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## LIST OF ABBREVIATIONS AND ACRONYMS

AGBT	Above-ground biomass per tree
AGBM	Above-ground biomass per m <sup>2</sup>
C	Clone
CW	Crown width
DF	Degree of freedom
G	Genus
LA	Leaf area
LAR	Leaf area ratio
LB	Leaf biomass
N	Nitrogen
N <sub>area</sub>	Nitrogen content
PAR	Photosynthetically active radiation
PNUE <sub>amb</sub>	Photosynthetic N-use efficiency in the ambient light
P <sub>n</sub>	Net photosynthesis
RB	Root biomass
RCD	Root collar diameter
RGR	Relative growth rate
RSR	Root shoot ratio
S	Spacing
SB	Stem biomass
SLA	Specific leaf area
SR	Slenderness ratio
SRF	Short rotation forestry
T	Time

## ABSTRACT

The main objective of this study was to determine morphological and physiological characteristics of clones driving to superior growth and yield performance under increased plant competition and N level. Seven hybrid poplars (915319; *Populus maximowiczii* × *P. balsamifera*, 747210; *P. balsamifera* × *P. trichocarpa*, 1081; *P. deltoides* × *P. balsamifera*, 2782; *P. tremuloides* × *P. tremula*, DN-74; *P. deltoides* × *P. nigra*, NM06; *P. maximowiczii* × *P. nigra*, and Walker; *P. deltoides* × *P. petrowskyana*) and five willows (HAN-A5; *Salix amygdaloides*, LEV-D5; *S. discolor*, SHE-E9; *S. eriocephala*, LAF-I4; *S. interior*, and TCH-N4; *S. nigra*) were grown in greenhouse conditions for 13 weeks under three spacings [20 (20×20), 35 (35×35), and 60 (60×60) cm] and two nitrogen (N) levels (20 and 200  $\mu\text{g g}^{-1}$ ).

Superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 had 143 % greater leafless aboveground biomass per tree (AGBT), compared to inferior clones 747210, 915319, LEV-D5, and LAF-I4. Greater productivity of superior clones was primarily explained by 100 % greater leaf area (LA) as LA was significantly related to plant growth for most clones. In particular, superior clones showed 16 % and 28 % greater leaf area ratio (LAR) and specific leaf area (SLA), respectively, suggesting that superior clones had greater LA production relative to plant and leaf biomass for maximizing total photosynthetic area per tree. In addition, superior clones showed greater AGBT per unit increases in LA and leaf N concentration ( $N_{\text{area}}$ ), indicating greater shoot growth capacity under the decrease in spacing where the amount of  $N_{\text{area}}$  and LA were smaller. The greater shoot growth capacity of superior clones resulted in 24 % lower root-shoot ratio (RSR). However, photosynthetic N-use efficiency in the ambient light condition ( $\text{PNUE}_{\text{amb}}$ ) was similar between superior and inferior clones, concluding that greater productivity of superior clones was not owing to the photosynthetic capacity of individual leaves in the ambient light conditions but due to greater LA production capacity. Compared to low N and 60 cm spacing, high N and 20 cm spacing showed similar RCD, CW, and LA values and even greater height for all clones, concluding that an addition of N can play an essential role in increasing plant growth under the intensive competition. The increase in N level significantly increased  $N_{\text{area}}$  by 23 % but had no significant effect on  $P_n$  and even reduced  $\text{PNUE}_{\text{amb}}$ , indicating that an addition of N had little effect on photosynthetic capacity under ambient light conditions.



## RÉSUMÉ

L'objectif principal de ce mémoire était de déterminer les caractéristiques morphologiques et physiologiques permettant au peuplier hybride et au saule d'avoir une meilleure croissance en conditions de taillis à courte rotation (compétition élevée et faible disponibilité en azote dues à un espacement très faible entre les arbres). Sept clones de peuplier hybride (915319; *Populus maximowiczii* × *P. balsamifera*, 747210; *P. balsamifera* × *P. trichocarpa*, 1081; *P. deltoides* × *P. balsamifera*, 2782; *P. tremuloides* × *P. tremula*, DN-74; *P. deltoides* × *P. nigra*, NM06; *P. maximowiczii* × *P. nigra*, and Walker; *P. deltoides* × *P. petrowskyana*) et cinq clones de saule (HAN-A5; *Salix amygdaloides*, LEV-D5; *S. discolor*, SHE-E9; *S. eriocephala*, LAF-I4; *S. interior*, and TCH-N4; *S. nigra*) ont été cultivés en serre pendant trois mois sous trois espacements (20 × 20, 35 × 35, et 60 × 60 cm) et deux niveaux d'azote (N) (20 et 200  $\mu\text{g g}^{-1}$ ).

Les meilleurs clones (2782, NM06, HAN-A5, SHE-E9, et TCH-N4) ont produit 143 % de plus de biomasse aérienne par arbre (AGBT) par rapport à la moyenne des clones qui ont eu une croissance faible (747210, 915319, LEV-D5, et LAF-I4). La plus grande productivité des meilleurs clones a été principalement expliquée par 100% de plus de surface foliaire (LA), et au fait que LA était positivement liée à la croissance des plantes pour la plupart des clones. En particulier, les clones supérieurs avaient un plus grand rapport de surface foliaire (LAR; 16%) et une plus grande surface foliaire spécifique (SLA, 28%). Les clones supérieurs ont montré une plus grande AGBT par augmentation de LA et de concentration en N ( $N_{\text{area}}$ ) des feuilles indiquant une plus grande capacité de croissance avec une diminution de l'espacement où la quantité d' $N_{\text{area}}$  et LA sont plus petits. La plus grande croissance des tiges des clones supérieurs a résulté en une diminution du ratio racine:tige (RSR) de 24% par rapport aux clones les moins productifs. Cependant, l'efficacité photosynthétique de l'utilisation de l'azote à lumière ambiante ( $\text{PNUE}_{\text{amb}}$ ) était similaire entre les clones supérieurs et inférieurs, ce qui suggère que la plus grande productivité des clones supérieurs n'était pas due à la capacité photosynthétique des feuilles individuelles dans les conditions de lumière ambiante, mais en raison d'une plus grande capacité de production de feuillage. Comparé au bas niveau de N et à l'espacement de 60 × 60 cm, les arbres avec le haut niveau de N et l'espacement 20 × 20 cm avaient des valeurs de RCD, CW, et LA similaires, mais les arbres étaient plus grands pour tous les clones, concluant que l'addition de N peut jouer un rôle essentiel dans l'augmentation de la croissance des plantes dans les plantations à espacements très serrés. L'augmentation du niveau N a augmenté  $N_{\text{area}}$  de 23%, mais n'a eu aucun effet significatif sur  $P_n$  et réduit  $\text{PNUE}_{\text{amb}}$ , ce qui indique que l'addition de N a eu peu d'effet sur la capacité de photosynthèse dans des conditions de lumière ambiante.

Mots clés : *Peuplier hybride et saule, clones supérieurs, productivité, capacité photosynthétique*

## CHAPTER I

### GENERAL INTRODUCTION

#### 1.1 Poplar and willow utilization in Canada

There is growing interest in hybrid poplar and willow production under short-rotation forestry (SRF) management across Canada. First, their rapid growth rate can produce pulp fibre within a relatively short-rotation period of fewer than 10-12 years (Thomas *et al.*, 2000). This can secure fibre sources to satisfy future needs for the Industry. Second, the high productivity of SRF crops provides renewable bio-energy and bio-fuel products that are increasingly used as a substitute for fossil-fuel based products. SRF crops are expected to revitalize the rural economy by providing a potential source of revenue for woodlot owners and employment opportunities. Bio-energy products from SRF may also be environmentally effective to counteract a growing concern over global CO<sub>2</sub> emissions; As photosynthesis sequesters carbon from atmospheric CO<sub>2</sub> in tree biomass, SRF crops can contribute to reduce global CO<sub>2</sub> emissions as carbon-neutral energy sources (Weih, 2004). Third, the fast growth of hybrid poplar crops can produce high-value lumber products within a shorter rotation period (15-30 years) compared to traditional forest trees such as spruce (*Picea* spp.), which matures after 40-50 years (Weih, 2004).

Poplars and willows are also used for the purpose of environmental protection; Phytoremediation using hybrid poplars and willows is applied to reduce pollutant concentrations at contaminated mine sites, landfills, and oil sands because of their high absorption capacity of heavy metal ions (Isebrands and Karnosky, 2001). Hybrid poplars and willows are also planted in riparian buffer areas to provide soil stability as well as to protect water sources by keeping potential pollutants from entering water bodies (Isebrands and Karnosky, 2001). When planted as shelterbelts near dairy, hog

and poultry facilities, they can provide a variety of benefits, including important ecosystem habitats for many species, flora and fauna biodiversity, and greatly diminishing livestock stress by providing shade and protection from hot winds (Isebrands and Karnosky, 2001).

## 1.2 Breeding and selection of *Populus* clones

In Canada, most bioenergy willow plantations are still under experimental phase and the majority of applications are used for environmental applications (Derbowka, 2012). On the other hand, hybrid poplar clones have been exclusively managed for the purpose of supplying pulp fibre or logs for engineered wood products (Derbowka, 2012). There were selection and breeding programs for hybrid poplars supported by companies such as Ainsworth Engineered Canada LP in Alberta, Alberta-Pacific Forest Industries in Alberta, Kruger Products Limited in British Columbia and by two governmental organizations, the Provincial Ministry of Natural Resources and Wildlife in Quebec and the Agri-Environment Services Branch (AESB) in Saskatchewan (Derbowka, 2012). These programs evaluated hybridization of poplar species including *Populus deltoides*, *P. balsamifera*, *P. maximowiczii*, *P. trichocarpa*, and *P. nigra* and hybridized aspen including *P. alba* × *P. grandidentata* and *P. tremula* × *P. tremuloides*. The breeding and selection processes consist of making numerous crosses of promising native and introduced species, which may show useful effects of heterosis to maximize productivity. With respect to heterosis in SRF, clones should be selected based on fast growth rates despite high plant density, disease and insect resistance, and local site adaptability (e.g., cold and drought tolerance).

### 1.2.1 Effects of spacing

Response to tree spacing is an important determinant as it is directly related to plant yield. Numerous *Populus* species have been shown to acclimate well to high

intensity of plant competition (0.5-3 m spacing between the trees), contributing to higher productivity per hectare during early growth periods (e.g., Larocque, 1999; Proe *et al.*, 2002; Benomar *et al.*, 2011). Larocque (1999) reported that decreased spacing from 1.5 to 1.0 m increased biomass production per hectare by 15 % for hybrid poplar DN-74 (*Populus deltoides* × *P. nigra*) after 4 years in Canada. Proe *et al.* (2002) also reported an increase in productivity of poplar and alder by 56 % when tree spacing was reduced from 1.5 to 1.0 m after 5 years of plantation establishment in central Scotland. However, appropriate plant spacing largely depends on the desired end product (e.g., timber or energy). Pronounced increases in biomass production under close spacings are largely related to capacity of trees to physiologically and morphologically acclimate to intensive tree competition where canopy occupancy would not cause crown-base recession (Bergkvist and Ledin, 1998; Fang *et al.*, 1999; Benomar *et al.*, 2012).

Trees increase photosynthetic rate under wider tree spacings as they can intercept more solar radiation. High irradiance conditions increase the portion of nitrogen (N) content to leaves (Evans and Poorter, 2001). Higher leaf N content is primarily used to produce Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) that is well correlated with photosynthetic capacity (Seemann *et al.*, 1987). Leaves grown in high light conditions also have a higher light-saturated photosynthetic capacity per unit leaf area than leaves grown in low light conditions (Kozlowski and Pallardy, 1997). Although the decrease in spacing reduces incoming light levels and associated leaf N content by competition between the trees, faster-growing plants can acclimate to close spacing by increasing specific leaf area (SLA: leaf area/leaf biomass) for maximizing net photosynthesis along the gradient of light conditions (Benomar *et al.*, 2011). The increase in SLA contributes to 1) light interception of the foliage by expanded leaf area and light transmittance by decreased leaf thickness (Vile *et al.*, 2005); 2) reducing N allocation to leaf construction costs, such as producing cell walls, but increasing N allocation to thylakoids and chlorophyll and hence light

absorption (Evans and Poorter, 2001; Feng *et al.*, 2008); and 3) lowered light compensation points (Kozlowski and Pallardy, 1997). Thus, these leaves can adjust rapidly to short periods of irradiation under shaded conditions through efficient photosynthetic induction such as fast stomata opening and high electron transport at low photon flux density (Kozlowski and Pallardy, 1997). As a result, the increase in SLA under intensive tree competition would improve light-and nitrogen-use efficiency for photosynthesis for maximizing carboxylation capacity and consequently increase biomass productivity (Feng *et al.*, 2008; Benomar *et al.*, 2011; 2012).

More productive or superior trees under crowded conditions should have increased slenderness ratio (SR: stem height/DBH or root collar diameter) for the efficient development of stem length as spacing decreases (Debell *et al.*, 1996). The increase in stem length can allow plant leaves to have more light in the upper crown under high competition, resulting in having longer (indeterminate) shoots with more foliage at the top of crowns (Dickmann, 2001). These sun leaves would play a vital role in the partitioning of carbohydrates to tree growth (Kozlowski and Pallardy, 1997). It is also likely that trees with longer stem length would produce more sylleptic branches with the increase in leaf area, which increases canopy size and then total shoot biomass (Marron *et al.*, 2006). Thus with these adaptations, superior plants could improve photosynthetic crown capacity under conditions of high competition.

It is thought that a decrease in spacing intensifies root competition and that trees allocate more carbon belowground to compete for limiting resources (Litton *et al.*, 2003). Thus, the trees would increase their root-to-shoot ratios (RSR) as spacing decreases. However, superior trees may rather increase density and longevity of fine roots characterized by an enhanced rate of N uptake per unit root mass in response to competition (Casper and Jackson, 1997). Therefore, efficient N uptake would allow plants to allocate more N to rapid shoot growth relative to growth of roots (Litton *et*

*al.*, 2003). As a consequence, superior trees could even reduce RSR even under close spacings.

Close spacing may also contribute to suppressing the competing ground vegetation, due to the accelerated canopy closure (Weih, 2004). In boreal regions, poplar trees are reported as being very sensitive to belowground competition where native weeds are well adapted to the growing conditions (Bowersox *et al.*, 1992; Labrecque *et al.*, 1994). As a result, inter-specific competition by weeds often limits stand productivity of poplars and willows for a longer period in boreal regions (Weih, 2004). Therefore, close spacing appears to be more advantageous in boreal regions in terms of producing large biomass yields as well as minimizing the use of herbicides. However, intense inter-plant competition may increase the risk of damage from pests and diseases due to plants being subjected to greater stress at closer spacing (Mitchell, 1995).

### 1.2.2 Nitrogen effects

Among environmental factors that affect tree growth, fertilization, particularly N availability, is also an essential factor as trees require a large quantity of nitrogen (N) for growth. However, boreal forest regions of Canada are characterized by low mineralization rates (Larsen, 1980). These sites could not generally meet the high nutrient requirements of hybrid poplars. Therefore, N supply plays a decisive role in attaining high productivity. As most of the N is distributed to the photosynthetic apparatus (Kozłowski and Pallardy, 1997), the increase in N level would increase the number and size of leaves by enhancing leaf photosynthetic capacity (Evans, 1989). As a result, the increase in N would lead to an expansion of the crown (Ceulemans *et al.*, 1990). N fertilization would also delay the rate of leaf maturation, which allows plants to expand more leaf area during the growing period (Kozłowski and Pallardy, 1997). On the other hand, low N availability would accelerate leaf senescence and reduce canopy size (Kozłowski and Pallardy, 1997). Studies reported that the growth

response to N varies among species and their hybrids (Guillemette and DesRochers, 2008; van den Driessche *et al.*, 2008), primarily due to their different patterns of N partitioning into leaves along the canopy light gradient (Field, 1983). Species-specific information is required to assess nitrogen-use efficiency for plant growth.

The effect of N on biomass production can be modified by various factors; When native soil fertility is high, N fertilization is less effective to increase tree growth (Guillemette and DesRochers, 2008). N fertilization would also be less effective on finer textured soils such as clay, clay loam, and silty clay loam, due to poor aeration and drainage (Dickmann and Stuart, 1983). These soils create saturated and waterlogged conditions easily, which starve the root system of oxygen and cause drought-like symptoms (Dickmann and Stuart, 1983). Consequently, nitrogen-uptake capacity per unit root mass would decrease under these heavy soils. Soil pH also affects the effect of N on tree growth; The increase in soil pH with NO<sub>3</sub> fertilization would decrease the rate of phosphorus (P) uptake, causing inhibition of tree growth, net photosynthesis, and stomatal conductance (DesRochers *et al.*, 2007). Lastly, the effect of N would be dependent on the balance with other nutrients; For example, a recent study revealed that N only promoted the sensitivity of hybrid poplars to drought-stress and adding P was required to withstand this effect (DesRochers *et al.*, 2006). Potassium (K) applied with P and N would reduce mortality rate by increasing resistance to diseases (Guillemette and DesRochers, 2008).

### 1.2.3 Growth-determinant information

An efficient approach is required to accelerate the selection process as the availability of new hybrid poplar or willow clones increases. Conventional selection methods have been based on the comparison of growth performance of new clones using biomass or volume production or tree growth under low stand density. As a consequence, this experimental approach requires considerable time and investment for the selection of superior clones. To minimize time and cost, it is beneficial to



identify the physiological and morphological characteristics of species leading to fast growth, such as crown architecture, root development, and photosynthetic efficiency of foliage under high competitive conditions. This growth information would provide clone-specific acclimation patterns to competition at the relatively early stage after experimental establishment, thereby achieving early clone selection based on desirable morphological and physiological traits. Thus, in addition to saving time for the selection, this can also end up reducing associated silviculture costs.

### 1.3 General characteristics of species used in this study

#### 1.3.1 Poplars

*P. balsamifera* (balsam poplar, *Tacamahaca* section) is primarily a species of boreal climates (Dickmann, 2001). Therefore, hybridization with this species is expected to show high resistance to low temperatures. In Quebec, although balsam poplar could show varied degree of resistance to *Septoria* canker, it seems more resistant than either *P. maximowiczii* (Japanese poplar, *Tacamahaca* section) or *P. trichocarpa* (black cottonwood, *Tacamahaca* section) (Newcombe *et al.*, 2001). Also the high capacity of balsam poplar to regenerate vegetatively from roots and buried branch fragments (cuttings) could achieve the ease propagation of its hybrids (Dickmann, 2001).

*P. deltoides* (eastern cottonwood, *Aigeiros* section) is distributed in eastern and midwestern US and southern Canada (Dickmann, 2001). Root development of eastern cottonwood is generally poor so that it is necessary to hybridize with species that have better rooting capacities (Eckenwalder, 1996). Eastern cottonwood has been reported to show high resistance to canker disease in southern Quebec (Dickmann, 2001). Clone 1081 (*P. balsamifera* × *P. deltoides*, *P. × jackii*) is commonly planted in the Canadian prairies and showed a high relative growth rate of stem biomass of  $8.99 \text{ g/day/g} \times 10^2$  compared to  $8.71 \text{ g/day/g} \times 10^2$  for clone DN2 (*P. deltoides* × *P. nigra*) in the first 45 days after planting, with higher net photosynthesis

rate per unit leaf area (Tschaplinski and Blake, 1989). However, *P. × jackii* (Northwest) is known for high susceptibility to poplar bud gall mite and *Melampsora* rust (Eckenwalder, 2001).

*P. maximowiczii* (Japanese poplar, *Tacamahaca* section) is originally distributed in Northeast Asia from Japan to eastern Siberia so that it has a high cold resistance (Dickmann, 2001). Japanese poplar can also adapt well to medium fertility and acidic soils (pH 4.8) in Quebec, indicating suitability for planting in boreal forest regions where many forest sites have low-medium fertility and acidic soils (Riemenschneider *et al.*, 2001). Although this species generally appears to have good resistance to insects and diseases, it is highly susceptible to stem cankers caused by *Septoria musiva* (Riemenschneider *et al.*, 2001). Clone 915319 (*P. balsamifera* × *P. maximowiczii*) produced intermediate yields of 6.15 Mg ha<sup>-1</sup> year<sup>-1</sup> under 1 × 1 m spacing after 6 years under boreal forest conditions of eastern Canada (Benomar *et al.*, 2012).

*P. nigra* (black poplar, *Aigeiros* section), native to Europe, southwest and central Asia, and northwest Africa, may reach 40 m in height and 2 m in diameter (Dickmann, 2001). It sprouts vigorously from stumps and suckers from roots and propagates easily from stem cuttings (Dickmann, 2001). Black poplar appears to have good resistance to foliar diseases and insect damages, and medium resistance to *Septoria musiva* in Quebec (Riemenschneider *et al.*, 2001). Traditionally DN hybrids (*P. deltoides* × *P. nigra*) have been used in North America and Europe for the longest time periods (van Oosten, 2001). However, many DN clones have shown susceptibility to *Septoria* stem canker (van Oosten, 2001). In the boreal forest regions where the canker is not a risk, cold hardiness of DN clones may not be enough to withstand cold winters (van Oosten, 2001). Clone NM06, *P. nigra* × *P. maximowiczii* hybrid, is very tolerant to *Septoria* stem canker and can produce reasonable volumes (van Oosten, 2001). For example, Pearson *et al.* (2010) reported

average growth rates of  $8.9 \text{ Mg ha}^{-1} \text{ year}^{-1}$  for NM06 under a  $2.44 \times 2.44 \text{ m}$  spacing during a 6-year production cycle at Fuita, Colorado, U.S. In an abandoned farmland near Montreal, annual yield of NM06 reached  $18.05 \text{ Mg ha}^{-1} \text{ year}^{-1}$  at a density of 18,000 plants  $\text{ha}^{-1}$  for 4 years (Labrecque and Teodorescu, 2005). Like DN clones, cold hardiness of NM06 clones may not be enough to withstand boreal winters (van Oosten, 2001).

*P. \times petrowskyana* (Russian poplar) is a hybrid of introduced laurel poplar (*P. laurifolia*) with *P. nigra*. This hybrid was introduced and widely distributed in western Canada about 100 years ago (Ronald and Steele, 1974). Walker clone is a hybrid of *P. deltoides* with *P. \times petrowskyana*, and has been planted in prairie shelterbelts over years in Saskatchewan, due to its rapid growth rate and high tolerance to cold temperatures (Kort, 2000). Walker is generally resistant to poplar bud gall mite and *Septoria* canker (Steppuhn *et al.*, 2008).

*P. tremuloides* (trembling aspen, *Populus* section) is the most widely distributed poplar in North America from Canada to central Mexico while *P. tremula* (eurasian aspen, *Populus* section) is also widely distributed at cool temperate regions of Europe and Asia (Dickmann, 2001). The inter-specific 2782 hybrid clone (*P. tremuloides*  $\times$  *P. tremula*) has shown superior growth performance than their intra-specific crosses because of hybrid vigour (heterosis). For example, height growth of clone 2 (*P. tremuloides*  $\times$  *P. tremula*) was 512 cm in the 5<sup>th</sup> year while that of *P. tremula* was 325 cm (Yu *et al.*, 2001). Hybrid aspen shows a susceptibility to *Melampsora* leaf rust and *Septoria* stem canker that negatively affect growth and yield (Tullus *et al.*, 2012).

*P. trichocarpa* (black cottonwood, *Tacamahaca* section) is a fast-growing species that grows in moist bottomland or alluvial ecosystems (Dickmann, 2001). Since the habitat ranges of black cottonwood overlaps with balsam poplar in Alberta and the northern Rocky Mountains of the U.S., their hybrids have been naturally occurring in the overlapping zone (Dickmann, 2001). Morphology between the two is also very

similar except the capsules that split in 2 parts for balsam poplar and in 3 parts for black cottonwood (Dickmann, 2001). Like balsam poplar, black cottonwood can be also easily propagated from unrooted cuttings (Dickmann, 2001). Similarly to clone 915319, clone 747210 (*P. balsamifera* × *P. trichocarpa*) showed an intermediate yield of 5.15 Mg ha<sup>-1</sup> year<sup>-1</sup> under 1 × 1 m spacing at boreal forest region in Canada (Benomar *et al.*, 2012).

### 1.3.2 Willows

*Salix amygdaloides* (peachleaf willow) is widely distributed over North America (Fryer, 2012). Comparison studies revealed low biomass production rates of this species (0.26 kg/species) compared to that of *S. eriocephala* (Missouri river willow) (1.36 kg) under 1 × 1 m spacing at 1 year old (Mosseler *et al.*, 1988). However, in the same study, 1-year-old coppice biomass of peachleaf willow showed 46 % of the biomass growth from a 2.5 years unrooted cutting, demonstrating beneficial effects on biomass production from coppicing. This species is susceptible to *Melampsora* leaf rust (Royle and Hubbest, 1992).

*S. discolor* (Pussy willow) is widely distributed across Canada and some northern part of the United States (Gucker, 2007). In eastern Canada, this species has demonstrated significant growth potential in intensive culture, particularly using wastewater sludge. Pussy willow has displayed about 12.5 Mg ha<sup>-1</sup> year<sup>-1</sup> at a density of 30,000 plants ha<sup>-1</sup>, using sludge at the nursery of the Montreal Botanical garden, Quebec (Labrecque *et al.*, 1997). Even without using sludge, this species showed high biomass production (13.62 Mg ha<sup>-1</sup> year<sup>-1</sup>) at a density of 18,000 plants ha<sup>-1</sup> for 4 years in abandoned farmland sites, southwest of Montreal (Labrecque and Teodorescu, 2005). According to a two-year field disease assessment, there was a low susceptibility of pussy willow to *Melampsola* leaf rust (Pei *et al.*, 2008).

*S. eriocephala* is widely distributed in central-eastern North America from Florida to Quebec (Lin *et al.*, 2008). Missouri river willow can potentially yield high productivity compared to the other willows (Mosseler *et al.*, 1988; Serapiglia *et al.*, 2012). However, Missouri river willow clones have also shown a high susceptibility to *Melampsora* leaf rust and beetle damage that negatively affected their yield (Serapiglia *et al.*, 2012). Since *Melampsora* resistance is highly heritable, hybridization with *S. schwerinii* is recommended to increase rust resistance (Pei *et al.*, 2008).

*S. interior* (sandbar willow) is widely distributed across North America and freely hybridized with other willow species for higher biomass productivity (Froiland, 1962). For example, the aboveground biomass of the hybrid (*S. interior* × *S. eriocephala*) produced an average of 9.3 Mg ha<sup>-1</sup> year<sup>-1</sup> at a density of 18,000 plants ha<sup>-1</sup> for 4 years on abandoned farmland, southwest of Montreal (Labrecque and Teodorescu, 2005). In North America, this species is often infected with *Melampsora* leaf rust (Smith and Blanchette, 2004).

*S. nigra* (black willow) is widely distributed over central-eastern North America and commonly found in floodplain and bottomland hardwood forests (Tesky, 1992). It is an excellent species for erosion control because it is propagated easily from cuttings, rapidly produces adventitious roots, and provides soil stabilization (Pezeshki *et al.*, 2004). Black willow has shown high susceptibility to *Melampsora* leaf rust (Smith and Blanchette, 2004).

#### 1.4 Objectives

The primary objective of this study was to determine morphological and physiological characteristics of clones leading to superior growth and yield performance under intensive plant competition, and to see how these characteristics vary under high or low nitrogen (N) levels. The hypotheses corresponding to the first

question were that although the decrease in spacing would negatively affect growth performance due to limited available resources such as light (PAR) and nutrients, clones with superior growth performance under the closer spacing would 1) show no significant changes in leafless aboveground biomass per tree (AGBT) compared to the wider spacing, 2) greater AGBT per unit increase in LA and leaf N concentration ( $N_{\text{area}}$ ), 3) exhibit greater amounts of leaf area (LA) and greater amount of LA per unit plant biomass (LAR), 4) have greater slenderness ratio (SR), 5) allocate more biomass to stems than roots, 6) show no significant changes in  $P_n$  compared to the wider spacing, 7) have greater SLA, and 8) show greater photosynthetic N-use efficiency in the ambient light condition ( $PNUE_{\text{amb}}$ ). We also hypothesized that negative effects of competition, such as crown recession or decreased RCD and LA, would be reduced with N fertilization with 1) increased LA and 2) increased  $P_n$  associated with greater leaf N content per unit LA ( $N_{\text{area}}$ ).

## CHAPTER II

### SHORT-TERM GROWTH AND MORPHOLOGICAL RESPONSES TO PLANT DENSITY AND NITROGEN AVAILABILITY IN HYBRID POPLAR AND WILLOW CLONES

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Mamashita T made the experiment, data analyses, and wrote this manuscript.

## 2.1 Abstract

The main objective of this study was to determine morphological characteristics of clones driving to superior growth compared to other slower growing-clones under increased plant competition, with low or high nitrogen (N) availability. Seven hybrid poplar (915319; *Populus maximowiczii* × *P. balsamifera*, 747210; *P. balsamifera* × *P. trichocarpa*, 1081; *P. deltoides* × *P. balsamifera*, 2782; *P. tremuloides* × *P. tremula*, DN-74; *P. deltoides* × *P. nigra*, NM06; *P. maximowiczii* × *P. nigra*, and Walker; *P. deltoides* × *P. petrowskyana*) and five willow (HAN-A5; *Salix amygdaloides*, LEV-D5; *S. discolor*, SHE-E9; *S. eriocephala*, LAF-I4; *S. interior*, and TCH-N4; *S. nigra*) clones were grown under greenhouse conditions for 13 weeks at three spacings [20 (20×20), 35 (35×35), and 60 (60×60) cm] and two N levels (20 and 200 µg g<sup>-1</sup>). Compared to inferior clones 747210, 915319, LEV-D5, and LAF-I4, superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 had 143 %, 244 %, 129 %, 100 % and 16 % greater leafless aboveground biomass per tree (AGBT), AGBT under low N level, leafless aboveground biomass per m<sup>2</sup> (AGBM), leaf area (LA), and leaf area ratio (LAR), respectively, and 24 % lower root-to-shoot ratios (RSR). More productive clones also showed greater AGBT per unit increase in LA, suggesting greater shoot-growth capacity. Trees under the closest spacing and high N level had similar RCD, CW, and LA values and greater height than trees under the largest spacing and low N level, indicating that an addition of N can play an important role in increasing plant growth under tight spacings.



## Résumé

L'objectif principal de cette étude était de déterminer les caractéristiques morphologiques des clones conduisant à une croissance supérieure en conditions de compétition sévère avec de l'azote en faible ou haute (N) disponibilité. Sept peuplier hybride (915319; *Populus maximowiczii* × *P. balsamifera*, 747210; *P. balsamifera* × *P. trichocarpa*, 1081; *P. deltoides* × *P. balsamifera*, 2782; *P. tremuloides* × *P. tremula*, DN-74; *P. deltoides* × *P. nigra*, NM06; *P. maximowiczii* × *P. nigra*, and Walker; *P. deltoides* × *P. petrowskyana*) et cinq saule (HAN-A5; *Salix amygdaloides*, LEV-D5; *S. discolor*, SHE-E9; *S. eriocephala*, LAF-I4; *S. interior*, and TCH-N4; *S. nigra*) clones ont été cultivés en serre pendant 13 semaines sous trois espacements [20 (20 × 20), 35 (35 × 35), et 60 (60 × 60) cm] et deux niveaux (20 et 200 µg g<sup>-1</sup>) de N. Par rapport aux clones à plus faible croissance 747210, 915319, LEV-D5, et LAF-I4, les clones supérieurs 2782, NM06, HAN-A5, SHE-E9 et TCH-N4 ont produit 143%, 244%, 129% et 100% de plus de biomasse aérienne (sans feuillage) par arbre (AGBT), de AGBT sous le faible niveau de N, de biomasse aérienne par m<sup>2</sup> (AGBM) et de surface foliaire par arbre (LA). Le rapport de surface foliaire par unité biomasse produite (LAR) des clones supérieurs étaient 16% plus élevés que pour les clones moins productifs alors que le ratio racine:tige (RSR) était inférieur de 24%. Les clones les plus productifs avaient également une plus grande AGBT par augmentation d'unité de LA, ce qui suggère une plus grande capacité de croissance de la tige. Les arbres sous l'espacement de 20 cm et le haut niveau de N plus avaient des valeurs de RCD, CW, et LA similaires, et une plus grande hauteur, que les arbres dans l'espacement 60 cm sous le faible niveau de N, ce qui indique que l'ajout de N peut jouer un rôle important dans la croissance des arbres sous espacements réduits.

## 2.2 Introduction

There is growing interest in short rotation forestry (SRF) plantations of hybrid poplars and willows on agricultural or degraded forest lands across Canada. Woody crop biomass for bioenergy may potentially 1) enhance energy security as a fossil fuel substitute, 2) offset greenhouse gases produced by the use of fossil fuels, and 3) revitalize rural economies by increasing employment opportunities in the forest industry and providing a locally available and renewable energy source (Messier *et al.*, 2003; Tan *et al.*, 2008; Levin *et al.*, 2011). However, for economically feasible SRF plantations, silviculture costs (e.g., site preparation and maintenance costs) must be reduced and biomass production must be maximized by using the best available clones (McKenney *et al.*, 2011).

Among the factors that affect growth and associated biomass production, stand density and site nutritional status are critical for maximizing the productivity of plantations. When spacing decreases from 3 to 0.5 m between the trees, hybrid poplars (*Populus* spp.) and willows (*Salix* spp.) can show greater biomass production per unit area on short rotation period (Kopp *et al.*, 1996; Larocque, 1999; Fang, *et al.*, 1999; Proe *et al.*, 2002; Benomar *et al.*, 2012). When tree competition occurs in dense stands where available resources per tree are limited, large amounts of carbohydrates are generally allocated to height growth at the expense of diameter growth in *Populus* spp., resulting in an increase in slenderness ratio (SL) (= stem height/diameter at breast height or root collar diameter) (DeBell *et al.*, 1996; Benomar *et al.*, 2012). As a result, these trees can achieve better light interception by sun leaves on the long (indeterminate) shoots of the upper crowns for increasing photosynthetic capacity under high plant competition (DeBell *et al.*, 1996; Dickmann *et al.*, 2001). *Populus* trees can also increase their photosynthetic capacity by optimizing spatial distribution of foliage for irradiance interception within a crown (Ceulemans *et al.*, 1990; Mitchell *et al.*, 1992; Casella and Sinoquet, 2003) and

efficiently increase whole-plant leaf area (LA) per unit plant biomass (LAR) under high competition (Larocque, 1999). However, intense plant competition can also cause nutritional deficiencies, particularly in nitrogen (N), resulting in decreasing amounts of harvestable biomass per tree (Hansen *et al.*, 1988). Thus, the addition of N fertilization can contribute to reduce negative effects of competition on plant growth as N can increase leaf area and overall plant growth and decrease the ratio of root-to-shoot under close spacing (< 3 m) of SRF management (Isebrands and Nelson, 1983; Dickson, 1989; Pregitzer *et al.*, 1990; Coleman *et al.*, 2004; Brown and van den Driessche, 2005).

Currently, there is a lack of understanding on growth responses of hybrid poplars and willows when growing under different intensities of competition and N availability. Previous studies reported that various clones demonstrated differences in growth patterns under changes in tree density or N availability, partly resulting from genotypic differences in carbohydrate partitioning (Pregitzer *et al.*, 1990; Fang *et al.*, 1999; Weih and Rönnerberg-Wästjung, 2007; Benomar *et al.*, 2012). For example, clone MB915 (*P. maximowiczii* × *P. balsamifera*) showed faster growth than clone BT747 (*P. balsamifera* × *P. trichocarpa*) under relatively close spacing (1 × 1 m), because MB915 allocated a greater proportion of biomass to longer stems and branches supporting more leaf area, resulting in greater total above-ground biomass (leafless) (Benomar *et al.*, 2012). Plants that can display plastic responses of biomass allocation to their environment can enhance growth efficiency and maximize productivity (Pregitzer *et al.*, 1990).

Growth information using desirable morphological and physiological traits may be useful for early clone selection and thereby save time and expenses (Lamhamedi *et al.*, 2007). Conventional selection processes to identify the most productive hybrid poplars and willows are based on field comparisons for biomass productivity over several years. However, this traditional approach is time consuming and requires

considerable silviculture investments. For northern latitudes, short growing seasons (3 to 4 months) decrease the duration of the selection process, particularly in the boreal forest (Weih, 2004). Therefore, the development of methodologies and procedures that could accelerate the selection process of the most productive clones would have considerable benefits. Using greenhouse facilities, it is possible to perform experimentation all year long. Although field studies remain necessary to examine adaptability to local field conditions and disease resistance, the primary advantage of controlled environments is to be able to provide more consistent results than field studies. Greenhouse conditions can reduce the amplitude of natural variability compared to field conditions (William *et al.*, 1999), which facilitates the relationships between growth and morphological attributes.

The objective of this study was to determine morphological characteristics of clones leading to superior growth performance under intensive plant competition, and to see how these characteristics vary under high or low N level. We hypothesized that the clones with superior yield performance under high competition would 1) show no significant changes in leafless aboveground biomass per tree (AGBT) under a tight spacing, 2) have high AGBT per unit increase in LA, 3) exhibit greater leaf area (LA), LA per unit plant biomass (LAR), slenderness ratio (SR), and lower root-to-shoot ratios (RSR) than least performing clones. We also hypothesized that negative effects of competition, such as crown recession or decreased RCD, would be reduced with N fertilization.

## 2.3 Materials and methods

### 2.3.1 Experimental design and measurements

The research trial took place in a greenhouse at the Laurentian Forestry Centre in Quebec (46°46'N, 71°16'W). The experimental design was set up as an incomplete block design with seven hybrid poplar clones (915319; *Populus maximowiczii* × *P. balsamifera*, 747210; *P. balsamifera* × *P. trichocarpa*, 1081; *P. deltoides* × *P.*

*balsamifera*, 2782; *P. tremuloides* × *P. tremula*, DN-74; *P. deltooides* × *P. nigra*, NM06; *P. maximowiczii* × *P. nigra*, and Walker; *P. deltooides* × *P. petrowskyana*) and five willow clones (HAN-A5; *Salix amygdaloides*, LEV-D5; *S. discolor*, SHE-E9; *S. eriocephala*, LAF-I4; *S. interior*, and TCH-N4; *S. nigra*). Within each repetitive block (growing period of 13 weeks), each combination of 6 clones, 2 fertilization levels [20 and 200  $\mu\text{g g}^{-1}$  of nitrogen (N)] and 3 spacings [20 (20 × 20), 35 (35 × 35), 60 (60 × 60) cm] was assigned to 36 plant pots, which were randomly positioned in the greenhouse. The plant pot sizes were 1 × 1 × 0.4 m for the 20 cm spacing, 1.4 × 1.4 × 0.4 m for the 35 cm spacing, and 1.2 × 1.2 × 0.4 m for the 60 cm spacing. Each combination of clones, spacings and fertilizer levels was replicated 3 times, once in each of 3 different blocks. For the 20 and 35 cm spacings, 16 cuttings of 10 cm in length were planted per plant pot and, while 5 cuttings were planted per pot for the 60 cm spacing. Trees from clone 2782 (hybrid aspen) were 20 cm long rooted plants from in-vitro culture as aspens are difficult to root from cuttings (Stanturf *et al.*, 2001).

All macro and micro nutrients were supplied twice a week by an irrigation system (O'Jet Olson Bleu 300, Harnois Inc., St-Thomas, QC, Canada). Two levels of N (20 and 200  $\mu\text{g g}^{-1}$ ) were applied as ammonium-nitrate ( $\text{NH}_4\text{NO}_3$ ). A 100 mL aliquot per tree supplied 2 mg N (low level) or 20 mg N (high level) per tree. Phosphorus (1.5 g P) was provided as monobasic calcium orthophosphate [ $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ] for each cutting. Calcium (1.55 g Ca) per cutting was supplied as above for calcium orthophosphate as well as  $\text{CaCO}_3$  for pH control. Potassium (50 mg K), magnesium (11.9 mg Mg), and sulfur (15.7 mg S) of macronutrient solutions were supplied on each tree with  $\text{K}_2\text{SO}_4$  and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . Micronutrients were also provided per cutting, including 1.35 mg of iron (chelated Fe), 0.5 mg of manganese (Mn), 0.013 mg of molybdenum (Mo), 0.04 mg of copper (Cu) and 0.39 mg of boron (B).

Temperature was set at 24 C° during daylight and 18 C° during dark period. Daily photoperiod was set at 16 h (0600-2200 h) using natural light, but supplemented with artificial light (400 W high pressure sodium bulbs, Litmore Distributors Ltd., Edmonton, Alta.) when necessary. Soil pH in the boxes ranged 5.5-7.

Root collar diameter (RCD) ( $\pm 1$  mm), height ( $\pm 1$  cm), and crown width (CW: the average length of two perpendicular lines across the crown area) ( $\pm 1$  cm) were measured on 4 central cuttings in the 20 and 35 cm spacings and one central cutting in the 60 cm spacing, every two weeks. Cuttings that had failed to root in the first few weeks after planting were replaced to maintain competition levels but the replaced trees were not included in the analyses. After three months, the cuttings in the centre of the plant pots were harvested and stems (including branches), roots and leaves were separated. Leaf area (LA) was measured with a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NE, USA). Then, stems, roots and leaves were oven-dried at 55°C until no change in mass was detected.

### 2.3.2 Data analyses

Relative growth rate (RGR) is a growth-efficiency measure that calculates the amount of growth per unit of time adjusted by the accumulated size (Kozłowski and Pallardy, 1997). RGR was calculated as:

$$(\ln W_2 - \ln W_1) / (T_2 - T_1) \dots \dots \dots \text{Eq 1}$$

where  $W_2$  and  $W_1$  represent RCD or height at the time of growing period  $T_2$  and  $T_1$ , respectively. The slenderness ratio (SR), calculated as height (cm)/RCD (mm), is a competitive status indicator of individual tree (Benomar *et al.*, 2012). Leaf area ratio (LAR) estimates photosynthetic biomass relative to respiring biomass (Hunt, 1982). It was calculated as the ratio of leaf area (cm<sup>2</sup>) to total biomass (g). The root shoot ratio (RSR), which represents the proportion of absorbing surface to transpiring

surface (Kozłowski and Pallardy, 1997), was calculated as root biomass (g)/stem and leaf biomass (g). For the proportion of biomass allocation, percentages of biomass for leaves, stems, and roots were calculated by dividing the biomass of each component by the total biomass. All the analyses were performed at the genus and clonal levels. Genus level represented the respective means of hybrid poplar and willow clones.

All data were analyzed using the Mixed Procedure in SAS (version 9.2, SAS Institute, Cary, NC, USA). The model contains fixed effects for genus or clone, nitrogen level, spacing, and their interactions in a factorial treatment structure. In this model, the dependent variables were RCD, height, CW, morphological measures, above-ground biomass, and proportion (%) of biomass allocated to stems, roots, and leaves measured at the end of growing period. The general linear model was as follows:

$$Y_{BGCNSP} = \mu + E_B + \beta_{GC} + \beta_N + \beta_S + \beta_{GC*N} + \beta_{N*S} + \beta_{GC*S} + \beta_{GC*N*S} + E_P + E_{GC*N*S*P} + E_r \dots \dots \dots \text{Eq2}$$

where  $Y_{BGCNSP}$  is the dependent variable,  $\mu$  the overall mean,  $\beta_{GC}$  the fixed effect of genus or clone,  $\beta_N$  the fixed effect of nitrogen,  $\beta_S$  the fixed effect of spacing,  $\beta_{GC*N}$  the genus or clone by nitrogen interaction,  $\beta_{N*S}$  the nitrogen by spacing interaction,  $\beta_{GC*S}$  the genus or clone by spacing interaction, and  $\beta_{GC*N*S}$  the genus or clone by nitrogen by spacing interaction.  $E_B$ ,  $E_P$ , and  $E_{GC*N*S*P}$ , are the random effects for block, plant pot, and tree, respectively.  $E_r$  is the residual error.

RCD, height, CW, RCD RGR, and height RGR measured every two weeks were also subjected to repeated-measures analysis using the following mixed model:

$$Y_{BGCNSPT} = \mu + E_B + \beta_{GC} + \beta_N + \beta_S + \beta_{GC*N} + \beta_{N*S} + \beta_{GC*S} + \beta_{GC*N*S} + E_P + E_{GC*N*S*P} + \beta_T + \beta_{GC*T} + \beta_{N*T} + \beta_{GC*N*T} + \beta_{S*T} + \beta_{N*S*T} + \beta_{GC*S*T} + \beta_{GC*N*S*T} + E_{P*T} + E_r \dots \dots \dots \text{Eq3}$$

where  $Y_{\text{BGCNSPT}}$  is the dependent variable,  $\beta_T$  is the fixed effect of time,  $\beta_{\text{GC}^*T}$  the genus or clone by time interaction,  $\beta_{\text{N}^*T}$  the nitrogen by time interaction,  $\beta_{\text{GC}^*\text{N}^*T}$  the genus or clone by nitrogen by time interaction,  $\beta_{\text{S}^*T}$  the spacing by time interaction,  $\beta_{\text{N}^*\text{S}^*T}$  the nitrogen by spacing by time interaction,  $\beta_{\text{GC}^*\text{S}^*T}$  the genus or clone by spacing by time interaction,  $\beta_{\text{GC}^*\text{N}^*\text{S}^*T}$  the genus or clone by nitrogen by spacing by time interaction, and  $E_{\text{P}^*T}$  the random time specific for plant pot. The other parameters are defined in Eq 2.

Linear regression analysis was undertaken to verify relationships between LA and RCD or height or CW of all treatments and each clone. Slopes among treatments were compared using analysis of covariance (ANCOVA). Based on morphological characteristics such as leafless aboveground biomass per tree (AGBT), leafless aboveground biomass per  $\text{m}^2$  (AGBM), LA, LAR, SR, and RSR, more productive or superior and less productive or inferior clones as a group were identified. Then, linear regression analysis was also used to verify relationships between AGBT and LA for superior clones and inferior clones as a group. ANCOVA was also used to compare the slopes between superior and inferior clones.

## 2.4 Results

### 2.4.1 Growth development

At the end of the 13 weeks growing period, hybrid poplars at the genus level had significantly greater RCD and smaller CW than willow clones, while there was no significant difference in height between the two genera (Tables 2.1; 2.2). The greatest RCD, height, and CW were 13.5 mm for SHE-E9, 162 cm for DN-74 and HAN-A5, and 101 cm for HAN-A5, respectively, in the 60 cm spacing and  $200 \mu\text{g g}^{-1}$  N (Table 2.2).



The increase in N significantly increased RCD, height and CW after the 5<sup>th</sup> weeks of growing period, followed by greater differences between high and low N level by the end of growing period (significant  $N \times T$  interaction, Fig. 2.1ace; Table 2.1). The increase in spacing also increased RCD after 7 weeks of growing period, height after 11 weeks of growing period, and CW after 5 weeks of growing period (significant  $S \times T$  interaction, Fig. 2.1bdf; Table 2.1). Both hybrid poplar and willow groups responded similarly in RCD, height, and CW to changes in N and spacing level (non-significant  $G \times N$ ;  $G \times S$  interactions, Table 2.1), although there were variations among individual clones in RCD and height with N level and spacing (significant  $C \times N$ ;  $C \times S$  interactions, Table 2.1). There were also significant three way interactions ( $C \times N \times S$ ) on RCD, height, and CW at the clonal level (Tables 2.1; 2.2).

During the growing period, the pattern of change in RCD RGR differed significantly between hybrid poplars and willows and among clones ( $G \times T$ ;  $C \times T$  interactions, Fig. 2.2ab and Table 2.1). RGR for hybrid poplars peaked in the first 3 weeks, followed by a rapid decrease and subsequently, relatively similar values for the remainder of the growing period (Fig. 2.2a). Clone 2782 initially showed the most rapid development of RCD RGR (Fig. 2.2b). On the other hand, willow clones as a group initially had slow development of RCD RGR, followed by rapid increases (Fig. 2.2a). This trend was noticeably evident for willow clone SHE-E9 (Fig. 2.2b). Height RGR for both hybrid poplars and willows initially peaked and then, gradually declined over time (Fig. 2.2c). However, there were slight but significant variations in height RGR over time between hybrid poplars and willows under the different spacings ( $G \times S \times T$  interaction, Fig. 2.3ab; Table 2.1). At the clonal level, height RGR for individual clones significantly differed over time ( $C \times T$  interaction, Fig. 2.2d and Table 2.1).

Table 2.1  $F$  values, and associated probabilities for root collar diameter (RCD), height, crown width (CW), RCD relative growth rate (RGR), and height RGR at the genus and clonal levels.

Source of variation	DF	RCD		Height		CW		RCDRGR		HeightRGR	
		$F$	$P$ value	$F$	$P$ value	$F$	$P$ value	$F$	$P$ value	$F$	$P$ value
G	1	26.9	<b>0.0002</b>	1.5	0.2498	6.9	<b>0.01</b>	23.5	<b>&lt;0.0001</b>	4.5	<b>0.03</b>
N	1	36.5	<b>&lt;0.0001</b>	37.2	<b>&lt;0.0001</b>	24.3	<b>0.0005</b>	32.3	<b>&lt;0.0001</b>	58.0	<b>&lt;0.0001</b>
S	2	3.3	0.0741	0.8	0.4714	7.9	<b>0.0077</b>	13.4	<b>&lt;0.0001</b>	17.5	<b>&lt;0.0001</b>
N × S	2	0.3	0.7483	0.1	0.8776	1.0	0.4048	0.3	0.7804	0.7	0.4984
G × N	1	0.6	0.4481	0.3	0.6281	2.2	0.1699	0.0	0.9234	0.0	0.8655
G × S	2	1.1	0.3543	0.2	0.8226	0.5	0.6239	1.3	0.2863	0.7	0.519
G × N × S	2	1.3	0.3007	0.7	0.5322	2.6	0.1177	0.7	0.4937	0.5	0.6292
T	5	553.3	<b>&lt;0.0001</b>	169.7	<b>&lt;0.0001</b>	152.2	<b>&lt;0.0001</b>	52.0	<b>&lt;0.0001</b>	555.9	<b>&lt;0.0001</b>
G × T	5	10.6	<b>&lt;0.0001</b>	0.8	0.5188	5.1	<b>0.0001</b>	54.9	<b>&lt;0.0001</b>	4.8	<b>0.0002</b>
N × T	5	6.1	<b>&lt;0.0001</b>	10.9	<b>&lt;0.0001</b>	5.0	<b>0.0002</b>	0.9	0.487	2.4	<b>0.03</b>
S × T	10	3.5	<b>0.0002</b>	2.9	<b>0.0014</b>	3.8	<b>&lt;0.0001</b>	0.9	0.5431	3.0	<b>0.0009</b>
N × S × T	10	0.2	0.9931	0.2	0.9975	0.7	0.7568	1.0	0.4897	0.6	0.7877
G × N × T	5	0.3	0.8907	0.3	0.9407	1.5	0.1902	0.4	0.8759	1.6	0.163
G × S × T	10	0.1	0.9998	0.4	0.9494	0.3	0.9847	0.5	0.8829	1.9	<b>0.0356</b>
G × N × S × T	10	0.5	0.9109	0.4	0.9311	0.7	0.758	0.8	0.6473	0.2	0.9955
C	11	14.4	<b>&lt;0.0001</b>	13.0	<b>&lt;0.0001</b>	13.6	<b>&lt;0.0001</b>	17.8	<b>&lt;0.0001</b>	10.2	<b>&lt;0.0001</b>
C × N	11	4.7	<b>&lt;0.0001</b>	2.5	<b>0.006</b>	0.8	0.5969	0.8	0.6734	0.5	0.899
C × S	22	2.8	<b>&lt;0.0001</b>	1.7	<b>0.02</b>	1.1	0.3283	0.5	0.9774	0.4	0.9938
C × N × S	22	2.7	<b>0.0001</b>	2.4	<b>0.0004</b>	2.7	<b>0.0001</b>	0.5	0.9531	0.2	1
C × T	55	9.0	<b>&lt;0.0001</b>	6.2	<b>&lt;0.0001</b>	22.4	<b>&lt;0.0001</b>	23.7	<b>&lt;0.0001</b>	13.0	<b>&lt;0.0001</b>
C × N × T	55	1.3	0.1024	0.5	0.9995	1.6	0.0809	0.3	0.984	0.3	0.984
C × S × T	110	0.6	0.9994	0.4	1	2.0	<b>0.0029</b>	0.8	0.75	0.4	0.997

Notes: In source of variation, G: genus (hybrid poplars vs willows), N: nitrogen, S: spacing, T: time, and C: clone. Significant  $P$  values are indicated in bold.

Table 2.2 Growth characteristics of seven hybrid poplar and five willow clones at three spacings (20, 35, and 60 cm) and two nitrogen levels (20 and 200  $\mu\text{g g}^{-1}$ ) after 13 weeks of growth.

Clone	Spacing (cm)-N ( $\mu\text{g g}^{-1}$ )					
	20-20	20-200	35-20	35-200	60-20	60-200
<b>Root collar diameter (mm)</b>						
1081	7.4 <sup>a</sup>	7.3 <sup>a</sup>	8 <sup>a</sup>	8.6 <sup>a</sup>	7.6 <sup>a</sup>	8.4 <sup>a</sup>
2782	7.9 <sup>a</sup>	8.3 <sup>ac</sup>	7.9 <sup>ab</sup>	9.8 <sup>ad</sup>	10.9 <sup>bcd</sup>	12.5 <sup>d</sup>
747210	5.3 <sup>a</sup>	6.3 <sup>a</sup>	7.5 <sup>a</sup>	8.1 <sup>a</sup>	6.1 <sup>a</sup>	7.1 <sup>a</sup>
915319	6.4 <sup>a</sup>	7.2 <sup>ab</sup>	6.1 <sup>a</sup>	9.4 <sup>b</sup>	6.3 <sup>a</sup>	8.9 <sup>ab</sup>
DN-74	5.7 <sup>a</sup>	9 <sup>bc</sup>	7.6 <sup>ab</sup>	11.1 <sup>bc</sup>	7.2 <sup>ab</sup>	12.9 <sup>f</sup>
NM06	7.1 <sup>a</sup>	9.1 <sup>ab</sup>	8.3 <sup>ab</sup>	11 <sup>b</sup>	9.7 <sup>ab</sup>	10.7 <sup>b</sup>
Walker	6.3 <sup>a</sup>	7.7 <sup>ab</sup>	6.3 <sup>a</sup>	11.7 <sup>c</sup>	9.2 <sup>ac</sup>	10.4 <sup>bc</sup>
<b>Hybrid poplar mean</b>	<b>6.6<sup>a</sup></b>	<b>7.9<sup>b</sup></b>	<b>7.4<sup>ab</sup></b>	<b>10<sup>c</sup></b>	<b>8.1<sup>b</sup></b>	<b>10.1<sup>c</sup></b>
HAN-A5	5.8 <sup>a</sup>	7.2 <sup>a</sup>	8.1 <sup>ab</sup>	7.6 <sup>ab</sup>	5.5 <sup>a</sup>	10.6 <sup>b</sup>
LEV-D5	4.9 <sup>a</sup>	7.5 <sup>ac</sup>	5.2 <sup>ab</sup>	8.4 <sup>c</sup>	6.2 <sup>ac</sup>	7.9 <sup>bc</sup>
SHE-E9	10.2 <sup>ab</sup>	8.5 <sup>a</sup>	10.5 <sup>ab</sup>	11 <sup>ab</sup>	13.5 <sup>b</sup>	12.6 <sup>b</sup>
LAF-I4	5.7 <sup>ab</sup>	7.7 <sup>a</sup>	6.1 <sup>ab</sup>	7.7 <sup>a</sup>	4 <sup>b</sup>	7.6 <sup>a</sup>
TCH-N4	6.4 <sup>a</sup>	7.8 <sup>ab</sup>	6.9 <sup>ab</sup>	8.5 <sup>b</sup>	8.8 <sup>ab</sup>	9.8 <sup>b</sup>
<b>Willow mean</b>	<b>6.6<sup>a</sup></b>	<b>7.8<sup>bc</sup></b>	<b>7.3<sup>ab</sup></b>	<b>8.6<sup>d</sup></b>	<b>7.6<sup>ab</sup></b>	<b>9.7<sup>d</sup></b>
<b>Height (cm)</b>						
1081	88.4 <sup>a</sup>	87.2 <sup>a</sup>	88.8 <sup>a</sup>	99.2 <sup>a</sup>	76 <sup>a</sup>	96.3 <sup>a</sup>
2782	123 <sup>a</sup>	140.9 <sup>a</sup>	117.6 <sup>a</sup>	152 <sup>a</sup>	152.3 <sup>a</sup>	159 <sup>a</sup>
747210	66 <sup>a</sup>	100.8 <sup>a</sup>	105.8 <sup>a</sup>	109.4 <sup>a</sup>	77.7 <sup>a</sup>	94 <sup>a</sup>
915319	85.5 <sup>ab</sup>	100.6 <sup>b</sup>	73.1 <sup>ab</sup>	114.4 <sup>b</sup>	62.3 <sup>a</sup>	107.7 <sup>b</sup>
DN-74	77.4 <sup>a</sup>	129.7 <sup>bc</sup>	98.7 <sup>a</sup>	156.9 <sup>c</sup>	102.7 <sup>ab</sup>	162 <sup>c</sup>
NM06	106.3 <sup>a</sup>	138.4 <sup>ab</sup>	101 <sup>a</sup>	159.6 <sup>b</sup>	129.3 <sup>ab</sup>	146.3 <sup>b</sup>
Walker	74 <sup>a</sup>	114.5 <sup>bc</sup>	66.6 <sup>a</sup>	157.4 <sup>c</sup>	100 <sup>ab</sup>	146 <sup>bc</sup>
<b>Hybrid poplar mean</b>	<b>89.4<sup>a</sup></b>	<b>116.9<sup>b</sup></b>	<b>93.8<sup>a</sup></b>	<b>135.6<sup>b</sup></b>	<b>100.1<sup>a</sup></b>	<b>129.4<sup>b</sup></b>
HAN-A5	93.5 <sup>a</sup>	125.1 <sup>ab</sup>	124.1 <sup>ab</sup>	126 <sup>b</sup>	88.7 <sup>a</sup>	162 <sup>b</sup>
LEV-D5	49.2 <sup>a</sup>	87.3 <sup>bc</sup>	61.6 <sup>ab</sup>	94.2 <sup>c</sup>	67.8 <sup>ab</sup>	114 <sup>c</sup>
SHE-E9	109 <sup>a</sup>	116.3 <sup>a</sup>	125.7 <sup>a</sup>	137.1 <sup>a</sup>	145.4 <sup>a</sup>	145.8 <sup>a</sup>
LAF-I4	73.7 <sup>ab</sup>	110.8 <sup>b</sup>	88.4 <sup>b</sup>	117.9 <sup>b</sup>	43.8 <sup>a</sup>	100.6 <sup>b</sup>
TCH-N4	85.9 <sup>a</sup>	111.6 <sup>ab</sup>	89.6 <sup>ab</sup>	111.9 <sup>b</sup>	108 <sup>ab</sup>	125.2 <sup>b</sup>
<b>Willow mean</b>	<b>82.1<sup>a</sup></b>	<b>110.6<sup>bc</sup></b>	<b>96.1<sup>ab</sup></b>	<b>117.4<sup>c</sup></b>	<b>90.8<sup>ab</sup></b>	<b>129.5<sup>c</sup></b>
<b>Crown width (cm)</b>						
1081	35.9 <sup>a</sup>	35.5 <sup>a</sup>	38.2 <sup>a</sup>	43 <sup>a</sup>	39 <sup>a</sup>	46.8 <sup>a</sup>
2782	29.2 <sup>a</sup>	27.9 <sup>a</sup>	36.3 <sup>ab</sup>	47.7 <sup>bc</sup>	63.8 <sup>bc</sup>	67.2 <sup>c</sup>
747210	28.3 <sup>a</sup>	34 <sup>ab</sup>	34.9 <sup>ab</sup>	36.6 <sup>ab</sup>	32.7 <sup>ab</sup>	47 <sup>b</sup>
915319	25.4 <sup>a</sup>	32 <sup>ab</sup>	25.8 <sup>a</sup>	56.5 <sup>b</sup>	28.3 <sup>ab</sup>	36.8 <sup>b</sup>
DN-74	26.2 <sup>a</sup>	32.5 <sup>ab</sup>	33.3 <sup>a</sup>	37.9 <sup>ab</sup>	31.8 <sup>a</sup>	47.2 <sup>b</sup>
NM06	30.2 <sup>a</sup>	34.3 <sup>ab</sup>	28.9 <sup>a</sup>	37.5 <sup>ab</sup>	41.3 <sup>ab</sup>	43 <sup>b</sup>
Walker	28.3 <sup>ab</sup>	29.5 <sup>ab</sup>	25 <sup>a</sup>	38.9 <sup>b</sup>	34 <sup>ab</sup>	40 <sup>b</sup>
<b>Hybrid poplar mean</b>	<b>29.1<sup>a</sup></b>	<b>32.3<sup>ab</sup></b>	<b>32<sup>ab</sup></b>	<b>43.4<sup>c</sup></b>	<b>39<sup>bc</sup></b>	<b>47.2<sup>c</sup></b>
HAN-A5	37.9 <sup>a</sup>	48.8 <sup>b</sup>	56.3 <sup>b</sup>	46.8 <sup>b</sup>	33.5 <sup>a</sup>	101.4 <sup>c</sup>
LEV-D5	19.1 <sup>a</sup>	28.3 <sup>ab</sup>	21.5 <sup>a</sup>	32.2 <sup>b</sup>	25.7 <sup>ab</sup>	29.8 <sup>b</sup>
SHE-E9	39.8 <sup>a</sup>	37.5 <sup>a</sup>	51.4 <sup>a</sup>	42.6 <sup>a</sup>	44.8 <sup>a</sup>	48.9 <sup>a</sup>
LAF-I4	28.4 <sup>ab</sup>	36.7 <sup>ab</sup>	32.5 <sup>ab</sup>	39.6 <sup>b</sup>	27.3 <sup>a</sup>	38.7 <sup>b</sup>
TCH-N4	41.3 <sup>a</sup>	45.4 <sup>ab</sup>	51.2 <sup>bc</sup>	64 <sup>cd</sup>	81.8 <sup>cd</sup>	79.8 <sup>d</sup>
<b>Willow mean</b>	<b>33.2<sup>a</sup></b>	<b>39.6<sup>b</sup></b>	<b>41<sup>b</sup></b>	<b>45.1<sup>b</sup></b>	<b>42.6<sup>b</sup></b>	<b>59.7<sup>c</sup></b>

Note: Within a row, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

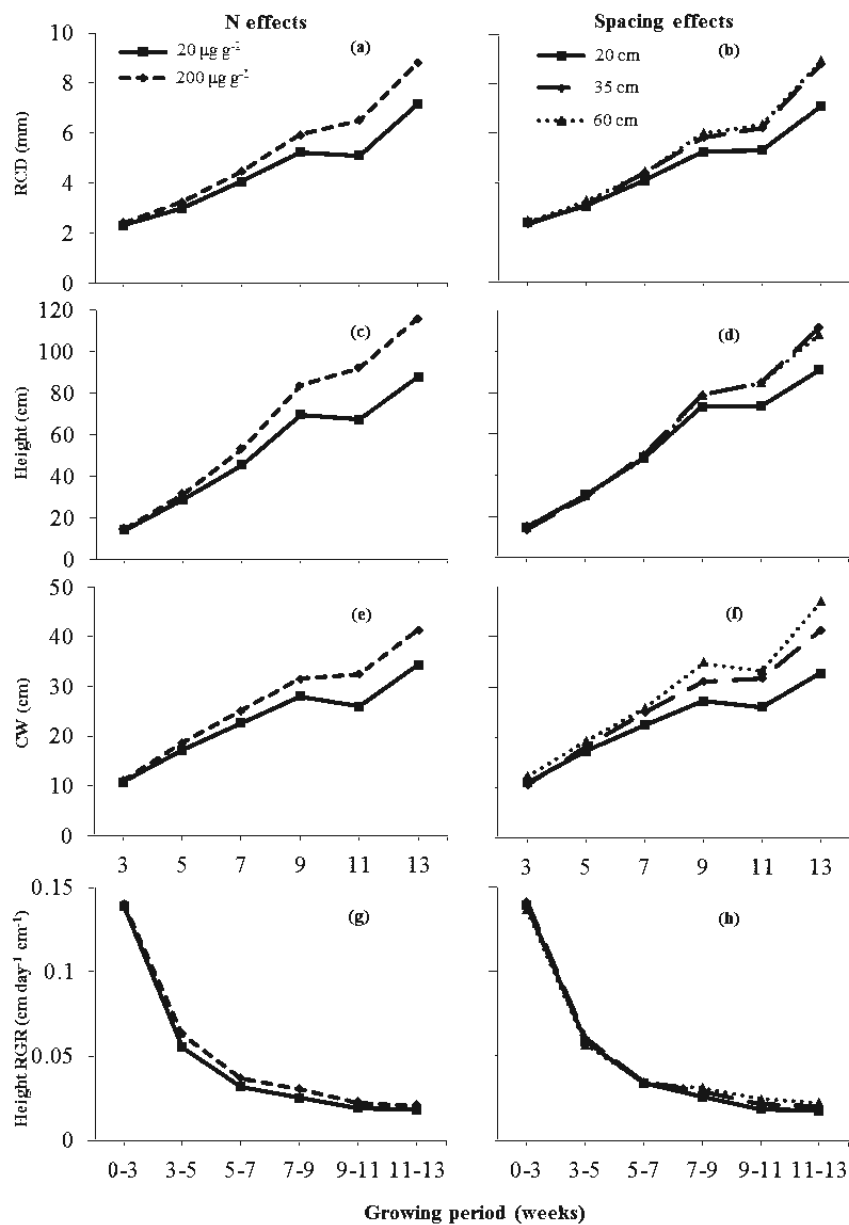


Figure 2.1 Changes in mean root collar diameter (RCD), height, crown width (CW), and height relative growth rate (RGR) for all clones under two N levels (20 and 200  $\mu\text{g g}^{-1}$ ) (a) (c) (e) (g) and three spacings (20, 35, and 60 cm) (b) (d) (f) (h) during 13 growing weeks.

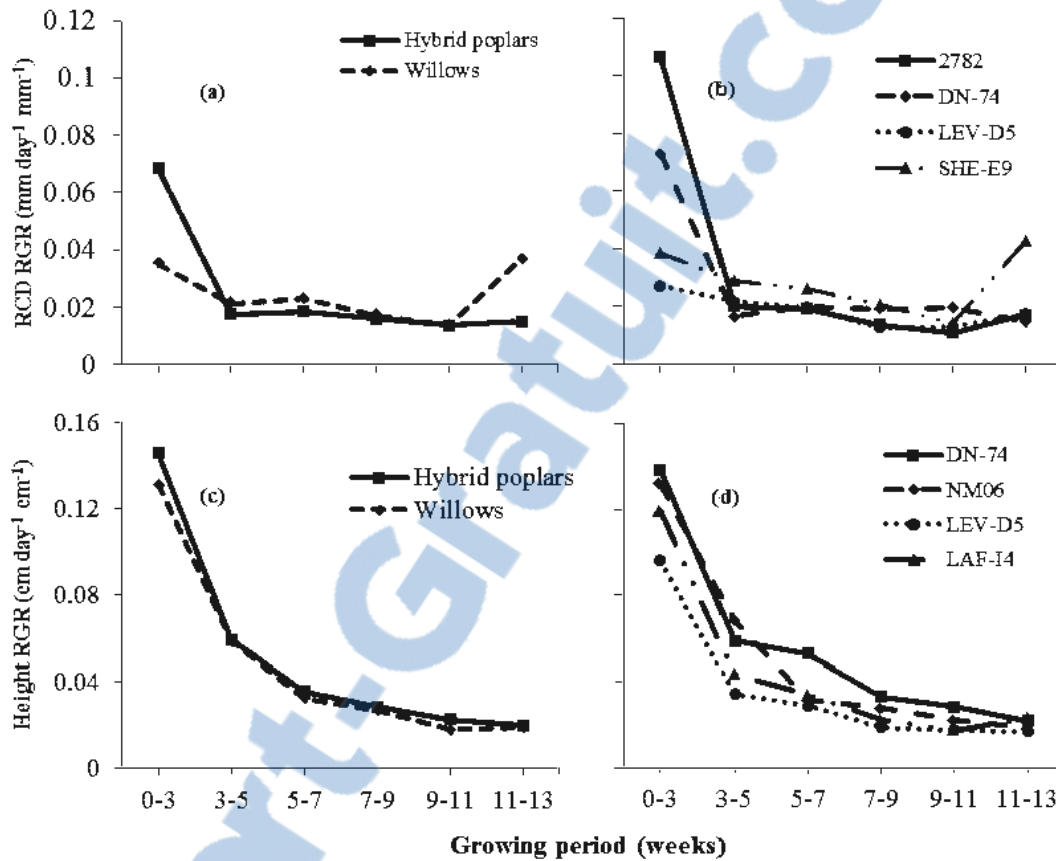


Figure 2.2 Changes in mean root collar diameter (RCD) relative growth rate (RGR) and height RGR for hybrid poplars and willows (a) (c) and for four hybrid poplar and willow clones (b) (d) during 13 growing weeks.

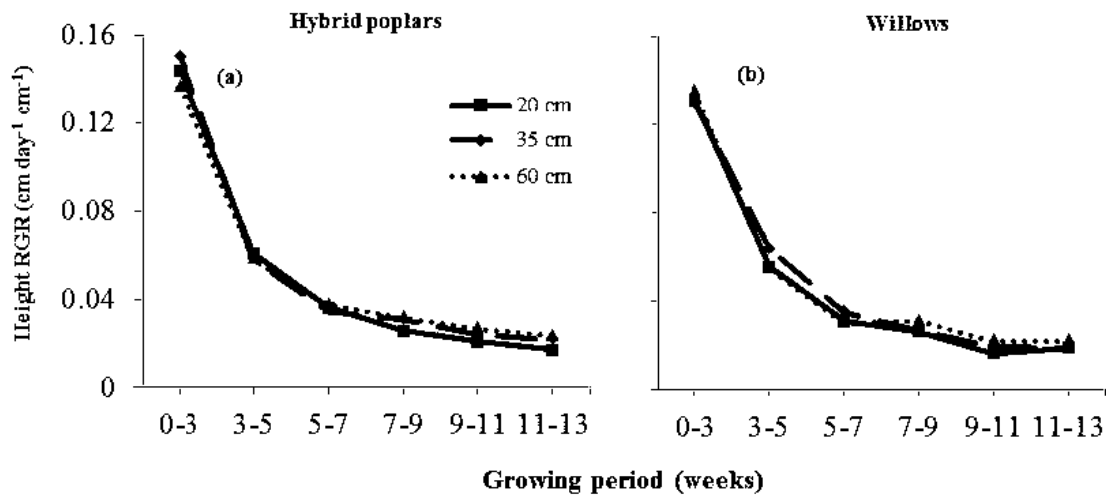


Figure 2.3 Changes in height relative growth rate (RGR) under three spacings (20, 35, and 60 cm) for hybrid poplars (a) and willows (b) during 13 growing weeks.

#### 2.4.2 Morphological characteristics

The high level nitrogen (N) addition caused an increase in leaf area (LA) differently under the different spacings such that it had a greater effect under the large spacing (N × S interaction; Fig. 2.4 and Table 2.3). LA significantly decreased with the decrease in spacing by 50 % from 60 to 20 cm spacing, but increased with N level by 30, 47, and 88 % in the 20, 35, and 60 cm spacings, respectively (Fig. 2.4). At the genus level, there was no significant difference in LA between hybrid poplars and willows (Table 2.3). At the clonal level, there were significant differences in LA among clones from 1229.6 cm<sup>2</sup> for LEV-D5 to 4146.8 cm<sup>2</sup> for NM06 (Table 2.4). Although LA significantly increased with higher N level, the amount of increasing LA varied among clones (Table 2.5). The higher N level significantly increased LA for 747210, 915319, DN-74, Walker, HAN-A5, LEV-D5, and LAF-14.

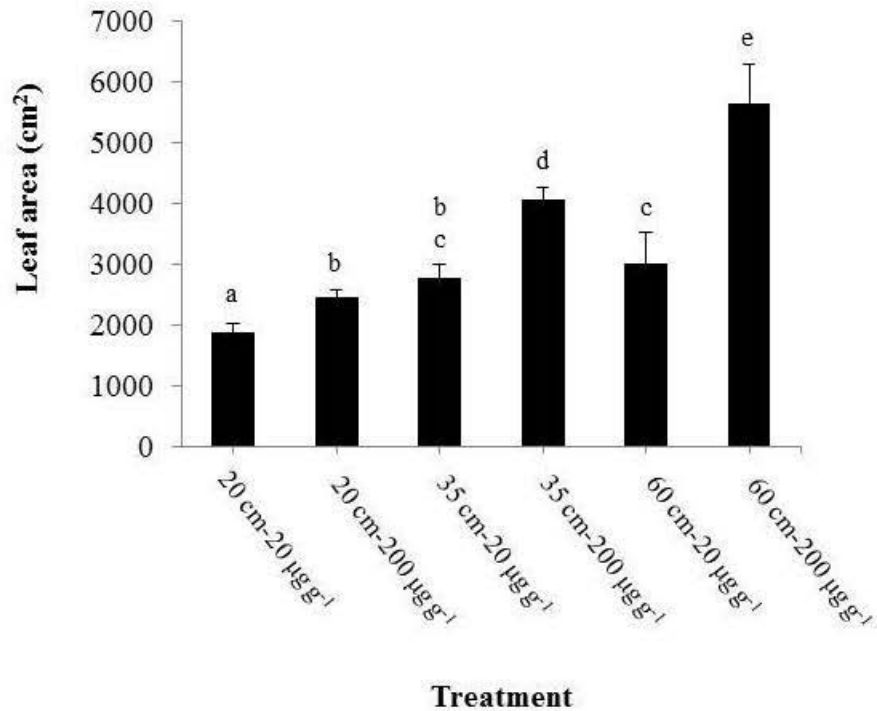


Figure 2.4 Mean leaf area for all clones under three spacings (20, 35, and 60 cm) and two N levels (20 and 200  $\mu\text{g g}^{-1}$ ) after 13 growing weeks. Means followed by the same letter on the bar do not significantly differ at  $\alpha = 0.05$ .

Table 2.3 Analysis of variance giving sources of variation, degrees of freedom (DF), *F* values, and associated probabilities for morphological characteristics of leaf area (LA), leaf area ratio (LAR), slenderness ratio (SR), and root-to-shoot ratios (RSR).

Source of variation	DF	LA		LAR		SR		RSR	
		<i>F</i>	<i>P value</i>	<i>F</i>	<i>P value</i>	<i>F</i>	<i>P value</i>	<i>F</i>	<i>P value</i>
G	1	3.2	0.073	58.7	< <b>0.0001</b>	20.6	< <b>0.0001</b>	0.0	0.884
N	1	65.8	< <b>0.0001</b>	6.9	<b>0.009</b>	10.8	<b>0.001</b>	33.3	< <b>0.0001</b>
S	2	33.8	< <b>0.0001</b>	0.5	0.589	3.7	<b>0.02</b>	0.5	0.626
G × N	1	0.0	0.8773	0.9	0.337	0.0	0.968	0.2	0.632
G × S	2	2.1	0.128	1.3	0.278	3.1	<b>0.04</b>	1.6	0.203
N × S	2	4.3	<b>0.01</b>	0.1	0.869	0.6	0.56	0.0	0.959
G × N × S	2	1.7	0.186	0.4	0.666	0.7	0.523	0.4	0.645
C	11	5.0	< <b>0.0001</b>	6.7	< <b>0.0001</b>	4.7	< <b>0.0001</b>	6.1	< <b>0.0001</b>
C × N	11	2.2	<b>0.016</b>	0.9	0.584	0.7	0.7552	1.8	0.056
C × S	22	1.0	0.426	1.3	0.165	0.6	0.922	0.9	0.649
C × N × S	22	1.4	0.14	1.2	0.234	0.7	0.844	0.6	0.941

Notes: In source of variation, G: genus (hybrid poplars vs willows), N: nitrogen, S: spacing, and C: clone. Significant *P* values are indicated in bold.



Table 2.4 Clonal differences in mean leaf area (LA, cm<sup>2</sup>), leaf area ratio (LAR, cm<sup>2</sup> g<sup>-1</sup>), slenderness ratio (SR, cm mm<sup>-1</sup>), root shoot ratio (RSR, g g<sup>-1</sup>), leafless above-ground biomass per tree (AGBT, g), proportion (%) of leaf biomass (PLB), proportion (%) of stem biomass (PSB), and proportion (%) of root biomass (PRB) under all three spacings (20, 35, and 60 cm) and two N levels (20 and 200 µg g<sup>-1</sup>) after 13 growing weeks.

Clone	LA	LAR	SR	RSR	AGBT	PLB	PSB	PRB
Hybrid poplars								
1081	3831.7	143.1	11.2	0.12	11.9	47	43.3	9.7
2782	3617.1	118.5	16.4	0.16	18	41.9	45.1	13
747210	2380.4	114.6	13.7	0.19	7.5	53.4	33.2	13.4
915319	2367.4	118.2	12.6	0.11	8.2	50.3	40.7	9
DN-74	3763.3	143.2	13.4	0.1	13	46.1	45	8.9
NM06	4146.8	118.4	14.3	0.07	18.2	48.2	45.7	6.1
Walker	2514.1	123.4	12.6	0.18	11.4	40.3	45.3	14.4
Mean	<b>3231.5</b>	<b>125.6</b>	<b>13.5</b>	<b>0.13</b>	<b>12.6</b>	<b>46.7</b>	<b>42.6</b>	<b>10.6</b>
Willows								
HAN-A5	3678.3	95.4	16.6	0.12	21.3	38.3	50.9	10.9
LEV-D5	1229.6	83	11	0.21	7	45.4	37.9	16.7
SHE-E9	3586.4	100.1	13.3	0.09	21.9	43.4	48.5	8.1
LAF-I4	1248.6	81	14.5	0.1	8.4	47.2	44.3	8.5
TCH-N4	2788.4	138	13.9	0.12	15.6	41.7	47.7	10.7
Mean	<b>2627.1</b>	<b>106.6</b>	<b>13.6</b>	<b>0.14</b>	<b>14.8</b>	<b>43.8</b>	<b>45.3</b>	<b>10.9</b>

Table 2.5 Mean leaf area ( $\text{cm}^2$ ) for seven hybrid poplar and five willow clones under two nitrogen levels (20 and 200  $\mu\text{g g}^{-1}$ ) and all three spacings (20, 35, and 60 cm) combined after 13 growing weeks.

Clone	Leaf area ( $\text{cm}^2$ )	
	20 $\mu\text{g g}^{-1}$	200 $\mu\text{g g}^{-1}$
Hybrid poplars		
1081	3833.8 <sup>a</sup>	3829.6 <sup>a</sup>
2782	3152.4 <sup>a</sup>	4081.7 <sup>a</sup>
747210	1759.7 <sup>a</sup>	3131.9 <sup>b</sup>
915319	1464.2 <sup>a</sup>	3270.6 <sup>b</sup>
DN-74	2757.6 <sup>a</sup>	4900.2 <sup>b</sup>
NM06	3842.2 <sup>a</sup>	4451.3 <sup>a</sup>
Walker	1318.7 <sup>a</sup>	3709.4 <sup>b</sup>
Willows		
HAN-A5	3215.4 <sup>a</sup>	4055.5 <sup>b</sup>
LEV-D5	663.8 <sup>a</sup>	1795.3 <sup>b</sup>
SHE-E9	3428.9 <sup>a</sup>	3743.9 <sup>a</sup>
LAF-I4	596.2 <sup>a</sup>	1901.0 <sup>b</sup>
TCH-N4	2241.7 <sup>a</sup>	3335.1 <sup>a</sup>

Note: Within a row, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

LA was positively related to RCD, height, and CW for all treatments (Table 2.6). RCD and height per unit increase in LA generally increased from 60 to 20 cm spacing, while CW per unit increase in LA did not show any changes within all treatments (Table 2.6). LA was also significantly related to RCD, height, and CW for all clones except RCD and CW of clone SHE-E9 (Table 2.7). RCD per unit increase in LA was greatest for LEV-D5 and lowest for 1081 and 915319; height per unit increase in LA was greatest for LEV-D5 and lowest for HAN-A5; and CW per unit increase in LA was greatest for 915319 and lowest for 1081 (Table 2.7).

Table 2.6 Linear regression analysis between leaf area and RCD (root collar diameter), height, and CW (crown width) under three spacings (20, 35, and 60 cm) and two nitrogen levels (20 and 200  $\mu\text{g g}^{-1}$ ) after 13 growing weeks.

Treatment	Intercept	Slope	R <sup>2</sup>	F	P
<b>RCD</b>					
20 cm-20 $\mu\text{g g}^{-1}$	4.9	0.001 <sup>b</sup>	0.42	100.3	< <b>0.0001</b>
20 cm-200 $\mu\text{g g}^{-1}$	5.9	0.001 <sup>b</sup>	0.34	68.6	< <b>0.0001</b>
35 cm-20 $\mu\text{g g}^{-1}$	5.4	0.0007 <sup>b</sup>	0.44	103.1	< <b>0.0001</b>
35 cm-200 $\mu\text{g g}^{-1}$	6.7	0.0007 <sup>b</sup>	0.29	53.8	< <b>0.0001</b>
60 cm-20 $\mu\text{g g}^{-1}$	5.1	0.0008 <sup>b</sup>	0.45	29	< <b>0.0001</b>
60 cm-200 $\mu\text{g g}^{-1}$	7.6	0.0004 <sup>a</sup>	0.2	9.2	<b>0.005</b>
<b>Height</b>					
20 cm-20 $\mu\text{g g}^{-1}$	59.6	0.015 <sup>c</sup>	0.56	180	< <b>0.0001</b>
20 cm-200 $\mu\text{g g}^{-1}$	78	0.015 <sup>c</sup>	0.38	79.3	< <b>0.0001</b>
35 cm-20 $\mu\text{g g}^{-1}$	63.4	0.011 <sup>b</sup>	0.6	192.8	< <b>0.0001</b>
35 cm-200 $\mu\text{g g}^{-1}$	84.4	0.011 <sup>b</sup>	0.36	73.4	< <b>0.0001</b>
60 cm-20 $\mu\text{g g}^{-1}$	61	0.012 <sup>bc</sup>	0.59	49	< <b>0.0001</b>
60 cm-200 $\mu\text{g g}^{-1}$	90.2	0.007 <sup>a</sup>	0.45	26.6	< <b>0.0001</b>
<b>CW</b>					
20 cm-20 $\mu\text{g g}^{-1}$	23.2	0.002 <sup>a</sup>	0.33	68.7	< <b>0.0001</b>
20 cm-200 $\mu\text{g g}^{-1}$	30	0.002 <sup>a</sup>	0.11	17.1	< <b>0.0001</b>
35 cm-20 $\mu\text{g g}^{-1}$	28.7	0.003 <sup>a</sup>	0.21	36.2	< <b>0.0001</b>
35 cm-200 $\mu\text{g g}^{-1}$	30.8	0.003 <sup>a</sup>	0.11	15.8	< <b>0.0001</b>
60 cm-20 $\mu\text{g g}^{-1}$	25.1	0.005 <sup>a</sup>	0.29	14.8	<b>0.001</b>
60 cm-200 $\mu\text{g g}^{-1}$	32.2	0.004 <sup>a</sup>	0.24	11.1	<b>0.002</b>

Notes: Within a column, slope values followed by the same letter do not significantly differ at  $\alpha = 0.05$ . Significant *P* values are indicated in bold.

Table 2.7 Linear regression analysis of leaf area versus RCD (root collar diameter), height, and CW (crown width) for seven hybrid poplar and five willow clones under all three spacings (20, 35, and 60 cm) and two N levels (20 and 200  $\mu\text{g g}^{-1}$ ) after 13 growing weeks.

Clone	Intercept	Slope	R <sup>2</sup>	F	P
<b>RCD</b>					
1081	5.3	0.0007	0.75	148.3	< <b>0.0001</b>
2782	6.0	0.0008	0.58	72.0	< <b>0.0001</b>
747210	4.4	0.0010	0.74	114.8	< <b>0.0001</b>
915319	5.6	0.0007	0.52	57.4	< <b>0.0001</b>
DN-74	4.4	0.0011	0.87	302.4	< <b>0.0001</b>
NM 06	4.1	0.0012	0.80	202.2	< <b>0.0001</b>
Walker	4.8	0.0014	0.78	124.8	< <b>0.0001</b>
HAN-A5	5.9	0.0004	0.28	18.3	< <b>0.0001</b>
LEV-D5	4.0	0.0021	0.72	129.3	< <b>0.0001</b>
SHE-E9	9.8	0.0002	0.01	0.5	0.5032
LAF-I4	5.4	0.0012	0.29	23.0	< <b>0.0001</b>
TCH-N4	5.2	0.0009	0.49	51.1	< <b>0.0001</b>
<b>Height</b>					
1081	54.4	0.0093	0.69	110.9	< <b>0.0001</b>
2782	100.6	0.0097	0.37	32.4	< <b>0.0001</b>
747210	52.2	0.0164	0.76	128.4	< <b>0.0001</b>
915319	64.9	0.0117	0.59	78.5	< <b>0.0001</b>
DN-74	56.9	0.0156	0.80	183.6	< <b>0.0001</b>
NM 06	71.7	0.0138	0.67	101.3	< <b>0.0001</b>
Walker	53.6	0.0205	0.78	121.5	< <b>0.0001</b>
HAN-A5	103.0	0.0045	0.16	9.8	<b>0.003</b>
LEV-D5	31.6	0.0358	0.74	142.7	< <b>0.0001</b>
SHE-E9	79.8	0.0126	0.49	52.8	< <b>0.0001</b>
LAF-I4	64.4	0.0256	0.42	40.2	< <b>0.0001</b>
TCH-N4	73.5	0.0103	0.40	36.6	< <b>0.0001</b>
<b>CW</b>					
1081	30.9	0.0020	0.43	38.8	< <b>0.0001</b>
2782	21.4	0.0048	0.28	22.0	< <b>0.0001</b>
747210	25.6	0.0035	0.60	63.6	< <b>0.0001</b>
915319	6.5	0.0119	0.58	74.0	< <b>0.0001</b>
DN-74	22.0	0.0029	0.74	137.8	< <b>0.0001</b>
NM 06	21.1	0.0030	0.64	93.3	< <b>0.0001</b>
Walker	23.3	0.0031	0.69	77.9	< <b>0.0001</b>
HAN-A5	36.3	0.0037	0.29	20.8	< <b>0.0001</b>
LEV-D5	19.8	0.0051	0.35	28.4	< <b>0.0001</b>
SHE-E9	41.8	-0.00007	0.00	0.0	0.95
LAF-I4	31.6	0.0024	0.10	4.5	<b>0.04</b>
TCH-N4	32.2	0.0079	0.47	48.9	< <b>0.0001</b>

Note: Significant *P* values are indicated in bold.

Spacing had no effect on leaf area ratio (LAR), while the increase in N level significantly increased LAR by 4 % (Tables 2.3; 2.8). Hybrid poplars had higher LAR than willows (Tables 2.3; 2.4), and LAR also varied among clones from 81.0 for LAF-I4 to 143.2 cm<sup>2</sup> g<sup>-1</sup> for DN-74 (Tables 2.3; 2.4). Slenderness ratio (SR) significantly increased with an increase in N by 10 % (Table 2.8). At the genus level, SR for hybrid poplars significantly increased from 60 to 20 cm spacing while there were no changes for willows ( $G \times S$  interaction, Fig. 2.5 and Table 2.3). At the clonal level, there were significant changes in SR among clones from 11 for LEV-D5 to 16.6 cm mm<sup>-1</sup> for HAN-A5 (Tables 2.3; 2.4). Root-to-shoot ratios (RSR) significantly decreased by 44 % with an increase in N level (Table 2.8). At the clonal level, there were significant changes in RSR among clones from 0.07 for NM06 to 0.21 g g<sup>-1</sup> for LEV-D5 (Tables 2.3; 2.4).

Table 2.8 The effects of N level on mean leaf area ratio (LAR), slenderness ratio (SR), root-to-shoot ratios (RSR), leafless above-ground biomass per tree (AGBT), leafless above-ground biomass per m<sup>2</sup> (AGBM), proportion (%) of stem biomass (PSB), and proportion (%) of root biomass (PRB) for all clones after 13 growing weeks.

Growth trait	Nitrogen level	
	20 µg g <sup>-1</sup>	200 µg g <sup>-1</sup>
LAR (cm <sup>2</sup> g <sup>-1</sup> )	112.2 <sup>a</sup>	116.6 <sup>b</sup>
SR (cm mm <sup>-1</sup> )	12.9 <sup>a</sup>	14.2 <sup>b</sup>
RSR (g g <sup>-1</sup> )	0.16 <sup>b</sup>	0.09 <sup>a</sup>
AGBT (g)	10.8 <sup>a</sup>	16.5 <sup>b</sup>
AGBM (g m <sup>-2</sup> )	143.3 <sup>a</sup>	215.5 <sup>b</sup>
PSB	41.2 <sup>a</sup>	47 <sup>b</sup>
PRB	12.9 <sup>b</sup>	8.4 <sup>a</sup>

Note: Within a row, means followed by the same letter do not significantly differ at  $\alpha = 0.05$

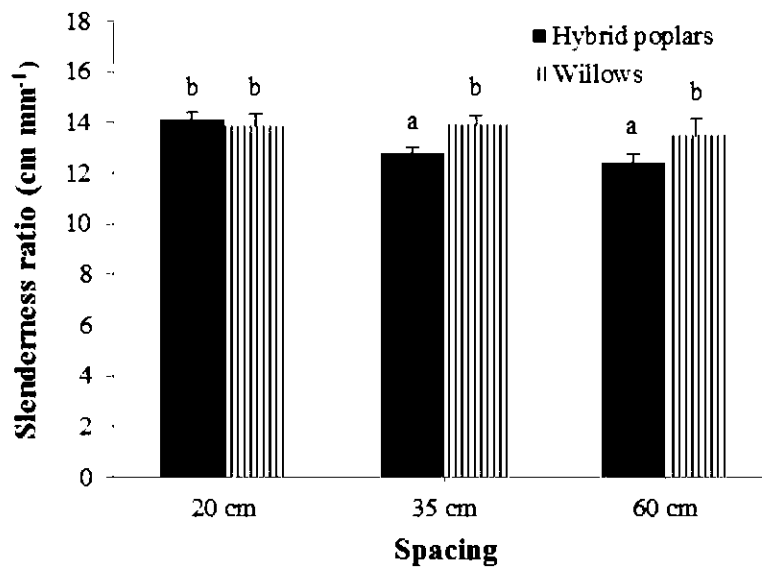


Figure 2.5 The effects of spacing on mean slenderness ratio for hybrid poplars and willows after 13 growing weeks. Means followed by the same letter on the bar do not significantly differ at  $\alpha = 0.05$ .

#### 2.4.3 Biomass productivity and allocation

The increase in N level significantly increased leafless above-ground biomass per tree (AGBT) by 53 % and leafless above-ground biomass per m<sup>2</sup> (AGBM) by 50 % after the growing period (Tables 2.8; 2.9). Spacing had no effect on AGBT (Table 2.9), which resulted in a significant increase in AGBM with the decrease in spacing for the both hybrid poplars and willows (Fig. 2.6). However, in the 20 cm spacing, willows had greater AGBM than hybrid poplars (Fig. 2.6). At the clonal level, there were significant differences in AGBT among clones from 7 for LEV-D5 to 21.9 g for SHE-E9 (Tables 2.4; 2.9).

Hybrid poplars had a greater proportion (%) of leaf biomass (PLB) than willows (Tables 2.4; 2.9). However, PLB significantly varied among clones depending on changes in spacing (C  $\times$  S interaction, Tables 2.9; 2.10). Generally, PLB for most clones increased with an increase in spacing and clones 2782 and HAN-A5 showed

significantly greater PLB (Table 2.10). The proportion (%) of stem biomass (PSB) significantly increased with an increase in N by 14 % (Tables 2.8; 2.9). At the genus level, PSB was greater for willows compared to hybrid poplars (Tables 2.4; 2.9). At the clonal level, PSB significantly varied among clones from 33.2 % for 747210 to 50.9 % for HAN-A5 (Tables 2.4; 2.9). Although there were no significant changes in PRB between hybrid poplars and willows, PRB for individual clones varied with N level (C × N interaction, Tables 2.9; 2.10). The increase in N level generally decreased PRB for all clones and significantly decreased for clones 2782, 915319, DN-74, and LEV-D5 (Table 2.10).

Table 2.9 Analysis of variance giving sources of variation, degrees of freedom (DF), *F* values, and associated probabilities for leafless above-ground biomass per tree (AGBT), leafless above-ground biomass per m<sup>2</sup> (AGBM), proportion (%) of leaf biomass (PLB), proportion (%) of stem biomass (PSB), and proportion (%) of root biomass (PRB).

Source of variation	DF	AGBT		AGBM		PLB		PSB		PRB	
		<i>F</i>	<i>P value</i>	<i>F</i>	<i>P value</i>	<i>F</i>	<i>P value</i>	<i>F</i>	<i>P value</i>	<i>F</i>	<i>P value</i>
G	1	2.4	0.122	2.9	0.091	27.3	< 0.0001	39.4	< 0.0001	1.5	0.217
N	1	38.2	< 0.0001	8.6	0.04	0.4	0.524	24.1	< 0.0001	37.9	< 0.0001
S	2	2.7	0.069	41.8	< 0.0001	3.3	0.03	2.2	0.108	0.5	0.622
G × N	1	0.7	0.418	0.0	0.948	0.2	0.623	0.1	0.73	0.1	0.816
G × S	2	0.6	0.557	3.1	0.05	0.1	0.895	1.2	0.312	1.2	0.293
N × S	2	1.5	0.234	1.3	0.285	0.3	0.713	1.0	0.383	0.3	0.726
G × N × S	2	0.1	0.907	0.2	0.85	0.3	0.782	0.1	0.895	0.6	0.545
C	11	3.5	0.0001	2.0	0.092	9.5	< 0.0001	10.6	< 0.0001	7.2	< 0.0001
C × N	11	1.0	0.44	0.3	0.871	1.5	0.113	1.4	0.163	2.0	0.03
C × S	22	0.4	0.994	0.7	0.672	1.7	0.03	1.2	0.229	1.1	0.374
C × N × S	22	0.6	0.943	0.1	0.997	1.2	0.206	1.2	0.211	0.5	0.956

Notes: In source of variation, G: genus (hybrid poplars vs willows), N: nitrogen, S: spacing, and C: clone. Significant *P* values are indicated in bold.

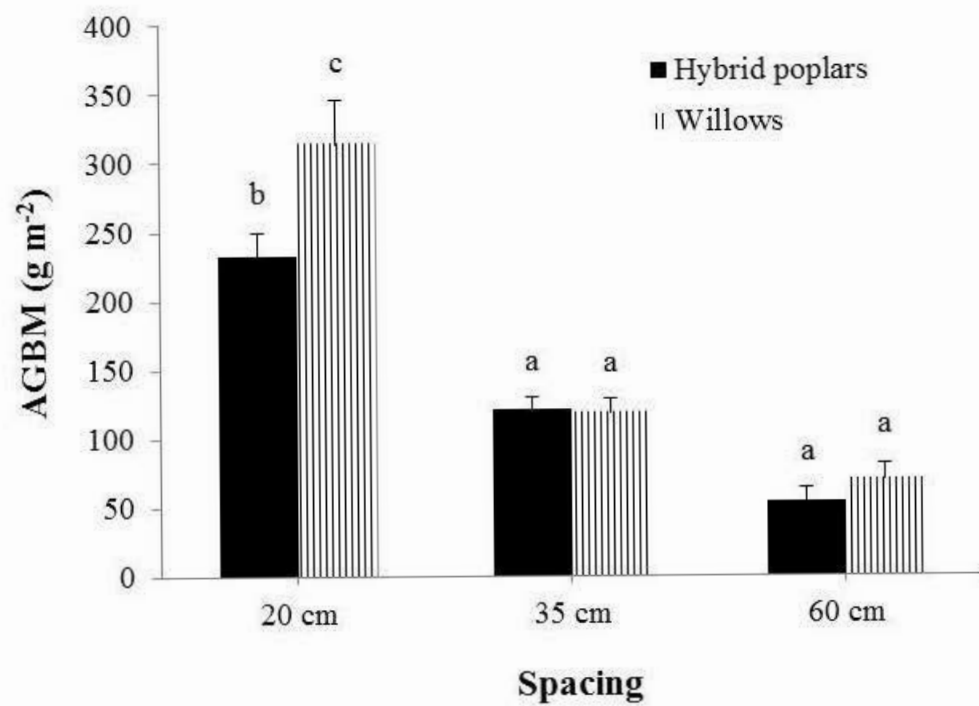


Figure 2.6 The effect of spacing on mean leafless above-ground biomass per m<sup>2</sup> (AGBM, g) for hybrid poplar and willow clones after 13 growing weeks. Means followed by the same letter on the bar do not significantly differ at  $\alpha = 0.05$ .



Table 2.10 Changes in mean proportion (%) of leaf biomass (PLB) under three spacings (20, 35, and 60 cm) and proportion (%) of root biomass (PRB) under two N levels (20 and 200  $\mu\text{g g}^{-1}$ ) for individual clone after 13 growing weeks.

Clone	PLB			PRB	
	20 cm	35 cm	60 cm	20 $\mu\text{g g}^{-1}$	200 $\mu\text{g g}^{-1}$
Hybrid poplars					
1081	45.8 <sup>a</sup>	47.4 <sup>a</sup>	50.8 <sup>a</sup>	11.5 <sup>a</sup>	7.6 <sup>a</sup>
2782	38.2 <sup>a</sup>	46.1 <sup>b</sup>	39.8 <sup>ab</sup>	15.2 <sup>b</sup>	10.8 <sup>a</sup>
747210	50.4 <sup>a</sup>	55 <sup>a</sup>	59.6 <sup>a</sup>	18.8 <sup>a</sup>	6.8 <sup>a</sup>
915319	48.4 <sup>a</sup>	51.5 <sup>a</sup>	53.3 <sup>a</sup>	12.4 <sup>b</sup>	5.4 <sup>a</sup>
DN-74	45.1 <sup>a</sup>	46.9 <sup>a</sup>	47.2 <sup>a</sup>	9.2 <sup>a</sup>	8.6 <sup>a</sup>
NM06	47.9 <sup>a</sup>	48.8 <sup>a</sup>	47.2 <sup>a</sup>	7.3 <sup>a</sup>	4.9 <sup>a</sup>
Walker	43 <sup>a</sup>	37.8 <sup>a</sup>	39.1 <sup>a</sup>	16.4 <sup>a</sup>	12.4 <sup>a</sup>
Willows					
HAN-A5	33.1 <sup>a</sup>	43 <sup>b</sup>	42.5 <sup>b</sup>	12.3 <sup>a</sup>	9.7 <sup>a</sup>
LEV-D5	46.4 <sup>a</sup>	43.5 <sup>a</sup>	48.4 <sup>a</sup>	20.8 <sup>b</sup>	12.6 <sup>a</sup>
SHE-E9	44.8 <sup>a</sup>	42.1 <sup>a</sup>	42.9 <sup>a</sup>	9.5 <sup>a</sup>	6.7 <sup>a</sup>
LAF-I4	45.4 <sup>a</sup>	47.5 <sup>a</sup>	53.6 <sup>a</sup>	10.5 <sup>a</sup>	6.8 <sup>a</sup>
TCH-N4	41.3 <sup>a</sup>	42.9 <sup>a</sup>	38.2 <sup>a</sup>	12.6 <sup>a</sup>	8.7 <sup>a</sup>

Note: Within a row, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

#### 2.4.4 Morphological characteristics of superior clones

This study showed great genotypic differences in morphological growth among clones. Characteristics of more productive or superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 showed 143 %, 244 %, 129 %, 100 %, and 16 % greater AGBT, AGBT under low N, AGBM, LA, and LAR, respectively and 24 % lower RSR compared to less productive or inferior clones 747210, 915319, LEV-D5, and LAF-I4 (Table 2.11). Superior and inferior clones showed significant relationships

between AGBT and LA ( $P < 0.05$ ) and superior clones showed significantly greater AGBT per unit increase in LA compared to inferior clones (Fig. 2.7).

Table 2.11 Differences in mean leafless aboveground biomass per tree (AGBT), AGBT under low N (Low N AGBT), on leafless above-ground biomass per m<sup>2</sup> (AGBM), leaf area (LA), leaf area ratio (LAR), slenderness ratio (SR), and root-to-shoot ratios (RSR) between superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group and inferior clones 747210, 915319, LEV-D5, and LAF-I4 as a group after 13 growing weeks.

Variable	Superior clones	Inferior clones
AGBT (g)	18.9 <sup>b</sup>	7.8 <sup>a</sup>
Low N AGBT (g)	16.6 <sup>b</sup>	4.8 <sup>a</sup>
AGBM (g m <sup>-2</sup> )	247 <sup>b</sup>	107.9 <sup>a</sup>
LA (cm <sup>2</sup> )	3556.8 <sup>b</sup>	1778.1 <sup>a</sup>
LAR (cm <sup>2</sup> g <sup>-1</sup> )	114.4 <sup>b</sup>	98.5 <sup>a</sup>
SR (cm mm <sup>-1</sup> )	14.8 <sup>a</sup>	12.9 <sup>a</sup>
RSR (g g <sup>-1</sup> )	0.11 <sup>a</sup>	0.15 <sup>b</sup>

Note: Within a row, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

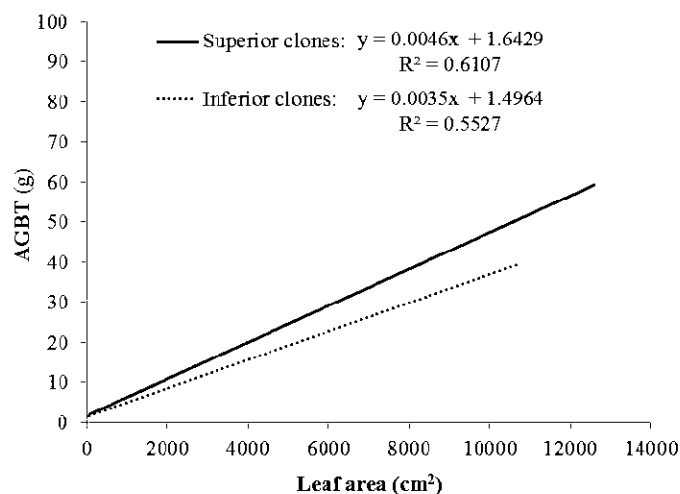


Figure 2.7 Relationship between leafless aboveground biomass per tree (AGBT) and leaf area for superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group and inferior clones 747210, 915319, LEV-D5, and LAF-I4 as a group after 13 growing weeks.

## 2.5 Discussion

For the selection process, it is desirable to identify clones that can grow well under intensive plant competition and low N level so that these clones can maximize biomass production per unit area with reduced fertilization cost. In this study, spacing did not significantly affect AGBT for all clones (Table 2.9), indicating that all clones acclimated well to the closer spacing. As there were no clone  $\times$  spacing interactions on LA, LAR, SR, and RSR, growth acclimation to different spacings was similar among clones. The greater acclimation to the closer spacings for all clones was generally explained by increased RCD and height per unit increase in LA (Table 2.6) despite significant decreases in LA by 50 % from 60 to 20 cm spacing (Fig. 2.4), indicating an efficient photosynthate allocation to leading shoot growth. Under intensive competition, a leading shoot requires more carbohydrate to attain its

potential length than diameter growth (Lanner, 1985). Consequently, the decrease in spacing resulted in a significant increase in SR (Table 2.3). This efficient increase in height may have achieved more light interception in the upper crown under competition by means of longer (indeterminate) shoots with more foliage on the top of crowns (Dickmann *et al.* 2001). These sun leaves would play an essential role in the partitioning of carbohydrate to tree growth (Kozlowski and Pallardy 1997). Therefore, this efficient height growth likely contributed to increasing shoot growth, thereby leading to relatively small decrease in height and the absence of significant changes in AGBT even with decreased spacing (Tables 2.2; 2.9). This relatively insensitive height growth under changes in spacing has been reported by Fang *et al.* (1999); however, others observed variations in height under changes in spacing, depending on genotypes, the range of tested spacing treatments, or a length of the growing period (Larocque, 1999; Benomar *et al.*, 2012). This study also showed clonal differences in height under changes in spacing and N level, which may have been caused by differences in nitrogen-uptake capacity related to root morphology (Al Afas *et al.*, 2008) or production capacity of leaf area (Scarascia-Mugnozza *et al.*, 1999; Cooke *et al.*, 2005).

Genotypic differences caused greater changes in biomass productivity in this study. Compared to the inferior clones 747210, 915319, LEV-D5, and LAF-I4 as a group, superior clones 2782, NM06, SHE-E9, HAN-A5, and TCH-N4 as a group showed 143 %, 244 %, and 129 % greater AGBT, AGBT with low N level, and hence AGBM, respectively (Table 2.11). Therefore, these superior clones may have better growth performance even under the decrease in spacing and N level. Compared to inferior clones, the greater productivity of superior clones can be primarily explained by 100 % greater LA as LA was significantly related to RCD, height, and CW under all treatments and most clones (Tables 2.6; 2.7; 2.11). Indeed, LA is an important determinant for plant growth under competition as the relationship between LA and plant growth is widely recognized because the production of forest stands has been

strongly associated with total amount of intercepted irradiance (Barigah *et al.*, 1994). Also, 24 % lower RSR of superior clones was explained by greater AGBT per unit increase in LA (Fig. 2.7; Table 2.11), indicating greater shoot production capacity. This greater shoot production capacity resulted in 16 % more efficient increase in total photosynthetic area per tree (LAR) (Table 2.11).

The increase in N level caused smaller increases in LA with the decrease in spacing (Fig. 2.4). Since crown recession rarely occurred during the short growing period of this study, the smaller increase in LA with the increase in N level in the closer spacing may have been due to limited length of sylleptic branch development corresponding to reduced available growing space (Benomar *et al.*, 2012). Sylleptic branch development may have played an important role in explaining clonal differences in LA development with an increase in N as longer sylleptic branches directly allow clones to have more leaf area per branch (Ceulemans *et al.*, 1990; Wu and Hinckly, 2001; Rae *et al.*, 2004; Cooke *et al.*, 2005). In fact, Benomar *et al.* (2012) reported that long branches of clone MB915 (*Populus maximowiczii* × *P. balsamifera*) had greater leaf area and biomass production compared to short branches of clone BT747 (*P. balsamifera* × *P. trichocarpa*). On the other hand, the increase in N level caused similar increases in AGBT under all spacings (non N × S interaction; Table 2.9). Since the increase in height per unit increase in LA was greater in the 20 cm spacing and high N compared to the 60 cm spacing and high N (Table 2.6), greater stem-growth efficiency in the 20 cm spacing and high N may have resulted in an efficient increase in AGBT despite greater limited resource availability between the trees. In particular, compared to 60 cm spacing and low N, 20 cm spacing and high N showed similar RCD, CW, and LA values and even greater height (Fig. 2.4; Table 2.2), indicating that the negative impact of competition was reduced by increasing N fertilization. As a consequence, AGBM was greatest in the 20 cm spacing and 200 µg g<sup>-1</sup> N (Fig. 2.6; Tables 2.8), indicating that trees under the

closer spacing and the greater N level can maximize biomass production with a greater number of stems per unit area before crown recession occurs.

N fertilization also increased LAR (Table 2.8), indicating an efficient increase in leaf area per unit plant mass. Thus, resource allocation to leaf area production was prioritized with an increase in N level (Cooke *et al.*, 2005). The increase in N level also increased the proportion of stem biomass and reduced the proportion of root biomass, resulting in lower RSR (Table 2.8). Previous studies also reported lower RSR as availability of N increases (Isebrands and Nelson, 1983; Axelsson and Axelsson, 1986; Pregitzer *et al.*, 1990). The lower RSR with an increase in N level may be explained by several factors: Firstly, as biomass allocation is prioritized based on the acquisition of limiting resources (McCarthy and Enquist, 2007), greater PRB under low N level may be due to increased requirement for further soil N uptake (Casper and Jackson, 1997). Secondly, as this study used very close spacings of 20-60 cm, the increase in N possibly intensified root competition, which limited root development between trees as poplar and willow species are known to be very sensitive to root competition (Sage, 1999; Messier *et al.*, 2009; Bilodeau-Gauthier *et al.*, 2011). Lastly, a weekly-basis N application probably did not require trees to produce further root development for N uptake as contact area for N fertilization was limited around trees by the irrigation system. Thus, the addition of N favoured shoot biomass allocation rather than root development.

The differences in crown morphology between hybrid poplars and willows generally explained their different growth patterns; Hybrid poplars such as DN-74, NM06, and Walker showed a long, narrow crown, greater RCD and height per unit increase in LA compared to the other clones (Table 2.7). Hybrid poplars allocated resources to a few dominant stems that enabled trees to achieve rapid shoot growth and also to distribute to the weaker sink of diameter growth (Ceulemans *et al.*, 1996), resulting in greater RCD, RCD RGR, and height RGR compared to willows (Fig. 2.2; Table 2.2).

The advantage of using these clones under SRF management is an efficient vertical shoot growth with a small CW under limited space that can delay crown occupancy and consequently leaf senescence (Dickmann *et al.*, 2001). Willows such as HAN-A5 and TCH-N4 had greater number of stems ( $> 3$ ) (data not shown) compared to hybrid poplars, resulting in the relatively greater CW per unit increase in LA (Table 2.7). The initial slower RCD RGR for willows compared to hybrid poplars was thus probably influenced by a greater number of stems per tree for willows (Fig. 2.2ab). Multiple-stem growth for willows reduced biomass allocation to each stem per tree, which increased a demand for the growth of leading shoots at the expense of diameter growth (Ceulemans *et al.*, 1996). Thus, willows had significantly greater SR and PSB than hybrid poplars (Table 2.4), which also explained the greater AGBM of willows in the 20 cm spacing despite the lower LA compared to hybrid poplars (Fig. 2.6). Later in the growing period, willows rapidly increased RCD RGR (Fig. 2.2ab), as willow leaves mature more slowly and show longer leaf retention (Ceulemans *et al.*, 1996).

Willow clones LEV-D5 and LAF-I4 demonstrated the greatest height increase per unit in LA (Table 2.7), but also showed the lowest height and LA of all clones (Tables 2.2; 2.4). This may be explained by physiological changes in foliage after aphid attack that frequently occurred during the growing period; Aphids generally cause leaf senescence and abscission, but they also enrich leaf nitrogen on the remaining leaves because of introduced metabolic compounds into host plants (Dixon, 1971; Collins *et al.*, 2001). This study also showed that mean leaf N concentration ( $N_{\text{area}}$ ) of LEV-D5 and LAF-I4 was the highest ( $1.2 \text{ g m}^{-2}$  and  $1.3 \text{ g m}^{-2}$ , respectively) of all clones (data not shown). As a result, since leaf N content is mostly allocated to photosynthetic apparatus (Evans, 1989), they had higher net photosynthesis per unit leaf area at a given irradiance (data not shown). This compensatory photosynthesis after aphid feeding has been also reported by previous studies (Way and Cammell, 1970; Collins *et al.*, 2001). Therefore, the greatest height per unit increase in LA for

LEV-D5 and LAF-I4 may not be owing to inherent growth capacity under competitive stress but likely higher photosynthetic capacity influenced by aphid attack.

## 2.6 Conclusion

Leaf area was the most important characteristic to explain plant growth under intensive plant competition as LA was significantly associated with RCD, height, and CW under all treatments and most clones in this study. However, the increase in N level caused a smaller degree of increased LA under the closer spacing, suggesting the sensitivity of LA development under the closer spacing with an addition of N. The decrease in spacing increased RCD and height per unit increase in LA, suggesting greater height-growth efficiency under closer spacing. This greater height-growth efficiency under closer spacing maximized shoot growth, thereby leading to no significant changes in AGBT for all clones even with the decrease in spacing. Also, because there were no clone  $\times$  spacing interactions on LA, LAR, SR, and RSR, growth acclimation to different spacings was similar among clones and all clones increased AGBM under the closer spacing. Hybrid poplars showed a faster shoot growth such as greater RCD, RCD RGR, and height RGR compared to willows at the end of the experimental period despite no significant changes in AGBT and AGBM between hybrid poplars and willows as a group. On the other hand, greater number of stems ( $> 3$ ) of willows resulted in relatively greater CW per unit increase in LA and greater AGBM in the closest spacing, compared to hybrid poplars. However, genotypic differences caused changes in AGBT; Compared to inferior clones 747210, 915319, LEV-D5, and LAF-I4 as a group, superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group showed 143 %, 244 %, and 129 % greater AGBT, AGBT under low N, and AGBM, respectively. The greater biomass productivity of superior clones was mainly explained by 100 % greater leaf area. In addition, compared to inferior clones, superior clones showed 16 % greater



LAR, which efficiently increased total photosynthetic area per tree. Superior clones also had lower RSR, which resulted in greater AGBT per unit increase in LA, indicating a greater shoot production capacity. Compared to low N and 60 cm spacing, high N and 20 cm spacing showed similar RCD, CW, and LA values and even greater height, concluding that an addition of N can play an essential role in increasing plant growth under the intensive competition. Lastly, although a 3 month study period is relatively short, this study showed clonal differences in morphological characteristics under intensive competition, illustrating the possibility for early clone selection.

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## CHAPTER III

### SHORT-TERM PHOTOSYNTHETIC ACCLIMATION OF HYBRID POPLARS AND WILLOWS TO DIFFERENT PLANTING DENSITIES AND NITROGEN AVAILABILITY

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Mamashita T made the experiment, data analyses, and wrote this manuscript.

### 3.1 Abstract

Photosynthetic acclimation of hybrid poplars and willows under different plant densities and nitrogen availability was studied to determine leaf photosynthetic characteristics leading to superior productivity. Seven hybrid poplar (915319; *Populus maximowiczii* × *P. balsamifera*, 747210; *P. balsamifera* × *P. trichocarpa*, 1081; *P. deltoides* × *P. balsamifera*, 2782; *P. tremuloides* × *P. tremula*, DN-74; *P. deltoides* × *P. nigra*, NM06; *P. maximowiczii* × *P. nigra*, and Walker; *P. deltoides* × *P. petrowskyana*) and five willow (HAN-A5; *Salix amygdaloides*, LEV-D5; *S. discolor*, SHE-E9; *S. eriocephala*, LAF-I4; *S. interior*, and TCH-N4; *S. nigra*) clones were grown in greenhouse conditions for three months under three spacings [20×20, 35×35, and 60×60 cm] and two nitrogen (N) levels (20 and 200  $\mu\text{g g}^{-1}$ ). Compared to inferior clones 747210, 915319, LEV-D5, and LAF-I4, superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group showed 143 % greater leafless aboveground biomass per tree (AGBT). This was because superior clones had significantly greater AGBT and leaf area (LA) per unit increase in leaf N concentration ( $N_{\text{area}}$ ). Superior clones also showed 28 % greater LA production per unit leaf biomass (SLA). The increase in N level had no significant effect on  $P_n$  and even reduced  $\text{PNUE}_{\text{amb}}$ , indicating that an addition of N had little effect on photosynthetic capacity under ambient light conditions.

## Résumé

L'acclimatation photosynthétique de peupliers hybrides et de saules a été étudiée sous différentes densités de plantation et disponibilités en azote pour déterminer les caractéristiques physiologiques menant à une productivité supérieure. Sept clones de peuplier hybride (915319; *Populus maximowiczii* × *P. balsamifera*, 747210; *P. balsamifera* × *P. trichocarpa*, 1081; *P. deltoides* × *P. balsamifera*, 2782; *P. tremuloides* × *P. tremula*, DN-74; *P. deltoides* × *P. nigra*, NM06; *P. maximowiczii* × *P. nigra*, and Walker; *P. deltoides* × *P. petrowskyana*) et cinq clones de saule (HAN-A5; *Salix amygdaloides*, LEV-D5; *S. discolor*, SHE-E9; *S. eriocephala*, LAF-I4; *S. interior*, and TCH-N4; *S. nigra*) clones ont été cultivés en serre pendant trois mois sous trois espacements [20 × 20, 35 × 35, et 60 × 60 cm] et deux niveaux d'azote (N) (20 et 200 µg g<sup>-1</sup>). Par rapport aux clones à croissance inférieure 747210, 915319, LEV-D5, et LAF-I4, les clones supérieurs 2782, NM06, HAN-A5, SHE-E9 et TCH-N4 comme groupe ont produit en moyenne 143 % de plus de biomasse aérienne par arbre (AGBT). Les clones supérieurs avaient une plus grande AGBT et surface foliaire (LA) par unité d'augmentation de la concentration en N du feuillage (N<sub>area</sub>). Les clones supérieurs avaient également une plus grande surface foliaire spécifique (SLA; 28%). L'augmentation du niveau de N n'a eu aucun effet significatif sur la photosynthèse nette (P<sub>n</sub>) et même réduit l'efficacité d'utilisation de l'azote (PNUE<sub>amb</sub>), ce qui indique que l'ajout de N a eu peu d'effet sur la capacité photosynthétique dans des conditions de lumière ambiante.



### 3.2 Introduction

Short rotation forestry (SRF) plantations using hybrid poplars and willows have been generating interest in Canada for the manufacture of a variety of products and bioenergy production. They can produce appreciable amounts of wood fibre in less than 10 years (O'Neill *et al.*, 2010). Generally, energy-crop productivities of *Populus* spp. attain 10 dry Mg ha<sup>-1</sup> yr<sup>-1</sup>, with values approaching 20 dry Mg ha<sup>-1</sup> yr<sup>-1</sup> for genotypes that are well adapted to local site conditions (Zalesny *et al.*, 2012). To achieve these production targets, a number of hybrid poplar and willow clonal and progeny trials have been established to evaluate growth rates, adaptability, and disease resistance across Canada over decades (Dominy *et al.*, 2010). However, there is a continuous need to develop better clones that could show higher growth performance and productivity to maximize economic returns of investments in SRF plantations (McKenney *et al.*, 2011).

Since productivity is determined by genetic background and environmental factors such as initial tree spacing, site quality, weed management, fertilization, and local climate (Weih, 2004), it is necessary to better understand the variation in physiological and morphological traits of available plant material under different environmental conditions. In hybrid poplar and willow improvement programs, photosynthetic capacity is an important trait as it is highly correlated to plant growth and productivity (Dickmann *et al.*, 2001). Previous studies found that plantation density affected the photosynthetic capacity of trees, due in part to changes in light environment and nitrogen (N) distribution along the crown (Kopp *et al.*, 1996; Proe *et al.*, 2002; Benomar *et al.*, 2011). In large spacings, trees generally develop many thick leaves with low specific leaf area (SLA, leaf area:leaf mass) growing under high photosynthetically active radiation (PAR) (Evans and Poorter, 2001). This increases the amount of photosynthetic enzymes, which improves the photosynthetic capacity per unit leaf area. Although the decrease in spacing generally reduces available

resources such as PAR and leaf nitrogen (N) content within the crown due to competition among trees, plants may show different photosynthetic acclimation depending on crown section (Benomar *et al.*, 2011). In the upper canopy, increased competition may result in a greater proportion of leaf nitrogen allocated to newly produced leaves to maintain greater photosynthetic capacity (Knops and Reinhart, 2000; Benomar *et al.*, 2011). In the lower canopy, the reduced PAR and leaf N content might induce an increase in SLA, which increases PAR- and N-use efficiency for photosynthesis under low light conditions (Rosati *et al.*, 1999; 2000). The increase in SLA is associated with larger leaf area per unit of leaf biomass that intercepts more PAR and decreases leaf thickness for better PAR transmittance, which allow plants to capture low PAR effectively under shaded condition (Vile *et al.*, 2005). The increase in SLA would also contribute to making use of small fraction of N more effectively for photosynthesis by increasing N allocation to photosynthetic machinery (Evans and Poorter, 2001). As a result, higher SLA may partially offset negative effect of competition by increasing light-use efficiency under low PAR (Benomar *et al.*, 2011). However, as SLA is inversely associated with leaf life-span (Reich *et al.*, 1992), an increase in plant competition might cause earlier loss of high SLA or shaded leaves (Fang *et al.*, 1999).

N fertilization generally increases leaf N content as a great portion of N is distributed to leaves, particularly Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), a critical enzyme for photosynthesis (Evans, 1989). Thus, positive correlations between leaf N content and net photosynthesis ( $P_n$ ) were often reported by previous studies (e.g., Reich *et al.*, 1991; Sheriff and Nambiar, 1991; Thompson and Wheeler, 1992). This increased whole photosynthetic capacity would also lead to greater biomass productivity of plants (Cooke *et al.*, 2005; Weih and Rönnerberg-Wästjung, 2007). However, the distribution of leaf N content per unit leaf area ( $N_{area}$ ) largely varies among species or depending on environmental factors such as irradiance and temperature, which also cause variations in  $P_n$  (Evans, 1989; Pointeau and Guy,

2014). There are few studies about how soil N availability influences the distribution of  $N_{\text{area}}$  among fast-growing hybrid poplars and willows under high plant competition and particularly how it affects  $P_n$  and photosynthetic N-use efficiency under ambient light conditions ( $PNUE_{\text{amb}}$ ) and under low PAR where plants grow at high plant density.

The primary objective of this study was to determine photosynthetic leaf characteristics leading to superior productivity under different plant density and N levels. We hypothesized that clones that show greater productivity would acclimate well to competition with no significant changes in  $P_n$  under tight spacings as these clones can enhance photosynthetic capacity with greater SLA and  $PNUE_{\text{amb}}$ . Also, these clones could have greater leafless aboveground biomass per tree (AGBT) and leaf area (LA) per unit increase in  $N_{\text{area}}$ . Adding N under intensive competition could increase  $P_n$  with a general increase in  $N_{\text{area}}$  within the crown, which can minimize the negative impact of competition.

### 3.3 Materials and methods

The study area was located in a greenhouse at the Laurentian Forestry Centre in Quebec (46°46'N, -71°16'W). The experiment was designed as an incomplete block design with seven hybrid poplar (915319; *Populus maximowiczii* × *P. balsamifera*, 747210; *P. balsamifera* × *P. trichocarpa*, 1081; *P. deltoides* × *P. balsamifera*, 2782; *P. tremuloides* × *P. tremula*, DN-74; *P. deltoides* × *P. nigra*, NM06; *P. maximowiczii* × *P. nigra*, and Walker; *P. deltoides* × *P. petrowskyana*) and five willow (HAN-A5; *Salix amygdaloides*, LEV-D5; *S. discolor*, SHE-E9; *S. eriocephala*, LAF-I4; *S. interior*, and TCH-N4; *S. nigra*) clones. Within each repetitive block (cycles of 13 weeks of growing period), each combination of 6 clones, 2 nitrogen (N) levels (20 and 200  $\mu\text{g g}^{-1}$  of nitrogen) and 3 spacings [20 (20 × 20), 35 (35 × 35), 60 (60 × 60) cm] was allocated to 36 plant pots randomly in the greenhouse. Thus, each clone was replicated 3 times, once in each of 3 different blocks. For the 20 and 35 cm spacings,

16 dormant, unrooted cuttings of 10 cm in length were planted per plant pot and, for the 60 cm spacing, 5 cuttings. Clone 2782 (hybrid aspen) were 20 cm long rooted plants from in-vitro culture as aspens are difficult to root from cuttings (Stanturf *et al.*, 2001).

Two nitrogen (N) levels (20 and 200  $\mu\text{g g}^{-1}$ ) were applied as ammonium-nitrate ( $\text{NH}_4\text{NO}_3$ ) twice a week. 2 mg N (low level) or 20 mg N (high level) with a 100 mL aliquot per tree was supplied. Phosphorus (1.5 g P) was provided as monobasic calcium orthophosphate [ $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ ] for each cutting. Calcium (1.55 g Ca) per cutting was supplied as above for calcium orthophosphate as well as  $\text{CaCO}_3$  for pH control. Potassium (50 mg K), magnesium (11.9 mg Mg), and sulfur (15.7 mg S) of macronutrient solutions were supplied on each tree with  $\text{K}_2\text{SO}_4$  and  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . Micronutrients were also provided per cutting, including 1.35 mg of iron (chelated Fe), 0.5 mg of manganese (Mn), 0.013 mg of molybdenum (Mo), 0.04 mg of copper (Cu) and 0.39 mg of boron (B). These nutrients were provided by the irrigation system using O'jet olson bleu 300 (Harnois Inc., St-Thomas, QC, Canada).

Temperature was maintained at 24 C° during the day period, and 18 C° during the night period. Daily photoperiod was set to 16 h (0600-2200 h) by natural and artificial light (400 W high pressure sodium bulbs, Litmore Distributors Ltd., Edmonton, Alta.). Soil pH in the boxes ranged 5.5-7.

Leaf gas exchange measurements were done on one of four central trees that was randomly selected in the 20 and 35 cm spacings and one central tree in the 60 cm spacing at the end of growing period. The crown of each sampled tree was divided equally into three layers from the bottom to the top of the crown from which two leaves per crown section were randomly selected for leaf gas exchange measurements. Net photosynthesis ( $P_n$ ) and photosynthetically active radiation (PAR) in the ambient light conditions were conducted, using a portable photosynthesis system LI-6400 (LI-COR Inc., Lincoln, NE, USA). Flow rate and

CO<sub>2</sub> concentration in the leaf cuvette were maintained at 200 mL min<sup>-1</sup> and 400 μmol mol<sup>-1</sup>, respectively. Measurements were made at 50-75 % relative humidity (RH) and 0.8-1.8 kPa vapour pressure deficit (VPD). All measurements were taken between 11h00 and 13h00.

After three months, the cuttings in the centre of pots were harvested and stems (including branches), roots and leaves were separated. Leaf area was measured with a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NE, USA). Then, stems, roots and leaves were oven-dried at 55°C until no change in mass was detected. Specific leaf area (SLA) was calculated as the ratio of leaf area (cm<sup>2</sup>) to leaf dry mass (g). Leaf N concentration (mg g<sup>-1</sup>) was measured using a NA-2000 dry combustion N-analyzer (Fissons Instruments, Milan, Italy), and N concentration per unit dry mass (mg g<sup>-1</sup>) was then converted to N content per unit leaf area (N<sub>area</sub>, g m<sup>-2</sup>). Photosynthetic N-use efficiency under ambient light condition (PNUE<sub>amb</sub>) was calculated as the ratio of P<sub>n</sub> to N<sub>area</sub>.

Analyses for PAR, P<sub>n</sub>, PNUE<sub>amb</sub>, SLA, and N<sub>area</sub> were examined at the clonal and genus levels. Genus level represented the mean of hybrid poplar and willow clones. All data were analyzed using the Mixed Procedure in SAS (version 9.2, SAS Institute, Cary, NC, USA). The model contained fixed effects for genus or clone, nitrogen level, spacing, crown section, and their interactions in a factorial treatment structure, with three replicates per combination. In this model, the dependent variables are P<sub>n</sub>, PAR, SLA, and N<sub>area</sub> and the general linear model is as follows:

$$Y_{BCNSCWP} = \mu + E_B + \beta_{GC} + \beta_N + \beta_S + \beta_{CW} + \beta_{GC*N} + \beta_{N*S} + \beta_{GC*S} + \beta_{GC*CW} + \beta_{N*CW} + \beta_{S*CW} + \beta_{GC*N*S} + \beta_{GC*N*CW} + \beta_{N*S*CW} + \beta_{GC*S*CW} + \beta_{GC*N*S*CW} + E_P + E_{GC*N*S*P} + E_r \dots \dots \dots \text{Eq1}$$

where  $Y_{BCNSLP}$  is the dependent variable,  $\mu$  is the overall mean,  $\beta_{GC}$  is the fixed effect of genus or clone,  $\beta_N$  is the fixed effect of nitrogen,  $\beta_S$  is the fixed effect of spacing,

$\beta_{CW}$  is the fixed effect of canopy layer or crown section,  $\beta_{GC*N}$  is the genus or clone by nitrogen interaction,  $\beta_{N*S}$  is the nitrogen by spacing interaction,  $\beta_{GC*S}$  is the genus or clone by spacing interaction,  $\beta_{GC*CW}$  is the genus or clone by crown section interaction,  $\beta_{N*CW}$  is the nitrogen by crown section interaction,  $\beta_{S*CW}$  is the spacing by crown section interaction,  $\beta_{GC*N*S}$  is the genus or clone by nitrogen by spacing interaction,  $\beta_{GC*N*CW}$  is the genus or clone by nitrogen by crown section interaction,  $\beta_{N*S*CW}$  is the nitrogen by spacing by crown section interaction,  $\beta_{GC*S*CW}$  is the genus or clone by spacing by crown section interaction,  $\beta_{GC*N*S*CW}$  is the genus or clone by nitrogen by spacing by crown section interaction.  $E_B$ ,  $E_P$ , and  $E_{GC*N*S*P}$ , are the random effects for block, plant pot, and tree, respectively.  $Er$  is the residual error.

Linear regression analyses were used to examine the relationships between  $P_n$  and  $N_{area}$  for hybrid poplars and willows (GLM procedure in SAS, version 9.2). More productive or superior and less productive or inferior clones as a group were identified based on leafless aboveground biomass per tree (AGBT) and aboveground biomass per  $m^2$  (AGBM) as well as physiological characteristics such as  $PNUE_{amb}$  and SLA. Then, linear regression analyses were also undertaken to verify relationship between leafless aboveground biomass per tree (AGBT) and leaf area versus leaf N concentration ( $N_{area}$ ) for superior clones and inferior clones as a group. Slopes between superior and inferior clones were compared using analysis of covariance (ANCOVA). Relationships between  $P_n$  and PAR and between  $PNUE_{amb}$  and PAR were analyzed using the best-fit light response equation as follows:

$$y = a(1 - e^{-bx}) \dots \dots \dots \text{Eq2}$$

where  $y$  is  $P_n$  or  $PNR$ ,  $x$  is PAR, and  $a$  and  $b$  are fitted constants (NLIN procedure in SAS, version 9.2).

### 3.4 Results

#### 3.4.1 Crown light environments and photosynthetic capacity

The 20 and 35 cm spacings caused significant decreases in PAR of 70 % and 50 %, respectively from the top to bottom crown section, while there was no significant changes in PAR between the middle and bottom crown sections in the 60 cm spacing ( $S \times CWS$  interaction, Tables 3.1; 3.2).  $P_n$  decreased by 36 % from the top to the bottom crown sections (Fig. 3.1a; Table 3.1). At the genus level,  $P_n$  for willows was significantly greater than hybrid poplars (Tables 3.1; 3.3). The increase in N level significantly decreased  $P_n$  by 22 % for willows but showed no changes for hybrid poplars ( $N \times G$  interaction, Fig. 3.1b; Table 3.1). There were significant changes in mean  $P_n$  among clones from 2.3 in 747210 to 8.6  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in LAF-I4 (Tables, 3.1; 3.3). Photosynthetic N-use efficiency under ambient light conditions ( $PNUE_{amb}$ ) significantly decreased by 15 % from the top to the bottom crown section (Fig. 3.2a). The increase in N level significantly decreased  $PNUE_{amb}$  by 33 % for hybrid poplars and by 37 % for willows (Fig. 3.2b).  $PNUE_{amb}$  varied depending on clones and spacings ( $C \times S$  interaction, Table 3.1; 3.4). Although most clones showed no significant changes in  $PNUE_{amb}$  under the different spacings,  $PNUE_{amb}$  for clones 2782 and HAN-A5 were lower in the 35 cm spacing compared to the 20 and 60 cm spacings (Table 3.4).

Table 3.1 Analysis of variance showing sources of variation, degrees of freedom (DF),  $F$  values, and associated probabilities for photosynthetically active radiation (PAR), net photosynthesis ( $P_n$ ), photosynthetic N-use efficiency under ambient light conditions ( $PNUE_{amb}$ ), specific leaf area (SLA), and nitrogen content ( $N_{area}$ ) at the genus and clonal levels.

Source of variation	DF	PAR		$P_n$		$PNUE_{amb}$		SLA		$N_{area}$	
		$F$	$P$	$F$	$P$	$F$	$P$	$F$	$P$	$F$	$P$
G	1	0.1	1.0	5.1	<b>0.03</b>	1.8	1.78	15.1	<b>0.0002</b>	61.5	<b>&lt;.0001</b>
N	1	1.9	0.172	0.8	0.369	18.8	<b>&lt;.0001</b>	49.5	<b>&lt;.0001</b>	53.5	<b>&lt;.0001</b>
S	2	6.3	<b>0.003</b>	1.7	0.197	0.1	0.906	14.2	<b>&lt;.0001</b>	33.3	<b>&lt;.0001</b>
CWS	2	126.9	<b>&lt;.0001</b>	36.5	<b>&lt;.0001</b>	3.8	<b>0.02</b>	8.4	<b>0.0002</b>	117.0	<b>&lt;.0001</b>
G × N	1	0.6	0.440	4.6	<b>0.04</b>	0.4	0.557	0.4	0.555	2.3	0.136
G × S	2	1.6	0.211	0.3	0.722	0.1	0.898	2.5	0.09	5.8	<b>0.005</b>
G × CWS	2	0.1	0.950	0.5	0.627	0.4	0.67	0.3	0.722	2.6	0.08
N × S	2	1.3	0.284	1.1	0.33	0.4	0.661	0.2	0.7945	0.5	0.604
N × CWS	2	1.7	0.189	2.1	0.124	0.2	0.83	0.0	0.9914	4.0	<b>0.02</b>
S × CWS	4	10.3	<b>&lt;.0001</b>	2.3	0.061	1.7	0.143	6.0	<b>&lt;.0001</b>	6.0	<b>&lt;.0001</b>
G × N × S	2	0.1	\$0.878	0.6	0.531	1.7	0.195	0.2	0.792	0.2	0.827
G × N × CWS	2	0.1	\$0.980	0.1	0.962	0.1	0.942	0.4	0.676	1.3	0.275
G × S × CWS	4	0.6	\$0.641	0.6	0.653	0.4	0.821	1.1	0.3381	1.0	0.397
N × S × CWS	4	0.9	0.497	0.6	0.645	0.4	0.892	0.5	0.7583	1.3	2.662
G × N × S × CWS	4	0.2	0.930	0.5	0.77	1.0	0.396	0.3	0.854	0.6	0.683
C	11	0.9	0.574	3.7	<b>0.0005</b>	11.0	<b>&lt;.0001</b>	23.6	<b>&lt;.0001</b>	25.1	<b>&lt;.0001</b>
C × N	11	0.3	0.985	1.7	0.0953	1.1	0.346	1.4	0.168	1.5	0.235
C × S	22	1.3	0.232	1.3	0.2294	2.4	<b>0.018</b>	1.7	<b>0.03</b>	2.0	<b>0.006</b>
C × CWS	22	1.3	0.200	0.9	0.645	0.8	0.641	1.6	<b>0.045</b>	2.3	<b>0.036</b>
C × N × S	22	0.5	0.953	0.9	0.5754	1.4	0.217	0.9	0.681	0.5	0.984
C × N × CWS	22	0.4	0.990	0.4	0.996	0.5	0.835	0.9	0.635	0.3	0.999
C × S × CWS	44	1.0	0.550	0.7	0.946	0.8	0.726	0.8	0.78	0.4	0.999
C × N × S × CWS	44	0.6	0.960	0.6	0.952	0.5	0.967	0.9	0.741	0.4	0.999

Notes: In source of variation, G: genus (hybrid poplars vs willows), N: nitrogen, S: spacing, CWS: crown section, and C: clone. Significant  $P$  values are indicated in bold.



Table 3.2 Mean photosynthetically active radiation (PAR,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), specific leaf area (SLA,  $\text{cm}^2 \text{g}^{-1}$ ), and leaf nitrogen concentration ( $N_{\text{area}}$ ,  $\text{g m}^{-2}$ ) for all clones at each crown section under three spacings (20, 35, and 60 cm) after 13 growing weeks.

Variable	Crown section	Spacing		
		20 cm	35 cm	60 cm
PAR	Top	354.6 <sup>d</sup>	329.8 <sup>d</sup>	302.3 <sup>d</sup>
	Middle	200.9 <sup>c</sup>	207.9 <sup>c</sup>	205.7 <sup>bc</sup>
	Bottom	102.9 <sup>a</sup>	148.2 <sup>b</sup>	179 <sup>b</sup>
SLA	Top	255 <sup>b</sup>	259.5 <sup>b</sup>	248.6 <sup>a</sup>
	Middle	257.2 <sup>b</sup>	242.2 <sup>a</sup>	223.2 <sup>a</sup>
	Bottom	280.3 <sup>c</sup>	258.7 <sup>b</sup>	230.7 <sup>a</sup>
$N_{\text{area}}$	Top	1.1 <sup>c</sup>	1.3 <sup>d</sup>	1.3 <sup>d</sup>
	Middle	0.9 <sup>b</sup>	1.1 <sup>c</sup>	1.2 <sup>c</sup>
	Bottom	0.7 <sup>a</sup>	0.9 <sup>b</sup>	1.1 <sup>c</sup>

Note: For each variable, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

Table 3.3 Mean net photosynthesis ( $P_n$ ,  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), photosynthetic N-use efficiency under ambient light conditions ( $\text{PNUE}_{\text{amb}}$ ,  $\mu\text{mol CO}_2 \text{s}^{-1} \text{g}^{-1} \text{N}$ ), specific leaf area (SLA,  $\text{cm}^2 \text{g}^{-1}$ ), and leaf nitrogen concentration ( $N_{\text{area}}$ ,  $\text{g m}^{-2}$ ) for each clone for all spacings (20, 35, and 60 cm) and N levels (20 and 200  $\mu\text{g g}^{-1}$ ) combined after 13 growing weeks.

Clone	Variable			
	$P_n$	$\text{PNUE}_{\text{amb}}$	SLA	$N_{\text{area}}$
<b>Hybrid poplars</b>				
1081	5.5	7.5	318.9	0.8
2782	5.1	6.5	275.5	0.9
747210	2.3	2.5	218.3	1
915319	4.7	5.4	240.4	1
DN-74	3.2	3.5	329	1
NM06	3.6	5	263	1
Walker	3.6	4.4	303.6	0.9
Mean	<b>4</b>	<b>4.8</b>	<b>278.4</b>	<b>0.9</b>
<b>Willows</b>				
HAN-A5	4.6	4.1	266.4	1.1
LEV-D5	5.8	5	195.3	1.2
SHE-E9	3.8	4.4	238.3	1
LAF-I4	8.6	6.4	169.8	1.3
TCH-N4	4.9	5.9	275	1
Mean	<b>5</b>	<b>5</b>	<b>246.7</b>	<b>1.1</b>

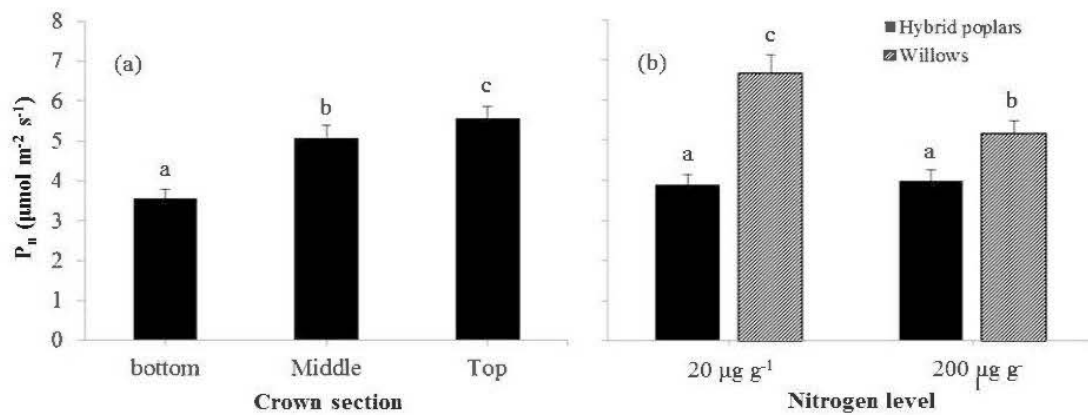


Figure 3.1 Mean net photosynthesis ( $P_n$ ) under three crown sections (bottom, middle, and top) for all clones (a) and under two N levels ( $20$  and  $200 \mu\text{g g}^{-1}$ ) comparing between hybrid poplars and willows (b) after 13 growing weeks. Means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

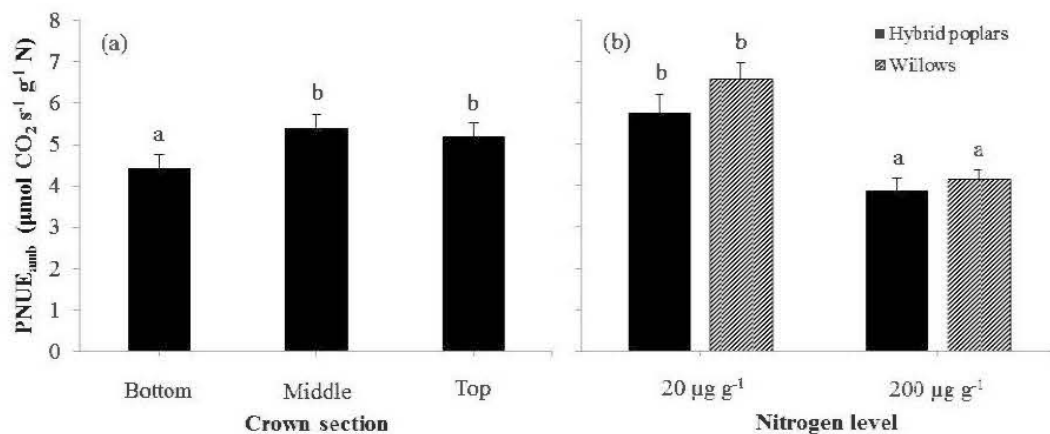


Figure 3.2 Mean photosynthetic N-use efficiency under ambient light conditions ( $\text{PNUE}_{\text{amb}}$ ) for all clones under three crown sections (bottom, middle, and top) (a) and for hybrid poplars and willows under two N levels ( $20$  and  $200 \mu\text{g g}^{-1}$ ) (b) after 13 growing weeks. Means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

Table 3.4 Mean photosynthetic N-use efficiency under ambient light condition ( $\text{PNUE}_{\text{amb}}$ ,  $\mu\text{mol CO}_2 \text{ s}^{-1} \text{ g}^{-1} \text{ N}$ ) for each hybrid poplar and willow clone and spacing (20, 35, and 60 cm) after 13 growing weeks.

Clone	Spacing		
	20 cm	35 cm	60 cm
1081	6 <sup>a</sup>	8.5 <sup>a</sup>	8.2 <sup>a</sup>
2782	6.7 <sup>b</sup>	4.1 <sup>a</sup>	8.8 <sup>b</sup>
747210	3.8 <sup>a</sup>	1.6 <sup>a</sup>	2.1 <sup>a</sup>
915319	5.5 <sup>a</sup>	6.1 <sup>a</sup>	4.6 <sup>a</sup>
DN-74	3.7 <sup>a</sup>	3.7 <sup>a</sup>	3.1 <sup>a</sup>
NM06	6.5 <sup>a</sup>	3.9 <sup>a</sup>	4.8 <sup>a</sup>
Walker	4.5 <sup>a</sup>	4.3 <sup>a</sup>	4.5 <sup>a</sup>
HAN-A5	5.5 <sup>b</sup>	2.4 <sup>a</sup>	4.4 <sup>b</sup>
LEV-D5	4.6 <sup>a</sup>	6.2 <sup>a</sup>	4.3 <sup>a</sup>
SHE-E9	5.3 <sup>a</sup>	3.8 <sup>a</sup>	4.2 <sup>a</sup>
LAF-I4	6.3 <sup>a</sup>	6.3 <sup>a</sup>	6.6 <sup>a</sup>
TCH-N4	6 <sup>a</sup>	5.6 <sup>a</sup>	6 <sup>a</sup>

Note: Within a row, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

#### 3.4.2 Specific leaf area (SLA) and leaf nitrogen concentration ( $\text{N}_{\text{area}}$ )

Trees growing under the high N level had 14 % greater SLA values, from 239 to 273  $\text{cm}^2 \text{ g}^{-1}$  (Fig. 3.3; Table 3.1). SLA significantly varied depending on crown section and spacing: It was greater in the 20 cm spacing from the top to bottom crown sections (+ 10 %), in the 35 cm spacing from the middle to bottom crown section (+ 7 %), and showed no significant changes within the crown in the 60 cm spacing ( $\text{S} \times \text{CWS}$  interaction, Tables 3.1; 3.2). Hybrid poplars had greater SLA compared to willows (Tables 3.1; 3.3). At the clonal level, clones Walker, LEV-D5, LAF-I4, and TCH-N4 had similar SLA in all spacings, while the others had increased SLA from

60 to 20 cm spacing ( $C \times S$  interaction, Tables 3.1; 3.5). There were significant increases in SLA from the top to bottom crown section for clones 1081 and HAN-A5, from the middle to bottom crown section for clones 2782 and Walker, and no significant changes for the other clones ( $C \times CWS$  interaction, Tables 3.1; 3.5).

$N_{area}$  decreased by 28 % from the top to bottom crown section under the low N level and decreased by 34 % under the high N level ( $N \times CWS$  interaction, Fig. 3.4a; Table 3.1).  $N_{area}$  also decreased from the top to bottom crown section by 37 and 30 % in the 20 and 35 cm spacing, respectively and showed no significant changes from the middle to bottom crown section in the 60 cm spacing ( $S \times CWS$  interaction, Tables 3.1; 3.2). At the genus level, hybrid poplars showed 26 and 11 % lower  $N_{area}$  in the 20 and 35 cm spacings, respectively, compared to willows, while the two genera had similar values in the 60 cm spacing ( $G \times S$  interaction, Fig. 3.4b; Table 3.1). At the clonal level, clones NM06, Walker, and LAF-I4 showed no significant changes in  $N_{area}$ , under spacing while the others exhibited lower  $N_{area}$  in the 20 cm spacing compared to the 35 or 60 cm spacing ( $C \times S$  interaction, Tables 3.1; 3.5). Although all clones showed significant decreases in  $N_{area}$  from the top to bottom crown section,  $N_{area}$  was lower from the middle to bottom crown section for all clones except SHE-E9 ( $C \times CWS$  interaction, Tables 3.1; 3.5).

Table 3.5 Mean specific leaf area (SLA,  $\text{cm}^2 \text{g}^{-1}$ ) and leaf nitrogen concentration ( $N_{\text{area}}$ ,  $\text{g m}^{-2}$ ) for each hybrid poplar and willow clone under the three spacings (20, 35, and 60 cm) and three crown sections (bottom, middle, and top) after 13 growing weeks.

Clone	Spacing			Crown section		
	20 cm	35 cm	60 cm	Bottom	Middle	Top
SLA						
1081	367.7 <sup>b</sup>	270.7 <sup>a</sup>	304.9 <sup>a</sup>	352.4 <sup>b</sup>	309.2 <sup>ab</sup>	290.1 <sup>a</sup>
2782	292.7 <sup>c</sup>	269.1 <sup>b</sup>	234.4 <sup>a</sup>	289.1 <sup>b</sup>	251.9 <sup>a</sup>	286.5 <sup>b</sup>
747210	237.5 <sup>b</sup>	201.3 <sup>a</sup>	200.1 <sup>a</sup>	226.9 <sup>a</sup>	216.1 <sup>a</sup>	212.1 <sup>a</sup>
915319	250.9 <sup>b</sup>	238.2 <sup>b</sup>	206.6 <sup>a</sup>	245.1 <sup>a</sup>	239.4 <sup>a</sup>	236.3 <sup>a</sup>
DN-74	319.9 <sup>b</sup>	357.1 <sup>c</sup>	272 <sup>a</sup>	348.5 <sup>a</sup>	336 <sup>a</sup>	306.4 <sup>a</sup>
NM06	281.3 <sup>b</sup>	249.5 <sup>a</sup>	236.7 <sup>a</sup>	268.3 <sup>a</sup>	249.7 <sup>a</sup>	271.5 <sup>a</sup>
Walker	295.9 <sup>a</sup>	307.4 <sup>a</sup>	319.2 <sup>a</sup>	300.4 <sup>b</sup>	280.4 <sup>a</sup>	329.9 <sup>b</sup>
HAN-A5	257.8 <sup>b</sup>	286.3 <sup>b</sup>	234 <sup>a</sup>	281.3 <sup>b</sup>	260.9 <sup>a</sup>	256.1 <sup>a</sup>
LEV-D5	196 <sup>a</sup>	195.5 <sup>a</sup>	192 <sup>a</sup>	198.8 <sup>ab</sup>	173.2 <sup>a</sup>	213.5 <sup>b</sup>
SHE-E9	251.1 <sup>b</sup>	232.5 <sup>b</sup>	212 <sup>a</sup>	234.7 <sup>ab</sup>	229.4 <sup>a</sup>	250.6 <sup>b</sup>
LAF-I4	168.7 <sup>a</sup>	174.6 <sup>a</sup>	154.9 <sup>a</sup>	175.1 <sup>a</sup>	170.3 <sup>a</sup>	164.1 <sup>a</sup>
TCH-N4	268.6 <sup>a</sup>	282.4 <sup>a</sup>	269.4 <sup>a</sup>	282.5 <sup>a</sup>	263.5 <sup>a</sup>	278.6 <sup>a</sup>
$N_{\text{area}}$						
1081	0.7 <sup>a</sup>	1 <sup>b</sup>	0.9 <sup>a</sup>	0.6 <sup>a</sup>	0.9 <sup>b</sup>	1.1 <sup>c</sup>
2782	0.8 <sup>a</sup>	0.9 <sup>ab</sup>	1.1 <sup>b</sup>	0.7 <sup>a</sup>	0.9 <sup>b</sup>	1 <sup>c</sup>
747210	0.7 <sup>a</sup>	1.1 <sup>b</sup>	1.2 <sup>b</sup>	0.7 <sup>a</sup>	0.9 <sup>b</sup>	1.2 <sup>c</sup>
915319	0.8 <sup>a</sup>	1.1 <sup>b</sup>	1.2 <sup>b</sup>	0.8 <sup>a</sup>	0.9 <sup>b</sup>	1.3 <sup>c</sup>
DN-74	0.8 <sup>a</sup>	1.1 <sup>b</sup>	1.2 <sup>b</sup>	0.8 <sup>a</sup>	0.9 <sup>b</sup>	1.2 <sup>c</sup>
NM06	0.9 <sup>a</sup>	1 <sup>a</sup>	1.1 <sup>a</sup>	0.8 <sup>a</sup>	1 <sup>b</sup>	1.2 <sup>c</sup>
Walker	0.8 <sup>a</sup>	1 <sup>a</sup>	1 <sup>a</sup>	0.8 <sup>a</sup>	1 <sup>b</sup>	1 <sup>c</sup>
HAN-A5	1.1 <sup>a</sup>	1.2 <sup>b</sup>	1.2 <sup>a</sup>	1 <sup>a</sup>	1.1 <sup>b</sup>	1.3 <sup>c</sup>
LEV-D5	1.1 <sup>a</sup>	1.3 <sup>b</sup>	1.2 <sup>ab</sup>	1.1 <sup>a</sup>	1.2 <sup>b</sup>	1.3 <sup>b</sup>
SHE-E9	0.9 <sup>a</sup>	1 <sup>ab</sup>	1.2 <sup>b</sup>	0.9 <sup>a</sup>	0.9 <sup>a</sup>	1.1 <sup>b</sup>
LAF-I4	1.3 <sup>a</sup>	1.3 <sup>a</sup>	1.6 <sup>a</sup>	1 <sup>a</sup>	1.3 <sup>b</sup>	1.7 <sup>c</sup>
TCH-N4	0.9 <sup>a</sup>	1 <sup>ab</sup>	1.2 <sup>b</sup>	0.8 <sup>a</sup>	1 <sup>b</sup>	1.2 <sup>c</sup>

Note: Within a row under spacing and crown section, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

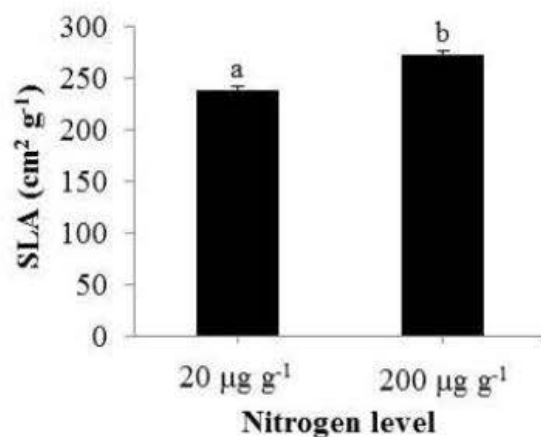


Figure 3.3 The effect of N level on mean specific leaf area (SLA) for all clones after 13 growing weeks.

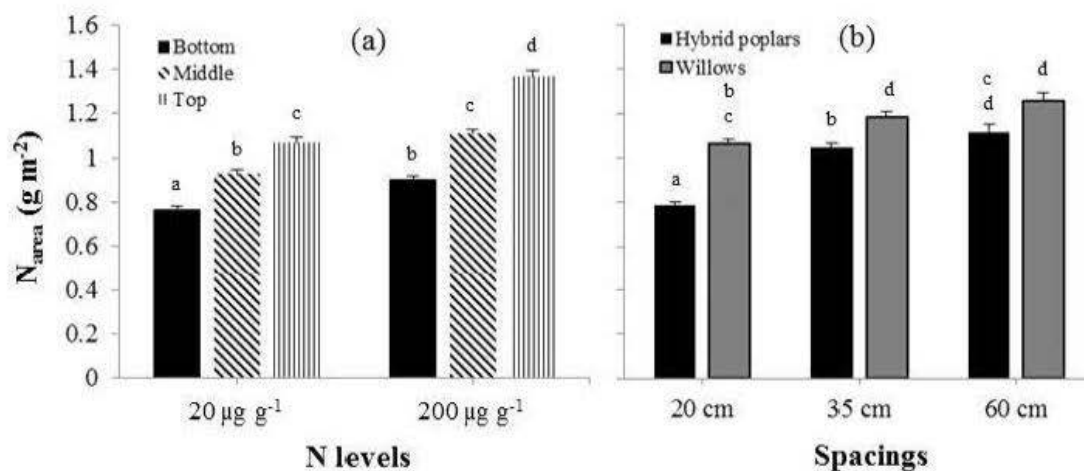


Figure 3.4 Mean nitrogen concentration ( $N_{area}$ ) for each nitrogen levels (20 and 200  $\mu g\ g^{-1}$ ) and crown section (bottom, middle, and top) (a) under three spacings (20, 35, and 60 cm) for hybrid poplars and willows (b) after 13 growing weeks. In each figure, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .



### 3.4.3 Leaf trait relationships

Mean photosynthetic N-use efficiency under ambient light conditions ( $\text{PNUE}_{\text{amb}}$ ) was always higher under the low N level (Fig. 3.5a). Spacing had little effect on the response of  $\text{PNUE}_{\text{amb}}$  to PAR (Fig. 3.5b). For any PAR,  $P_n$  for both hybrid poplars and willows were lower in the bottom crown section compared to the middle and top crown sections (Fig. 3.6ab). However, under lower PAR ( $< 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ),  $\text{PNUE}_{\text{amb}}$  in the bottom crown section for hybrid poplars and willows was relatively higher than the top crown sections (Fig. 3.6cd). Hybrid poplars and willows showed different acclimations to the received PAR;  $\text{PNUE}_{\text{amb}}$  for willows reached a plateau at  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR for all crown sections (Fig. 3.6d) while  $\text{PNUE}_{\text{amb}}$  for hybrid poplars did not level off in the ambient light conditions (Fig. 3.6c). Hybrid poplars and willows also showed different  $P_n$ - $N_{\text{area}}$  relationships, as  $P_n$  was positively related to  $N_{\text{area}}$  for willows ( $P < 0.05$ ) (Fig. 3.7), but not the hybrid poplars ( $P > 0.05$ ).

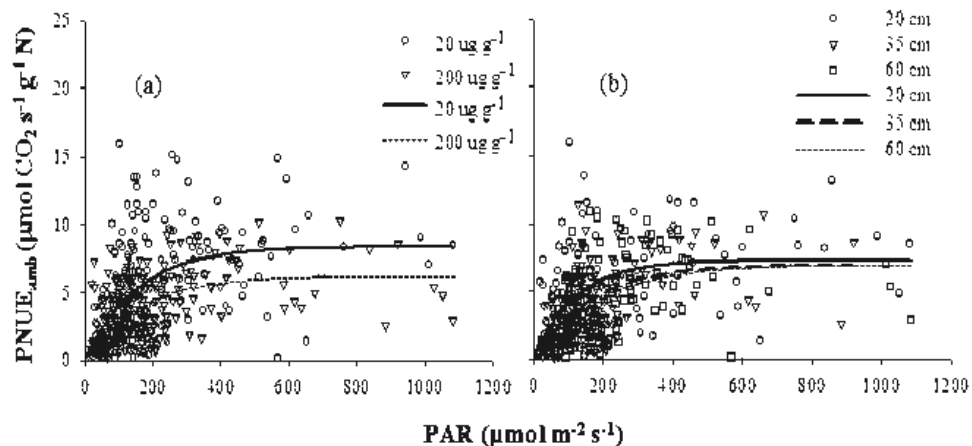


Figure 3.5 Photosynthetic N-use efficiency under ambient light conditions ( $\text{PNUE}_{\text{amb}}$ )-photosynthetically active radiation (PAR) response curve under two nitrogen (N) levels (20 and  $200 \mu\text{g g}^{-1}$ ) (a) and three spacings (20, 35, and 60 cm) (b) after 13 growing weeks.  $P < 0.001$  and  $R^2 > 0.3$  for all regressions.



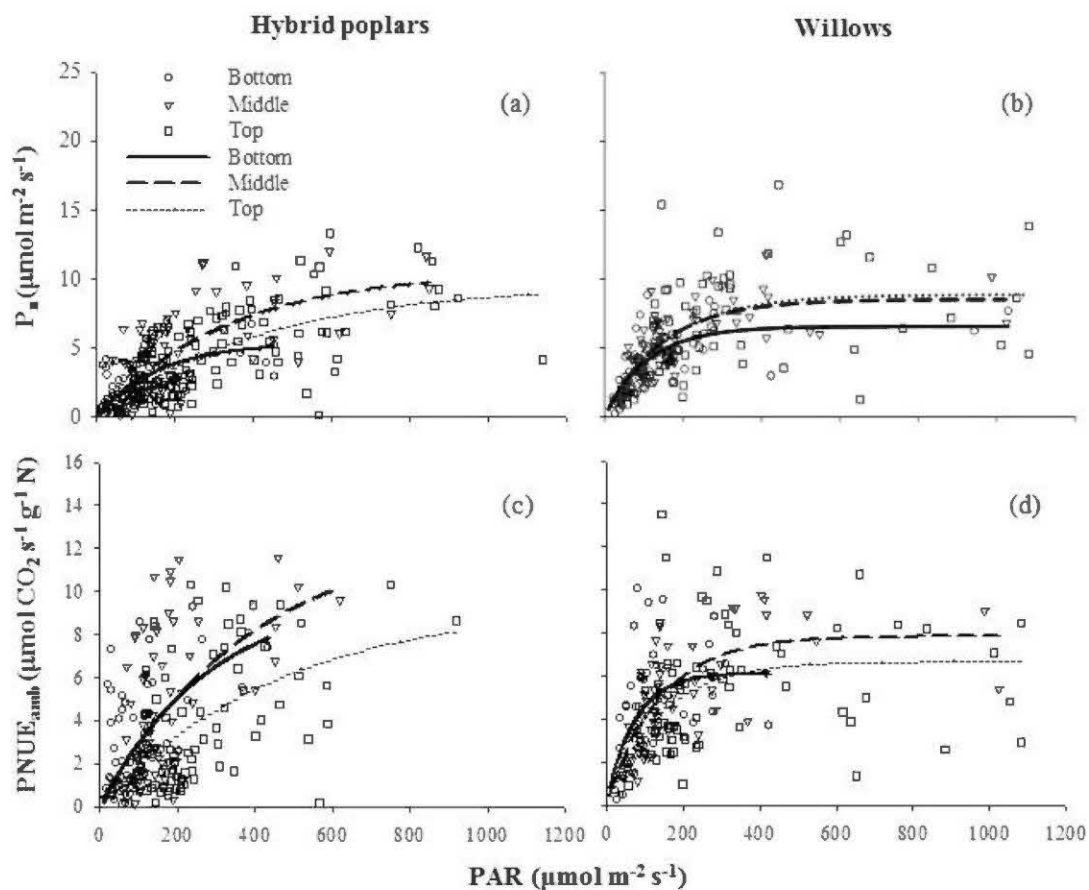


Figure 3.6 Net photosynthesis ( $P_n$ )- photosynthetically active radiation (PAR) response curve for the three crown sections (bottom, middle, and top) for hybrid poplars (a) and willows (b) and photosynthetic N-use efficiency under ambient light condition ( $\text{PNUE}_{\text{amb}}$ )-PAR response curve under three crown sections for hybrid poplars (c) and willows (d) after 13 growing weeks.  $P < 0.001$  and  $R^2 > 0.3$  for all regressions.

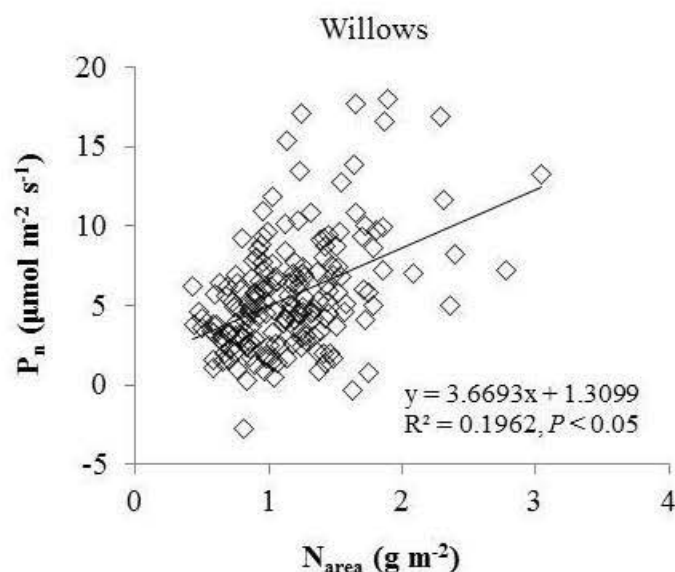


Figure 3.7 Relationships between net photosynthesis ( $P_n$ ) and nitrogen concentration ( $N_{\text{area}}$ ) for willows after 13 growing weeks. No significant relationships for hybrid poplars.

#### 3.4.4 Characteristics of superior clones

Genotypic differences caused great differences in plant morphology and physiology. Compared to less productive or inferior clones 747210, 915319, LEV-D5, and LAF-I4, more productive or superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group had 143 %, 244 %, 129 %, and 28 % greater leafless aboveground biomass per tree (AGBT), greater AGBT with low N level, leafless aboveground biomass per  $\text{m}^2$  (AGBM), and SLA, respectively (Table 3.6). Superior clones also had 6 % greater  $\text{PNUE}_{\text{amb}}$  than inferior clones but it was not significantly different (Table 3.6). In addition, superior clones had greater AGBT and leaf area per unit increase in  $N_{\text{area}}$  (Figs. 3.8; 3.9).

Table 3.6 Mean leafless aboveground biomass per tree (AGBT), AGBT under low N (Low N AGBT), leafless aboveground biomass per m<sup>2</sup> (AGBM), specific leaf area (SLA), and photosynthetic N-use efficiency in the ambient light condition (PNUE<sub>amb</sub>) of superior clones (2782, NM06, HAN-A5, SHE-E9, and TCH-N4) compared to inferior clones (747210, 915319, LEV-D5, and LAF-I4) as a group after 13 growing weeks.

Variable	Superior clones	Inferior clones
AGBT (g)	18.9 <sup>b</sup>	7.8 <sup>a</sup>
Low N AGBT (g)	16.6 <sup>b</sup>	4.8 <sup>a</sup>
AGBM (g m <sup>-2</sup> )	247 <sup>b</sup>	107.9 <sup>a</sup>
SLA (cm <sup>2</sup> g <sup>-1</sup> )	263.6 <sup>b</sup>	205.3 <sup>a</sup>
PNUE <sub>amb</sub> (μmol CO <sub>2</sub> s <sup>-1</sup> g <sup>-1</sup> N)	5.2 <sup>a</sup>	4.9 <sup>a</sup>

Note: Within a row, means followed by the same letter do not significantly differ at  $\alpha = 0.05$ .

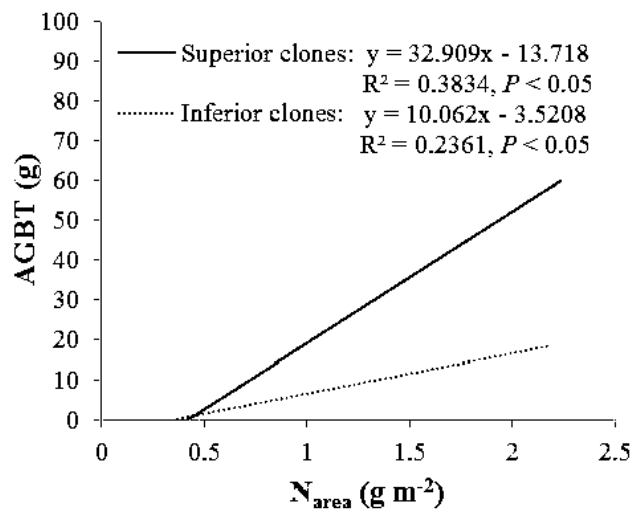


Figure 3.8 Relationship between leafless aboveground biomass per tree (AGBT) and leaf N concentration (N<sub>area</sub>) for superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group and inferior clones 747210, 915319, LEV-D5, and LAF-I4 as a group after 13 growing weeks.

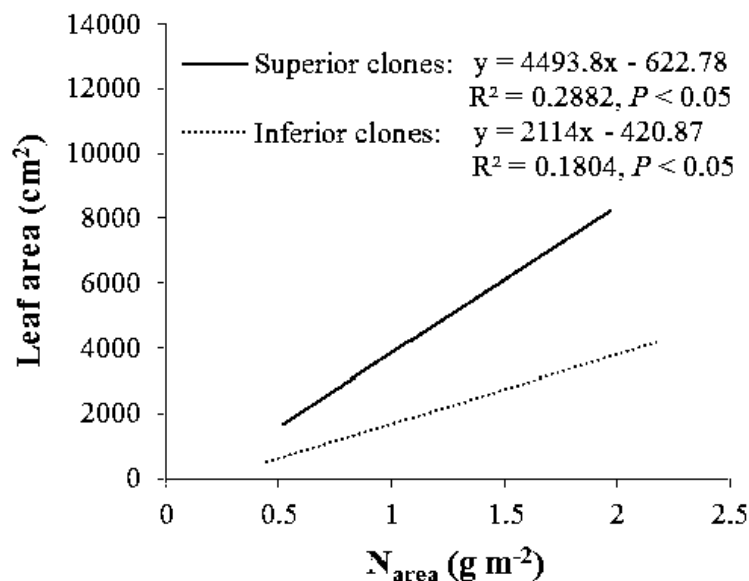


Figure 3.9 Relationship between leaf area and leaf N concentration (N<sub>area</sub>) for superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group and inferior clones 747210, 915319, LEV-D5, and LAF-I4 as a group after 13 growing weeks.

### 3.5 Discussion

It is desirable to identify clones that can grow well under intensive plant competition and low N level so that these clones can maximize biomass productivity per unit area with reduced fertilization and land acquisition costs. As all clones did not show any significant changes in AGBT with the decrease in spacing (data not shown), they acclimated their growth well to the intensive competition where resource availability was more limited (Table 3.1). Similar clonal responses of AGBT to spacing were due to similar responses of  $P_n$ ,  $PNUE_{amb}$ , SLA, and N<sub>area</sub> for most clones to spacing (Tables 3.1; 3.4; 3.5). The greater growth capacity to the closer spacing for all clones was primarily explained by greater photosynthetic acclimation under competitive conditions. In this study, leaves from the upper canopy or in the largest spacing received higher PAR, which resulted in thicker leaves with lower SLA and higher

$N_{\text{area}}$  (Table 3.2), probably due in part to greater N allocation in extra layers of palisade or longer palisade cells (Evans and Poorter, 2001). The development of palisade cells increases the number of chloroplasts and the amount of photosynthetic enzymes (Evans and Poorter, 2001) and thereby enhances photosynthetic capacity. In this study,  $P_n$  significantly increased from the bottom to the top crown section with greater  $N_{\text{area}}$  and PAR (Fig. 3.1a and Table 3.2). On the other hand, in spite of the great decrease in PAR in the bottom crown section in the 20 cm spacing, shade leaves utilized N efficiently for greater  $P_n$  compared to sun leaves (Fig. 3.6cd). This photosynthetic acclimation under low PAR resulted from thin leaves with higher SLA and lower  $N_{\text{area}}$  (Table 3.2). This may have induced a greater proportion of N allocated to photosynthetically derived compounds such as Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Hirosaka and Terashima, 1995), thereby increasing photosynthetic capacity (Poorter and Evans, 1998; Evans and Poorter, 2001). It is widely recognized that shade or high-SLA leaves have greater photosynthetic nitrogen-use efficiency at either ambient light or saturating light conditions (Poorter and Evans, 1998; Evans and Poorter, 2001). These results show that the shade leaves partially offset the negative impact of competition on  $P_n$  by maximizing PAR- and N-use efficiency for  $P_n$ .

Genotypic differences caused significant changes in plant productivity as superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group had greater AGBT, AGBT under low N level, and hence AGBM compared to inferior clones 747210, 915319, LEV-D5, and LAF-I4 as a group (Table 3.6). As superior clones showed significantly greater AGBT and leaf area (LA) per unit increase in  $N_{\text{area}}$  compared to inferior clones (FigS. 3.8; 3.9), the greater N-use efficiency for biomass production was due to greater N-use efficiency for LA expansion. Therefore, superior clones had greater photosynthetic area per tree even under the closer spacing where  $N_{\text{area}}$  per unit LA was smaller. In addition, superior clones had 28 % greater LA production per unit leaf biomass (SLA) (Table 3.6), indicating greater LA development capacity

under the decrease in spacing where leaf biomass was significantly reduced (data not shown). However,  $PNUE_{amb}$  was not significantly different between superior and inferior clones (Table 3.6), concluding that greater productivity of superior clones was not owing to the photosynthetic capacity of individual leaves under ambient light conditions but due to greater LA production capacity.

Compared to a field study using the same hybrid poplar clones 747210 and 915319 (Benomar *et al.*, 2011), this greenhouse study showed greater values of SLA, 200-251  $cm^2 g^{-1}$ , compared to 69-142  $cm^2 g^{-1}$  in the field. We also had lower  $N_{area}$  0.7-1.3  $g m^{-2}$  values compared to 1.1-3.4  $g m^{-2}$  in the field (Benomar *et al.*, 2011); Although the field study used wider spacings of  $1 \times 1$  to  $5 \times 5$  m, the measurements were taken 5 years after plantation establishment where trees were under fairly competitive conditions (Benomar *et al.*, 2011). Since greenhouse light conditions may represent 60 to 80 % of field light conditions (Sharpe *et al.*, 1999; Keyhaninejad *et al.*, 2012), the higher SLA and lower  $N_{area}$  may be primarily owing to much lower light levels in the greenhouse. In this study, the highest PAR measured was 1082  $\mu mol m^{-2} s^{-1}$  (data not shown). However, in the open field, PAR often reaches up to 1500  $\mu mol m^{-2} s^{-1}$  in North America (Fredericksen *et al.*, 1996; Cadenasso *et al.*, 1997).

The addition of N significantly increased  $N_{area}$  at each crown section (Fig. 3.4a), which was consistent with other studies as a great portion of N may have been distributed to leaves, particularly Rubisco, a critical leaf protein for photosynthesis (Rosati *et al.*, 2000; Clearwater *et al.*, 2001; Dickmann *et al.*, 2001). In this study, willows had a greater amount of leaf N within the canopy compared to hybrid poplars (Fig. 3.4b). Greater  $N_{area}$  for willows may have been due to greater nitrogen uptake capacity in relation to root morphology (Al Afas *et al.*, 2008) or distribution of root biomass (Johnson and Biondini, 2001), or to a greater N fixation capacity with the presence of diazotrophic microorganisms in willows (Doty *et al.*, 2009). Curiously, although N is an important nutrient for photosynthesis, the significant relationship

between  $P_n$  and  $N_{area}$  observed for willows was not found for hybrid poplars (Fig. 3.7). Others studies using several deciduous and coniferous trees also showed significant relationships between leaf N concentration and the rate of photosynthesis (e.g., *Acer Saccharum* Marsh., Reich *et al.*, 1991; *Eucalyptus globulus* Labill., Sheriff and Nambiar, 1991; *Pinus radiant*, Thompson and Wheeler, 1992; *Acer Saccharum* and *Betula alleghaniensis*, Delagrange, 2011) while other found non-significant relationships (e.g., several hybrids of *Populus deltoides*, *P. trichocarpa* and *P. maximowiczii*, Ceulemans *et al.*, 1987; *Pseudotsuga menziesii* (Mirb.), Mitchell and Hinckley, 1993). The different  $P_n$ - $N_{area}$  relationships between hybrid poplars and willows may be explained by variations in the proportion of N allocated to photosynthetic versus non-photosynthetic functions among species (Poorter and Evans, 1998). Therefore, willows may have allocated a greater proportion of N to photosynthetic functions compared to hybrid poplars. Also, as the activation state of Rubisco differs among species (Poorter and Evans, 1998), the activity of Rubisco for willows may have better responded to leaf N changes.

As the increase in N level increased leaf area by 47 % (data not shown), the decreased  $P_n$  for willows likely resulted from an increased degree of self-shading with N fertilization. In fact,  $P_n$  patterns were paralleled with light environment where willow clones reduced PAR by 10 % and hybrid poplars by 2 % following increased N level (data not shown). Under any of the measured PAR levels,  $PNUE_{amb}$  for high N level was lower than low N level (Fig. 3.5a), indicating that the increased amount of  $N_{area}$  under the high N level may have overinvested the production of photosynthetic proteins that were not efficiently utilized for photosynthesis under the available PAR (Boadman, 1977). This may be because the benefit of higher amount of Rubisco in photosynthesis are partially lessened by a lower Rubisco activity state in high-N leaves (Cheng and Fuchigami, 2000; Ray *et al.*, 2003).

Hybrid poplars and willows showed different degrees of shade tolerance under tree competition. Willows exhibited relatively higher light saturation point ( $5.8 \mu\text{mol m}^{-2} \text{s}^{-1} P_n$ ) at  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR in the bottom crown section, compared to hybrid poplars that reached lower light saturation point ( $5.0 \mu\text{mol m}^{-2} \text{s}^{-1} P_n$ ) at  $400 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. This may have indicated a greater shade tolerance for willows as leaves with greater shade tolerance can enhance light-use efficiency for photosynthesis at lower PAR (Kozlowski and Pallardy, 1997). The greater light-use efficiency for willows may be also explained by narrow leaves for willows that could help having better light interception in the lower canopy by reducing the degree of leaf aggregation compared to roundly triangular leaves for hybrid poplars (Percy and Yang, 1998; Takenaka *et al.*, 2001; Casella and Sinoquet, 2007). In this study, willows had greater ratios of leaf length to leaf width and relatively greater PAR for willows ( $132 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) compared to hybrid poplars ( $110 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in the bottom crown section (data not shown). In fact, narrow leaves can increase light harvesting during the establishment year (Niinemets 1998; Casella and Sinoquet, 2007), indicating a beneficial impact on photosynthetic capacity under intensive competition. Under ambient light conditions,  $\text{PNUE}_{\text{amb}}$  for willows was greater up to  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR compared to hybrid poplars (Fig. 3.6cd). However, hybrid poplars had greater  $\text{PNUE}_{\text{amb}}$  after  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (Fig. 3.6cd), indicating greater shade intolerance of hybrid poplars under higher PAR.

Willow clones LEV-D5 and LAF-I4 exhibited the highest mean  $N_{\text{area}}$  and relatively greater  $P_n$  compared to the other clones. Greater  $P_n$  of these clones could have been the result of compensatory photosynthesis after aphid feeding that frequently occurred during growing period as aphids may have enriched leaf nitrogen resulting from introduced metabolic compounds into host plants (Collins *et al.*, 2001). Similarly, an increase in net photosynthesis after aphids was also reported by previous studies (Way and Cammell 1970; Collins *et al.*, 2001). Also, the lowest SLA for these clones in the bottom crown sections may have resulted from enhanced



light penetration within the crown as aphid caused a large loss of leaves (Mattson *et al.*, 2001).

### 3.6 Conclusion

The decrease in spacing reduced available resources such as PAR and  $N_{area}$ , and trees generally responded by increasing SLA and increasing  $PNUE_{amb}$  under lower PAR, thereby increasing photosynthetic capacity under intensive competition. Consequently, spacing did not cause significant changes in  $P_n$  for all clones indicating that all clones photosynthetically acclimated to the decrease in spacing. Compared to inferior clones 747210, 915319, LEV-D5, and LAF-I4, superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group had greater AGBT, AGBT under low N level, and AGBM. Superior clones also showed greater AGBT and LA per unit increase in  $N_{area}$ , suggesting greater shoot-growth capacity under the closer spacing where  $N_{area}$  per unit LA was smaller. Superior clones had 28 % greater SLA, compared to inferior clones, suggesting a more efficient LA production per unit leaf biomass. However,  $PNUE_{amb}$  was similar between superior and inferior clones, concluding that greater productivity of superior clones was not owing to the photosynthetic capacity of individual leaves in the ambient light conditions but due to greater LA production capacity. The increase in N increased  $N_{area}$  and SLA within the crown, but had little effect on  $P_n$  and even decreased  $PNUE_{amb}$  under different spacings. Thus, N fertilization did not play an important role in increasing photosynthetic capacity.

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## CHAPTER IV

### GENERAL CONCLUSION

The primary objective of this study was to investigate the morphological and physiological characteristics leading to superior growth performance of clones under high plant competition and different nitrogen (N) levels. Through this study, I answered two specific questions: Firstly, if the decrease in spacing caused variations in growth performance of clones under high or low N level, what were the morphological and physiological characteristics that lead to greater growth performance? Secondly, how much did soil N availability reduce the negative impacts of competition on growth? The hypotheses corresponding to the first question were that although a decrease in spacing would reduce available resources such as light (PAR) and nutrients, clones with superior growth performance under competition would 1) show no significant changes in leafless aboveground biomass per tree (AGBT), 2) have greater AGBT per unit increase in LA and leaf N concentration ( $N_{area}$ ), 3) exhibit greater amounts of leaf area (LA) and greater amount of LA per unit plant biomass (LAR), 4) have greater slenderness ratios (SR), 5) allocate more biomass to stems compared to roots, 6) show no significant changes in  $P_n$  under reduced spacing, 7) have greater SLA, and 8) greater photosynthetic N-use efficiency under ambient light conditions ( $PNUE_{amb}$ ). We also hypothesized that negative effects of competition, such as crown recession or decreased RCD and LA, would be reduced with N fertilization with 1) greater LA and 2) greater  $P_n$  associated with greater leaf N content per unit LA ( $N_{area}$ ).

For the first question, although genotypic differences caused significant changes in RCD and height, clones had similar AGBT under the different spacings, indicating that they acclimated well to intensive plant competition for the study period, 13

weeks. To achieve this, clones increased SR and height per unit LA with the decrease in spacing, increasing shoot growth under limited available space. Similarly, there were no significant changes in  $P_n$  for all clones under the different spacings despite significant decreases in PAR and  $N_{area}$  from 60 to 20 cm spacing, indicating a greater photosynthetic acclimation to the decrease in spacing. This was because  $PNUE_{amb}$  was generally greater under low PAR and SLA also increased from 60 to 20 cm spacing, which increased photosynthetic capacity under shaded conditions. Therefore, all clones showed relatively similar growth responses to spacing. Although acclimation to competition did not vary among clones, genotypic differences caused significant changes in AGBT in this study. Compared to inferior clones 747210, 915319, LEV-D5, and LAF-14 as a group, superior clones 2782, NM06, HAN-A5, SHE-E9, and TCH-N4 as a group 143 %, 244 %, and 129 % greater AGBT, AGBT under low N, and AGBM, respectively after 13 growing weeks, which also indicates their greater growth performance in the field. The greater biomass productivity of superior clones was mainly explained by 100 % greater leaf area (LA) as LA was significantly related to plant growth of most clones. In particular, compared to inferior clones, superior clones prioritized greatly to increase LA production for maximizing total photosynthetic area per tree as LAR and SLA for superior clones were greater. In addition, superior clones showed greater AGBT per unit increases in LA and  $N_{area}$ , indicating greater shoot growth capacity under the decrease in spacing where the amount of  $N_{area}$  and LA were smaller. The greater shoot growth capacity of superior clones resulted in lower RSR. However,  $PNUE_{amb}$  was not significantly different between superior and inferior clones, concluding that greater productivity of superior clones was not owing to the photosynthetic capacity of individual leaves under ambient light conditions but due to greater LA production capacity. For economically feasible SRF plantations, silviculture costs including site preparation, maintenance costs, and fertilization must be reduced as much as possible to achieve great economic returns with best clones. Thus, these superior clones can be candidate ones to achieve greater biomass production per unit area with reduced N fertilization



cost. Thus, these clones should be tested in the field if these selected ones can maximize productivity under local climate conditions and show resistance to pest and diseases.

Nitrogen fertilization could be an effective silvicultural treatment under intensive competition as N was an important element to increase tree growth. Compared to low N and 60 cm spacing, trees growing under high N and 20 cm spacing showed similar RCD, CW, and LA values and even greater height, concluding that an addition of N can play an essential role in increasing plant growth under the intensive competition with greater LA. Although the increase in N increased  $N_{area}$ , soil N availability had little effect on  $P_n$  and even decreased  $PNUE_{amb}$ . Thus, N fertilization did not play an important role in increasing photosynthetic capacity.

This study also addressed similarities and differences between hybrid poplar and willow clones for their suitability for SRF management. The both hybrid poplars and willows maximized AGBM in the closest spacing, indicating great acclimation to the intensive plant competition where resource availability (e.g., light, nutrients) is limited. Therefore, both hybrid poplars and willows seemed suitable for SRF management to maximize biomass production. The greater AGBM in the closer spacing was primarily explained by greater SR for stem elongation and SLA for leaf area expansion relative to leaf biomass, which achieved greater light-interception capacity. There were also no significant changes in AGBT and AGBM between hybrid poplars and willows. This was because hybrid poplars and willows increased shoot growth in a different way corresponding to their morphology and physiology. Morphological differences in hybrid poplars and willows were, for the most part, explained by crown architecture; Hybrid poplars generally had a long vertical crown with narrow crown width, while willows had greater crown width with more stems. Fewer stems of hybrid poplars had relatively greater height per unit an increase in LA, resulting in greater relative growth rate in height compared to willows. In addition,

greater LA of hybrid poplars resulted from a greater LAR and biomass proportion to leaves, indicating better light-interception capacity. On the other hand, greater number of stems for willows had greater biomass allocation to stems, which resulted in greater crown width per unit increase in LA compared to hybrid poplars. Photosynthetic capacity also varied among hybrid poplars and willows; Under ambient light conditions,  $\text{PNUE}_{\text{amb}}$  for willows was greater up to  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, while hybrid poplars had greater  $\text{PNUE}_{\text{amb}}$  after  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. This indicated that willows had greater photosynthetic capacity under low PAR, likely due to greater shade tolerant characteristics of leaves and hybrid poplars showed greater photosynthetic capacity under higher PAR, due to greater shade intolerant of leaves.

Despite no significant differences in the total AGBM between hybrid poplars and willows, willows had greater AGBM in the 20 cm spacing compared to hybrid poplars. The greater AGBM of willows may be explained by several factors: Firstly, willows had greater capacity to produce AGBT per unit increase in LA as willows had the lower LA and greater biomass allocation to stems compared to hybrid poplars. Secondly, willows had greater photosynthetic capacity ( $\text{PNUE}_{\text{amb}}$ ) under low PAR, which allowed willow trees to increase AGBT efficiently under shaded conditions in the closest spacing. Thirdly, as willows had greater  $N_{\text{area}}$  in the 20 cm spacing compared to hybrid poplars, greater  $N_{\text{area}}$  enhanced photosynthetic capacity under the shade condition in the closest spacing.

Lastly, to accelerate selection processes in tree improvement programs, we ask ourselves: Which variables should be measured and how? This study suggests that all clones showed similar productivity responses to spacing, indicating that the growth acclimation to competition did not vary a lot among clones. However, genotypic differences caused significant changes in clonal productivity. In particular, LA production capacity played an essential role in greater productivity as AGBT was significantly related to LA. Therefore, greater LA production per unit increase in

$N_{area}$  could be a useful measure to evaluate greater production capacity of clones because superior clones would show greater LA under the decrease in spacing where  $N_{area}$  generally decreased. Based on this result, I suggest a methodology to achieve earlier selection as follows: at first, clones are planted in the 20 cm spacing as these clones will be under competitive status over 3 months of growing period. Although clones in the both 20 and 35 cm spacings were under competitive conditions in this study, the 20 cm spacing had significantly less LA which can minimize time for measuring LA compared to the 35 cm spacing. Secondly, total LA and  $N_{area}$  per tree would be measured and regressed. Then, clones with greater LA per unit increase in  $N_{area}$  could be selected as superior clones as these clones also have greater shoot growth per unit increase in  $N_{area}$ .

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