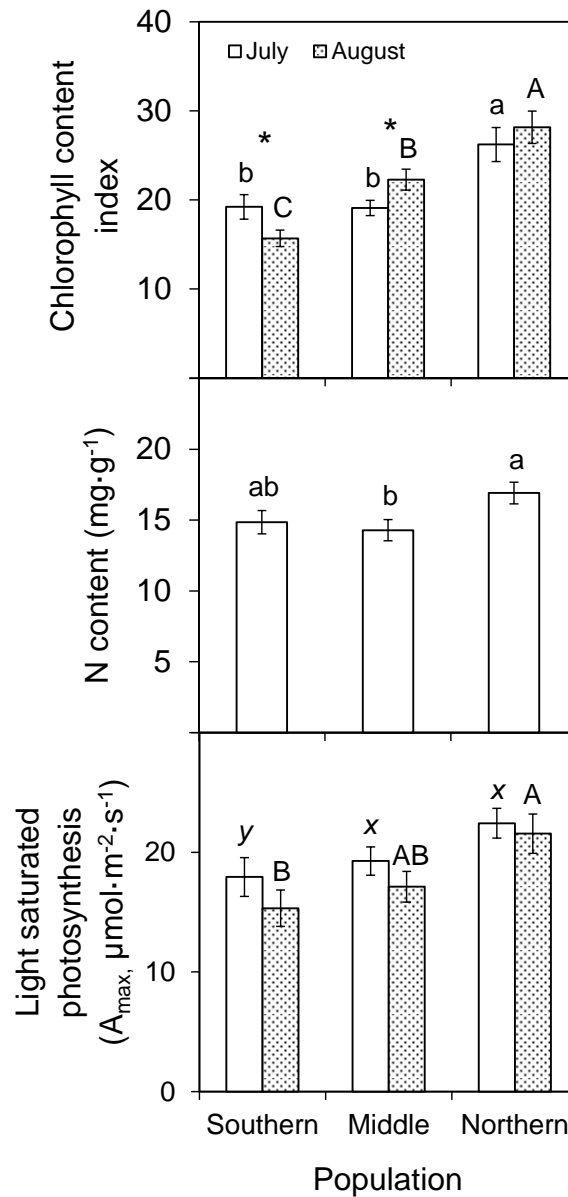


Figure 3.8 The chlorophyll content index, N content and the light saturated photosynthesis ( $A_{\max}$ ) of narrowleaf cottonwoods for northern, middle and southern populations grown at Lethbridge, AB ( $n=20$  from each P). Different letters (A, B, C) indicate significant differences ( $P<0.05$ ) and letters  $x$  and  $y$  indicate trends in differences ( $P<0.1$ ) for individual traits within months. Lower case letters are for July and upper case letters are for August. Asterisks (\*) indicate July versus August is significantly different ( $P<0.05$ ).

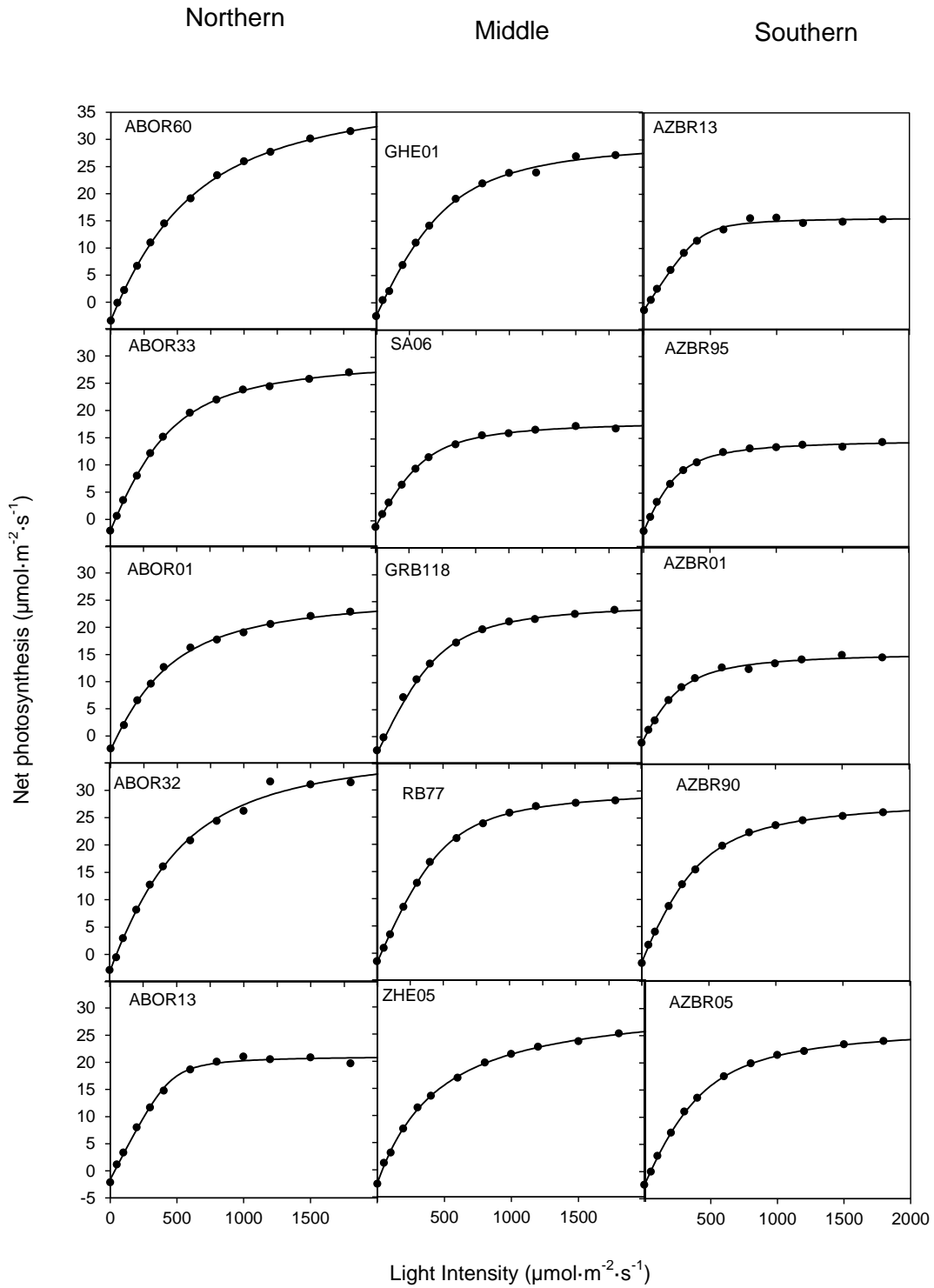


Figure 3.9 Light response curves developed for genotypes from the northern, middle and southern populations.

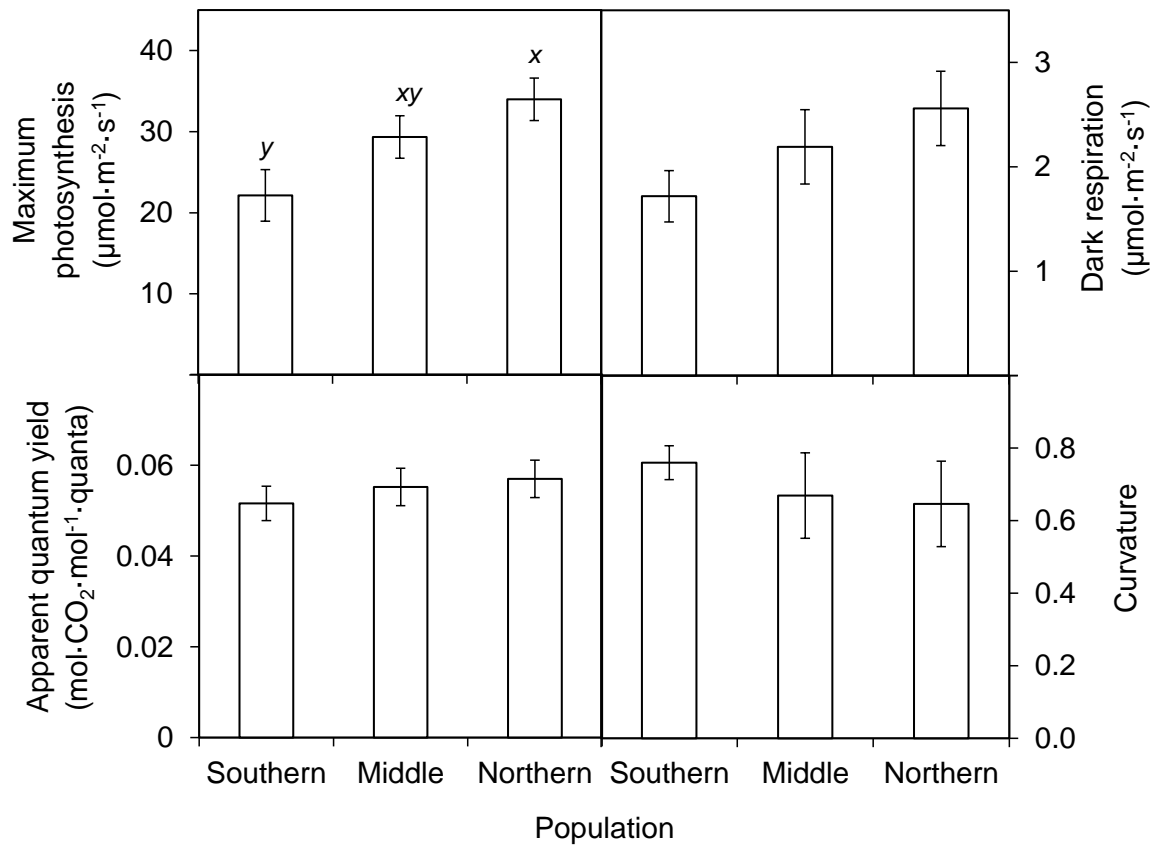


Figure 3.10 Maximum photosynthesis, dark respiration rate, apparent quantum yield and curvature derived from light response curves for narrowleaf cottonwoods from southern, middle and northern populations grown at Lethbridge, AB (n=5 from each population). Different letters (*x* and *y*) indicate trends in differences ( $P<0.1$ ) for individual traits.

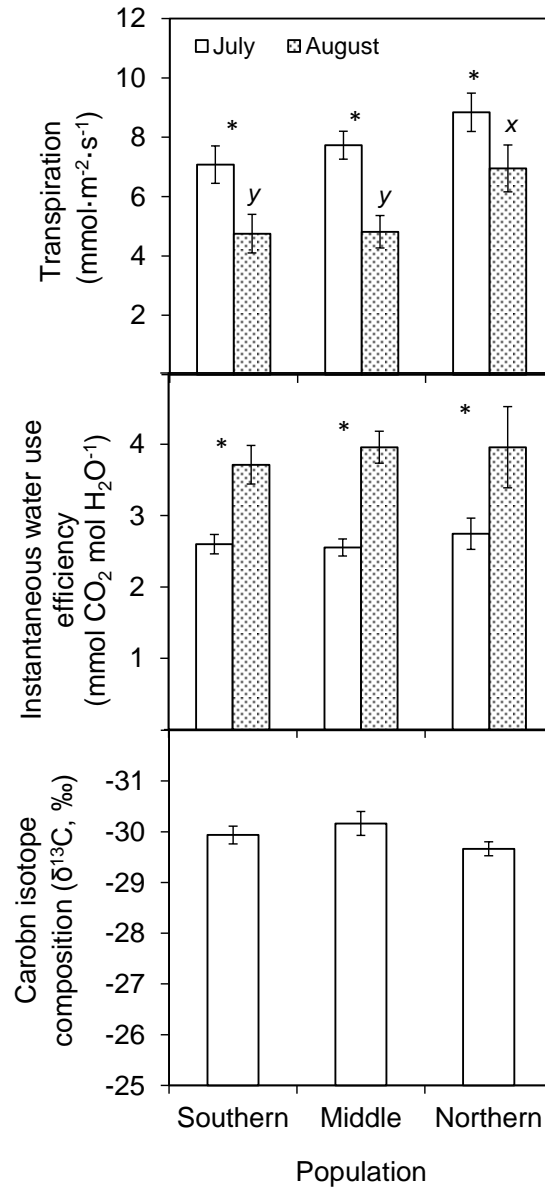


Figure 3.11 Transpiration ( $E$ ), instantaneous water use efficiency ( $WUE_e$ ,  $A_{max}/E$ ) and leaf carbon isotope composition ( $\delta^{13}C$ ), for narrowleaf cottonwood populations from southern, middle and northern populations grown at Lethbridge, AB ( $n=20$  from each population). Different letters ( $x$  and  $y$ ) indicate trends in differences ( $P<0.1$ ) for individual traits within months. Asterisks indicate July versus August is significantly different. \*-  $P<0.05$ .

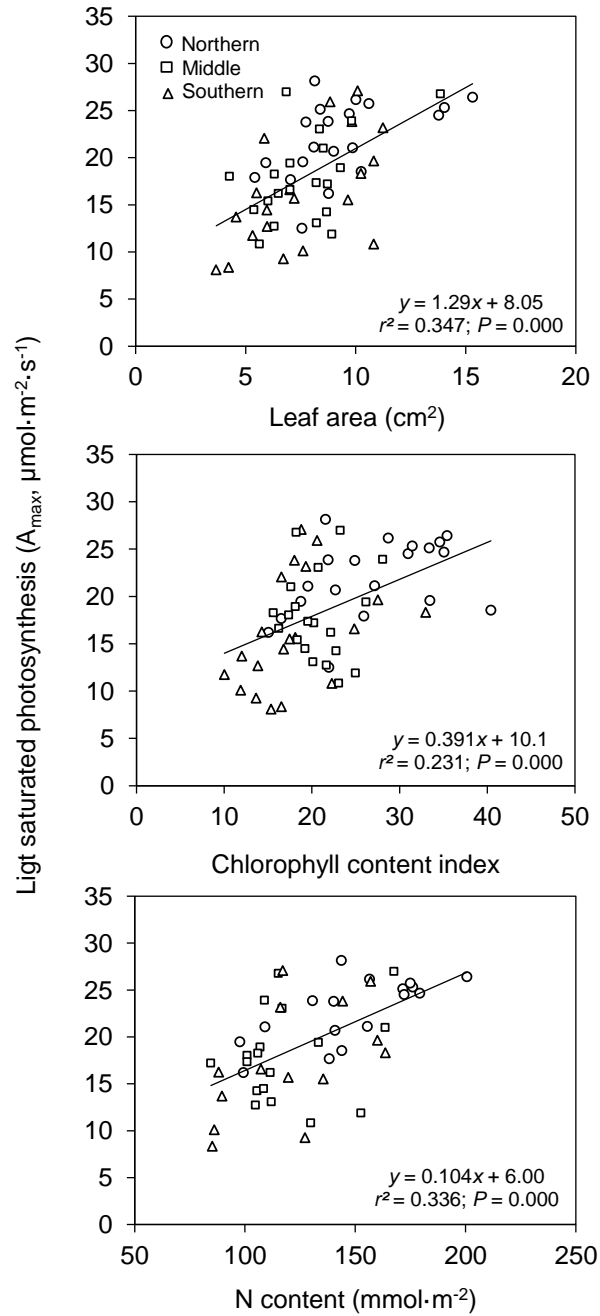


Figure 3.12 The light-saturated photosynthesis ( $A_{max}$ , averaged July and August) versus leaf area, chlorophyll content index (averaged July and August) and N content for narrowleaf cottonwoods ( $n=60$ ) grown at Lethbridge, AB.

## Chapter 4. Conclusion

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For deciduous trees, the growth season progressively become shorter with increasing latitude or elevation. More northern trees may have developed characteristics to increase physiological capacity, to overcome the challenge of growth and reproduction within the limited interval of optimal growth conditions. We investigated growth season compensation with *P. angustifolia* genotypes grown in a northern common garden at Lethbridge, AB, Canada.

### 4.1 Latitudinal compensation

The first part of the study (Chapter 2) involved 167 *P. angustifolia* genotypes representing the full latitudinal range (16°) of the species' distribution. In a northern common garden (University of Lethbridge, Lethbridge, Canada), growth, some foliar morphological and biochemical characteristics were closely associated with the latitude of origin but no associations were found with the elevation of origin. Therefore we accepted the primary hypothesis (Chapter 2,  $H_1$ ), that there is a close association of growth, foliar morphological and biochemical characters with the latitude of origin and rejected the null hypothesis (Chapter 2,  $H_0$ ), that there is no association of growth, foliar morphological and biochemical characters with the latitude of origin or with the elevation of origin and the secondary hypothesis (Chapter 2,  $H_2$ ), that there is a positive association of growth, foliar morphological and biochemical characters with the elevation of origin.

## **4.2 Population comparison**

The second part of the study (Chapter 3) involved 20 *P. angustifolia* genotypes from each of the three populations from the southern, middle and northern parts of the full latitudinal range of the species distribution. In this part of the study, we further investigated latitudinal compensation because of the close associations found between growth and foliar characteristics with the latitude of origin in the previous part of the study (Chapter 2). We compared the foliar morphological, biochemical and gas exchange characteristics of three populations from along the latitudinal range from south to north.

Table 4.1 summarises the results of foliar morphological, biochemical and physiological traits investigated for narrowleaf cottonwoods in this study.

Table 4.1 Significant associations of growth, foliar morphological, biochemical and physiological characteristics with latitude and southern, middle and northern populations comparison of these traits of *P. angustifolia* grown in a northern common garden at Lethbridge, Canada. Significant ( $P < 0.05$ ) positive associations are marked as ( $\uparrow$ ) and negative associations are marked as ( $\downarrow$ ). Trends in differences ( $P < 0.1$ ) in Chapter 2 results are indicated with a letter *t*. Different letters (*a* and *b*) indicates significant ( $P < 0.05$ ) differences. Trends in differences ( $P < 0.1$ ) in Chapter 3 are indicated as letters *x* and *y*.

Traits	Latitudinal compensation (Chapter 2)	Population comparison (Chapter 3)		
		Southern	Middle	Northern
<i>Growth</i>				
Height	$\downarrow$			
Diameter	$\downarrow$			
<i>Foliar morphological characteristics</i>				
Leaf length	-	-	-	-
Leaf width	$\uparrow^t$	<i>b</i>	<i>b</i>	<i>a</i>
Leaf area	-	<i>y</i>	<i>xy</i>	<i>x</i>
Leaf mass	$\uparrow$	<i>b</i>	<i>b</i>	<i>a</i>
Leaf mass per area (LMA)	$\uparrow$	<i>b</i>	<i>ab</i>	<i>a</i>
Adaxial stomata density (ADSD)		<i>b</i>	<i>a</i>	<i>a</i>
Abaxial stomata density (ABSD)		<i>a</i>	<i>b</i>	<i>b</i>
Total stomata density		<i>a</i>	<i>b</i>	<i>ab</i>
Q ratio (ADSD/ABSD)		<i>b</i>	<i>a</i>	<i>a</i>
<i>Foliar biochemical characteristics</i>				
Chlorophyll <i>a</i>		<i>b</i>	<i>b</i>	<i>a</i>
Chlorophyll <i>b</i>		-	-	-



Traits	Latitudinal compensation (Chapter 2)	Population comparison (Chapter 3)		
		Southern	Middle	Northern
Carotenoids		<i>b</i>	<i>b</i>	<i>a</i>
Chlorophyll content (CCI)	↑	<i>b</i>	<i>b</i>	<i>a</i>
N content	↑	<i>ab</i>	<i>b</i>	<i>a</i>
Stable isotope composition	-	-	-	-
<i>Physiological characteristics</i>				
Light saturated photosynthesis		<i>b</i>	<i>b</i>	<i>a</i>
Maximum photosynthesis from light response curve		<i>y</i>	<i>xy</i>	<i>x</i>
Dark respiration from light response curve		-	-	-
Apparent quantum yield from light response curve		-	-	-
Curvature from light response curve		-	-	-
Transpiration		<i>b</i>	<i>a</i>	<i>a</i>
Instantaneous water use efficiency		-	-	-

### **4.3 Study Conclusion**

Growth, foliar morphological and biochemical characteristics of narrowleaf cottonwoods grown at Lethbridge, AB, were associated with the latitude of origin. Foliar morphological and biochemical characteristics we investigated were significantly different between northern and southern populations, as well as the light saturated photosynthesis.

Therefore I conclude that growth, foliar morphological and biochemical as well as some gas exchange characteristics of narrowleaf cottonwoods grown in a northern common garden associated significantly with the latitude of origin. This provides further evidence for latitudinal compensation as a part of counter gradient variation of deciduous trees in the northern hemisphere.

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