THE RESPONSES OF FEMALE AND MALE COTTONWOOD SAPLINGS TO
FLOODING

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DEDICATION

I would like to dedicate this thesis to my parents, Hugo and Lynda Nielsen, who, although not with us anymore, were able to guide me through the challenging and rewarding time of graduate school at the University of Lethbridge. This small contribution to the vast body of knowledge we refer to as science will not alter the world, but for me it has changed my perception and attitude towards our involvements that work to make the world a better place, enhance life and learning and promote the accumulation of knowledge. I see fit in dedicating my work to the two people who gave me unconditional support and encouragement throughout my academic, and personal, endeavors and who instilled in me the thirst for knowledge, the importance of making a difference (no matter how small) and the value of life. Their presence in my life surpasses their deaths and will continue to guide me through such gratifying challenges, as it has done thus far.

ABSTRACT

Cottonwoods are poplar trees that are adapted to riparian zones that are naturally occasionally flooded. Like all *Salicaceae*, cottonwoods are dioecious and prior studies have indicated that males are more drought-tolerant than females and found more often in poorer, drier sites. We investigated sex differentiation of cottonwoods in response to the opposite water-stress, flood, and predicted that the increased water-stress tolerance of males in drought would also apply to flood-stress. Twenty-one clones of male and female narrowleaf cottonwoods (*Populus angustifolia*) were grown in a greenhouse along with three female clones of the hybrid native lanceleaf cottonwood (*P. x acuminata*) for comparison. It was anticipated that the hybrids would show the fastest growth owing to the genetic contribution from the *P. deltoides* parent and its rapid intrinsic growth rate.

Flood reduced heights and the numbers and sizes of leaves and roots, and consequently dry weights, abaxial stomatal conductance and leaf chlorophyll. Inundation increased carbon:nitrogen, but did not alter stomatal density, leaf water potential, or $\delta^{13}C$. The hybrid saplings were much larger than the narrowleaf saplings but their proportional growth reduction with flooding was greater than in the female *P. angustifolia*, suggesting higher flood-tolerance of the narrowleaf cottonwood. *P. angustifolia* sexes performed similarly under reference conditions but the males were proportionally more inhibited by flood, suggesting sex differentiation in flood-tolerance. This study indicates that riparian cottonwoods are reasonably flood-tolerant but slight differences exist between the sexes and to a greater extent, across taxa. While prior studies have indicated males are apparently more tolerant of drought, females are probably more flood-tolerant.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

- A, Net assimilation (g/unit leaf area)
- CDW, Cutting dry weight (g)
- Chl, Relative abundance of chlorophyll (CCl units)
- C: N, Carbon to nitrogen abundance
- $\delta^{13}C$, Carbon isotope composition
- DG, Diameter growth (cm)
- F, Female
- Group, Either *Populus x acuminata* females, *P. angustifolia* females or *P. angustifolia* males
- g_s, Stomatal conductance (mmol $H_20/m^2/s$)
- H, Height (cm)
- LA, Leaf area (cm^2)
- LAR, Leaf area ratio (total leaf area/whole plant dry weight) (cm^2/g)
- LDW, Leaf dry weight (g)
- LN, Leaf number
- LS, Leaf size (cm)
- M, Male
- n, Number of replicates present in a sample
- Ψ_L, Leaf water potential (MPa)
- PO x ac, *Populus* x *acuminata*
- PO ang, *Populus angustifolia*
- RA, Root area (cm^2)
- RA_l, Lower root area (cm^2)
- RA_u, Upper root area (cm^2)
- RDW, Root dry weight (g)
- RN, Root number
- RN_l, Lower root number
- RN_u, Upper root number
- SD, Stomatal density (per mm^2)
- ShDW, Shoot dry weight (g)
- SLA, Specific leaf area (leaf area/leaf dry weight) (cm^2/g)
- StDW, Stem dry weight (g)
- TDW, Total sapling dry weight (g)
- WUE, Water use efficiency (net assimilation/stomatal conductance)

CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1 Introduction

Southern Alberta riparian woodlands, the natural habitat of the long-lived cottonwoods (*Populus*), experience seasonal flood events which are an integral component in the trees life history (Rood et al. 2003). Foothill and prairie rivers draining the eastern slopes of the Rocky Mountains whose flows have been manipulated by dams, weirs and diversion canals have shown a departure from the timing and magnitude of natural seasonal discharge events, such as floods (Rood and Mahoney 1990; Rood et al. 2005). Changes in the seasonal moisture regime and continual disruption of water availability (experienced as persistently dry or prolonged inundated conditions) have significant repercussions on the establishment, growth, reproductive capacity and survival of riparian cottonwood groves, which rely on instream flows (Rood and Mahoney 2000; Rood et al. 2008). Recent field findings of male-biased sex ratios in *Populus* in southern Alberta and Washington state (Rood unpublished data; Braatne et al. 2007) have led to speculation that regulation of river flows may result in soil moisture conditions and water stress which cause differences in growth rate and survival between the sexes of *Populus*.

The mechanism of differential responses of the sexes of *Populus* to various moisture conditions is not known. Requiring attention is the speculation that differences in water relations between the sexes are due to sexual dimorphism in physiological processes and morphology. The literature has revealed support for various concepts including higher drought tolerance in woody riparian males over that of females, higher

1

performance of females than males in mesic habitats displaying a stable water regime, and sex-specific water-use strategies based on genetics and spatial distributions within the local environment. In addition, prior research has discovered sexual dimorphism affecting plant water-use and regulation to be strongly correlated with habitat condition, specifically moisture availability, which is governed by river flows in the riparian environment (Table 1).

Table 1. Studies showing physiological or morphological differences, or both, between the sexes of *Populus*, *Salix* and *Acer* juveniles and adults under differing field (natural) and lab moisture regimes

Study (Authors and Date)	Species	Findings of physiological/morphological divergence between the sexes (F=female, M=male)
Hughes et al. 2000	*Populus nigra*	Low level of physiological and genetic variation. Overlap in water and nutrient requirements
Xu et al. 2008a	*P. cathayana*	Physiological and structural divergence in drought which negatively influence F's growth and photosynthetic capacity, indicating their greater sensitivity to water deficit
Xu et al. 2008b	*P. cathayana*	Significant physiological and morphological disparities under drought and photosynthetic response under well-watered conditions. Drought-stress limits photosynthetic capacity to a greater degree in F's
Dawson and Bliss 1989	*Salix arctica*	Physiological variation in g_s, Ψ_L and ^{12}C: ^{13}C and hydraulic architecture (tissue elasticity) over mesic to dry moisture conditions. Conjectured sex-specific differences have an underlying genetic basis
Dudley and Galen 2007	*S. glauca*	Dry conditions induced greater water stress (a larger decline in Ψ_L and higher g_s) in F's. Genetic physiological divergence occurs in the absence of reproductive sinks and is not detectable at sexual maturity under natural conditions
Dawson and Ehleringer 1993	*Acer negundo*	Sex-specific physiology, growth and carbon allocation differences contribute to disparities in size and age of the sexes within a population and occur across a range of moisture conditions
Ward et al. 2002	*A. negundo*	Under high water availability (mesic sites) physiological divergence resulted in higher growth rates (greater stem diameters) in F's and water uptake and use
Dawson et al. 2004	*A. negundo*	Under favourable (unlimited water) conditions divergence in g_s and carbon isotope discrimination occurred between F's and M's of juveniles and adults. Under drought, F's showed greater reduction in g_s and photosynthetic rate and displayed higher abaxial stomatal density and leaf nitrogen per unit area. Changing moisture conditions may decouple physiological responses in F's and M's to different extents
Hultine et al. 2007	*A. negundo* and *P. fremontii*	Significant differences in photosynthetic rate, g_s and sap flux density of adults lending to higher growth rates of F's in wetter environments. The same is observed for M's in dry habitats
Hultine et al. 2008	*A. negundo*	Under wet, natural conditions physiological processes differed over a growing season for adult (reproductive) trees, resulting in differential water-use between F's and M's

3

To address the speculation surrounding sex-based differences and their

relationship to moisture conditions (specifically water-stress) and their effects on

physiological response and growth, the greenhouse study was initiated. The intent of the

study was to establish if the sexes (at a juvenile life stage) of a native cottonwood species

would show divergent physiological responses or structure, or both, under reference

(unlimited moisture) or flood (water-stress) conditions, or both. In recognition of prior

research regarding sex-specific responses in *Populus*, *Salix* (willow) and *Acer* (maple) to

different water availabilities, we predicted distinctive water-use between the sexes to

differentially influence physiological response, growth and possibly survival of the

female and male saplings in mesic reference conditions and in flood in the greenhouse

study. The objectives of the study were recognized as directly applying to physiologically

based plant research involving water-stress and tree sex, and enhancement of initiatives

in riparian ecosystem management.

1.2 Human-induced changes to river flow regimes and the relationship between biased
sex ratios in Salicaceae and habitat moisture conditions

Studies in the fields of hydro-ecology, plant physiology and environmental

science have demonstrated an urgent need to better understand the relationship between

riparian tree sex and tree-water relations, and specifically, to elucidate that relationship

under various moisture conditions and water stresses. Different water-use strategies of

females and males of woody, dioecious riparian species have the potential to contribute to

the erosion of the riparian woodland system's health, resilience and ecological integrity

under present-day ecosystem conditions (Hultine et al. 2007). Riparian cottonwoods, of

the genus *Populus* within the *Salicaceae* family (Farrar 1995), are keystone tree species

of the river valley woodlands and floodplain forests of the Rocky Mountain headwaters,

4

foothills and prairies (Rood et al. 2005). In the semi-arid region of southern Alberta, and into the western United States, human-induced riverine ecosystem modification and riparian habitat removal resulting from dams and diversions have contributed to significantly altering the seasonality and magnitude of discharge of rivers that drain the eastern slopes of the Rocky Mountains (Rood and Mahoney 1990; Barnett et al. 2008). Subsequently, the geomorphological condition, resulting from natural riverine ecosystem processes and events, such as flood, which is responsible for sustaining streamside woodlands, has been altered as a result of the spatial and temporal changes in those processes. As obligate phreatophytes, riparian cottonwoods are dependent upon the physical riparian environment and its connections to the hydrologic regime. Cottonwoods obtain approximately 80% of their water requirements from alluvial groundwater sources (Gazal et al. 2006), which are governed by instream flows that contribute to groundwater recharge.

Evidence for this phreatophyte's susceptibility to water-stress (originating from altered flows) has been documented by observations of the absence of mature female *Populus* in field populations (Rood, unpublished data; Braatne et al. 2007) and a decline in riparian woodland health along rivers in southern Alberta and the western United States (Rood and Mahoney 1990; Busch and Smith 1995; Rood et al. 1995). The collapse of cottonwood populations downstream from dams and irrigation diversions has been documented by Rood and Mahoney (2000), Williams and Cooper (2005) and Braatne and others (2007). The most recent demise of *Populus* groves, primarily along dammed or diverted river systems, is also associated with a lower abundance of female trees along rivers that have experienced prolonged decreases and changed seasonality in flow (Rood

unpublished data; Braatne et al. 2007). It is unclear whether the male bias has resulted

from a higher mortality of mature female trees or if from a lower initial percentage of

juvenile females germinating and establishing. Various studies of *Populus* have shown

male-biased sex ratios to dominate populations, often regardless of habitat moisture and

nutrient condition. These include Pauley (1949) (*Populus* spp.), Kaul and Kaul (1984)

and Farmer (1964) (*P. deltoides*), Pauley and Mennel (1957), Einspahr (1962), Lester

(1963), Grant and Mitton (1979) and Burton (2004) (*P. tremuloides*), and Carev (1969)

(*P. tremula*).

In contrast, balanced sex ratios of *Populus* have also been reported, for example

by Valentine (1975) (*P. tremuloides*), Comtois et al. (1986) (*P. balsamifera*), Gom and

Rood (1999b) (*P. angustifolia, P. balsamifera* and *P. deltoides*), and Rowland and

Johnson (2001) (*P. deltoides*). Opposite to the apparently more common male-biased sex

ratios observed in various current *Populus* field populations are the extensively

documented female-biased sex ratios of the closely related willows (*Salix* spp.). Studies

that have shown that female-biased ratios predominate in the willows include those of

Faliski (1980) (*Salix* spp.), Crawford and Balfour (1983) (*S. polaris, S. herbacea*),

Alliende and Harper (1989) (*S. cineria*), Shafroth and others (1994) (*S. x rubens*),

Dormann and Scarpe (2002) (*S. polaris*) and Ueno and others (2007) (*S. sachalinensis*).

The alteration of flow of southern Alberta rivers by dams and diversions coupled

with the ever-increasing demand for river water has put into jeopardy the distinct

streamside habitat niches of the *Populus* sexes. The habitat condition (soil moisture and

nutrient balance) of these riparian niches has been conceived to have influenced the sex's

ecophysiology and unique adaptations to moisture stress (Freeman et al. 1976), possibly

6

reflected in biased sex ratios. Differences in resource availability, particularly moisture, of the habitat niches which are occupied by the cottonwood and willow sexes have been correlated with differentiation in female and male growth rate and survival (performance). For example, a spatial pattern dependent on specific site moisture conditions has been observed for the sexes (Bierzychudek and Eckhart 1988).

1.3 Sex-based differences influencing water relations within the Salicaceae: Theories and concepts relative to water-stress tolerance and a pattern linked to moisture regime

The effects of short-term and prolonged drought or flood on riparian trees and shrubs of many species have been the subject of numerous global studies (Kozlowski 1984; Busch and Smith 1995; Rood et al. 1995; Retuerto et al. 2000; Amlin and Rood 2001; Li et al. 2004; Zhang et al. 2004; Monclus et al. 2006; Braatne et al. 2007). Research aimed at determining the effects of water-stress on the *sexes* of species within the *Salicaceae* (specifically of the *Populus and Salix* genera) as well as in *Acer,* which intends to elucidate sex-specific responses (and thus physiological divergence between the sexes), is deemed in need after review of the literature (Table 1). Recent studies (Hultine et al. 2007) have noted that theory involving sexual dimorphism requires considerable evaluation and research in order to determine if the sexes of riparian trees have developed distinct hydraulic architecture through evolutionary pressures that corresponds to habitat condition (resource availability) and preference. Differential physiological responses of the sexes of *Populus* to various soil moisture regimes and levels of water stress result in disparate growth rates and percent survival between the sexes (Zhang et al. 2004; Xu et al. 2008a, b). It is unclear whether differences in physiological processes, morphology and survival of the sexes reflect innate genetic

divergence or differential development of hydraulic architecture (Zimmermann 1978) and structure influenced by resource availability (Dudley and Galen 2007).

Following Fisher's law (Fisher 1930) most dioecious trees and shrubs maintain sex ratios that do not depart from unity, or males are more numerous than females (Opler and Bawa 1978; Bullock 1982). It may be speculated that males could maintain a higher tolerance (to moisture and nutrient stress) lending to a higher growth and survival rate over that of females and an enhanced competitive ability, based on their greater frequency across populations in general. Studies and preliminary observations have revealed that females of various riparian species occupy a habitat niche with favourable moisture conditions (tending towards an unlimited water supply or a mesic environment), whereas males are more likely to be found in more water-stressed, xeric conditions in the natural environment (Freeman et al. 1976; Cox 1981; Zimmerman and Lechowicz 1982; Bierzychudek and Eckhart 1988; Dawson and Bliss 1989). Prior studies have theorized that males generally maintain conservative water-use strategies and females are more speculative or less cautious in their water-use across a broad range of moisture conditions (Dawson and Bliss 1989; Dawson and Ehleringer 1993; Dawson et al. 2004).

The mechanism behind higher drought-tolerance observed in males of woody shrubs and trees has been explained through "reproductive allocation theory" (Charlesworth and Charlesworth 1978; Charnov 1982; Charlesworth and Morgan 1991). This theory proposes that females demand for moisture, nutrients and minerals is greater than that of males, resulting from females requirements for seed and fruit production and maintenance exceeding males' for pollen and sex organ development (Freeman et al. 1976; Cox 1981; Waser 1984; Obeso 2002; Li et al. 2004). This theory has led to the

8

prediction that functional (physiological) traits will diverge between the sexes of mature trees in order to support differing resource demands, thus contributing differentially to each sex's growth and survival under environments of shifting moisture conditions (Dudley and Galen 2007).

This differential resource utilization by the sexes of dioecious plants has been linked with the observed phenomenon of spatial segregation of the sexes (SSS) (Bierzychudek and Eckhart 1988). The concept of SSS has been explained as female plants being more commonly found in wet, higher nutrient habitats, or rich microsites of the local environment, and males in more xeric, nutrient poor habitats that are often prone to drought (Freeman et al. 1976; Dawson and Bliss 1989; Hultine et al. 2008). Tradeoffs between male and female competitive ability occur where greater drought tolerance of males comes in lieu of decreased competitive ability in regions of stable water supply or in wet seasons (Hultine et al. 2007). Under circumstances of stable, abundant moisture, females have been observed to out-perform males through greater above ground growth due to enhanced resource uptake capacity and use (Dawson and Ehleringer 1993; Hultine et al. 2007, 2008). The mechanism behind reproductive allocation theory and the niche-based spatial distributions of the sexes is yet to be fully uncovered and explained, although can be predicted to support the hypothesis that physiological and structural divergence between the sexes of mature, and possibly juvenile, *Populus* occurs when moisture conditions inhibit growth and survival.

The inference regarding disparate water-use as a result of sexual dimorphism between juvenile *Populus* females and males and its dependence on habitat condition (water availability) is supported by an additional theory, the "sexual allocation theory"

9

which originated from the historic hypotheses of Darwin (1877). The theory suggests that dimorphism in the sexes of dioecious plants can be imparted by natural selection and is consequently of evolutionary origin (Darwin 1877; Willson 1983). In accepting that dioecious plant species have evolved disparate physiology which has the ability to differentially affect plant water relations and carbon balance, the question arises "what physiological differences between the sexes are responsible for the lower drought tolerance of females, their enhanced competitive ability in mesic environments (Dawson and Bliss 1989), and a possible lower sensitivity of males to moisture-stressed conditions in general?" Recently stated by Retuerto and others (2000) and Xu and others (2008a), previous studies are few (Table 1) which elucidate sex-specific physiology and biological responses of dioecious riparian tree species to water stress or moisture regime. However, common patterns in physiological processes and distinctive structural characteristics influencing water uptake and use between the adult sexes have been discovered within the *Salicaceae* and have been used to explain occurrences of biased sex ratios and the concept of SSS (Bierzychudek and Eckhart 1988; Dawson and Bliss 1989; Dudley and Galen 2007; Hultine et al. 2007, 2008; Xu et al. 2008a, b).

1.4 Studies of sexual divergence within Populus, Salix and Acer: influence of moisture condition on female and male response and water-use strategy

As documented by the concept of SSS, it has been hypothesized that sex-specific physiological adaptations have allowed the mature sexes of riparian tree species to specialize and thrive in spatially segregated habitats which differ in their moisture and nutrient regimes (Dawson and Geber 1999). Previous work has determined that reproductive females possess the physiological characteristics which enable them to maximize resource acquisition capacity, where as males do not employ the same

10

physiological responses which exploit resources to a maximum. On the contrary, males have shown to maintain greater resource use efficiency through cautious and efficient water and nutrient acquisition and use, which has been most prevalent when resources are scarce, or limiting (Dawson and Ehleringer 1993). Differential carbon balances (expressed through growth rates) between the sexes are conceived to be a result of differences mainly in water acquisition rate and capacity, transport and assimilation and loss, all of which are physiological processes (Dawson and Ehleringer 1993) and the hydraulic architecture of a tree (the distribution of hydraulic resistances in the conducting system, Zimmermann, 1978). Studies that have detected physiological differences between males and females in the genera of *Populus, Salix* and *Acer* may explain differences found in growth and survival in populations subjected to water stresses.

Physiological differences between the sexes of *Acer negundo*, a woody, dioecious tree, have been documented at the leaf level, where females have exhibited higher stomatal conductance (g_s), net carbon assimilation (A), and carbon isotope discrimination (Δ) and lower water use efficiency (WUE=A/g_s) than males, regardless of habitat (Dawson and Ehleringer 1993; Dawson et al. 2004; Hultine et al. 2007, 2008).

Previous research has investigated genetic divergence in physiology between the sexes in the absence of sex-specific reproductive structures, which have been thought to skew resource requirements in favour of females. In comparing physiological responses to drought stress in *Salix glauca* (northern or grayleaf willow) males and females, Dudley and Galen (2007) determined that sex-specific genetic differences occur in the absence of reproductive sinks and that the discrepancies in water relations between the sexes are in fact not detectable at sexual maturity under natural conditions. Dudley and Galen's

11

(2007) study reinforces the theory that diverging physiological processes and structure between the sexes may only develop and be distinguishable under water-stress and at a juvenile life stage or in non-flowering trees. Furthermore, it is possible that sex-specific responses and traits could be recognized as being intrinsic to the sexes (genetically based) and not a result of differential resource allocation due to reproductive sink requirements or habitat quality.

Prior studies have determined *Populus* sexes do not consistently follow a pattern whereby efficient use of water is greater in one sex, but that physiological differences are dependent on environmental condition, are plastic traits and that sexual dimorphisms influencing plant water relations can be genetically based (Grant and Mitton 1979; Hughes et al. 2000; Rowland 2001; Xu et al. 2008a, b). Rowland (2001) notes that plasticity in physiological (and morphological) traits of *Populus* may, under certain environmental conditions, override genetics in determining differences in water relations between the sexes and amongst populations. Letts and others (2008) note that either persistent limited or excessive moisture induces distinct physiological responses leading to differences in water-use efficiency (WUE) between the sexes, and often opposing water-use strategies occur amongst different species within the same genus.

Previous studies have indicated mechanisms of natural selection working through time to produce sexual dimorphism affecting water relations. For example, males of *Salix arctica,* arctic willow, have shown higher leaf elasticity and greater responsive osmotic adjustment over those of females, which tend to lack (or not employ) these water-stress mechanisms (Dawson and Bliss 1989). Dawson and Ehleringer (1993) determined males of *Acer negundo* exhibit greater stomatal sensitivity to water deficit, closing stomates and

avoiding water loss at the expense of carbon gain more frequently than females. Some divergences in gender physiology are typical across dioecious species (i.e.; females maintain a slower growth rate over males and are smaller in vegetative size at any age) (Lloyd and Webb 1977; Zimmerman and Lechowicz 1982) and are theorized to be environmentally based or reflect interspecific variation in the cost of reproduction to males and females, having the ability to mask genetically based divergence (Delph 1999; Dudley and Galen 2007). For the *Salicaceae* in general, divergence between the sexes in physiological traits and processes (i.e. rates of photosynthesis, stomatal conductance, carbon isotope discrimination and leaf water potential) and in tree structure has revealed a pattern of sex-based performance which is expressed, and is most pronounced, in water-stressed conditions. The observation of males maintaining higher tolerance to stressed (resource poor) environments in comparison to females enhanced performance and competitive ability in high moisture and nutrient habitats (of stable water supply), reflects a common pattern, within the *Salicaceae*, of the sexes which emerges across moisture (and resource) gradients.

1.5 The greenhouse study: Intent, design and expectations

In juvenile *Populus* sex is indeterminable, in the absence of genetic analyses, (refer to Yin and others (2008) for recent evidence of a sex chromosome emerging in *Populus*) leading to only speculation about sex ratios in immature populations and whether the inference that likely divergence in physiology and hydraulic architecture between mature females and males could also be applied to juveniles. In recognition of the observed differences in growth and survival of the sexes as being particular to the moisture availability of an environment, divergence in physiology and morphology

13

would have to occur between the sexes of juvenile *Populus* under flood-stress and possibly in mesic moisture conditions with unlimited water. Through a greenhouse study we intended to establish if the sexes of juvenile cottonwood develop divergent physiology and structural characteristics resulting from differential response to primarily flood-stress. If disparate responses occurred, it was speculated that differences in physiology and morphology would be expressed as significant differences in growth and survival under specific water availabilities.

The taxa chosen for the study, both native to southern Alberta riparian woodlands, were the narrowleaf cottonwood, *Populus angustifolia* James, and the lanceleaf cottonwood, *P. x acuminata* Rydb., a cross between *P. angustifolia* and the prairie or plains cottonwood, *P. deltoides* Bartr. ex Marsh. *P. angustifolia* is included in the *Populus* section *Tacamahaca* Spach., whereas *P. x acuminata* is an intersectional hybrid and is placed between the sections of *Tacamahaca* and *Aigeiros* Duby (Floate 2004). The narrowleaf cottonwood was chosen as the central species for female/male comparisons while the hybrid (all females) served as an out-group, providing a relevant comparison and serving as a means by which to gauge the sensitivity of the study design and the effectiveness of water treatments. Variation in responses of *P. angustifolia* and *P. deltoides* to different moisture conditions reflect divergent species-specific physiology which governs water-stress resistance, whereby such differences have been proposed to have developed in accordance to environmental (climatic and physical) conditions of that species' niche (Pallardy and Kozlowski 1981). The inclusion of *P. deltoides* into the hybrid's genome holds the potential for specialized traits and physiology to emerge which enhance its adaptability, resilience and survival in drier conditions (Kalischuk et

al. 2001; Pearce et al. 2006). Through natural selective processes, *P. deltoides* are better adapted to more xeric environments which characterize its native range (Kalischuk et al. 2001; Rood et al. 2003; Pearce et al. 2006) than *P. angustifolia,* as a result of developing physiological functions and morphology conducive to survival in habitats prone to drought and chronic low water availability (Pallardy and Kozlowski 1981).

The species of the *Aigeiros* section and *P. x acuminata* most often occur in riparian zones in semi-arid environments where they are the *Populus* species most adapted to the driest conditions found in geographic range the genus (Rowland and Johnson 2001; Rood et al. 2003). *P. angustifolia* is found in regions of a semi-arid to arid climate that support mesic to xeric site conditions and a broad range of temperatures (Farrar 1995; Rood et al. 2003). Narrowleaf cottonwood commonly occurs at low elevations adjacent to the river channel in a zone that is frequently flooded and characterized by a shallow water table (Karrenberg et al. 2002).

The greenhouse study involved two moisture conditions: 1) unlimited water or mesic moisture (the reference treatment) and 2) flood (the water-stress treatment). We proposed that female performance would be higher than that of males in mesic (reference) conditions, and males' growth and survival would exceed that of females in the flood treatment, if experienced as a water-stress. We hypothesized females' enhanced performance in moisture conditions promoting growth (unlimited water) due to prior research revealing increased water-use, rapid growth and higher numbers of females in wetter environments (Dawson and Ehleringer 1993). We speculated that the higher drought tolerance of males would contribute to greater overall moisture-stress tolerance and thereby raise their competitive ability under inundation in comparison to females',

especially since flood has been observed to induce similar physiological responses to those of drought (Kozlowski 1984). Continuous flooding, leading to soil anoxia, has been determined to adversely affect growth rates and physiological responses of *Populus* resulting in decreased height, diameter growth, leaf formation and leaf expansion, and severely inhibited basal root permeability and growth (Kozlowski 1984; Liu and Dickmann 1992; Francis et al. 2005). A decline in root formation, expansion and permeability will decrease and inhibit water acquisition, which is comparable to the decreased water uptake that occurs in drought. Common to plants experiencing drought-stress are declines in stomatal conductance, chlorophyll, leaf turgidity and carbon assimilation (photosynthetic rate), which most often occur during the initial stages of flooding, and in the absence of immediate and effective physiological response inducing plant-water recovery, growth and eventually survival in waterlogged (and dry) conditions will decline (Tang and Kozlowski 1982, 1984; Will et al.1995).

We expected chronic stress from flooding to reduce growth, and possibly survival, in the narrowleaf sexes and in the lanceleaf hybrid, although by different magnitudes which would show *P. angustifolia* to be less sensitive to flood, and males to maintain the highest flood tolerance of all three groups. Exposure of woody, riparian species to prolonged inundation has shown to commonly result in decreased growth and survival of all individuals (Harrington 1987; Liu and Dickmann 1992; Will et al.1995; Cao and Conner 1998; Francis et al. 2005). From review of the literature, research involving sex-specific response (acclimation, growth and survival) of *Populus* to flood stress has yet to be fully examined, allowing for the greenhouse study to provide novel data in relation to sex-related traits responses and survival under inundated conditions.

1.6 Greenhouse study objectives

Primary objective: Establish if physiological processes or morphological characteristics, or both, of clonally propagated *P. angustifolia* saplings differ between the sexes when grown in reference (mesic conditions of unlimited water) or flood conditions. If disparities in physiological processes or morphological attributes, or both, exist determine whether those differences have affected a sex's growth or survival, or both, in a specific water treatment.

Secondary objectives: a) Verify the morphological and physiological differences that exist between *P. angustifolia* and *P. x acuminata* under reference and flooded conditions. b) Establish the effectiveness of the water treatments on sapling growth and survival and gauge the sensitivity of the response of *P. angustifolia* to moisture stress (through a comparison with the hybrid out-group). This objective serves as a validation of the study design and species responses to treatments.

1.7 Greenhouse study hypotheses and predictions

Growth and survival predictions for the reference water treatments

   P. angustifolia females and males and the hybrid will show higher growth and survival in the mesic conditions of the reference treatments over reduced performance expected in flood. *P. angustifolia* females will show greater growth and competitive ability in reference conditions over that of males. Females of *Acer,* with unlimited water supply, have shown to maintain higher xylem transport efficiency and develop larger leaf area than sapwood or root area, or both, which is more efficient and effective in water transport thereby exceeding that of males (Dawson and Ehleringer 1993; Ward et al.

17

2002; Hultine et al. 2008). These characteristics of females lend to greater whole plant water use and a less cautious water-use strategy than that of males, thus promoting a rapid growth rate, and have been observed to be uncoupled with water availability (Freeman et al. 1976; Dawson and Bliss 1989; Dawson and Ehleringer 1993; Hultine et al. 2008).

The hybrid will display the highest growth with unlimited water, exceeding the size of the narrowleaf females, the most relevant comparison, but also that of the males. *P. angustifolia* saplings, including propagated clones, have tended to exhibit slower shoot growth in height and radial increments (initial diameter growth) than that observed in *P. deltoides* and *P. balsamifera* across a range of field conditions (Campbell et al.1993; Kalischuk et al. 2001; Willms et al. 2006). We attribute the expected higher growth rate of the hybrid, potentially leading to greater survival, to dominance resulting from the introgression of *P. deltoides* traits (notably its rapid intrinsic growth rate) into the hybrid genome. *P. deltoides* traits have been thought to enhance the hybrid's competitive ability and survival over a range of moisture conditions (Campbell et al. 1993). The intrinsically superior growth rate of *P. deltoides* (related to its reproductive life history and geographic distribution) and the comparatively slower growth of *P. angustifolia* seedlings have been documented in the field and lab (Campbell et al. 1993; Kalischuk et al. 2001). It was speculated that vigorous parent genotypes could also contribute to a higher growth rate in the *P. x acuminata* saplings. In relation to dominance expressed by the hybrid, Campbell and others (1993) demonstrated that heterosis does not systematically occur in southern Alberta populations, where natural hybrids (such as *P. x acuminata*) have shown interbreeding across many generations. Such long-term, extensive introgression has been

hypothesized to result in native species sharing overlapping gene pools, thus decreasing the occurrence of heterosis (expressed as hybrid vigor) which is presumed to rely on the crossing of parents from genetically distinct populations, enabling adequate heterozygosity (Rood et al. 1986; Campbell et al. 1993).

Growth and survival predictions for the flood treatment

Exposure to constant inundation will reduce growth rate, and with a significant enough decline in growth, possibly inhibit survival for each taxon. *P. angustifolia* males will show greater growth over *P. angustifolia* females and as such could survive better in flood. It is predicted *P. angustifolia* males will exceed the hybrid females in growth, although only under inundation. Flood-stress is expected to cause a decline in soil oxygen which coupled with waterlogged substrate will decrease root formation, growth and permeability, and as such will impede water uptake by the roots (Kozlowski 1984; Francis et al. 2005). We propose that for all taxa stress acclimation responses to inundation will only slightly improve physiological response and hindered growth rates over the course of the study.

P. x acuminata females will have decreased growth, which if inhibited significantly by inundation, also reduced survival relative to the speculated lower mortality in reference conditions and to the expected higher survival of *P. angustifolia* in flood. We propose that *P. x acuminata* saplings will display disproportionately greater declines in growth reflecting a lower flood-tolerance than *P. angustifolia* which we expect to be less sensitive to inundation. The flood treatment is speculated to generate general plant water-stress acclimation responses such as hypertrophied lenticels,

adventitious roots at or above the saturated soil surface, and decreased stomatal conductance or a decline in water potential, or both, (Kozlowski 1984) for both taxa. *P. angustifolia* are more adapted to slightly wetter environments that are more prone to flooding (Amlin and Rood 2001), and we assume that *P. x acuminata* are adapted to survival in dry environments due to the genetic influence of *P. deltoides*, which are better suited to drier soils in semi-arid climates where exposure to flooding is less frequent (Rowland and Johnson 2001; Pearce et al. 2006).

In turn, it is anticipated that the narrowleaf males and females, through pre-conditioning to wetter habitats, would have developed physiological acclimation responses which effectively mitigate the effects of inundation and enhance their survival under flood-stress, which they are more likely to experience within their native geographic range and localized streamside niche (Amlin and Rood 2001). In comparison, *P. x acuminata* has been influenced by pre-conditioning to a different climatic niche in which their natural distribution commonly includes areas of xeric moisture regime which are less prone to seasonal flooding (zones to which *P. deltoides* are adapted) and inhabit areas at higher elevations along the river margin, making this taxon less likely to acclimate to flood-stress with rapid, effective physiological process.

1.8 Relevance of the greenhouse study to research and its application in ecosystem management

Current trends in *Populus* grove establishment, expansion, health and longevity support the need for studies which attempt to determine and explain the physiological mechanisms inherent to the cottonwood sexes that govern their unique water relations. If distinct physiological processes or disparities in morphology (and specifically hydraulic

architecture), or both, are found to occur between the sexes of juveniles of *P. angustifolia,* such findings may corroborate patterns of growth and survival found in cottonwood field populations with skewed sex ratios. Determination of sex-specific physiological response and water-use strategies in juveniles will most likely occur through lab or greenhouse studies, whose findings will contribute to the conservation and management of young, establishing riparian cottonwood populations in the field.

CHAPTER 2

MATERIALS AND METHODS

2.1 Plant Materials: Cottonwood genotypes

In the spring of 2007, male and female source trees of two taxa were selected for the greenhouse study. The parent trees were reproductively mature, similarly-sized and apparently healthy, and were situated in a 3-ha native riparian cottonwood grove at 'Pearce Corner', situated along the south side of the Oldman River (49°51'03" N, 113°15'18" W). Pearce Corner is at an elevation of 915 metres asl and located approximately 30 km northwest and upstream of Lethbridge, Alberta. The roughly 450 x 80 m grove is located on a floodplain of the constrained 200-250 m wide valley, and trees were located at a distance of 50 to 120 m from the bank. River, geomorphological and woodland conditions at this site have been further described by Willms and others (2006). To avoid clonal ramets, the individual trunks were at a minimum 30 m apart or were different sexes. In addition, the trees may have displayed discrete bud-flushing phenology if the two other conditions were not met in prevention of selecting clonal ramets (Gom and Rood 1999a).

Tree selection

Eight of the trees selected were four female and four male narrowleaf cottonwoods, *Populus angustifolia* James, that were previously studied relative to growth, photosynthesis and water relations (Letts et al. 2008). These trees were predominantly *P. angustifolia* but displayed leaf shapes and crown structure that indicated some introgression from black cottonwood, *P. trichocarpa* Torr. & A. Gray

22

(syn. *P. balsamifera*, ssp. *trichocarpa* Brayshaw), that is sparse at the site but predominant upstream (Floate 2004; Berg et al. 2007). We selected eight additional male trees that were also predominantly *P. angustifolia*. Eight similarly-sized female trees were also selected but females were considerably less abundant than males in this woodland and there were few additional females with crown forms indicating relatively pure *P. angustifolia*. We selected the eight additional trees of both sexes at the onset of catkin flushing to identify the sexes, and after leaf flushing it was determined by comparison with the foliar shapes of Gom and Rood (1999a), that three of the additional females were lanceleaf cottonwoods, *P. x acuminata* Rydb., hybrids between *P. angustifolia* and the prairie or plains cottonwood, *P. deltoides* Bartr. *P. deltoides* is rare in the Pearce Corner grove but occurs commonly along the Oldman River near Lethbridge and further downstream (Gom and Rood 1999a; Floate 2004). The study thus included three groups of cottonwood genotypes: twelve male *P. angustifolia*, nine female *P. angustifolia,* and three female *P. x acuminata* (Table 2).

Table 2. Greenhouse study clone identification showing assigned numbers and names for each genotype and taxonomic group (*P. angustifolia* females (PO ang F), males (PO ang M) or *P. x acuminata* females (PO x ac F)) to which it belongs.

Genotype Number	Genotype Name	Group
1	F 10	PO x ac F
2	F 6	PO ang F
3	F 8	PO x ac F
4	F 9	PO x ac F
5	Bertha	PO ang F
6	Maria	PO ang F
7	Elchup	PO ang F
8	F 11	PO ang F
9	F 12	PO ang F
10	F 5	PO ang F
11	F 7	PO ang F
12	Rosa	PO ang F
13	Juan	PO ang M
14	M 1	PO ang M
15	M 10	PO ang M
16	M 11	PO ang M
17	M 14	PO ang M
18	M 2	PO ang M
19	M 3	PO ang M
20	M 4	PO ang M
21	M 5	PO ang M
22	Carlos	PO ang M
23	Pedro	PO ang M
24	Rico	PO ang M

Cutting Collection

From the 24 genotypes, branch cuttings were harvested with a pole pruner and ladder on three separate days between April 25 and May 5, 2007. Approximately twenty, 15 to 20 cm long apical cuttings were taken per tree, generally from vegetative shoots occurring in the mid- to upper crown. The cuttings were placed in plastic bags and stored at 4°C until all had been collected and were then refrigerated for a further week in an attempt to coordinate bud phenology.

2.2 Experimental design and conditions

Establishment; cutting preparation and propagation

Following refrigeration, the cuttings were cut, soaked and stuck in pots containing a gravel-sand mixture. Propagation took place in the greenhouse at the University of Lethbridge (49°40'42" N, 112°51'50" W), Lethbridge, Alberta, Canada. The cuttings were cut into 10 cm long pieces with an apparently healthy bud approximately one cm below the apical end (Figure 1). After bud flushing, buds and undeveloped non-dominant shoots were removed, 26 days after sticking (June 6), to leave one shoot per cutting. The eighteen most uniform cuttings from each of the 24 genotypes were assigned to three moisture treatments (two reference and a flood) which were replicated six times using six round, plastic pools with diameter and depth of 1.2 m x 0.30 m respectively, which served as the experiment replicates (Amlin and Rood 2001). The 18 cuttings from a single genotype were randomly divided into three cuttings per pool. Within each pool, one cutting of each genotype was randomly assigned to each of three moisture treatments.

Once assigned a moisture treatment, a cutting was randomly assigned a position in one of

six pots, with four positions per pot. On May 12 (day 1 of sticking timeline), after

soaking in room temperature water for 12 to 24 hours, all cuttings were stuck to a depth

of 5 cm into the substrate (Figure 2).
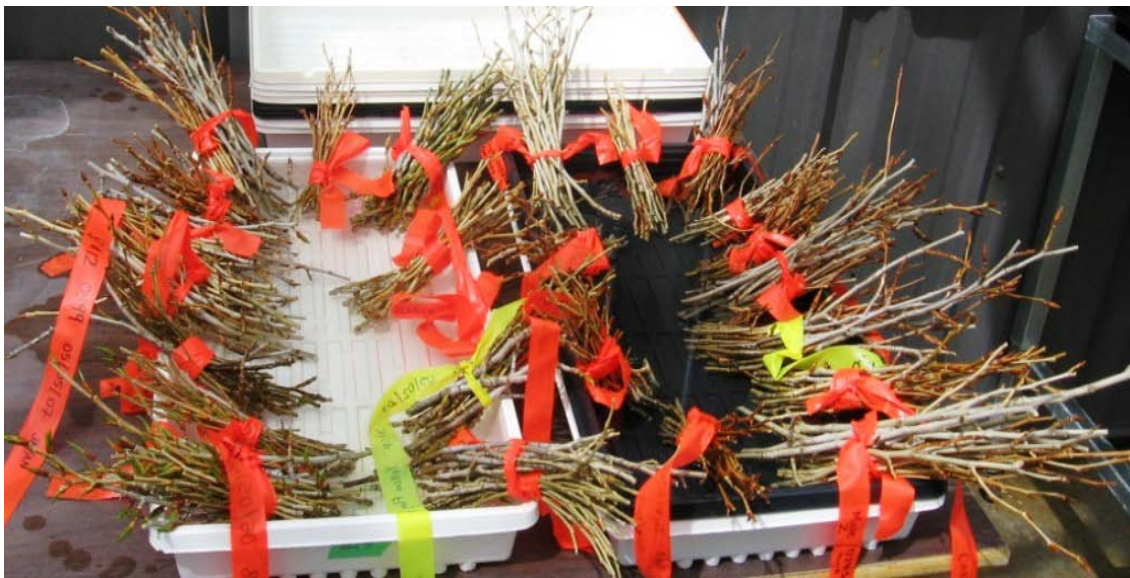
Figure 1. Photograph of cuttings while soaking on day 1 (May 12) of the greenhouse
study, when sticking occurred.

Figure 2. Photograph showing cuttings in the pre-treatment phase, while pots were subjected to the Water 2 treatment, two days after sticking.

Substrate in which the cuttings were grown consisted of a 1:1.5 pea gravel to coarse sand mixture (of size classes 1.0 to 1.2 cm and 3.0 mm respectively) excavated by volume from the Oldman River valley at Lethbridge and sorted by Tollestrup Construction Inc., Lethbridge. A layer of 2.5 cm washed gravel, 2 centimetres deep, was placed in the bottom of 20.3 cm by 14.0 cm deep round pots to keep the gravel-sand mixture in place.

Following the sticking of cuttings, all pots were placed in 7 cm of water in the pools (pre- treatment or establishment moisture conditions) allowing for adequate moisture to promote cutting establishment and growth before experimental moisture treatments were implemented (Figure 3). Pools were raised approximately one metre off

the ground on separate tables, located in six different positions in the greenhouse (Figure

4). Pots within the pools were removed from each pool and rotated weekly between

pools. This was done to minimize the effects of differences in temperature, light and air

flow in the greenhouse.

Figure 3. Photograph of pots in the establishment phase, 24 days after sticking (June 4).

Figure 4. Photograph of the greenhouse design showing pools and data logger (relative humidity and light sensors) during the establishment phase, 47 days after sticking (June 27).

During propagation of the cuttings, 36 g/pool of fertilizer (Peters Professional Peat-Lite-Special, 20-10-20 with micronutrients, TerraLink Horticulture, Abbotsford, B.C., Canada) was added to the standing water starting 17 days after sticking (May 28). The solution was changed weekly with the same amount of fertilizer added. After implementation of the different moisture treatments (49 days after sticking on June 29), the fertilizer dosage was doubled.

During cutting establishment and propagation, newly expanded, preformed leaves were used to confirm species identity by leaf shape, size, edge serration profile, angle of leaf base at petiole attachment and petiole length. The species/hybrid leaf-identification guide from Gom and Rood (1999a) was used to distinguish taxa based on leaf characteristics.

Moisture treatments and greenhouse environmental conditions

Moisture treatments were started 49 days after sticking and were achieved by maintaining a constant water level in the pool then using brick (10 cm x 20 cm x 6 cm) and tile (7 cm x 16 cm x 0.6 cm) combinations to raise pots to two of the three water levels (Figure 5) (Amlin and Rood 2001). The moisture treatments were described as Water 1 (pots placed to a depth of 1 cm), Water 2 (pots at a depth of 7 cm; pre-treatment conditions) and Flood (pots with a water level maintained to 3 cm below the rim; placed to a depth of 11 cm). Each of six pools contained 18 randomly distributed pots, with six pots per moisture treatment (Figure 6).
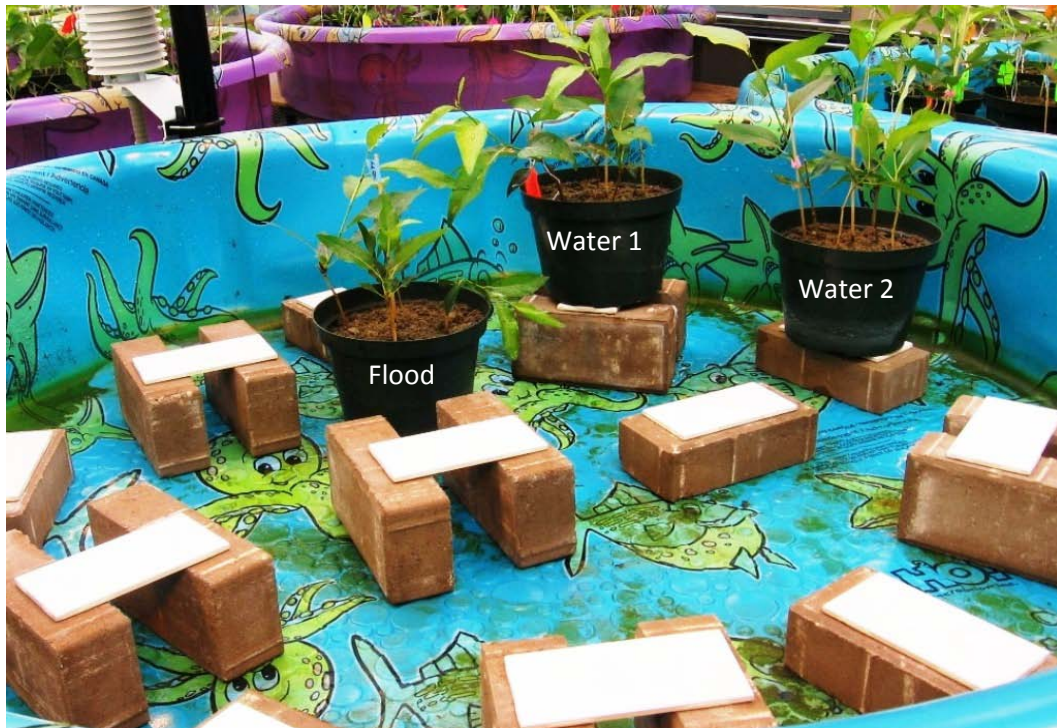
Figure 5. Photograph of brick and tile combinations used for Water 1 and 2 treatments, taken 49 days after sticking (June 29) during pot rotation between pools.

Figure 6. (Left to right) Photograph of pots in Flood, Water 1 and Water 2 treatments, 77 days after sticking (July 27).

Commencing on June 20, greenhouse ambient conditions were recorded using a CR23X data logger (Campbell Scientific, Edmonton, Alberta, Canada). Greenhouse ambient light intensity (photosynthetically active radiation) was captured by a LI190 quantum sensor (Li-Cor Biosciences, Lincoln, Nebraska, USA) and air temperature (°C) and relative humidity were measured with a Vaisala HMP45c temperature and humidity probe (Campbell Scientific, Edmonton, Alberta). Atmospheric pressure was measured with a CS105 Vaisala PTB101 barometric pressure sensor (Campbell Scientific, Edmonton, Alberta). To avoid effects of seasonal change in daylength on plant growth, a uniform day length was provided: on June 25 greenhouse lights were turned on to provide four hours (0530 to 0730 and 1930 to 2130 h) per day of supplementary illumination (Sylvania S50 250W Lumalux bulbs). Nine light bulbs were arranged in a 3 x 3 square ceiling pattern, 2 m apart and at a height of 2.5 m above the pots in the greenhouse.

Average light intensity at pool-rim height (1.2 m off the ground) was 46 ± 2 µmol/s/m^2 (mean $\pm$ S.E., n = 9) and 91 ± 10 µmol/s/m^2 (mean $\pm$ S.E., n = 9) at 1 metre above the pool rim.

Stomatal conductance and leaf water potential were measured during two time periods and for these greenhouse atmospheric conditions of air temperature (T), relative humidity (RH), vapour pressure deficit (D), and photosynthetic photon flux density (PPFD, light intensity) were calculated over three days (46, 47 and 48 days after sticking) during the pre-treatment phase, and over six days (74, 76, 77, 78, 81 and 83 days after sticking) during treatments. Stomatal conductance was measured during the two time periods. Leaf water potential was measured only during the treatment phase. Both were measured from 12:00 to 18:00 hrs *over* the three and six day courses, when the mean values for each atmospheric condition were determined. The mean value for each day was then used to determine an average for each time period. The following means were determined from hourly measurements for the hours of 12:00 to 18:00: (1) mean T for the time course 46 to 48 days after sticking was 27.1°C and 30.1°C for the periods 74, 76, 77, 78, 81 and 83 days after sticking; (2) mean RH during the pre-treatment phase and during treatments was 31.8% and 30.5% respectively; (3) means of VPD (determined by average vapour pressure subtracted from saturated vapour pressure) during pre-treatment and then treatment phases were 2.47 kPa and 3.10 kPa respectively; (4) mean PPFD for the pre-treatment phase and treatment phase was 564 µmol/m^2 and 518 µmol/m^2 respectively. PPFD was recorded from a light sensor situated in the centre of the greenhouse at approximately two metres height off the ground, and so did not record light intensity at the leaf.

2.3 Greenhouse study timeline

The greenhouse study occurred over 108 days in 2007 (Table 3), commencing May 12 (day 1) and ending August 27, when the last of the saplings were harvested.

Table 3. Timeline showing the four phases (in bold type; collection, establishment, treatments and harvest) of the greenhouse study and the corresponding morphological and physiological measurements

Day of Study	Date	Phase	Measurement
1 to 49	May 12 to June 29	**Post-collection and Establishment**: Sticking of cuttings, and propagation in Water 2 conditions	-Air temperature (T), light intensity (PPFD), relative humidity (RH), vapour pressure deficit (D) -Height, preformed and new leaf number, stomatal conductance (g_s)
49 to 96	June 29 to August 15	**Treatments:** Water 1 and Flood treatments implemented alongside Water 2 treatment pots	- T, PPFD, RH, D - Height, new leaf number, g_s, soil moisture content, relative abundance of leaf chlorophyll, stem diameter, stomatal density and length, leaf water potential
96 to 108	August 15 to August 27 Post August 27	**Harvest:** Saplings harvested from six pools sequentially, beginning with pool 1 **Post Harvest:** Air and oven drying of sapling material, leaf grinding for mass spectrometry	-Height, new leaf number, leaf area, root number, root area, cutting diameter, distance of root farthest from cutting base -Mass of dry matter accumulation (leaves, stem, cutting, roots), carbon: nitrogen ratio, carbon isotope composition

2.4 Measurements and instrumentation

2.4.1 Morphological measures

Fifteen morphological variables were measured which included pre-treatment (initial) stem diameter, height, stem dry weight, cutting dry weight, leaf number, leaf area, leaf dry weight, root number (with upper and lower root numbers independently),

root area (with upper and lower root areas independently), root dry weight and cutting (or shoot basal end) diameter at harvest. In addition, seven derivative values were calculated that included diameter growth, leaf size (total leaf area/ number of leaves), specific leaf area (leaf area/ leaf dry weight), leaf area ratio (leaf area/ total sapling dry weight), shoot dry weight (dry weight of stem + cutting + leaves), total plant dry weight (shoot + root) and shoot/ root ratio.

Stem Diameter Growth

Cutting diameter was measured using digital calipers at harvest, while stem diameter was measured approximately one week after moisture treatments started, 57 days post sticking (considered a pre-treatment measure), where the average of two cutting or two stem widths were taken. Cutting diameters were measured at mid-cutting length, and stem diameters were measured at the junction of the stem and petiole of the second new leaf up from the whorl of preformed leaves, located approximately 1 to 2 cm above the top of the cutting. For the purpose of calculating diameter growth, the cutting and base of the stem were considered equivalent parts. Cutting diameter minus stem diameter gave a measure of diameter growth (DG).

Height

Final height (H) was measured at harvest. The entire length of stem was measured from the top of the cutting to the stem apex.

Leaf Number

The number of new leaves (leaves formed and expanded to a size of 1 cm wide at mid point and 1 cm long) was recorded weekly commencing 45 days after sticking (June 25), four days prior to Water 1 and Flood treatment implementation, and total leaf number (LN) was determined at harvest. Weekly leaf counts were not reported although were summed and compared with LN counted at harvest.

Leaf Area

Total leaf area (LA) for each sapling was determined at harvest with a LI3000 Portable Area Meter with a LI3050A Transparent Belt Conveyer Accessory (Li-Cor Biosciences, Lincoln, Nebraska, USA). Leaf size, or average individual leaf area, (LS) was determined using LA and LN (LA/LN) measures taken at harvest.

Root Number

Root number (RN) of upper and lower roots (RN_u and RN_l respectively) were recorded at harvest. Root number provided a comparative estimate of a sapling's total number of roots due to the inability to retrieve all sapling roots at harvest as a consequence of roots being intertwined within the substrate. Root number is therefore representative of a root index. Lower roots were defined as being within 2 cm of the cutting base and upper roots were those above. Roots were counted as major roots, >3 mm diameter at the root base or as minor roots, $\leq$ 3 mm diameter.

Root Area

A comparative estimate of root area (RA) was determined with the LI3000 Area Meter. Root areas were separated into upper root area (RA_u) and lower root area (RA_l) and were measured to provide additional information to verify root dry weight and number, root size, composition and growth form.

Dry Matter Accumulation

Mass of dry matter accumulation per sapling, or total dry weight (TDW) was determined by accumulating leaf dry weight (LDW), stem dry weight (StDW), cutting dry weight (CDW) and root dry weight (RDW). Root dry weight values were an estimate of dry weight due to the inability to retrieve all sapling roots which were intertwined in the substrate at harvest. Total dry weight was therefore a comparative measure due to the inclusion of root dry weights (which were estimates of dry weight). Shoot dry weight (ShDW) included LDW, StDW and CDW together. The ratio of shoot to root dry weights (shoot/root ratio) was considered a shoot/root index due to root dry weight being a comparative estimate of dry weight. Plant material was dried in open-top paper bags in the greenhouse for about eight weeks then oven-dried at 80° C for 12 to 24 hours. It was determined that after four hours of oven-drying weights of the plant material did not decrease.

2.4.2 Physiological measures

Nine physiological variables were measured which included pre-treatment and in-treatment abaxial stomatal density, stomatal lengths, pre-treatment and in-treatment

stomatal conductance, leaf water potential, relative abundance of leaf chlorophyll, abundance of foliar carbon and nitrogen (yielding a carbon to nitrogen ratio) and carbon isotope composition.

Stomatal Density and Lengths

A subset of stomatal lengths were measured but not analysed or reported, as the reliability of the measurement of this parameter was greatly reduced due to poor quality epidermal peels resulting in difficulties in viewing. As such, the accuracy and consistency of stomatal density counts was also reduced, although not to the same extent, and was subsequently analysed and reported.

Abaxial (leaf underside) stomatal density (SD) was determined for leaves that developed and expanded during the establishment phase, or lower leaves, and for leaves that developed and grew during the treatment phase, or upper leaves. Lower leaves were mature (fully expanded) leaves greater than 1 cm wide and long, situated at the second leaf node above the preformed leaf whorl. Upper leaves were chosen as the first mature leaf, greater than 1 cm wide and long, in the section that developed during the period 75 to 82 days after sticking (July 25 to August 1). Lower and upper leaves were chosen to compare stomatal density within individual saplings as well as between sexes and across groups, as these leaves would have developed at the same time for each sapling and were considered to be fully expanded when imprints were taken. Epidermal impressions (imprints), in accordance with the methods employed by Ceulemans and others (1995), were taken using clear nail polish and transparent packing tape. The imprint of the leaf epidermis was preserved on a 1 mm thick, 25 X 75 mm microscope slide. One sapling per

genotype in each moisture treatment was sampled. Saplings in pools 1 to 5 were randomly selected for sampling. A Nikon Eclipse E600 microscope (Nikon Instruments Inc., Mississauga, Ontario, Canada) with a digital camera attachment was used to obtain approximately five photographs per imprint at 40X or 100X magnification. The program Image J (Rasband 2007) was used to count and measure stomata.

Stomatal Conductance

Light-saturated stomatal conductance (g_s) was measured with a LI-1600 Steady State Porometer (Li-Cor Biosciences, Lincoln, Nebraska, USA) during three days (46 to 48 days after sticking; June 26, 27, 28) before treatments, and six days (74, 76, 77, 78, 81 and 83 days after sticking; July 24, 26, 27, 28, 31 and August 2) after treatments were implemented. The abaxial (lower) surface of a single leaf was measured once per sapling. During the establishment phase, only three pools were sampled and as such a subset of the total number (432) of saplings was measured over three days. All 24 genotypes were sampled at least twice with some up to three times per water treatment for a total of 128 saplings sampled. During the treatment phase, three (different) pools were also sampled and subsequently a subset of the total saplings was again measured over six days. All genotypes were sampled at least twice with some up to three times per water treatment as previous. During treatments a total of 152 individual saplings were measured, but as a result of sapling mortality and pot position (shading during sampling hours), a total of 280 saplings had g_s measured over the nine days of sampling during establishment and treatment phases.

Due to sunlight angle and pool position, g_s measures were taken between 12:30 and 17:30. Prior to the treatment phase, leaves measured were those located on the second leaf node above the whorl of preformed leaves, formed 49 to 55 days after sticking. During treatments, leaves measured were those located second down from the last weekly leaf-count permanent stem mark and would have been formed and growing in the period 68 to 82 days after sticking. If a leaf to be measured was missing, the next expanded, intact leaf above that leaf was used. Leaves were allowed to equilibrate in full sunlight for a minimum of five minutes before g_s was measured. If a leaf was not already in full sunlight prior to measuring g_s, the sapling was moved from shade to direct sun within that pool.

Leaf Water Potential

Leaf water potential (Ψ_L) was measured in conjunction with g_s over six days (74, 76, 77, 78, 81 and 83 days after sticking) during the treatment phase. One leaf per sapling was removed and measured using a Model 1000 Pressure Chamber Instrument (PMS Instruments, Albany, Oregon, USA). Leaf water potential was taken between 12:30 and 17:30. Leaves removed were those located second down from a weekly leaf-count permanent stem mark. If a leaf to be measured was abscised, the next expanded, intact leaf above that was used. These leaves would have formed and grown while the saplings were subjected to treatments and as such were chosen to detect physiological changes, seen in responses of g_s and Ψ_L to moisture stress or a change in soil moisture.

All 24 genotypes in a pool were sampled, with three pools being measured over the six days. Each sapling was sampled once per moisture treatment for a total of 216 individuals being measured.

Relative Abundance of Chlorophyll

Relative abundance of leaf chlorophyll (Chl) was measured five times (53, 69, 76, 81 and 87 days after sticking; July 3, 19, 26, 31 and August 6) during the treatment phase using a CCM-200 Chlorophyll Content Meter (Opti-Sciences, Tyngsboro, Massachusetts, USA). At 53 days after sticking, one bottom leaf was measured from all saplings. This leaf was located on the second node above the whorl of preformed leaves, and formed 49 to 55 days after sticking. At 69, 76, 81 and 87 days after sticking, saplings from only three pools were sampled. Each sapling had two leaves measured, a bottom leaf (the same leaf as that measured 53 days after sticking), and remained the same leaf on each sapling for the five sampling days), and a top leaf, which was the second leaf located below a weekly leaf-count mark (the last one) on the stem. This was most often the second leaf (a mature leaf) below the apical bud. Each sapling's top leaves were formed during the same time period, which was in the week prior to the sampling date. Periods of growth for top leaves were: (1) July 3 leaves would have begun to develop after 49 days post sticking; (2) July 19 leaves would have developed after 55 days post sticking; (3) July 26 leaves would have developed after 68 days post sticking; (4) July 31 leaves would have developed after 75 days after sticking; and (5) August 6 leaves would have developed after 82 days post sticking.

Foliar Carbon and Nitrogen Natural Abundance and Carbon Isotope Composition

Carbon and nitrogen natural abundance in leaves (C: N, µgrams of carbon to µgrams nitrogen) and carbon isotope composition ($\delta^{13}C$) were obtained from C and N natural abundance mass spectrometry analysis performed at the University of California, Davis, Stable Isotope Facility. The instrument used was a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Leaves were harvested during two different time periods, with the first set of leaves having been formed and developed during the establishment phase and the second set having developed during the treatment phase.

Pre-treatment (establishment) leaves were removed 49 days after sticking (June 29) from saplings designated in either Water 1 or Flood pots from a single pool. A single leaf per sapling was removed, located on the second leaf node up the stem from the whorl of preformed leaves. These leaves were formed prior to 46 days post sticking (June 26), when g_s measures were taken on these leaves. A total of 39 saplings had leaves removed, as nine were absent due to mortality or unsuitable leaf sizes.

Leaves that developed during the treatment phase were removed from all saplings subjected to Water 1 and Flood treatments from all pools during harvest, 96 to 108 days after sticking, when pools were harvested sequentially starting with pool 1, each pool taking 2-3 days to harvest. One leaf per sapling was harvested and was located in the fifth (out of seven) weekly leaf-count stem section at approximately the 15th node up the stem from the whorl of preformed leaves. These leaves developed within the period 75 days to 82 days after sticking and therefore formed during treatments.

41

Leaves were cut at the base of the leaf petiole then placed in paper envelopes in an oven at approximately 80° C for 12 to 24 hours. Leaves were stored in a standard glass desiccator for six months prior to grinding. Individual leaves were cut to remove the mid-vein and petiole and the remaining leaf blades frozen in liquid nitrogen, then ground with a mortar and pestle into a fine powder. In preparation for mass spectrometry procedures, leaf tissue samples of 2-3 mg were weighed into tin capsules (8 x 5 mm) using a standard microbalance.

Carbon isotope composition ($\delta^{13}C$) was determined in relation to the Pee Dee Belemnite standard (PDB) $^{13}C/^{12}C$ ratio, derived from the carbonate formation located in South Carolina, USA (O'Leary 1981; Werner and Brand 2000). The $\delta^{13}C$ was calculated at the University of California, Davis, Stable Isotope Facility as the molar ratio of the isotopic composition of the sample (R_{sample}) to that of the PDB carbonate standard ($R_{standard}$) (Craig 1957) and expressed in parts per thousand (‰):

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

Where R_{sample} refers to the $^{13}C/^{12}C$ ratio of the foliar sample and $R_{standard}$ refers to the $^{13}C/^{12}C$ ratio of the PDB carbonate standard (Craig 1957; Kroopnick and Craig 1976).

2.5 Statistical analysis

Data management and statistical analyses for each morphological and physiological parameter measured and analysed commenced with an analysis of variance (ANOVA). An ANOVA was first performed to investigate the significance of genotypes, treatments and pool and their interactions. Pool was sometimes a significant effect due to

the sequential harvesting of pools resulting in a delayed harvest of saplings (dependent on pool number), hence a greater number of days of growth for those individuals. In these cases an analysis of co-variance (ANCOVA) was performed with pool as a covariate. Following an ANCOVA involving genotypes, an ANOVA was performed with the three *Populus* groups to investigate the significance of groups and treatments and their interaction. Genotype adjusted means (determined with pool as a covariate) were used in subsequent ANOVAs to determine group means. When significant differences regarding group or treatment, or both, or a significant interaction was found, or both, a one- or two-way ANOVA or a paired comparison test (Bonferroni), or both, were performed and followed by Tukey's Honestly Significant Difference (HSD) post hoc test (Bruning and Kintz 1977), in addition to the Bonferroni post hoc test (significance at the .05 level). When evidence of significant differences between *P. angustifolia* females and males was shown, a subsequent ANOVA (without the *P. x acuminata* group) was performed.

ANOVAs, ANCOVAs, paired comparison and post hoc tests were performed using the univariate General Linear Model (GLM) function in SPSS 16.0 (SPSS Inc., Chicago, Illinois) using *Type III sum of squares*. Morphological and physiological measures were treated as dependent variables. Water treatment, genotype, group, time of day and leaf position (when applicable for the latter two factors) were treated as fixed factors. Figures were prepared in SPSS 16.0 using *Chart Builder* and *Legacy Dialogue* graphing options.

CHAPTER 3

RESULTS

3.1 Morphological measures

Results of morphological measures will be presented according to the three anatomical groupings of shoot attributes (stem and leaves), root attributes, and whole plant attributes. Statistical analyses revealed that the majority of structural growth measures followed a pattern, defined as Pattern A, of group response within and across water treatments (Table 4). Sapling attributes which followed Pattern A displayed increased growth of *P. x acuminata* (the hybrid) over that of the narrowleaf sexes, which showed similar growth, in each water treatment. In addition, measurements of these traits were depressed in the Flood treatment for each group.

Table 4. Sapling attributes whose measurement results followed Pattern A, defined by group and water treatment responses. All measures following this pattern were morphological.

Sapling Anatomical Grouping	Morphological Measure
Stem	Diameter growth
	Height
	Stem dry weight
	Cutting dry weight
Leaves	Leaf size
	Leaf area
	Leaf dry weight
Roots	Root number
	Lower root number
	Root area
	Root dry weight
Whole Plant	Shoot dry weight
	Total dry weight

Stem

Initial stem diameters showed a trend amongst groups (Table 5) which related to the hybrid's, on average, slightly larger (thicker) cuttings. Stem diameter growth of the hybrid in all water treatments was significantly greater than that of the narrowleaf females or males, which displayed similar growth (Figure 7). Diameter growth in Water 1 and Water 2 treatments did not significantly differ, but was significantly depressed in the Flood treatment for all groups (Figure 7, Table 5). Other stem measures that followed this same pattern included height, stem dry weight, and cutting dry weight (Figure 7, Table 6). Stem dry weight displayed a significant group x water treatment interaction (Table 6), which was attributed to the hybrid's disproportionate decrease (large magnitude of decline) of stem dry weight in flood in comparison to the slighter declines observed in the narrowleaf sexes (Figure 7). This interaction was shown as a trend in cutting, leaf and shoot dry weights (Tables 6, 7 and 8 respectively), whereby the flood treatment showed a lesser discrepancy in dry weights between *P. x acuminata* and *P. angustifolia* than in Water 1 and 2 treatments.

Table 5. ANOVA results for pre-treatment (initial) stem diameter and diameter growth. Statistically significant ($p < 0.05$) effects are indicated in bold font.

Measure	Pre-treatment Stem Diameter			Diameter Growth		
Effect	df	F-value	p	df	F-value	p
Group (G)	2	2.816	0.083	2	21.9	**<0.0001**
Water treatment (W)				2	9.05	**<0.0001**
G x W				4	0.521	0.720
Error	21			63		
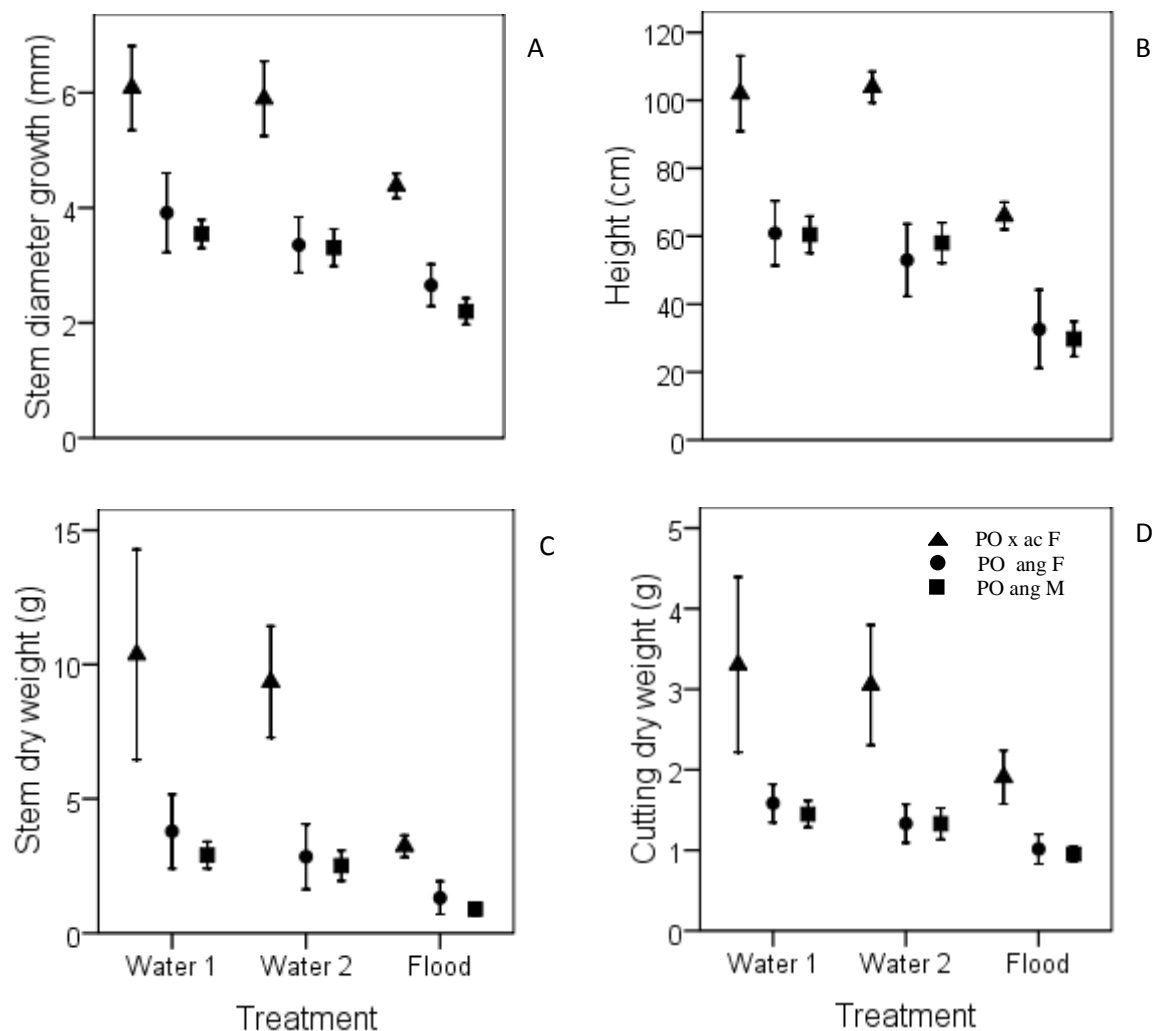
Figure 7. (A) Stem diameter growth, (B) height, (C) stem dry weight and (D) cutting dry weight (means $\pm$ S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three water treatments.

Table 6. ANOVA results for height, stem dry weight and cutting dry weight. Statistically significant ($p<0.05$) effects are indicated in bold font. Values for degrees of freedom (df) were the same for all measures.

Measure		Height		Stem Dry Weight		Cutting Dry Weight	
Effect	df	F-value	p	F-value	p	F-value	p
Group (G)	2	13.2	**<0.0001**	23.1	**<0.0001**	27.9	**<0.0001**
Water treatment (W)	2	11.1	**<0.0001**	12.0	**<0.0001**	7.38	**0.001**
G x W	4	0.399	0.809	2.98	**0.026**	2.07	0.096
Error	60						

Leaves

Leaf numbers were similar between the hybrid and narrowleaf females, which were significantly greater than those of the narrowleaf males (Figure 8). In a separate two-way analysis of variance involving the two *P. angustifolia* sexes, females displayed significantly higher leaf numbers than those of the males (F-value = 6.518, df = 1, p = 0.013). Water 1 and Water 2 treatments maintained similar leaf numbers and Flood numbers were comparatively reduced (Figure 8, Table 7).

The measures of leaf size, leaf area and leaf dry weight followed Pattern A (Figure 8). Water 1 and Water 2 treatments did not significantly differ, and Flood treatment values were significantly reduced (Figure 8, Table 7). Leaf size displayed a significant group x water treatment interaction, and leaf area a trend, attributable to the narrowleaf female's smaller declines of leaf area and number in flood, in comparison to the males' and the hybrid's larger reductions under inundation (Table 7). Specific leaf areas (leaf area/leaf dry weight), were approximately 200 cm^2/g and were similar for all groups in each water treatment (Table 7).
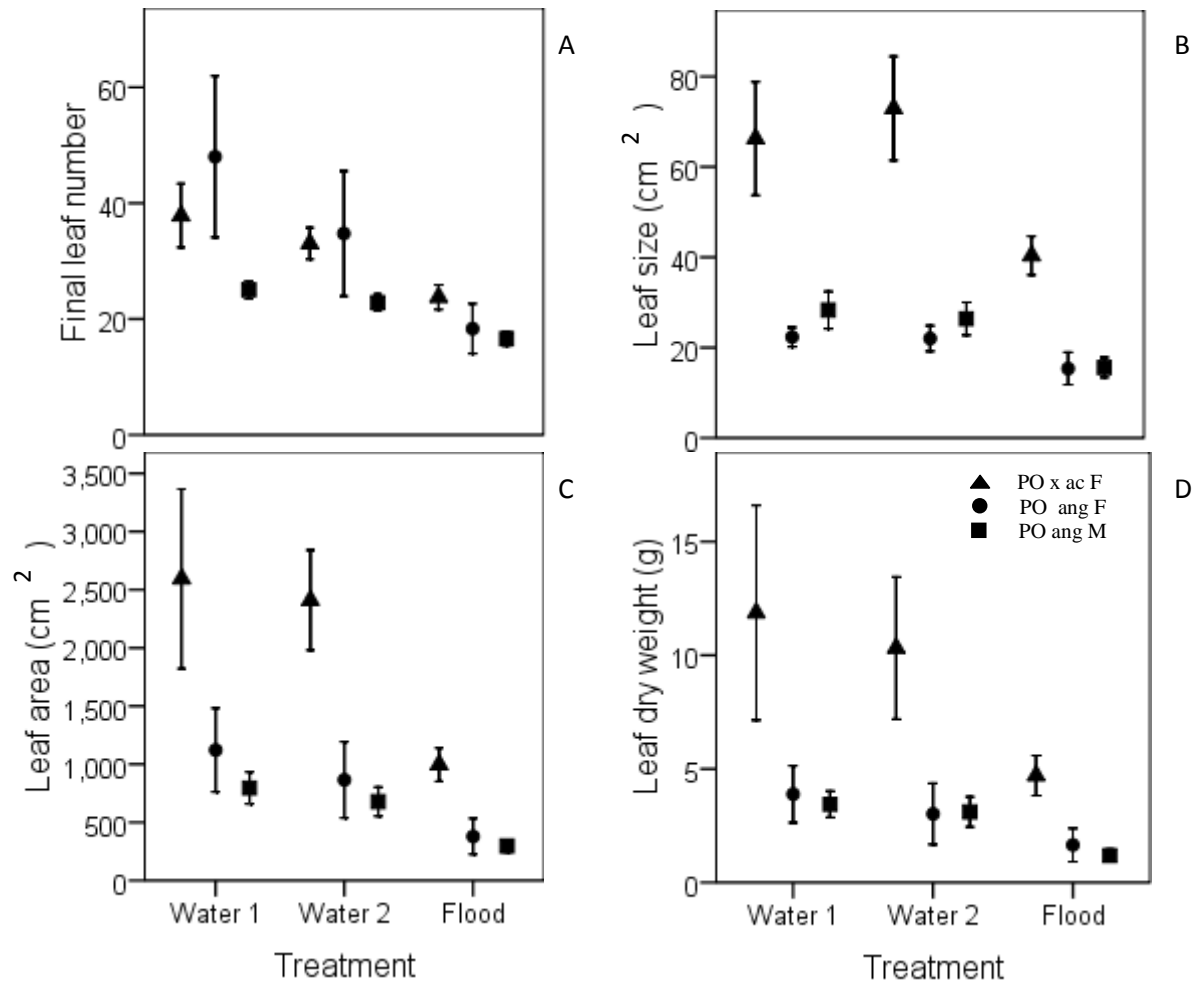
Figure 8. (A) Leaf number at harvest, (B) leaf size, (C) leaf area and (D) leaf dry weight (means ± S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments.

Table 7. ANOVA results for leaf number, area, size, dry weight and specific leaf area. Statistically significant (p< 0.05) effects are indicated in bold font. Values for df[1] were the same for leaf number, leaf dry weight and specific leaf area and for df[2] were the same for leaf area and leaf size.

Measure	df[1]	Leaf Number		df[2]	Leaf Area		Leaf Size		Leaf Dry Weight		Specific Leaf Area	
Effect		F-value	p		F-value	p	F-value	p	F-value	p	F-value	p
Group (G)	2	3.81	**0.028**	2	27.4	**<0.0001**	76.2	**<0.0001**	25.1	**<0.0001**	1.46	0.240
Water treatment (W)	2	4.18	**0.020**	2	13.9	**<0.0001**	17.3	**<0.0001**	9.00	**<0.0001**	0.267	0.767
G x W	4	0.699	0.595	4	2.15	0.086	3.24	**0.018**	2.350	0.065	0.342	0.849
Error	63			62								

49

Shoot dry weight results (stem + cutting + leaves) were consistent with Pattern A (Figure 9). The hybrid was significantly heavier than the narrowleaf females or males, which were similar (Figure 9, Table 8). Dry weights did not differ between Water 1 and 2 treatments but were reduced in Flood.
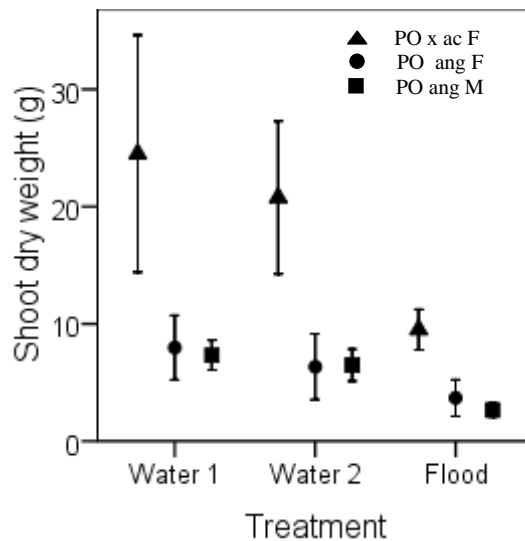
Figure 9. Shoot dry weights (means $\pm$ S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments.

Table 8. ANOVA results for shoot dry weight. Statistically significant (p<0.05) effects are indicated in bold font.

Measure		Shoot Dry Weight	
Effect	df	F-value	p
Group(G)	2	21.8	**<0.0001**
Water treatment (W)	2	8.34	**0.001**
G x W	4	2.37	0.063
Error	60		

3.1.2 Roots

In Water 2 and Flood, the hybrid maintained significantly higher total and upper root numbers than those of the narrowleaf males and females, which displayed similar numbers (Figure 10). Water 1 showed the hybrid and narrowleaf females to maintain similar total and upper root numbers which were greater than those of the narrowleaf males (Figure 10). Upper root numbers for all groups did not differ amongst treatments in contrast to total and lower root numbers which showed significant reductions from Water 1 to Flood (Figure 10, Table 9). The hybrid developed a greater number of lower roots in Water 1 and 2 in comparison to the narrowleaf sexes which showed similar lower root numbers, and all groups were reduced to similar numbers in Flood.
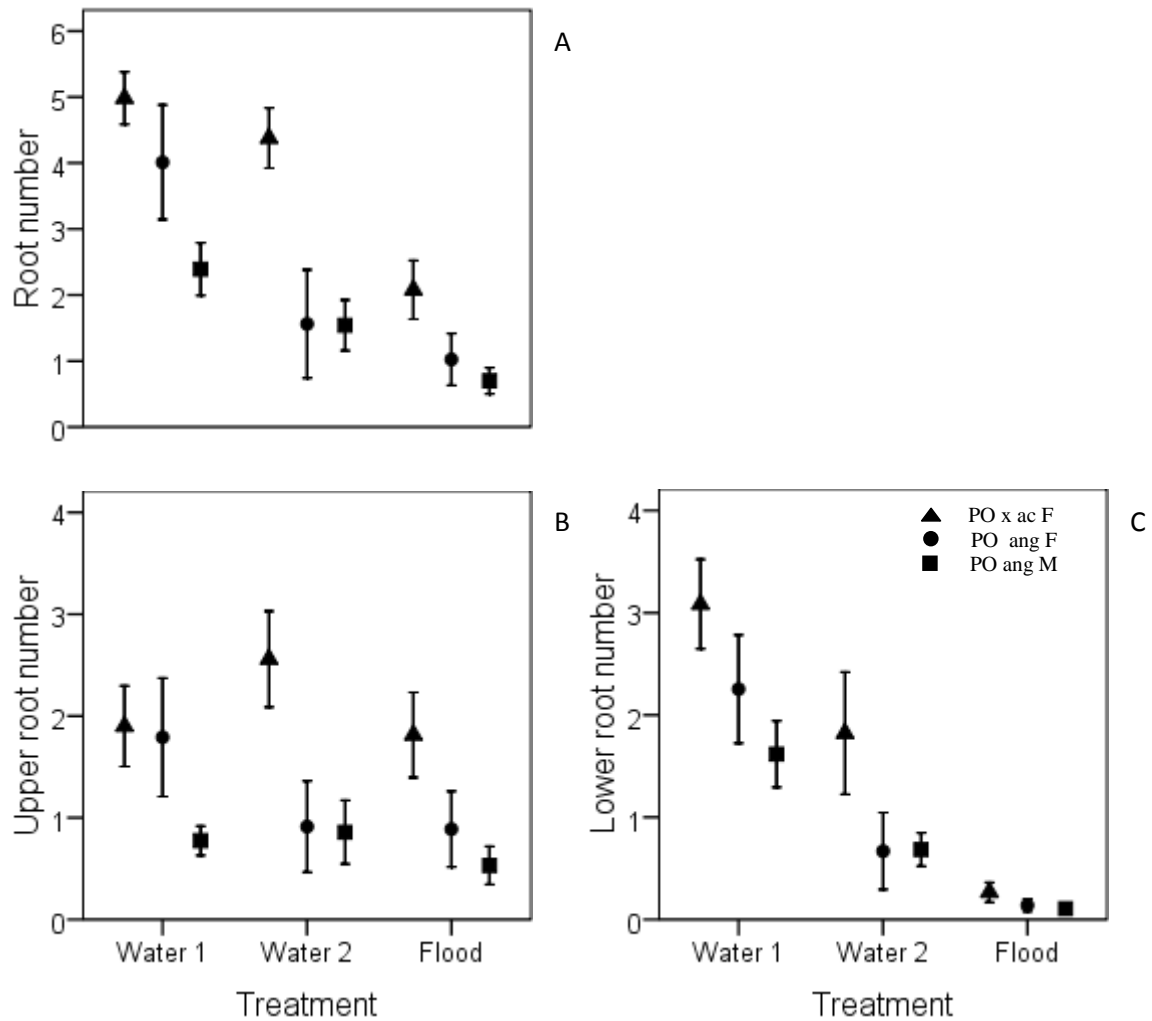
Figure 10. (A) Root number (total), (B) upper root number and (C) lower root number (means ± S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments.

Table 9. ANOVA results for upper and lower root number. Statistically significant (p<0.05) effects are indicated in bold font. Values for df were the same for both measures.

Measure		Upper Root Number		Lower Root Number	
Effect	df	F-value	p	F-value	p
Group (G)	2	5.23	**0.008**	5.02	**0.010**
Water treatment (W)	2	0.673	0.514	29.0	**<0.0001**
G x W	4	0.520	0.721	1.35	0.262
Error	63				

Total (upper + lower root areas) and upper root areas displayed group responses that followed Pattern A (Figure 11). Water 1 and 2 treatments did not differ in (total) root area, but values were reduced in Flood. Water treatments did not have a significant effect on upper root areas, in contrast to lower root areas which were reduced across treatments (Figure 11). Lower root areas in Water 1 were greater than those in Water 2, which were greater than those in Flood, and groups displayed similar areas in Water 2 and Flood, versus Water 1 where the hybrid showed the highest area (Table 10).

Root dry weight followed Pattern A, whereby the hybrid had significantly heavier dry weights than the narrowleaf females or males, which showed similar dry weights to each other (Figure 11). Water 1 and 2 treatments showed no significant differences, whereas Flood displayed reduced values (Table 11).
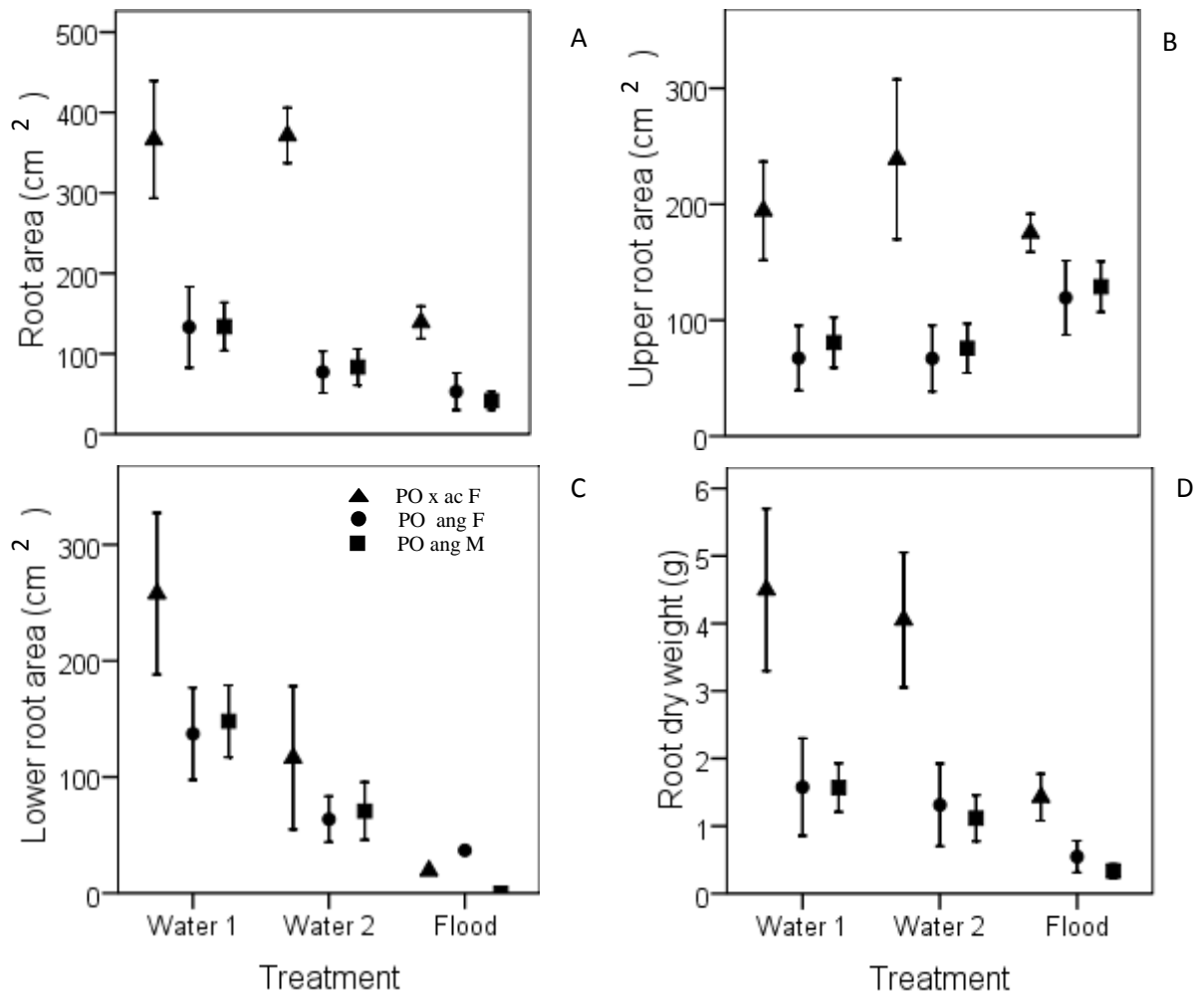
Figure 11. (A) Root area (upper + lower areas), (B) upper root area, (C) lower root area and (D) root dry weight (means $\pm$ S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments. Lower root area error bar calculations involved low sample numbers in the Flood treatment, consequently S.E. bars were extreme and therefore removed.

Table 10. ANOVA results for upper and lower root area. Statistically significant (p<0.05) effects are indicated in bold font.

Measure		Upper Root Area			Lower Root Area	
Effect	df	F-value	p	df	F-value	p
Group (G)	2	8.81	**0.001**	2	0.637	0.535
Water treatment (W)	2	0.235	0.792	2	9.51	**<0.0001**
G x W	4	0.530	0.714	4	1.74	0.162
Error	38			36		

54

Table 11. ANOVA results for root dry weight. Statistically significant (p<0.05) effects are indicated in bold font.

Measure		Root Dry Weight	
Effect	df	F-value	p
Group (G)	2	21.6	**<0.001**
Water treatment (W)	2	11.5	**<0.001**
G x W	4	1.73	0.156
Error	60		

3.1.3 Whole Plant

Total dry weight results followed Pattern A, which was consistent with stem, cutting, leaf, root and shoot dry weight results (Table 12, Figure 12). To check for any anomalies that might be caused by individual genotypes in the Water 1 or Flood treatments, total dry weights per genotype were arranged from heaviest to lightest in the Water 2 treatment according to group (Figure 12). Consistently, genotypes in Water 1 and 2 treatments displayed higher weights than those in Flood and Water 1 and 2 maintained similar weights for each genotype (Table 12). Genotype dry weights differed significantly within a treatment, although hybrid genotypes showed heavier weights, on average, than the narrowleaf females or males which displayed a proportionate number of genotypes that had similar weights for a treatment (Figure 12). Percent reduction in dry weight for the total sapling (TDW) from Water 2 to Flood was largest for the hybrid followed by the narrowleaf males, in contrast to the narrowleaf females which showed the lowest sensitivity to inundation (Table 13, Figure 13). Narrowleaf females displayed the lowest percent reduction in dry weight for stem, cutting, leaves and roots and the males showed the highest reduction in root dry weight, although males were similar to the hybrid for reduction in leaf dry weight (Table 13). Overall, Flood effectively reduced

TDW of the narrowleaf sexes to significantly different degrees and affected similar reductions in the narrowleaf males and the hybrid (Figure 13). The interaction of group x water treatment showed a trend in TDW (Table 12), attributed to the differential effects of flood on each group.

Shoot/root ratios displayed results that were the inverse of Pattern A whereby across the study, ratios of the hybrid tended to be lower than those of the narrowleaf sexes, which were similar (Figure 14). Water 1 and Water 2 treatments maintained similar ratios, but Flood values displayed a trend and tended to be elevated for each group (Table 12). Leaf area ratios, derived from leaf area/total sapling dry weight, were similar for all groups in Water 1 and Water 2 treatments, whereas the narrowleaf sexes had reduced ratios in the Flood treatment (Figure 15). A water treatment effect was not detected despite the reduced ratios of the narrowleaf sexes in Flood (Table 12).

Table 12. ANCOVA and ANOVA results of total sapling dry weight, shoot/root ratio and leaf area ratio. Statistically significant (p<0.05) effects are indicated in bold font. Values of df in the ANOVA for groups were the same for all measures.

Measure		Total Dry Weight		Shoot/Root Ratio		Leaf Area Ratio	
Effect	df	F-value	p	F-value	p	F-value	p
Genotype (Ge)	23	21.8	**<0.0001**				
Water treatment (W)	2	32.5	**<0.0001**				
Ge x W	46	2.25	**<0.001**				
Pool	1	1.90	0.169				
Error	358						
Group (G)	2	21.8	**<0.0001**	2.85	0.066	4.54	**.015**
Water treatment (W)	2	8.75	**<0.0001**	2.57	0.085	.659	.521
G x W	4	2.31	0.068	0.294	0.881	1.38	.253
Error	60						
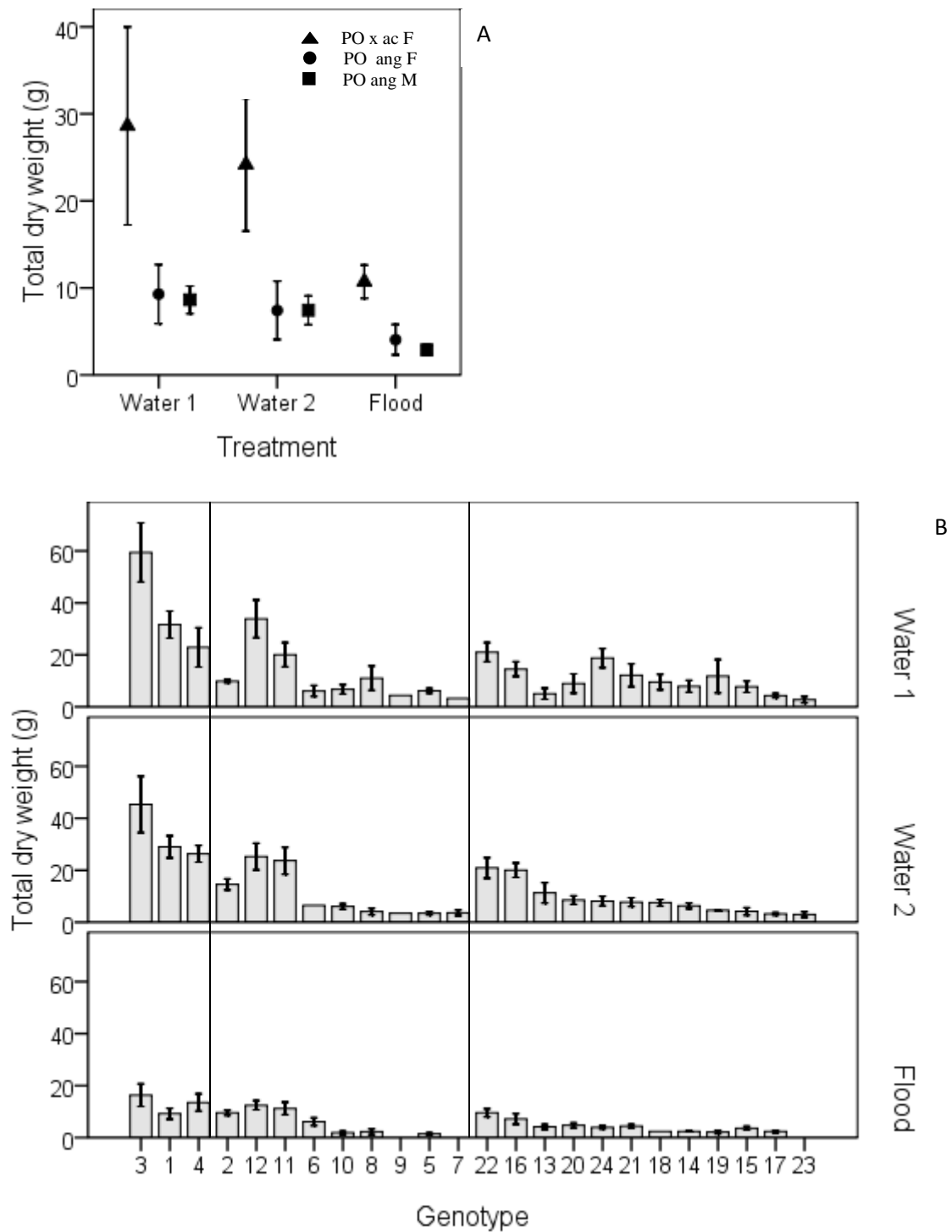
Figure 12. (A) Total sapling dry weights per group and (B) total dry weight per genotype (means ± S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments. Genotype designations for the three *Populus x acuminata* females are 1, 3 and 4, for the nine *P. angustifolia* females are 2, and 5-12 and for the twelve *P. angustifolia* males are 13-24, and are separated as such.

57

Table 13. Percent reduction in dry weights (g) of cutting, stem, leaves, roots and total dry weight (TDW) for three groups from Water 2 to the flood treatment, with the largest reduction per attribute indicated in bold. Percent reductions were derived from Water 2 and Flood dry weights means.

Group Sapling Attribute	PO ang M	PO ang F	PO x ac F
	Percent Reduction (%)		
Cutting	-25	-6	**-45**
Stem	-61	-37	**-71**
Leaves	-59	-24	**-60**
Roots	**-70**	-60	-64
Total Sapling (TDW)	-58	-23	**-62**
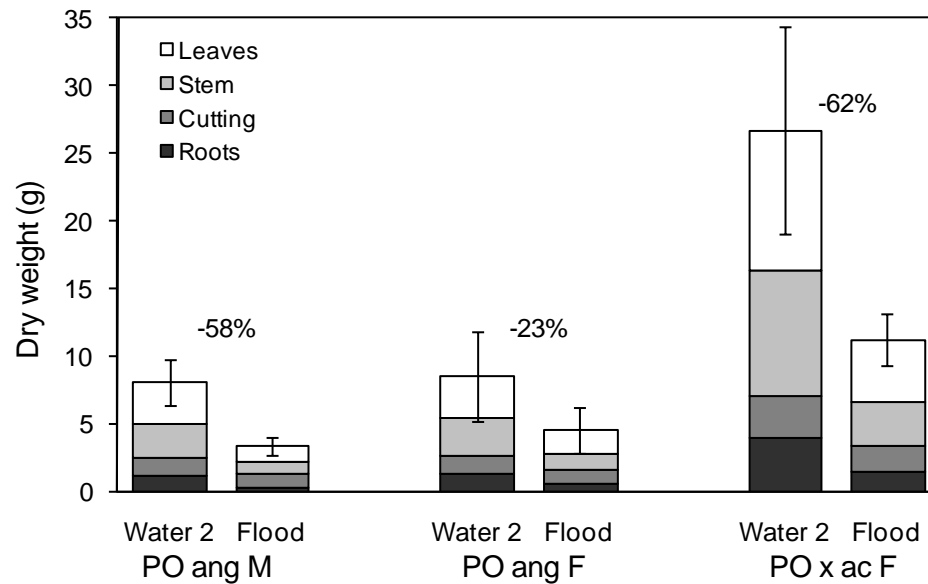
Figure 13. Total dry weights partitioned by leaves, stem, cutting and roots per group in Water 2 and Flood (means $\pm$ S.E. for TDW) and subsequent percent reductions.
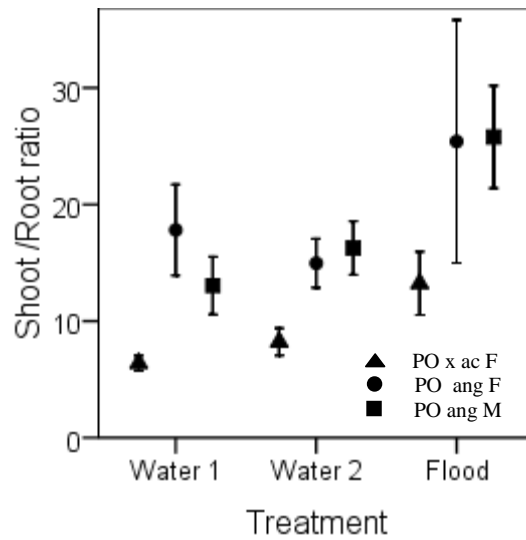
Figure 14. Shoot/root ratio (means $\pm$ S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments.
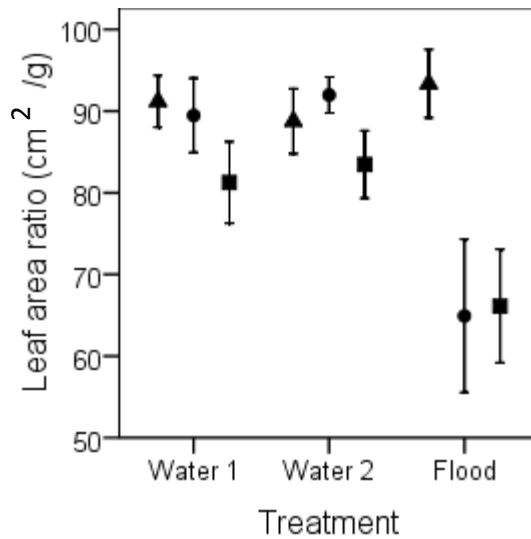
Figure 15. Leaf area ratio (means $\pm$ S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments.

3.2 Physiological Measures

Results of physiological measures will be presented according to the groupings: stomatal characteristics, leaf water potential and leaf traits (chlorophyll, C: N ratio and carbon isotope composition).

3.2.1 Stomatal Characteristics & Leaf Water Potential

In-treatment abaxial stomatal densities (SD) (those of leaves which formed during the treatment phase) of the hybrid and the narrowleaf females and males were similar, although the hybrid tended to have lower densities across treatments (Figure 16, Table 14). Water treatment did not significantly affect densities (Table 14).

Light saturated, abaxial stomatal conductance (g_s) taken over the period of July 24 to August 2 during treatments (74 to 83 days after sticking) did not significantly differ amongst the groups, although the hybrid tended to show lower g_s than that of the narrowleaf sexes in Water 1 (Figure 16, Table 15). Water treatments 1 and 2 deviated little and showed increased g_s relative to that in Flood, which displayed significantly lower gas exchange values (Table 15). Pre-treatment (during the establishment phase, 46 to 48 days after sticking; June 26 to 28) g_s compared to g_s taken during the period July 24 to August 2 measured the change in g_s between pre-treatment and in-treatment leaves. All groups showed significant change in g_s (Figure 17), while the hybrid had significantly reduced pre-treatment g_s compared to the narrowleaf sexes, which displayed similar pre-treatment values (data not shown).

60
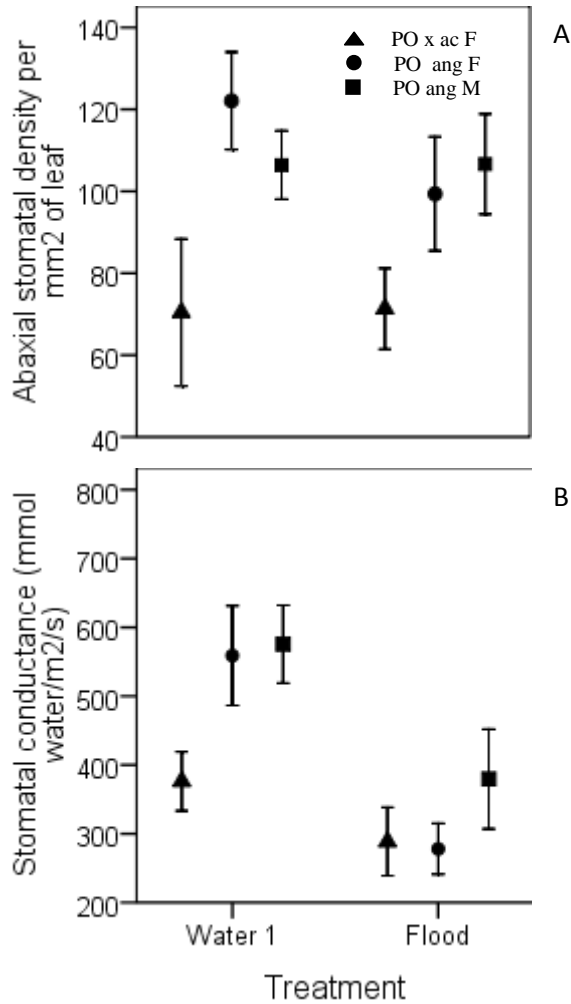
Figure 16. (A) In-treatment stomatal densies, (B) July 24 to August 2 abaxial stomatal conductance (g_s), (means $\pm$ S.E.) of a sample population of *Populus x acuminata* females and *P. angustifolia* females and males in two treatments.

Table 14. ANOVA results for in-treatment stomatal density. Statistically significant (p<0.05) effects are indicated in bold font.

Measure	In-treatment Stomatal Density		
Effect	df	F-value	p
Group (G)	2	1.97	0.153
Water treatment (W)	1	0.222	0.640
G x W	2	0.472	0.627
Error	42		

Table 15. ANOVA results for July 24 to August 2 g_s. Statistically significant (p<0.05) effects are indicated in bold font.

	Measure	July 24 to August 2 g_s	
Effect	df	F-value	p
Group (G)	2	1.74	0.189
Water treatment (W)	1	7.24	**0.010**
G x W	2	0.439	0.648
Error	39		
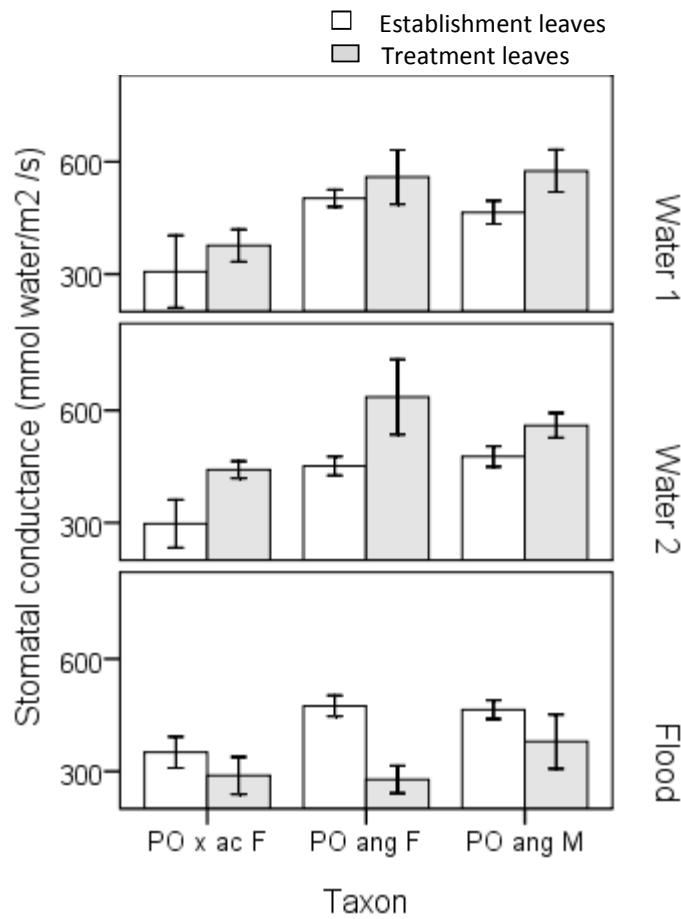
Figure 17. Pre-treatment g_s compared to July 24 to August 2 (in-treatment) g_s (means $\pm$ S.E.) of three groups in each treatment. Establishment leaves formed in Water 2 conditions, and treatment leaves during the treatment phase. Water 2 treatment leaves were formed and measured during treatments. Figure 16 (B) and 17 show the same data of in-treatment g_s.

Groups displayed similar leaf water potentials (Ψ_L) within and across treatments (Figure 18, Table 16).

Figure 18. Leaf water potentials (means $\pm$ S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments.

Table 16. Leaf water potential results from an ANOVA performed on the three groups.

Measure		Leaf Water Potential	
Effect	df	F-value	p
Group (G)	2	0.677	0.512
Water treatment (W)	2	0.009	0.991
G x W	4	0.237	0.916
Error	56		

3.2.2 Leaf Traits

Chlorophyll

Relative abundance of chlorophyll (Chl) taken July 3 and July 31 (53 and 81 days after sticking respectively) on leaves that formed during the establishment phase were similar amongst groups (Table 17). While water treatments did not affect chlorophyll

abundance taken July 3, July 31 Chl showed a treatment effect (Table 17). Chl taken on

leaves July 31 tended to be similar between the hybrid and the narrowleaf sexes in Water

1 and 2 treatments. Conversely, in Flood the hybrid maintained similar values to those in

Water 1 and 2, whereas the narrowleaf sexes showed depressed values (Figure 19). The

depressed values of the sexes in Flood resulted in a treatment effect (Table 17).

Table 17. ANOVA results for relative abundance of leaf chlorophyll (Chl). Lower leaves for July 3 and 31 (53 and 81 days after sticking) were the same leaves and formed during the establishment phase. Upper leaves formed during the treatment phase. Statistically significant ($p<0.05$) effects are indicated in bold font. Values of df were the same for lower and upper leaves measured July 31.

| Measure	Chl Lower Leaf July 3			Chl Lower Leaf July 31			Chl Upper Leaf July 31	
Effect	df	F-value	p	df	F-value	p	F-value	p
Group (G)	2	1.07	0.350	2	0.93	0.400	2.27	0.112
Water treatment (W)	2	1.26	0.291	2	3.51	**0.036**	6.77	**0.002**
G x W	4	0.046	0.996	4	1.040	0.392	0.409	0.802
Error	63			59				
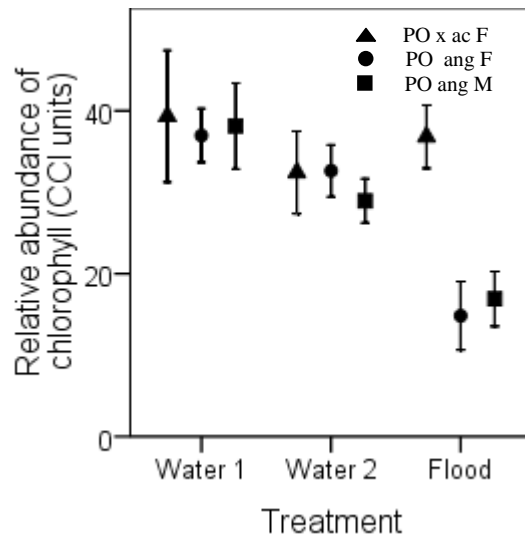
Figure 19. Relative abundance of chlorophyll of upper leaves on July 31 (means $\pm$ S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments.

*Foliar Carbon:Nitrogen Ratio & Carbon Isotope Composition*

Carbon to nitrogen ratio group results displayed the inverse to Pattern A, whereby ratios of the hybrid were significantly reduced compared to those of the narrowleaf females or males, which displayed similar ratios in Water 1, but not in the Flood treatment (Figure 20). The hybrid and narrowleaf males showed elevated values in the Flood treatment, resulting in a treatment effect (Table 18). The hybrid showed the largest percent increase in C: N under inundation, although similar to that of males (Table 19). Evidence of significant divergence between the narrowleaf males and females in the Flood treatment led to a one-way ANOVA excluding the hybrid. The ANOVA revealed the sexes to show a trend where males tended to have a higher C: N ratio than the females in Flood (F-value= 3.036, df= 1, p= 0.090). A treatment effect resulted from the significant percent increase in C:N of the narrowleaf males in Flood, compared to the

66

females which showed only a relatively slight percent increase from Water 1 to Flood, which was the lowest of the groups' (F-value= 5.115, df= 1, p= 0.030) (Table 19).

Foliar carbon isotope composition (δ^{13}C) of the hybrid was significantly greater (less negative) than that of the narrowleaf males and females, which were similar in both Water 1 and Flood (Figure 20, Table 18). δ^{13}C showed no change between Water 1 and Flood treatments (Table 18).
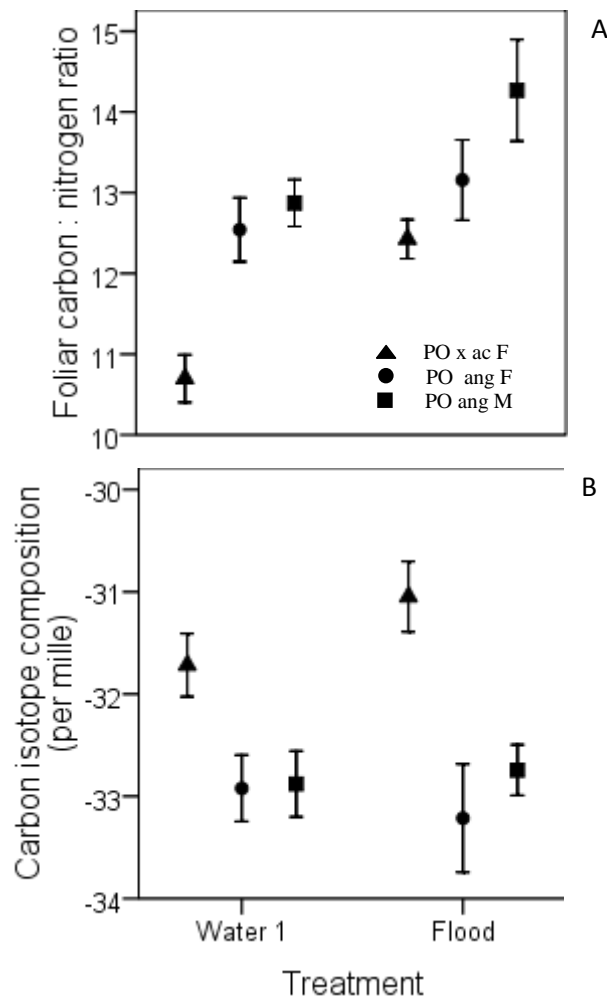
Figure 20. (A) Carbon:nitrogen ratios (C:N) and (B) foliar carbon isotope compositions (δ^{13}C, ‰) (means $\pm$ S.E.) of *Populus x acuminata* females and *P. angustifolia* females and males in two treatments.

Table 18. ANOVA results for C: N and $\delta^{13}C$. Statistically significant (p<0.05) effects are indicated in bold font. Values of df were the same for both measures.

Measure		Carbon : Nitrogen Ratio		Carbon Isotope Composition	
Effect	df	F-value	p	F-value	p
Group (G)	2	5.41	**0.008**	3.90	**0.028**
Water treatment (W)	1	6.46	**0.015**	0.119	0.732
G x W	2	0.336	0.717	0.090	0.914
Error	42				

Table 19. Means and percent increase in carbon: nitrogen ratio (C: N) for three groups from Water 1 to the Flood treatment.

Treatment	Water 1	Flood	
Group	C: N Means		Percent Increase (%)
PO ang M	12.9	14.3	11
PO ang F	12.4	13.1	6
PO x ac F	10.6	12.3	16

3.3 Percent Survival

For Water 1 and Flood treatments, percent survival at harvest for the hybrid was higher than that of the narrowleaf females or males, although were similar to males' in Water 2 (Figure 21). A two-way ANOVA (followed by Tukey's Honestly Significant Difference (HSD) post hoc test, in addition to the Bonferroni post hoc test) showed water treatment did not have a significant effect, in contrast to revealing a statistically significant group effect (Table 20). Due to evidence of significant differences between *P. angustifolia* females and males, a two-way ANOVA was performed which revealed a trend whereby the narrowleaf males tended to have greater percent survival over the females, most notably in Water 1 and 2 treatments (F-value = 3.839, df = 1, p = 0.055). For all three groups, percent survival was not significantly affected across water treatments, revealed in a one-way ANOVA (Table 20). In acknowledging that a different number (n) of genotypes comprised the survivorship means for a group, conclusions with

respect to group percent survival may be linked to the percent survival of one or more

particular genotypes of a group.

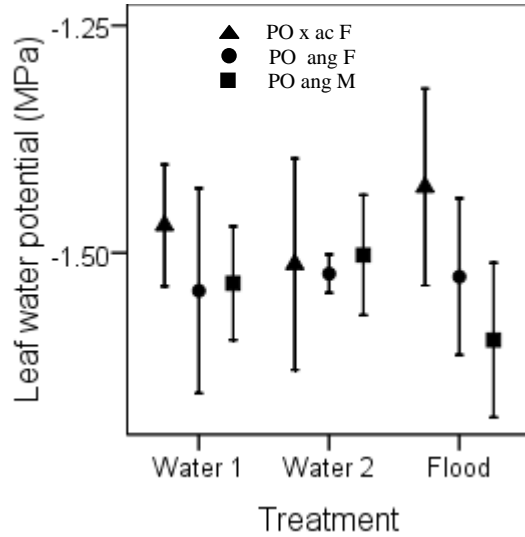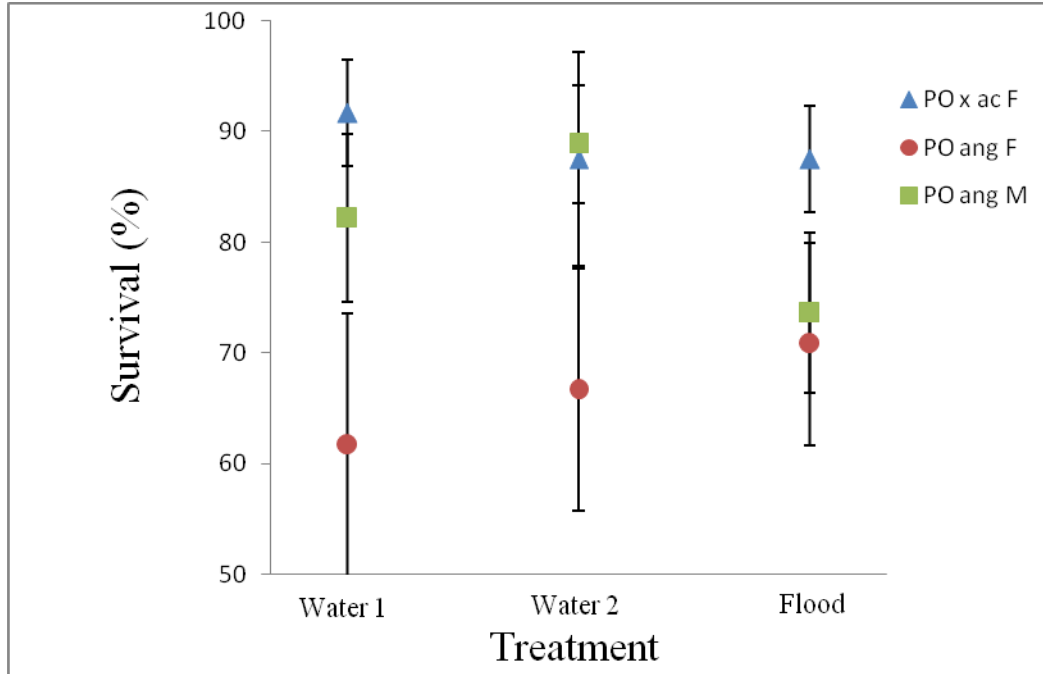Figure 21. Percent survival at harvest (means $\pm$ S.E.), of *Populus x acuminata* females and *P. angustifolia* females and males in three treatments.

Table 20. Percent survival results for three groups in each water treatment and across water treatments. Statistically significant (p<0.05) effects are indicated in bold font.

Measure	Percent Survival at Harvest per W			Percent Survival at Harvest across W		
Effect	df	F-value	p	df	F-value	p
Between Groups				2	1.471	0.252
Group (G)	2	3.520	**0.036**			
Water treatment (W)	2	0.112	0.894			
G x W	4	0.596	0.667			
Error	63			21		

In relation to specific declines in percent survival of a group at a certain number

of days since sticking, it was determined that a single genotype, or a select few, within a

group was not responsible for these declines (Figure 22). A marked decline in percent

survivorship of narrowleaf females in the Water 1 treatment up to 67 days after sticking

was not influenced by any one genotype but attributable to the mortality within six

genotypes during that time (Figure 23). Likewise, during the same time period in the

Water 2 treatment, narrowleaf males showed a decline in percent survival resulting from

the mortality within three genotypes (Figure 23). A different number of genotypes

influencing a decline in a group's percent survival were also observed in the Flood

treatment for both narrowleaf females and males, at days 74 and 81 post sticking (Figure

23). In turn, narrowleaf females and males showed reduced percent survival due to the

mortality of different genotypes within groups, and not within a single water treatment

but across all treatments, which resulted in no water treatment effect (Table 20, Figure

22).
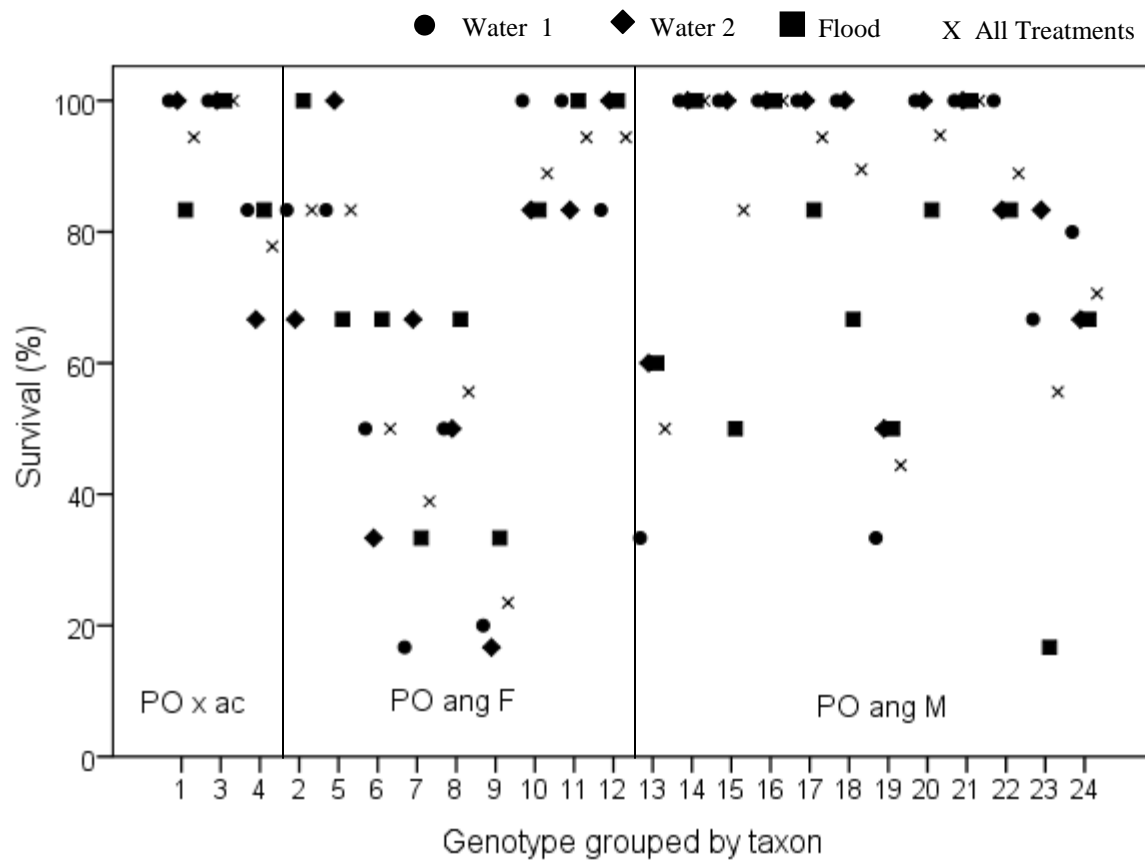
Figure 22. Percent survival at harvest (means), of four *Populus x acuminata* female genotypes, eight *P. angustifolia* female genotypes and twelve *P. angustifolia* male genotypes in each water treatment and across treatments.
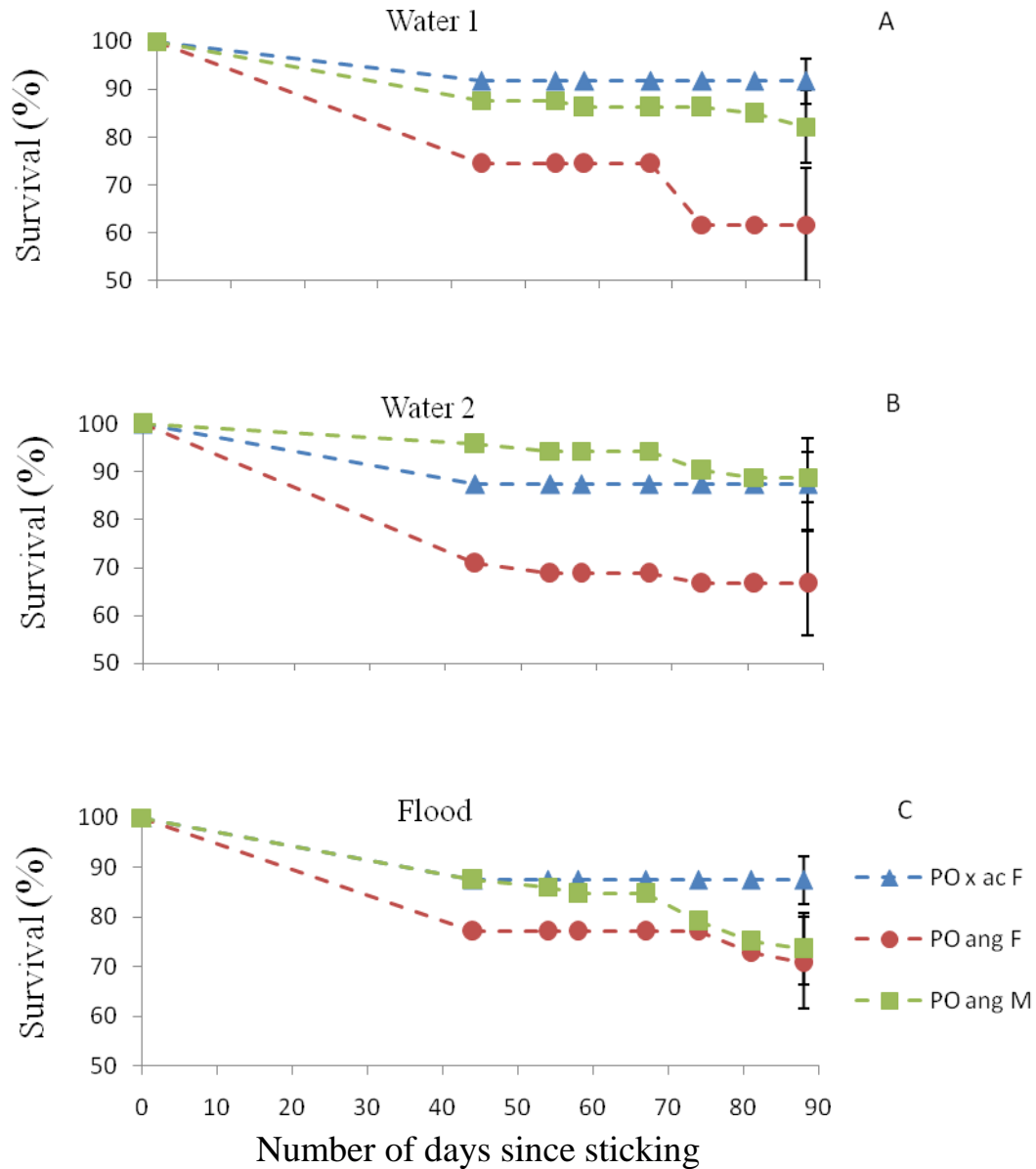
Figure 23. Cumulative percent survival (means and means $\pm$ S.E. at day 88), at different days since sticking of cuttings of *Populus x acuminata* females and *P. angustifolia* females and males in (A) Water 1, (B) Water 2 and (C) Flood treatments.

A Mann-Whitney U test revealed that in each water treatment no significant differences with respect to percent survival between the narrowleaf sexes during the establishment phase occurred (Mann-Whitney U 36.5, P = 0.202). The establishment phase was up until day 49 after sticking, when treatments began (Figure 23).

CHAPTER 4

DISCUSSION

4.1 Common taxa response across water treatments

Two patterns of taxa response across water treatments occurred and the majority, (thirteen) of the fifteen morphological variables measured, followed one of these patterns, called Pattern A. This pattern was repeatedly observed for sapling structural attributes in addition to derivative measures that were calculated from these variables. Pattern A showed similar growth of taxon groups in Water 1 and Water 2 treatments, and significantly depressed values in Flood. Within treatments, growth of the hybrid was significantly greater than that of the narrowleaf females or males, which displayed similar growth. A mechanism underlying this pattern became apparent after analysis of morphological traits data indicated Water 1 and 2 treatments to be of similar soil moistures. As such, Water 1 and 2 were shown to be experienced as relatively equivalent water treatments conducive to high(er) growth rates (over those shown in the flood treatment). Once roots reached the standing water level (unlimited water availability), set slightly lower in Water 1 pots than in Water 2, water uptake and soil moisture availability would have deviated minimally between these treatments. Slight differences between Water 1 and Water 2 treatments were correlated with the location of the capillary fringe (the zone of root growth and moist substrate) which surrounded the entire cutting in Water 1 pots, but was restricted closer to the upper section of the cutting in Water 2 pots (in which the cutting base was likely submersed in standing water) (Figure 24). As a result of the differences in elevation of the zone of substrate conducive to root formation

73

and growth, distribution of sapling roots (lower root numbers and lower root areas of the taxa) differed between the two treatments. Both Water 1 and 2 treatments displayed moist to saturated conditions with unlimited water availability which favoured establishment and growth, and subsequently both served as control treatments, representing a reference condition. Reduced growth in the Flood treatment verified it as a water-stress, in which saplings displayed common responses to flood-stress (adventitious roots at or above the saturated soil surface and decreased stomatal conductance).

Within treatments, vigorous growth of the hybrid exceeding that of the narrowleaf sexes, displayed by Pattern A, was attributed to the slower growth rate of *P. angustifolia* in comparison to other native *Populus* species (Campbell et al. 1993; Kalischuk et al. 2001), as well as dominance being expressed in *P. x acuminata* as a result of *P. deltoides* high intrinsic growth rate incorporated into the hybrid genome (Kalischuk et al. 2001).
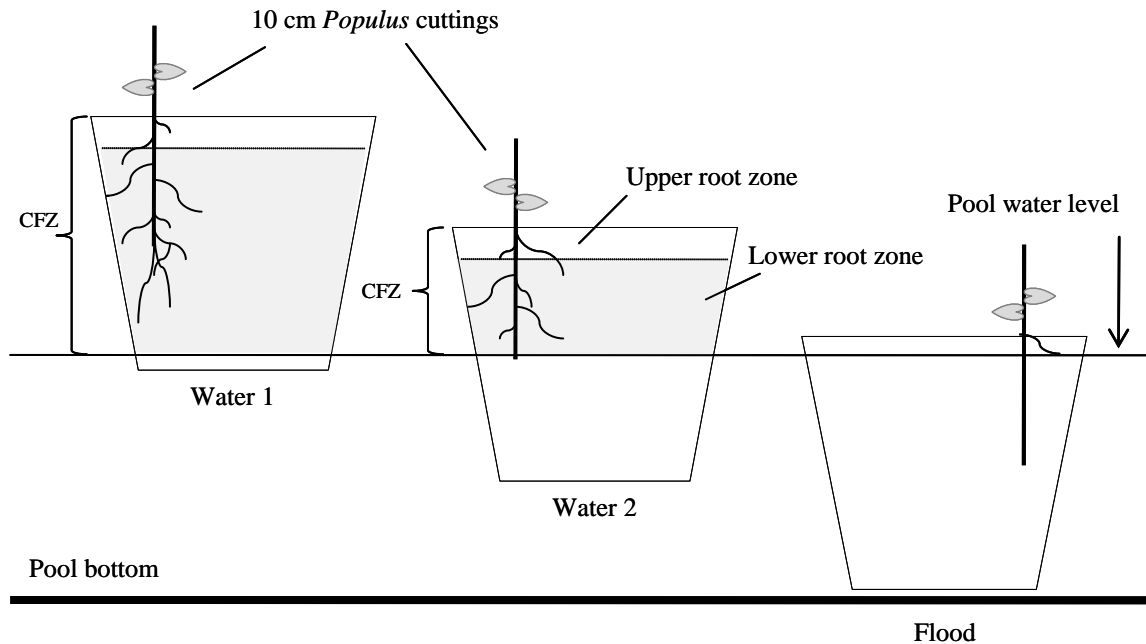
Figure 24. Schematic diagram (to scale) showing sapling root distribution within the capillary fringe zone (CFZ; upper + lower root zones) of pots in Water 1, 2 and Flood treatments. Upper and lower root zones represent substrate that is conducive to root growth.

4.2 Physiological processes and traits

Foremost, stomatal conductance (g_s) is a measure of the physiological process of stomatal regulation, which is most often considered a plant's first line of defense against excess water observed during environmental stress (Pereira and Kozlowski 1977; Kozlowski et al. 1991). Stomatal regulation governs carbon acquisition and subsequent carbon gain, which translates into overall plant growth acquired through photosynthesis. This gas exchange process is the regulator of water loss via transpiration and has direct effects on a plant's water balance through initiating an immediate response to water stress.

Abaxial g_s results showed in-treatment g_s of the hybrid and narrowleaf sexes to be similar in Water 1 and Flood (Figure 16, Table 15), although results were close to displaying a trend in which the hybrid maintained slightly lower g_s in reference conditions. Flood-stress reduced g_s for all groups, where increased leaf diffusive resistance has been determined a universal plant response to root zone oxygen deprivation (hypoxic or anoxic conditions) caused by flood (Pereira and Kozlowski 1977; Kozlowski 1984; Sojka 1992). At the onset of flood, g_s has shown immediate reduction which relates to its mediation by atmospheric humidity at the leaf surface and soil moisture availability at the roots (Aphalo and Jarvis 1991; Tardieu and Davies 1993; Fort et al. 1997). Reduction of g_s can be triggered by a decline in leaf turgor which is often a consequence of decreased absorption of water resulting from lowered root permeability in flooded conditions (Tang and Kozlowski 1984). *P. deltoides* have shown abrupt stomatal closure at the onset of flood and over time a decline in g_s, as a result of prolonged inundation (Regehr et al. 1975).

Slightly reduced g_s of the hybrid in reference conditions compared to that of the narrowleaf males and females may be correlated with observed differences in leaf abaxial stomatal densities and g_s between *P. angustifolia* and *P. x acuminata*, as well as between *P. deltoides*. *Populus*, an amphistomatous genus, has shown single surface stomatal conductances and densities to be correlated, but to differ among species (Pallardy and Kozlowski 1979; Ceulemans et al. 1984; Pearce et al. 2006). These differences have been attributed to reflect adaptations to physical habitat and climatic variations amongst native geographic ranges (Sparks and Black 1999; Dunlap and Stettler 2001; Pearce et al. 2006). *P. angustifolia* has been reported to have significantly higher stomatal densities on both

leaf surfaces than those of *P.x acuminata*, (which most often has greater leaf areas) and higher abaxial densities than *P. deltoides*, which have been shown to correspondingly affect stomatal conductance (Pearce et al. 2006). Pearce and others (2006) determined *P. angustifolia's* higher densities to be coupled with higher g_s and presumably greater stomatal sensitivity lending to higher stomatal control. In accordance with our results, the narrowleaf sexes displayed slightly higher g_s coupled with slightly higher abaxial stomatal densities (which were close to displaying a trend amongst groups) (Figure 16, Table 14). Relative to this may be the larger leaf areas of the hybrid which would have the effect of decreasing stomatal density. The hybrid response observed in these strongly heritable stomatal characteristics (Pallardy and Kozlowski 1979; Ceulemans et al. 1988) coincides with *P. x acuminata's* conditioning stemming from the distributional range of *P. deltoides* which has made it naturally more adapted to warm, dry conditions, over *P. angustifolia*.

A treatment effect with respect to stomatal density was not observed, as densities for all groups were not correlated (showed no change) with a drop in g_s shown in Flood (Figure 16). As discussed in the literature, for plants in general including *Populus,* stomatal density has been shown to increase in response to water stress (flood and drought) over time (Kozlowski 1984; Ridolfi et al. 1996; Banon et al. 2004; Xu and Zhou 2008). This has been speculated to occur because an increase in stomatal density could lead to greater stomatal control and therefore bring about tighter regulation of gas exchange and water loss under water stress. It may be speculated that stomatal regulation influenced the drop in g_s brought about by flood since stomatal densities did not show a corresponding increase.

The change in g_s between in- and pre-treatment leaves indicated flood to impose a water-stress on all groups (Figure 17), as gas exchange was significantly reduced in those leaves that were exposed to inundation over time, compared to those (pre-treatment) that developed and were measured during the establishment phase (subjected only to reference conditions). Somewhat different to the pattern observed for in-treatment leaves, g_s of establishment leaves of the hybrid was significantly lower than that of the narrowleaf sexes (Figure 17) indicating gas exchange rates, per unit leaf area on a single leaf surface, of the hybrid were inherently lower than those of *P. angustifolia*. Flood proportionately reduced g_s for each group. An intrinsically lower rate of g_s in *P. x acuminata* (in comparison to *P. angustifolia*) may be correlated to ecophysiological adaptation which the hybrid has developed in accordance to its native range of warmer, xeric climatic conditions. Lower gas exchange would promote water conservation in *P. x acuminata* whereby stomata would close more frequently in response to high evaporation and low soil water availability in hotter, dry climates. *P. angustifolia* male's slightly higher g_s in flood (and of both sexes in reference conditions), corresponds to physiological response conducive to cooler, wetter climates. As well, the slight trend in higher stomatal densities of the *P. angustifolia* females and males across water treatments were speculated to contribute to their higher gas exchange by enhancing carbon uptake capacity over the leaf surface.

Leaf water potential (Ψ_L) is a measure of stress within a plant which can reveal the effects of environmental (water) stress on growth. It is referred to as the driving force for the acquisition and distribution of water through the plant (Jarvis 1976). Underlying leaf water potential is the mechanism that provides efficient and effective water delivery

through the plant via initiation and maintenance of water absorption through the roots. In

flood, disruption in water absorption by roots and a decline in root conductance leading

to a water deficit developing within the plant has been hypothesized to decrease Ψ_L in

flood-intolerant woody species, if stomatal closure does not occur in response to

inundation (Kozlowski 1984; Tyree and Sperry 1988; Will et al. 1995). The relationship

between g_s and Ψ_L in water-stressed conditions has been shown to exist through a

feedback mechanism between the two variables which enables Ψ_L to be maintained above

a threshold potential (in isohydric species such as *Populus*), which inhibits a lethal drop

of Ψ_L to go below a minimum, cavitation-inducing potential (Tyree and Sperry 1988;

Sperry et al. 2002). As is most often the case in flood, water deficits in leaves or the

reduction of Ψ_L will occur as a result of transpirational water losses exceeding absorption

by the roots (Jarvis 1976). However, if acclimation responses to flooded conditions occur

early and rapidly, such as stomatal closure and growth of adventitious roots and

hypertrophied lenticels, increased water (and oxygen) uptake by the newly formed roots

and resumed g_s is likely, resulting in raised Ψ_L in flood (Kozlowski 1984; Tang and

Kozlowski 1984).

For all groups, there was no significant difference in Ψ_L between flooded and

well-watered saplings (Figure 18, Table 16). The flood treatment did not induce a drop

(or eventual increase) in Ψ_L for any group, which was maintained at approximately -1.5

MPa, and was speculated to be a result of effective stomatal regulation, observed as a

decline of g_s in flood (Figure 16). Frequent stomatal closure as an early response to flood-

stress, which was speculated to have occurred in all groups, would have contributed to

water preservation within the leaf, and maintenance of leaf turgor and leaf water potential

through sustained lowered (or ceased) g_s. Leaf water potential was measured in the afternoon across six days and subsequently did not account for diurnal fluctuations associated with vapour pressure deficit. These results indicate, on a given day, the level of water stress in a sapling, but give no information as to whether that level of stress is unusual for those conditions. Prior studies of *Populus* have found afternoon Ψ_L values of -1.4 MPa in mature *P.trichocarpa* (Pezeshki and Hinckley 1982) and in field grown *P. trichocarpa*, in *P. deltoides* and hybrids to range between -1.1 to -1.7 MPa (Schulte et al. 1987) when soil moisture was high, and in *P. angustifolia* branch cuttings exposed to water-stress Ψ_L dropped to -1.7 MPa (Tyree et al. 1994). These different values determined in mesic and water-stressed conditions correspond to the hybrid and narrowleaf's Ψ_L in reference and flood conditions. These comparisons indicate that the hybrid and narrowleaf sexes did not respond to the flood treatment with high enough sensitivity to stimulate a drop in Ψ_L, which was most likely maintained through frequent stomatal closure, triggered by a decline in water uptake and leaf turgor under saturation, resulting in effective regulation of transpirational water loss (Taiz and Zeiger 2006). Regehr and others (1975) determined *P. deltoides* were capable of offsetting decreased water absorption due to enhanced root resistance in response to flood, by a simultaneous increase in stomatal closure, thereby maintaining leaf turgidity and avoiding a drop in Ψ_L.

Leaf chlorophyll values represented relative abundance of chlorophyll (Chl), an essential leaf pigment, which would give insight into the photosynthetic capacity of leaves and as such its influence on plant growth. It has been reported that leaf chlorophyll content can be influenced by species, in addition to the type of leaf (i.e.; sun or shade), leaf area, leaf thickness and season (if plants are in a natural field setting)

(Demarez et al. 1999). The greenhouse study showed differences in Chl between taxa (Figure 19, Table 17), which could be correlated with significant differences in leaf areas between the hybrid and narrowleaf sexes. Studies of *Populus* as well as other species have shown moisture stress, and in particular flood, to reduce total chlorophyll (chlorophyll a and b) in leaves (Maranville and Paulsen 1970; Sena Gomes and Kozlowski 1988; Cao and Conner 1999; Prakash and Ramachandran 2000). This was observed in the greenhouse study for the narrowleaf sexes in flood (Figure 19).

Upper leaves, formed during the treatment phase, showed the hybrid to maintain Chl across water treatments, but the narrowleaf sexes to significantly decrease Chl in flood (Figure 19). With the narrowleaf sexes smaller leaf sizes, compared to those of *P. x acuminata*, observed in all treatments (Figure 8) it is possible a lower leaf area may have contributed to less chlorophyll being present in the smaller leaves and in the chlorotic leaves observed in *P. angustifolia* in flood. Reduction of total leaf area (hence smaller leaf size) has been coupled with a reduction in chlorophyll content, which has been linked to inhibition of photosynthesis in flood (Sena Gomes and Kozlowski 1980). Leaves that formed in establishment (reference) conditions showed similar Chl for all groups (Figure 19). These leaves, after approximately one month (28 days) showed an increase in Chl for the hybrid and narrowleaf sexes, regardless of water treatment (data not shown). Again, the increase in Chl over time is indicative of larger, and older, leaves maintaining greater chlorophyll content than smaller (and in this case younger) leaves, where there is more leaf area for chlorophyll to be produced and stored over time. Dickmann (1971) indicates that total chlorophyll in developing leaves of *P. deltoides*

increases linearly as the leaves age and expand, and is correlated with the onset of net photosynthesis.

Carbon:nitrogen ratio (C:N) showed an inverse to Pattern A (although was interpreted as a concurrent response to that of Pattern A) in which ratios of the hybrid were significantly reduced in comparison to those of the sexes in reference and flood conditions (Figure 20, Table 18). Flood raised C: N ratios for all groups, although a significantly large magnitude of increase was only shared by the hybrid and *P. angustifolia* males, as the narrowleaf females displayed a relatively slight increase in comparison (Figure 20). The enhancement of the C: N ratio in flooded conditions was speculated to be a combination of both foliar carbon and nitrogen changing in response to water stress. The elevated C: N ratios in flood are most likely attributable to the higher senescence of (mainly chlorotic) leaves brought about by waterlogging, where nitrogen content would decline in dehydrated, dying leaves due to translocation from older leaves to younger ones (Drew and Sisworo 1977; Cooke and Weih 2005). Previous research has indicated inhibited soil nitrogen uptake as a probable result of lowered root permeability and root die back in waterlogged substrate (Kozlowski 1984; Trought and Drew 1980). Root die-back was observed at harvest in the flood treatment and likely influenced a decrease in nitrogen uptake, which would not have been limiting in the substrate due to weekly fertilizer additions.

The lesser magnitude of increase in C: N of the narrowleaf females, compared to those of the males and the hybrid, in flood correlates to a lower sensitivity of the females to sustained inundation, as the ratio integrates a group's response to water stress over time, accounting for changes at the onset of flood and those during the later stages of

stress acclimation. Of particular interest were the significant differences between the

C: N ratios of the narrowleaf sexes shown only in flood. The males tended to have higher

ratios (which were similar to *P. x acuminata's* in flood) (Figure 20), indicating that the

males either maintained, per unit mass, elevated carbon or experienced a decline in

nitrogen, or both, over the period of inundation to a greater degree than the narrowleaf

females. It is speculated that this response may have resulted from slightly higher g_s of

the males over that of the narrowleaf females in flood allowing for higher carbon uptake

and subsequent assimilation.

A higher accumulation of carbon in leaves exposed to flood, most commonly

demonstrated by the narrowleaf males and the hybrid, was speculated to occur as a result

of lower investment of carbon in below-ground biomass (roots) and an increase in shoots

(stem and leaves), serving as a mechanism to offset a decrease in root growth and die-

back under anaerobic conditions. The literature reveals that translocation of

carbohydrates (carbon assimilates produced via photosynthesis in the leaves) is reduced

in flooded plants (Kramer and Kozlowski 1979), which would influence a build-up of

foliar carbon. Kozlowski (1984) proposes that soil anoxia due to flood leads to a

reduction in the rate of translocation of photosynthetic (carbon) products from leaves to

other parts of the plant and is attributable to the involvement of toxins produced by

anaerobic respiration.

Higher amounts of carbon allocated to above ground biomass was observed in the

shoot/root ratios for all groups which tended to be elevated in flood (Figure 14), which

was interpreted as a greater proportion of carbon allocated to leaves and stems versus

roots. The narrowleaf sexes displayed a trend where they tended to retain higher

shoot/root ratios than the hybrid, in reference and flood conditions (Figure 14, Table 12), possibly indicating a greater proportion of carbon allocation to stems and leaves than roots which is comparable to their higher foliar C: N ratios across treatments. It is speculated that the hybrid's slightly lower shoot/root ratio across treatments is likely a result of greater proportional root growth over that of shoot, especially in reference conditions, which is consistent with *P. x acuminata*'s ecophysiological adaptations to dryer soil environments (Pallardy and Kozlowski 1981; Kalischuk et al. 2001; Pearce et al. 2006). Likewise, lower root growth as opposed to that of shoot in *P. angustifolia* may be linked to the species natural range of distribution and consequently its inherent growth pattern enabling it to survive in a wetter, cooler climate (Rood et al. 2003; Pearce et al. 2006).

Correlated to the likely decline of foliar nitrogen in flood, was the substantial decrease in Chl of the narrowleaf sexes after several weeks of exposure to waterlogging (data not shown). The nitrogen element is an integral component in the synthesis of the chlorophyll molecule, where it forms covalent bonds with carbon (Taiz and Zeiger 2006), and thus over time a drop in nitrogen availability within the leaf could lead to a decline in chlorophyll production. In relative comparison, Liu and Dickmann's (1992) study of two hybrid *Populus* clones revealed *addition* of nitrogen in flooded conditions to enhance leaf chlorophyll. The narrowleaf sex's (particularly the male's) likely drop in absorption and conversion of nitrogen in flood could be correlated to their decreased root number and area (Figures 10, 11) which were lower than the hybrid's in reference and flood conditions and dropped to a greater extent in flood. A lesser amount of absorbing root

84

surface area in all groups may be translated into a decreased capacity for nitrogen uptake

compounding the effects of lowered root permeability.

Carbon isotope composition ($\delta^{13}C$) has been considered a reliable indicator of

water availability and is a time-integrated measure of water use efficiency (WUE; carbon

assimilation/g_s) (Dawson and Ehleringer 1993; Donovan and Ehleringer 1994), and as

such leaf $\delta^{13}C$ is indicative of average growth over a growing season (Pita et al. 2001).

Foliar $\delta^{13}C$ is regulated by g_s and the leaf enzymes involved in carbon fixation in

photosynthesis (Farquhar et al. 1989).

The hybrid displayed higher (less negative) values than those of the narrowleaf

sexes in reference and flood conditions (Figure 20), which could be translated into raised

amounts of ^{13}C in the hybrid leaves in comparison to that of the narrowleaf's. A greater

$\delta^{13}C$ in the hybrid may be a reflection of its lower stomatal conductance, although only in

reference conditions, indicating greater frequency in stomatal closure and subsequently

higher conversion of $^{13}CO_2$ into ^{13}C within the leaf. Closure of stomata would result in

the heavier $^{13}CO_2$ isotope becoming "trapped" within the leaf interior while preventing

the more abundant and more readily converted $^{12}CO_2$ isotope in photosynthesis to diffuse

into the leaf, altering ambient to internal carbon concentrations (Farquhar et al. 1989;

Taiz and Zeiger 2006). This results in the availability of $^{12}CO_2$ at the carboxylation site

becoming a limiting factor, lessening the discrimination against $^{13}CO_2$ by the leaf protein

rubsico (ribulose-1,5 biphosphate carboxylase-oxygenase) (Taiz and Zeiger 2006). This

closed system alters the rate of CO_2 diffusion into the leaf, the subsequent concentrations

of the carbon isotopes in the leaf and the carboxylation selectivity for $^{12}CO_2$, all of which contribute to increasing the abundance of ^{13}C fixed in leaves, relative to that of ^{12}C (Ehleringer et al. 1993; Taiz and Zeiger 2006). Although the slightly higher g_s of the narrowleaf sexes in reference conditions corresponds to their lower $\delta^{13}C$ values (compared to those of the hybrid), it is unlikely g_s was responsible in contributing to the narrowleaf's lower $\delta^{13}C$ in comparison to the hybrid's in flood because g_s was reduced to similar values for all groups in the flood treatment (Figure 16).

P. angustifolia females and males did not exhibit any differences in $\delta^{13}C$ in either reference or flood conditions, and moreover, flood did not effect a change in $\delta^{13}C$ for any group (Figure 20, Table 18). This indicated that flood-stress, as experienced for the duration of the study, was not effective enough to the point of effecting a change in the carbon isotope most readily used in the assimilation of leaf carbon. Leffler and Evans (2001) note that typically plants experiencing drought or flood are enriched in ^{13}C (hence raising $\delta^{13}C$), reflecting the tradeoff between carbon assimilation and water loss. It was speculated in the greenhouse study that over time physiological acclimation responses to persistent inundation may have occurred in all groups, whereby g_s was resumed inhibiting an increase in $\delta^{13}C$ and promoting the assimilation of $^{12}CO_2$ over $^{13}CO_2$ during carboxylation.

The hybrid maintained higher proportions of foliar ^{13}C over those of the narrowleaf sexes regardless of moisture condition, and as such was not correlated with a flood-stress response, although was indicative of greater frequency in stomatal closure. The determination that flood did not increase $\delta^{13}C$ for any group, coupled with consistency in Ψ_L across treatments, supported our interpretation that all groups were

86

fairly flood tolerant. This theory was consolidated by the determination of similar percent survival across water treatments for all groups (Figure 21, Table 20). *P. x acuminata's* lower flood-tolerance was reflected by its slightly lower g_s in addition to its higher $\delta^{13}C$ under inundation, compared to those of the narrowleaf's.

Studies of *Populus* under well-watered conditions have shown no significant changes in $\delta^{13}C$ versus values commonly observed in the field (most often around -28‰) (Zhang et al. 2004) indicating both taxa maintained considerably lower $\delta^{13}C$ in reference conditions in the greenhouse study than would be expected under similar natural conditions (Figure 20). Lower values of $\delta^{13}C$ (depleted foliar ^{13}C) for the hybrid and narrowleaf sexes may reflect the moisture availability of the reference treatment as being favorable and more conducive to growth (a less stressful regime) than a similar moisture environment in the field would present.

4.3 Morphological traits

Morphological variables which followed Pattern A were diameter growth, height, stem, cutting, leaf, shoot and root (stem + cutting + leaf) dry weights (Figures 7, 8, 9 and 11), leaf area and leaf size (Figure 8), root number and lower root number, root area (Figures 10 and 11) and total plant dry weight (Figure 12). Of all variables measured, lower root number (RN_l) (Figure 10, Table 9) and lower root area (RA_l) (Figure 11, Table 10), were the only traits which showed Water 1 and Water 2 treatments to differ, and as such showed Water 1 as being slightly more moisture limiting and having a deeper capillary fringe zone. Lower root number and area were the most sensitive variables to actual water level in the pots, sensing the slight discrepancy in substrate level inundation.

This was shown through RN_l and RA_l being greater in Water 1, as the basal end of the cutting would have been able to grow roots throughout the lower portion of the pot (which was not inundated) to access the 1cm of standing water at the bottom (Figure 24) . RN_l and areas were less in Water 2 due to the base of the cutting being submersed or located in an anoxic zone which would have promoted root growth higher up, in the capillary fringe zone.

In relation to the higher sensitivity of RN_l and RA_l and the capacity of these traits to distinguish between slightly differing soil moisture levels, root area (RA), root dry weight (RDW) (Figure 11) and to an extent root number (RN) (Figure 10), did not differ between Water 1 and Water 2 treatments for all groups. RDW and RN, which are composite measures integrating a number of traits (thus increasing their reliability and significance), verified that the two reference treatments were effectively equivalent moisture treatments, differing only with respect to the lower elevation of the capillary fringe in Water 1 (Figure 24). The responses seen in RA and RDW correlate with the concept that root growth of the totipotent cuttings is most likely to occur in the capillary fringe zone, where moisture and oxygen uptake are not impeded as a result of decreased root permeability and substrate hypoxia. In all groups, smaller fine auxiliary roots formed at the base of the shoot, where oxygen was available relative to the anoxic substrate conditions in flood, and were indicative of a general plant response to water-stress. Relative to root functionality in flood stress, it was speculated that aquaporins, protein ports that facilitate water movement across root membranes, could improve root permeability and water uptake in anoxic waterlogged conditions, and as such, improve root survival in flood.

88

Total sapling dry weight (TDW) was a fundamental measure that encompassed all above and below ground growth and thereby represented core plant biomass. TDW results verified similar, nearly equivalent, growth of the narrowleaf sexes across reference treatments for the majority (except one) of morphological traits (as shown for those that followed Pattern A), indicating corresponding physiological responses between the sexes to those water treatments. The flood treatment showed *P. angustifolia* females' TDW to be reduced less than the males' or the hybrid's, indicative of a slightly different physiological response in the narrowleaf females, to those of the males of *P. x acuminate,* to flood (Table 13). TDW also confirmed the greater growth of the hybrid over that of the narrowleaf sexes across treatments (Figure 12) and for the majority of traits measured.

Genotypes displayed a broad range of responses to a water treatment which resulted in a significant interaction between genotype and treatment. Moreover, all genotypes for a group contributed relatively similar degrees, although at consistently various extents, to the hybrid's heavier dry weights and the narrowleaf sex's similar dry weights across treatments (Figure 12). This confirmed that a group's response to a treatment did not result from a single or small subset of individuals showing anomalous growth and thus skewing the growth pattern of a group. The flood treatment significantly depressing growth for all groups (although to a lesser extent in the narrowleaf females) and Water 1 and 2 representing similar moisture conditions were verified by analysis of the genotypes, whereby all genotypes consistently displayed a lower TDW in flood, and similar weights in Water 1 and 2 (Figure 12).

With respect to the effects of flood on overall sapling growth, TDW and C: N (integrative measures of growth over sapling area and physiological response through

time, respectively) showed that growth was significantly hindered by the flood condition (Figure 13, Tables 13, 19). Flood affected the groups to different extents as predicted, whereby *P. x acuminata* displayed the greatest growth reductions under inundation expressed as significant declines in TDW (and its components), but also as raised C:N in flood. In contradiction to our theory of *P. angustifolia* males maintaining higher water-stress tolerance, was the narrowleaf female's lower sensitivity to inundation. *P. angustifolia* females displayed only slight TDW reduction, and an equivalent slight increase of C: N in flood, whereby these were the smallest changes out of all the groups (Tables 13, 19). The males showed a greater magnitude of decline in TDW and increase in C: N over the narrowleaf females' in flood, indicating their lower flood tolerance. The narrowleaf females different response to flood, displayed in its lesser reduction in TDW, was observed as a trend in the group x treatment interaction for TDW (Table 12). In addition, the similar growth (shown in the morphological traits that followed Pattern A as well as the components of TDW and C: N) of the narrowleaf sexes in reference conditions did not corroborate with the literature which details evidence of females' higher performance (ie; rapid growth) in mesic conditions with unlimited water. It is unclear why the narrowleaf females did not display enhanced performance (greater overall growth) in the wet reference treatment over that of males, although the similar responses between the sexes verifies relatively equivalent physiological response and morphology in reference (favourable) conditions. Most crucial of the study's findings were the different responses of the *P. angustifolia* sexes to flood, as summed by the measures of TDW and C: N, which reflect possible differences in physiology and hydraulic structure between females and males in flood. Such differences were then

expressed as distinct flood tolerance levels between the sexes with females maintaining a lower sensitivity to flood-stress. As displayed by Pattern A, narrowleaf females were not more sensitive to flood relative to a single measure of growth, but growth expressed collectively through TDW and integrated over time in the C: N ratio was shown to be less affected by flood in the females in comparison to the males and to the hybrid females. Single measures of growth that displayed either a significant group x treatment interaction (stem dry weight and leaf size), or a trend (leaf, cutting and shoot dry weight, and leaf area) supported a different response of the narrowleaf females to that of the collective under flood conditions, indicating a higher flood-tolerance as expressed by those measures.

4.4 Morphological traits that followed a second pattern of response

In addition to Pattern A, a second less common pattern was detected in which the derivative measure of leaf area ratio (LAR) (Figure 15) and Chl (Figure 19) followed within and across treatments. This pattern showed the hybrid to display similar growth to that of the narrowleaf sexes in reference conditions and sustain this level, and pattern, of growth in flood, resulting in a significant group effect. Conversely, *P. angustifolia* females and males showed significant reductions in growth (LA and Chl) in flood, which contradicted the theory that the sexes of *P. angustifolia* would show lower sensitivity to inundation over the course of the study. The decrease in LAR in flood (although a treatment effect was not observed for LAR but speculated to result from the hybrid's similar ratios across treatments) would have resulted from the narrowleaf sex's leaf areas (LA) declining to a greater extent than did TDW (a disproportionate decrease), which correlates to TDW being a composite measure of several structural traits, hence

91

representing an "average" measure of decline, versus just a single measured trait. Conversely, the hybrid's LA would have had a proportionate decline relative to its TDW, which was approximately 3-fold larger than the narrowleaf sexes declines in LA (Figure 8), and as such this larger decline was similar (proportional) to the hybrid's significant decrease in TDW in flood (larger than that of the narrowleaf females', but similar to that of the males) (Figure 12, Table 13). The larger declines, relative to the *P. angustifolia* sexes, in LA and TDW of the hybrid correlate to the theory of *P. x acuminata's* higher sensitivity to flood, whereby the less marked declines in TDW of the narrowleaf sexes supports *P. angustifolia's* lower flood-sensitivity. The greater decline in *P. angustifolia's* LA (over its TDW) was thought to be associated with the sexes' significant decrease in Chl. As such, the adverse effects of flood on LA were expressed through Chl whereby smaller leaves of *P. angustifolia* would maintain lower Chl (and lack the capacity to produce as much chlorophyll as larger leaves not exposed to flood-stress), hence a drop of Chl in flood that was comparable to its large decrease in LA. It was speculated that *P. x acuminata* did not show a parallel drop in Chl in comparison to that of *P. angustifolia's* in flood , as its LA did not decline to the extent that the narrowleaf's did, but displayed a decrease in LA that was comparable to the decrease in growth of other structural traits. Although flood did not adversely affect the hybrid's LA to the same degree as the narrowleaf sex's (which showed higher flood-sensitivity with a large magnitude of decline in LA relative to TDW), *P. x acuminata's* proportional significant declines of LA and TDW in flood are characteristic of its ecophysiological adaptations to a dryer climate and thus this response corresponds to the hypothesis of its greater sensitivity to inundation.

4.5 *Populus x acuminata* out-group performance

Pattern A and TDW and C: N analysis confirmed our hypotheses that the *P. x acuminata* females would display greater growth over *P. angustifolia* in reference conditions, and show hindered growth in flood which would reflect a higher sensitivity to inundation over that of the narrowleaf's. It was theorized in concurrence with Amlin and Rood (2001) and Rood and others (submitted) that *P. acuminata* are probably more drought-tolerant but less flood-tolerant than *P. angustifolia*, owing to the influence of *P.deltoides* into the hybrid genome, and as such a higher growth and flood-sensitivity would be displayed. Inconsistent with these predictions was the hybrid's greater percent survival over both narrowleaf sexes in flood (Figure 21, Table 20). The hybrid's higher survival at harvest in the flood treatment was associated with its high percent survival during the establishment phase which was similar to the males and greater than that of the narrowleaf females across treatments (Figure 23). The hybrid maintained initial lower mortality of saplings across all treatments (compared to the narrowleaf females) before treatments were implemented which would have contributed to *P. x acuminata's* higher percent survival at harvest in flood. In turn, *P. x acuminata's* greater percent survival under inundation was interpreted as the result of higher cutting establishment success (primarily over that of *P. angustifolia* females') and initial vigorous sapling growth influenced by dominance, and not to higher flood-tolerance. This conclusion is supported by the finding that water treatment did not have a significant effect on percent survival for any group, whereby flood did not decrease percent survival to a significant degree for the hybrid or narrowleaf sexes (Figure 21, Table 20).

A separate set of measurements of the greenhouse study saplings involving a subset of all groups and their individual leaf areas revealed the narrowleaf females response to flood to be the least sensitive of all groups, shown by the smallest declines in LN, LA and LS (Rood et al. submitted). In contrast, the hybrid showed the lowest flood-tolerance with the largest magnitude in decline in LN, LA and LS, whereby LA and LS had the most precipitous declines in flood (Rood et al. submitted). This greater sensitivity of the hybrid and the more modest response of the narrowleafs to flood is in accordance with the similar degrees of decline in TDW and increase in C: N in flood for the groups.

4.6 Measures in which the sexes of *Populus angustifolia* differed

Leaf number was the single trait that showed significant differences between the narrowleaf sexes. Narrowleaf females showed a significantly higher number of leaves (in which values were similar to those of the hybrid) in reference treatments, although females and males displayed similar, depressed LN in flood (Figure 8, Table 7). This may be correlated to findings regarding females of the *Salicaceae*, including *Populus,* which show enhanced growth (over males') and greater abundance of females in moist (unlimited water), rich environments (Freeman et al. 1976; Dawson and Bliss 1989). Dawson and Ehleringer (1993) hypothesized that a sex's specialization to a particular habitat would reduce growth and survival in the contrasting habitat, which would explain a likely trade-off between drought tolerance (and/or avoidance) and carbon acquisition in males and their lower performance (growth) in more mesic conditions.

A less conservative use of water by females, apparent when moisture is abundant but not an impediment to root development or function, has led to females of the

Salicaceae, many of which are phreatophytes like *Populus*, to maintain higher water-use rates and increased growth rates (Dawson and Ehleringer 1993; Dawson et al. 2004; Hultine et al. 2008). Higher water use of the narrowleaf females in reference conditions may have resulted in enhanced production of leaves, which would have promoted carbon capture and assimilation, as increased growth of roots would have resulted in little benefit under a mesic, nutrient rich regime. Wetter sites have been viewed as holding a greater advantage for females, as they would be more conducive to a liberal water-use strategy and rapid growth by facilitating the large photosynthetic capacity in females (Ueno and Seiwa 2003). Hultine and others (2008) note that trees adapted to mesic habitats generally have larger canopies, hence larger, more numerous leaves than those which most often occur and are adapted to dryer sites. This observation could be applied to the sexes of *Populus*, as it has also been shown that other riparian trees (*Acer negundo*) have females most commonly inhabiting persistently wet sites where they maintain more expansive canopies than co-occurring males (Dawson and Ehleringer 1993; Ward et. al 2002), and as such, produce more leaves.

Female's vigorous growth rates in mesic environments have been correlated with the theory of spatial segregation of the sexes (SSS) which suggests a strong dependence of performance of the sexes on habitat water availability and site richness (speculated to result from sex-specific physiology) (Opler and Bawa 1978; Cox 1981; Zimmerman and Lechowicz 1982; Bierzychudek and Eckhart 1988), coupled with the premise that females sustain a higher demand for resources due to their greater reproduction requirements over those of males (reproductive allocation theory) (Charlesworth and Charlesworth 1978; Cox 1981; Waser 1984; Obeso 2002). In addition, sexual allocation

theory serves to rationalize these concepts, proposing that physiological divergence leading to structural dimorphism in the sexes can be imparted to natural selection and is consequently of evolutionary (inherent) origin (Darwin 1877; Willson 1983).

It was hypothesized that the narrowleaf females only expressed greater growth in LN, and not in LS (or LA) and LDW, or both, or in other structural traits such as H and DG in reference conditions, due to its modest growth (similar to that of males) in the mesic environment being a possible trade-off for the capacity of the females to maintain lower sensitivity to flood.

The *P. angustifolia* sexes maintained similar LS, LA and LDW in all treatments (Figure 8) and leaf sizes were relatively equivalent across reference to flood conditions implying inundation had little effect on leaf expansion for the narrowleaf sexes. The responses shown by LA, LS and LDW support the hypothesis that narrowleaf cottonwood maintains a higher tolerance to flooding than the lanceleaf cottonwood. *P. x acuminata* showed marked declines in LA, LS and LDW from reference to flood conditions, over the modest changes shown by *P. angustifolia* (Figure 8). In contrast, leaf initiation (LN) was hampered by flood, expressed in the relatively parallel decline in LN of all groups to flood-stress, which reduced LN in the females to that of the males of *P. angustifolia*. The similar decline in LN of the narrowleaf females and males in flood was reflective of the females's only slightly higher flood-tolerance, whereby independently, individual measures did not all show lower sensitivity in the females to inundation.

It was also speculated that narrowleaf males could have produced similar LN to that of the females in reference conditions, but in the mesic moisture regime to which

96

their water-use strategy is not as conducive to, but more so to dryer environments, males abscised more leaves than females and compensated for this loss by sustaining LA and LDW equivalent to that of the female's in those conditions. This strategy could be viewed as an attempt that would compromise photosynthetic capacity in the least, whereby leaves that were fully expanded could not be reduced in area or dry weight (thickness), therefore a decrease in LN (and newly developing leaves size) could promote higher survival in wetter conditions.

In addition to LN, percent survival at harvest between the narrowleaf sexes differed slightly (showed a trend), although only under reference conditions. Males tended to have greater percent survival over that of the narrowleaf females in the two reference treatments (Figure 21). It was apparent after analysis of the narrowleaf sex's percent survival of genotypes that a single or few vigorous male genotypes did not skew the male's response in reference conditions which would have increased their percent survival over that of the females (Figure 22). Likewise, narrowleaf female genotypes displayed relatively similar contributions to the group's overall percent survival in both reference and flood conditions, hence their lower survival was not attributable to poor performance of a single or few genotypes (Figure 22).

Analysis of percent survival over time revealed contribution of initial disparities in establishment between the *P. angustifolia* females and males, which were displayed at 45 days since cuttings were stuck up until day 49 (Figure 23), to greatly influence survival at harvest. The narrowleaf female's lower percent survival in reference treatments was greatly affected by their higher mortality of saplings during the establishment period (Figure 23). Although the female and male differences in cutting establishment were not

statistically significant, the sexes final percent survival at harvest was indeed influenced by sapling survival before treatments were implemented. In contrast, the *P. angustifolia* sexes displayed similar percent survival in flood (Figure 21), which did not correlate to the female's higher mortality of saplings during the establishment phase in flood (Figure 23). It was speculated that the females did not show reduced percent survival over that of the males in flood as a result of the trend observed in growth responses of the sexes to inundation which showed TDW and C: N to be less adversely affected by flood-stress in the females. In turn, the females displayed a slight increase in percent survival in flood (exhibiting their lower flood-sensitivity), whereas the males showed a slight decline in survival, attributable to their significantly greater reductions of growth in flood (Figure 21, Table 13). This would have resulted in the narrowleaf sex's similar survivorship in flood and thus reflects differences in flood tolerance between the sexes, which were supported by different responses in TDW and C: N ratio under inundation (Table 13).

The greatest discrepancies in physiological processes and morphological traits were, as expected, manifested as different responses to water treatments by *P. angustifolia* and *P x. acuminata*, and not between the sexes of *P. angustifolia.* Since the narrowleaf females and males showed consistently similar responses and growth across treatments for nearly all measures, it is unclear as to why females showed lower (although not statistically significant) percent survival to that of all groups during the establishment phase in each treatment (Figure 23). It was postulated that in reference treatments, considered to be mesic conditions, narrowleaf females would possess higher growth rates during establishment and therefore have a competitive advantage over males, leading to greater survival. Moreover, establishment conditions, and not water

treatments, had the greatest effect on percent survival, which was displayed by the results of percent survival at harvest across treatments that showed flood did not significantly reduce survival for any group (Figure 21, Table 20).

4.7 Relevance to hypotheses and predictions

We found that the sexes of *P. angustifolia* do not show significant differences in growth or survival in reference conditions conducive to growth, which was interpreted as the sexes maintaining similar physiological response and morphology in mesic conditions. This was contrary to our hypothesis that females would sustain higher growth rates in reference conditions. However, it was determined that females and males differ slightly in flood-tolerance, although results were contrary to our prediction that males would be less sensitive to flood. In turn, we determined that physiological response and structural traits do have the capacity to differ between the sexes when under flood-stress. We confirmed that differences between *P. angustifolia* and *P. x acuminata* are consistent with species-specific adaptations, where the hybrid showed lower flood-tolerance and an enhanced growth rate which were in accordance with previous research findings. It was apparent these differences were detectable under unlimited water and flood conditions. We therefore determined that flood-stress was capable of inducing and sustaining divergence in water-stress responses between the sexes of *Populus*, and as such indicated different mechanisms inherent to females and males which regulate flood-tolerance.

CHAPTER 5

CONCLUSIONS

Relative to our primary study objective it can be deduced that female and male narrowleaf cottonwood clones (saplings) most likely do not significantly deviate in their physiological processes and morphology when grown under reference (wet substrate with unlimited water availability) or flooded conditions, in a controlled environment. The juvenile narrowleaf sexes showed similar growth and stress responses for the majority of traits measured across water treatments, as well as similar percent survival in flood. Although, some differences in survival (during the establishment phase and harvest) and growth existed between the sexes of *P. angustifolia*. Significant divergence in morphology, shown in greater LN in the females, and in percent survival, higher in males during harvest, were detected only in reference conditions. As such, it was concluded that flood-stress most likely does not induce significantly different physiological stress responses between the sexes of juvenile *Populus*, thus differences in water-use and structural traits would not be detected in flood.

The reference and flood treatments affected growth of all groups as predicted with the mesic reference conditions being the most conducive to growth and flood reducing growth rates (and inducing physiological stress response). Relative to decreased growth in flood, percent survival did not significantly decline for any group. It was determined that differences in survival during establishment, rather than an effect of water treatment, were mainly responsible for *P. x acuminata's* higher percent survival across treatments as well as the males' greater survival (over the narrowleaf females) in reference conditions at harvest. In addition to the hybrid's greater percent survival, versus the

narrowleafs, during the establishment phase across treatments it was proposed that *P. x acuminata's* rapid growth could have contributed to its high percent survival in flood. The hybrid's fast growth was thought to possibly counter-balance its high(er) sensitivity to flood by buffering significantly greater reductions in growth (TDW) which may have occurred without the influence of dominance in the hybrid. Concerning our secondary study objective, the expression of dominance in the hybrid was evident throughout the study as shown in *P. x acuminata's* greater growth over that of *P. angustifolia* and demonstrated in the sapling traits that followed Pattern A. The growth response of the lanceleaf cottonwood across water treatments confirmed the differences in physiological response, growth rate and structure that exist between *P. angustifolia* and *P. x acuminata* when exposed to unlimited moisture and inundated conditions.

The performance of the hybrid out-group indicated that the reference water treatments served as favourable moisture conditions conducive to increasing growth rates and that flood was experienced as a water-stress capable of hindering growth and stimulating general physiological stress responses. It was apparent, when comparing the hybrid's responses to the different treatments, that the narrowleafs (slower) growth rates and sensitivities to the different moisture conditions were generally consistent with study expectations (knowledge of species-specific ecophysiological adaptations and moisture stress acclimatization). The hybrid's higher sensitivity to inundation, shown in marked declines in TDW, raised C: N and higher δ^{13}C in flood, over the slighter changes in the narrowleaf sexes, are reflective of *P. x acuminata's* inherent physiology. *P. x acuminata*, developing in drier climates which characterize its native range, would likely not maintain enhanced adaptability and resilience in wet, especially flood, conditions. In

comparison, the sexes of *P. angustifolia* displayed higher flood tolerance, females more so than males, which is consistent with our prediction of the narrowleaf cottonwood maintaining a smaller proportional decline in growth under inundation in comparison to a larger reduction of growth by *P. x acuminata* in flood.

It was concluded that in flood conditions the narrowleaf sexes differed slightly in their physiological response, shown in the females comparatively small increase in C: N, and in morphology, shown by the females lesser reductions of TDW, which led to sex-based differences in flood-tolerance. In contradiction to our hypothesis that males would show higher tolerance to flood (or effective avoidance of flood effects), was the narrowleaf females lower flood-sensitivity, expressed through a collective measure of growth and integrated through time. The female's lower flood-sensitivity was speculated to relate to their high water-use and rapid growth as documented for females of the *Salicaceae* in more flood prone environments. As such, *Populus* females may be pre-conditioned to the effects of inundation, developing physiological stress responses and structure that are better suited to waterlogged substrates, which correlates to their less-conservative water-use strategy and lower drought-tolerance. In turn, we concluded that the similar responses and growth of the narrowleaf sexes in reference (mesic) conditions, where the females were predicted to sustain higher growth rates, may have resulted from a physiological tradeoff for the female's enhanced flood-tolerance capacity. If so, we speculated their physiology and hydraulic structure to be better suited to inundation (functioning the most effectively under flood-stress) resulting in females maintaining modest growth rates (not enhanced) in favourable moisture conditions.

Overall, all groups were thought to show a degree of moderate flood tolerance, which was supported by physiological measures that showed no change in flood ($\delta^{13}C$, Ψ_L and abaxial stomatal density), in addition to similar percent survival observed for each group across treatments. With respect to flood sensitivity, it was concluded more research is required to substantiate our findings that females of *Populus* maintain a higher tolerance to the adverse effects of inundation, contradictory to their drought-sensitivity. In addition, further studies are needed in order to determine if divergence in physiological response and structure occurs between the sexes of *Populus* under flood-stress at later life stages, such as in older saplings and reproductive adults.

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APPENDICES

Statistical analyses, ANCOVAs of genotypes

Table 21. Results of the ANCOVA analyzing genotype and treatment and their interaction for pre-treatment stem diameter and diameter growth. Statistically significant (p<0.05) effects are indicated in bold font.

Measure	Pre-treatment Stem Diameter			Diameter Growth		
Effect	df	F-value	p	df	F-value	p
Genotype (Ge)	23	10.7	**<0.0001**	23	14.1	**<0.0001**
Water treatment (W)				2	25.4	**<0.0001**
Ge x W				46	0.897	0.663
Pool	1	11.2	**0.001**	1	1.41	0.235
Error	320			260		

Table 22. Results of the ANCOVA analyzing genotype and treatment for height, stem dry weight and cutting dry weight. Statistically significant (p<0.05) effects are indicated in bold font.

Measure	Stem Height			Stem Dry Weight			Cutting Dry Weight		
Effect	df	F-value	p	df	F-value	p	df	F-value	p
Genotype (Ge)	23	16.1	**<0.0001**	23	15.6	**<0.0001**	23	16.9	**<0.0001**
Water treatment (W)	2	46.4	**<0.0001**	2	22.4	**<0.0001**	2	14.7	**<0.0001**
Ge x W	46	0.814	0.798	46	2.00	**<0.001**	46	1.45	**0.038**
Pool	1	3.54	0.061	1	4.08	**0.044**	1	0.065	0.799
	263			261			265		
Error									

Table 23. Results of the ANCOVA analyzing genotype and treatment for leaf number, area, size, dry weight and specific leaf area. Statistically significant (p<0.05) effects are indicated in bold font. Values for df^1 were the same for leaf number, leaf dry weight and specific leaf area and for df^2 were the same for leaf area and leaf size.

Measure	Leaf Number			Leaf Area			Leaf Size		Leaf Dry Weight			Specific Leaf Area	
Effect	df^1	F-value	p	df^2	F-value	p	F-value	p	df	F-value	p	F-value	p
Genotype (G)	23	16.2	**<0.0001**	23	22.5	**<0.0001**	43.4	**<0.0001**	23	19.9	**<0.0001**	3.23	**<0.0001**
Water treatment (W)	2	23.3	**<0.0001**	2	33.8	**<0.0001**	45.3	**<0.0001**	2	29.2	**<0.0001**	6.51	**0.002**
G x W	46	2.12	**<0.001**	45	2.04	**<0.001**	2.73	**<0.0001**	46	2.04	**<0.001**	1.26	0.136
Pool	1	3.54	0.06	1	6.02	**0.01**	0.07	0.788	1	2.32	0.128	0.067	0.796
Error	263			253					358				

116

Table 24. Results of the ANCOVA analyzing genotype and treatment for shoot dry weight. Statistically significant (p<0.05) effects are indicated in bold font.

Measure	Shoot Dry Weight		
Effect	df	F-value	p
Genotype (Ge)	23	21.2	**<0.0001**
Water treatment (W)	2	29.6	**<0.0001**
Ge x W	46	2.23	**<0.001**
Pool	1	2.46	0.117
Error	358		

Table 25. Results of the ANCOVA analyzing genotype and treatment for shoot/root ratio and leaf area ratio. Statistically significant (p<0.05) effects are indicated in bold font.

Measure	Shoot/Root Ratio			Leaf Area Ratio		
Effect	df	F-value	p	df	F-value	p
Genotype (Ge)	23	3.45	**<0.0001**	23	3.08	**<0.0001**
Water treatment (W)	2	8.67	**<0.001**	2	13.8	**<0.0001**
Ge x W	43	1.05	0.392	46	1.34	0.080
Pool	1	4.04	**0.046**	1	0.292	0.589
Error	232			578		

Table 26. Results of the ANCOVA analyzing genotype and treatment for foliar carbon: nitrogen ratio and carbon isotope composition. Statistically significant (p<0.05) effects are indicated in bold font.

Measure	Foliar Carbon : Nitrogen Ratio			Carbon Isotope Composition		
Effect	df	F-value	p	df	F-value	p
Genotype (Ge)	23	3.60	**<0.0001**	23	11.5	**<0.0001**
Water treatment (W)	1	16.0	**<0.0001**	1	0.375	0.541
Ge x W	23	1.11	0.337	23	1.78	**0.021**
Pool	1	0.541	0.463	1	3.62	0.059
Error	164			166		