

Article

# Tree Water Use, Water Use Efficiency, and Carbon Isotope Discrimination in Relation to Growth Potential in *Populus deltoides* and Hybrids under Field Conditions

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**Abstract:** We explored the relationship between tree growth, water use, and related hydraulic traits in *Populus deltoides* Bartr. ex Marsh. and hybrid clones, to examine potential trade-offs between growth and water use efficiency. Nine genotypes, six *P. deltoides* and three hybrid clones, that represented genotypes with high (Group H), intermediate (Group I), and low (Group L) growth performance were selected for study, based on year-two standing stem biomass in a replicated field trial. In year four, tree growth, transpiration ( $E_t$ ), canopy stomatal conductance ( $G_s$ ), whole-tree hydraulic conductance ( $G_p$ ), and carbon isotope discrimination ( $\Delta^{13}C$ ) were measured. Tree sap flux was measured continuously using thermal dissipation probes. We hypothesized that Group H genotypes would have increased growth efficiency (GE), increased water use efficiency of production (WUE<sub>p</sub>, woody biomass growth/ $E_t$ ), lower  $\Delta^{13}C$ , and greater  $G_p$  than slower growing genotypes. Tree GE increased with relative growth rate (RGR), and mean GE in Group H was significantly greater than L, but not I. Tree WUE<sub>p</sub> ranged between 1.7 and 3.9 kg biomass m<sup>3</sup> H<sub>2</sub>O<sup>-1</sup>, which increased with RGR. At similar levels of  $E_t$ , WUE<sub>p</sub> was significantly greater in Group H ( $2.45 \pm 0.20$  kg m<sup>-3</sup>), compared to I ( $2.03 \pm 0.18$  kg m<sup>-3</sup>) or L ( $1.72 \pm 0.23$  kg m<sup>-3</sup>). Leaf and wood  $\Delta^{13}C$  scaled positively with stem biomass growth but was not correlated with WUE<sub>p</sub>. However, at a similar biomass increment, clones in Group H and I had significantly lower leaf  $\Delta^{13}C$  than Group L. Similarly, Group H clones had a significantly lower wood  $\Delta^{13}C$  than Group L, supporting our hypothesis of increased WUE in larger trees. Tree physiological and hydraulic traits partially explain differences in WUE<sub>p</sub> and  $\Delta^{13}C$ , and suggest that clone selection and management activities that increase tree biomass production will likely increase tree and stand WUE. However, more research is needed to discern the underlying hydraulic mechanisms responsible for the higher WUE exhibited by large trees and distinct clones.

**Keywords:** poplar (*Populus*); hybrid; water-use efficiency; sap flow; productivity; carbon isotope discrimination; stomatal conductance

## 1. Introduction

Poplar (*Populus* spp.) and their hybrids, grown as short-rotation woody crops (SRWC), have multiple industrial applications, that provide solid wood products, veneer, pulp and paper, excelsior, chemicals, and feedstocks, for cellulosic energy and the biofuels industry [1,2]. The productive and economic potential of short-rotation poplar are directly tied to their use of site resources (nutrients and water) and tolerance to changes in climate, such as multi-year droughts, increased vapor pressure deficits,

tropical storms, and extended periods of heat or cold [3]. These systems will likely be located on marginal lands, unsuitable for agriculture; however, low site quality, combined with high establishment and maintenance costs, may limit profitability on these sites [4]. Due to rapid growth and high transpiration rates, short rotation poplars have high water use [5–8], and seasonal water deficits can strongly limit poplar productivity and yield [9–11]. Successful commercial production relies greatly on water availability [12,13] and selection of suitable genotypes for site conditions [14]. Potential tradeoffs between productivity and water use in short-rotation poplar are critical to understand, considering that management of these systems may need to account for multiple ecosystem services (e.g., biomass, water yield, waste management, carbon sequestration) [15,16].

An understanding of the physiological mechanisms that regulate tree growth and water use is important when predicting which poplar genotypes will maximize biomass production and water use efficiency. The plant hydraulic and carbon assimilation systems are intimately coordinated, such that carbon gain (photosynthesis) and growth are directly tied to the efficiency of the xylem to transport water [17,18] and high whole-plant hydraulic conductance (or low resistance) is thought necessary for high productivity in forest trees [19,20]. Water use efficiency (WUE), relates to the amount of water used to produce a unit of biomass, or a unit of fixed carbon, during photosynthesis. It is a key trait, linking the carbon and water cycles [3], and is a useful metric for studying the relationship between productivity, water use, and other ecosystem services [13,21]. Tree WUE can be assessed at different spatial and temporal scales using several different methods. Leaf-level WUE is often measured via gas exchange, as the ratio of photosynthesis ( $A$ ) and stomatal conductance ( $g_s$ ), and reflects intrinsic WUE ( $iWUE = A/g_s$ ) [22]. Leaf (or wood) stable carbon isotope abundance ( $\delta^{13}C$ ) or discrimination ( $\Delta^{13}C$ ) can be used as a proxy for  $iWUE$ , as it reflects a long-term or time-integrated ratio of  $A/g_s$  during the tissue development [23]. Tissue  $\Delta^{13}C$  has been used extensively to assess genetic variation in  $iWUE$  in poplars [13,24–29]. Whole-tree or stand water use efficiency can be defined in terms of growth, as biomass produced per unit time (day, month year), per unit water consumed or transpired, and is referred to as the WUE of production ( $WUEp$ ; e.g.,  $kg\ biomass\ m^{-3}\ H_2O^{-1}$ ) [22].

A robust relationship between WUE and tree growth would be useful for genotype selection and tree breeding [13,26,30]. Poplar spp. and their hybrids vary widely in growth and WUE [31], and utilize different structural and physiological adaptations to cope with limited water resources, particularly in areas prone to seasonal fluctuations in soil water availability [13,26,32–34]. For example, Shulte and Hinckley [35] found that *P. deltoides* was sensitive to drought, closing stomata with declining leaf water potential, in contrast to *P. trichocarpa* Torr. & A.Gray ex. Hook., which maintained high stomatal conductance at low leaf water potentials. The work of Braatne et al. [5] further showed that *P. trichocarpa*  $\times$  *deltoides* F1 hybrids carried more leaf area, were more sensitive to soil moisture deficits, and had greater survival under drought than their parental genotypes. Others have also found different water use strategies among closely related poplar species [36,37], populations [25], and hybrid crosses [34] growing in the same environment. Generally, there is a strong positive relationship between growth and water use, wherein fast growth is associated with high water use [38]. However, the link between productivity and WUE is unclear [39] and often contradictory [40]. This variation may be the result of the approach used to measure WUE (i.e.,  $iWUE$ ,  $\Delta^{13}C$ ,  $WUEp$ ) and to the type of plant material under study (leaves, wood, or whole-tree) [28]. Because of the inherent link between  $A$  and  $g_s$ , high  $iWUE$  is often associated with low transpiration and low productivity [41,42]. On the other hand, high productivity can result in increased WUE [39]. The work of Binkley et al. [43] proposed that resource use efficiency (water, light, or nutrients) is positively correlated with the magnitude of the resource used and that, as tree and stand growth increases, there is generally an increase in  $WUEp$  [44]. Poplar genotypes that are efficient in balancing water use and carbon gain, based on physiological traits associated with growth and WUE, would be useful for cultivating poplar in areas with low or variable water availability [5,26,45]. A mechanistic understanding of the variation in water relations, and their relationship to growth among poplar clones and hybrids, would help managers choose appropriate genotypes for cultivation, and aid in deployment decisions.

In the southeastern United States, *P. deltoides* and their hybrids are promising SRWC [46]. Successful deployment requires that genotypes be properly matched to site conditions [47,48]. Furthermore, adapting *Populus* culture to marginal sites, e.g., upland sites with seasonal soil water deficits, is important, to expand poplar as a SRWC in the region [49]. Understanding the physiological mechanisms that regulate water use and growth is critical for predicting which poplar genotypes will maximize biomass production and WUE under variable environmental conditions. In this study, we explored the relationship between individual tree water use, WUE<sub>p</sub>,  $\Delta^{13}\text{C}$ , hydraulic architecture, and growth, in four-year-old clones of *P. deltoides* and hybrids (*P. deltoides* × *P. maximowiczii* A. Henry and *P. trichocarpa* × *P. deltoides*), to assess and quantify trade-offs between tree growth, WUE, and the hydraulic efficiency in transporting water. The clones varied widely in growth performance. We hypothesized that faster growing genotypes would have greater growth efficiency, greater WUE<sub>p</sub>, lower  $\Delta^{13}\text{C}$ , and greater whole-tree hydraulic conductance. We further examined relationships between physiological and hydraulic traits, to better understand the regulation of tree water use, WUE<sub>p</sub>,  $\Delta^{13}\text{C}$ , and limitations to tree growth.

## 2. Materials and Methods

### 2.1. Site Description

The study site was located at North Carolina State University's Horticultural Field Laboratory (HLF) in Raleigh, NC (35.79°N, 78.70°W). Site elevation is 143 m. The climate is humid subtropical, with a mean annual temperature of 16 °C and precipitation of 1180 mm. The soil was a Cecil series (Fine, kaolinitic, thermic Typic Kanhapludults) with a gravely sandy loam surface (Natural Resource Conservation Service, <https://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>). The site was prepared by shallow soil ripping to 20 cm and applying 18.3 mL per hectare of pre-emergent herbicide (Oust®). Planting took place in March 2014. In April 2015, triple superphosphate (TSP) (0–45–0) was added to the soil. Glyphosate was applied to reduce unwanted vegetation, and insecticide (Sevin®) was sprayed to control cottonwood leaf beetles in May and July 2015.

### 2.2. Experimental Design

The study site was part of a larger study, designed to determine the feasibility of *Populus* as a short-rotation woody crop for bioenergy production in the Southeastern United States [50] (The Integrated Biomass Supply System Partnership, Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30410 from the USDA National Institute of Food and Agriculture.). The larger study contained 52 clones (ArborGen Inc. Summerville, South Carolina, USA) from three species combinations, consisting of *P. deltoides*, *P. trichocarpa*, and *P.s maximowiczii*; 43 were from *P. deltoides* × *P. deltoides* (DD) crosses, six from *P. trichocarpa* × *P. deltoides* (TD), and three from *P. deltoides* × *P. maximowiczii* (DM). The study design was a randomized complete-block, with eight blocks and one ramet of each clone in a block. Non-rooted, 33–38 cm cuttings were planted at a spacing of 1.52 × 1.52 m (4,328 trees ha<sup>-1</sup>). A single border row of *P. deltoides* was planted around the perimeter of the site.

### 2.3. Clone Selection for Water Use Study

For this study, nine clones were selected from the original 52, in three or four of the eight blocks. In order to cover the range of productivity, clones were chosen based on mean stem biomass after the second growing season [51] and represented three productivity groups: high, Group H (>90th percentile, clones DM8019, TD185, DD116), intermediate, Group I (45–55th percentile, clones DD428, DD109, DD115), and low, Group L (<10th percentile, clones DD224, TD187, DD402) (Table 1). Thirty-two trees were measured, as this was the maximum number of sensors the sap flux measurement system would allow (Table 1). During the fourth growing season, tree sap flux density and growth were measured over a 146 day period between June 1 (DOY 152) and October 25 (DOY 298) 2015.

**Table 1.** Tree biomass and canopy structural characteristics for nine *Populus* clones. Stem, foliage, and root biomass were estimated using stand specific allometric equations<sup>1</sup>. Initial stem biomass is for DOY 152 and stem growth and relative growth rate (RGR) are biomass accumulation over the 146 day study period. Foliage, root biomass, SLA, tree leaf area ( $A_l$ ), sapwood area ( $A_s$ ),  $A_l:A_s$ , the ratio of  $A_l$  to root biomass ( $A_l:Root$ ), and the hydraulic allometry index ( $A_H$ ) were estimated for tree diameter in late June. Growth efficiency (GE) is the ratio of stem growth and tree leaf area. Data are lsmeans (standard error). Genetic crosses are DD: *P. deltoides* × *P. deltoides*, DM: *P. deltoides* × *P. maximowiczii*, and TD: *P. trichocarpa* × *P. deltoides*. Cells with no shading, light shading, or dark shading, represent genotypes with high (H), intermediate (I), and low (L) growth potential, respectively.

Clone	Group	Height (m)	Initial DBH (cm)	Initial Stem Biomass (kg tree <sup>-1</sup> )	Stem Growth (kg tree <sup>-1</sup> )	RGR (g kg <sup>-1</sup> day <sup>-1</sup> )	Foliage Biomass (kg tree <sup>-1</sup> )	Root Biomass (kg tree <sup>-1</sup> )	SLA (cm <sup>2</sup> g <sup>-1</sup> )	Leaf Area ( $A_l$ , m <sup>2</sup> )	Sapwood Area ( $A_s$ , cm <sup>2</sup> )	$A_l:A_s$ (m <sup>2</sup> cm <sup>-2</sup> )	$A_l:Root$ (cm <sup>2</sup> g <sup>-1</sup> )	$A_H$ (cm <sup>2</sup> m <sup>-3</sup> )	GE (kg tree <sup>-1</sup> m <sup>-2</sup> )	n
DM8019	H	9.70 (0.62)	7.76 (0.59)	5.59 (0.70)	2.28 (0.32)	2.84 (0.33)	1.59 (0.18)	1.46 (0.17)	135.2 (7.0)	21.5 (2.4)	48.6 (5.46)	0.445 (0.003)	148.0 (1.2)	0.240 (0.018)	0.106 (0.011)	3
TD185	H	8.70 (0.54)	6.60 (0.50)	4.00 (0.60)	1.69 (0.28)	2.84 (0.28)	1.24 (0.15)	1.12 (0.14)	161.2 (6.1)	20.0 (2.1)	37.1 (4.65)	0.539 (0.003)	178.6 (1.0)	0.215 (0.017)	0.082 (0.010)	4
DD116	H	8.85 (0.54)	6.32 (0.50)	3.71 (0.60)	1.40 (0.28)	2.62 (0.28)	1.13 (0.15)	1.02 (0.14)	140.0 (6.1)	15.8 (2.1)	33.9 (4.65)	0.469 (0.003)	155.9 (1.0)	0.243 (0.017)	0.087 (0.010)	4
DD428	I	8.48 (0.54)	5.45 (0.50)	2.68 (0.60)	1.03 (0.28)	2.50 (0.28)	0.89 (0.15)	0.79 (0.17)	127.2 (6.1)	11.3 (2.1)	26.4 (4.65)	0.428 (0.003)	143.2 (1.01)	0.276 (0.017)	0.086 (0.010)	4
DD109	I	8.70 (0.54)	6.08 (0.50)	3.39 (0.60)	1.17 (0.28)	2.31 (0.28)	1.06 (0.15)	0.95 (0.14)	121.7 (6.2)	12.9 (2.1)	31.5 (4.65)	0.409 (0.003)	135.9 (1.0)	0.282 (0.017)	0.087 (0.010)	4
DD115	I	8.45 (0.54)	5.55 (0.50)	2.75 (0.60)	0.96 (0.28)	2.35 (0.28)	0.89 (0.15)	0.80 (0.14)	122.8 (6.1)	11.0 (2.1)	26.4 (4.65)	0.415 (0.003)	138.1 (1.0)	0.286 (0.017)	0.086 (0.010)	4
DD224	L	7.43 (0.62)	4.99 (0.59)	2.20 (0.70)	0.95 (0.32)	2.88 (0.33)	0.74 (0.18)	0.65 (0.17)	142.6 (6.1)	10.5 (2.4)	22.0 (5.47)	0.479 (0.003)	161.8 (1.2)	0.290 (0.018)	0.087 (0.011)	3
TD187	L	8.10 (0.62)	4.50 (0.59)	1.76 (0.70)	0.49 (0.32)	1.93 (0.33)	0.63 (0.18)	0.55 (0.17)	164.5 (6.2)	10.3 (2.4)	18.8 (5.47)	0.548 (0.003)	188.0 (1.2)	0.229 (0.018)	0.047 (0.010)	3
DD402	L	7.70 (0.62)	5.03 (0.59)	2.30 (0.70)	0.58 (0.32)	1.71 (0.28)	0.77 (0.18)	0.68 (0.17)	129.6 (6.1)	10.0 (2.4)	23.0 (5.47)	0.432 (0.003)	147.0 (1.2)	0.309 (0.018)	0.057 (0.011)	3
<i>p</i> > F																
Clone		0.345	0.017	0.020	0.016	0.157	0.017	0.017	<0.001	0.006	<0.001	<0.001	<0.001	0.001	0.041	
Group		0.027	<0.001	<0.001	<0.001	0.091	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.018	

<sup>1</sup>  $\ln(\text{stem biomass, kg}) = 4.617 + 2.122 \times \ln(\text{DBH, cm})$ ,  $R^2 = 0.92$ ,  $\text{MSE} = 0.136$ ,  $n = 35$ ;  $\ln(\text{foliage biomass, kg}) = 3.847 + 1.706 \times \ln(\text{DBH, cm})$ ,  $R^2 = 0.58$ ,  $\text{MSE} = 0.309$ ,  $n = 35$ ;  $\ln(\text{root biomass, kg}) = 3.639 + 1.744 \times \ln(\text{DBH, cm})$ ,  $R^2 = 0.74$ ,  $\text{MSE} = 0.230$ ,  $n = 18$ .

#### 2.4. Tree Sap Flow and Transpiration

Tree sap flow was measured continuously using a Dynamax FLGS-TDP XM 1000 Sap Flow Velocity System (Dynamax, Inc., Houston, TX, USA (the use of trade or firm names in this publication is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service)). One 30 mm thermal dissipation probe (TDP30; Dynamax, Inc., Houston, TX, USA) was installed, with needles five cm apart vertically in stem sapwood 30 cm above ground line, on the northern aspect of each tree. The top needle was a heat dissipation sensor powered by a constant 0.2 W of power. Probes were wrapped with ReflexTex thermal insulation to reduce thermal gradients. Temperature data were collected every 30 s, and 15 min averages recorded using a (CR1000 data logger; Campbell Scientific, Inc., Logan, UT, USA), in conjunction with an AM 16/32 Multiplexer (Campbell Scientific, Inc., Logan, UT, USA). Sap flux density ( $F_d$ ,  $\text{g m}^{-2} \text{s}^{-1}$ ) was empirically estimated, following Granier [52]. Additional measurements recorded by the data logger included photosynthetically active radiation (PAR, LI-190s, Licor, Lincoln, NE, USA), relative humidity (Vaisala HMP-60, Vantaa, Finland), air temperature, and soil volumetric water content in the top 30 cm (VWC,  $\text{m}^3 \text{m}^{-3}$ , CS-616 Campbell Scientific, Logan, UT, USA). Daily rainfall was recorded at a nearby weather station (Reedy Creek Field Laboratory, North Carolina State Climate Office). Vapor pressure deficit ( $D$ , kPa) was calculated from air temperature and air humidity following Jones [53]. Soil relative extractable water (REW, %) was calculated as:  $\text{REW} = (\text{VWC} - \text{VWC}_{\min}) / (\text{VWC}_{\max} - \text{VWC}_{\min})$ , where  $\text{VWC}_{\min}$  and  $\text{VWC}_{\max}$  are the minimum and maximum values of VWC measured during the study.

Tree transpiration ( $E_{t,\text{tree}}$ ) was calculated as the product of sap flux density ( $F_d$ ) and sapwood area ( $A_s$ ), and expressed in  $\text{L tree}^{-1}$  or  $\text{m}^3 \text{H}_2\text{O tree}^{-1}$ , depending on the interval of the measurement. In order to calculate  $A_s$  at the probe location, the diameter at DBH was taken every two weeks and related to diameter at the location of the probe, measured at the beginning and end of the study ( $4.57 + 1.12 \times \text{DBH}$ ;  $R^2 = 0.95$ ,  $n = 32$ ). Sapwood area was linearly correlated with stem diameter ( $A_s = 4.886 - 0.011 \times \text{DBH} + 0.007 \times \text{DBH}^2$ ;  $R^2 = 0.98$ ,  $n = 6$ ). We assumed uniform sap flux across the sapwood [54]. Other studies have found little or no reduction in sapwood conductivity or  $F_d$  with sapwood depth for small (<4.0 cm radius) *P. deltoides* trees [55,56]. Transpiration per unit leaf area ( $E_l$ ,  $\text{mmol m}^{-2} \text{leaf area s}^{-1}$ ) was estimated as the product of  $E_{t,\text{tree}}$  ( $\text{kg tree}^{-1}$ ) and ratio of sapwood area to leaf area ( $A_s:A_l$ ).

#### 2.5. Canopy Stomatal Conductance and Specific Hydraulic Conductance

Canopy stomatal conductance ( $G_s$ ,  $\text{mmol m}^{-2} \text{leaf area s}^{-1}$ ) was calculated from  $E_l$  and  $D$  as:

$$G_s = (\lambda \times E_l \times \gamma) / (\rho \times c_p \times D) \quad (1)$$

where  $\lambda$  is the latent heat of vaporization of water ( $2465 \text{ J g}^{-1}$ ),  $\gamma$  is the psychrometric constant ( $65.5 \text{ Pa K}^{-1}$ ),  $\rho$  is the density of air ( $1225 \text{ g m}^{-3}$ ),  $c_p$  is the specific heat of air ( $1.01 \text{ J g}^{-1} \text{ K}^{-1}$ ). Values were converted from  $\text{m s}^{-1}$  to  $\text{mmol m}^{-2} \text{s}^{-1}$ , following Nobel [57]. We followed the approach of Oren et al. [58] to analyze the sensitivity of  $G_s$  to  $D$ . Hourly  $G_s$  were fitted to the model:

$$G_s = G_{\text{ref}} \times -dG_s/d\ln D \quad (2)$$

where  $G_{\text{ref}}$  is reference  $G_s$  at  $D = 1 \text{ kPa}$  and  $-dG_s/d\ln D$  is the slope or sensitivity to  $D$ . Hourly values of  $G_s$  under high light (PAR >  $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) [54] and  $D > 0.6 \text{ kPa}$  were used to minimize error in  $G_s$  measurement at low  $D$  [59].

Leaf water potential was measured with a pressure chamber (PMS Instrument Corp., Corvallis, OR) on leaves collected from the upper third of the canopy at predawn ( $\Psi_{\text{pd}}$ , before 0600 h) and at midday ( $\Psi_{\text{md}}$ , 1200–1400 h). The difference between  $\Psi_{\text{pd}}$  and  $\Psi_{\text{md}}$  ( $\Psi_{\text{diff}}$ ) was used as a proxy for the gradient in  $\Psi$  from soil to leaf. Whole-tree hydraulic conductance normalized to sapwood area

( $G_p$ , mol m<sup>-2</sup> s<sup>-1</sup> MPa<sup>-1</sup>) was calculated for each tree as the quotient of  $F_d$  and  $\Psi_{diff}$ , corrected for the gravitational effects on  $\Psi$  with tree height [60–62].

## 2.6. Biometrics

Aboveground wood, foliage, and root biomass were determined from stand-specific allometric equations. Three to four trees of the same genotypes (35 trees) in the four remaining blocks were harvested in June 2016. Trees were cut 10 cm from the ground. Total height (m), diameter (cm) at 0.1 m and breast height (1.37 m, DBH), and crown length (m) were measured. Crowns were divided into thirds based on crown length. Coarse roots (>2 mm diameter) were measured in a subset of trees ( $n = 18$ ). Roots were extracted using shovels, pruners, and a small excavator in a meter square area centered on the stem. Foliage, branches, stems, and roots were dried at 60 °C until at a constant dry weight. There were no significant clone effects on stem, foliage, or root allometry, thus general equations were developed for aboveground wood biomass (stem + branch):  $\ln(\text{wood biomass, kg}) = 4.617 + 2.122 \times \ln(\text{DBH})$  ( $n = 35$ ,  $R^2 = 0.92$ ,  $\text{MSE} = 0.136$ ,  $p < 0.001$ ), foliage biomass:  $\ln(\text{foliage biomass, kg}) = 3.847 + 1.706 \times \ln(\text{DBH})$  ( $n = 35$ ,  $R^2 = 0.58$ ,  $\text{MSE} = 0.309$ ,  $p < 0.001$ ), and root biomass:  $\ln(\text{root, kg}) = 3.639 + 1.744 \times \ln(\text{DBH})$  ( $n = 18$ ,  $R^2 = 0.74$ ,  $\text{MSE} = 0.230$ ,  $p < 0.001$ ). Specific leaf area (SLA, cm<sup>2</sup> g<sup>-1</sup>) was determined for two fully expanded leaves sampled from each crown third. The average SLA was used to estimate tree leaf area ( $A_l$ , m<sup>2</sup>),  $A_l:A_s$ ,  $A_l:\text{Root}$ , and the hydraulic allometry index ( $A_H$ ,  $A_s$  divided by the product of  $A_l$  and stem height). Wood biomass growth was estimated as the difference between wood biomass at the beginning and end of the study. Relative growth rate (RGR, g g<sup>-1</sup> day<sup>-1</sup>) was estimated as the increase in biomass per unit biomass already present per unit of time. Growth efficiency (GE) and water use efficiency of production (WUE<sub>p</sub>) were estimated as the ratio of wood biomass growth and tree leaf area (kg biomass m<sup>-2</sup>) and  $E_{t,\text{tree}}$  (kg biomass m<sup>3</sup> H<sub>2</sub>O), respectively. Leaf N concentration (mmol m<sup>-2</sup>) was determined with a Carlo-Erba analyzer (Model NA 1500, Fison instruments, Danvers, MA).

## 2.7. Isotope-Wood and Foliar $\delta^{13}\text{C}$

A long-term integrated estimate of WUE (iWUE) was made, based on leaf and wood carbon isotope composition. The relative abundance of wood and leaf <sup>13</sup>C to <sup>12</sup>C ( $\delta^{13}\text{C}$ ) composition was measured on harvested trees in June 2016. Stem disks were collected at 1.37 m and foliage was sampled at mid-canopy. Tissue was dried at 60 °C for 72 h and ground. Ground samples were analyzed by the Duke Environmental Stable Isotope Laboratory (Nicholas School of the Environment, Division of Earth and Ocean Sciences, Durham, North Carolina, USA). Carbon isotope discrimination was calculated as  $\Delta^{13}\text{C} = (\delta^{13}\text{C}_{\text{air}} - \delta^{13}\text{C}_{\text{plant}})/(1 + \delta^{13}\text{C}_{\text{plant}})$ , where  $\delta^{13}\text{C}_{\text{air}} = -8\text{‰}$ .

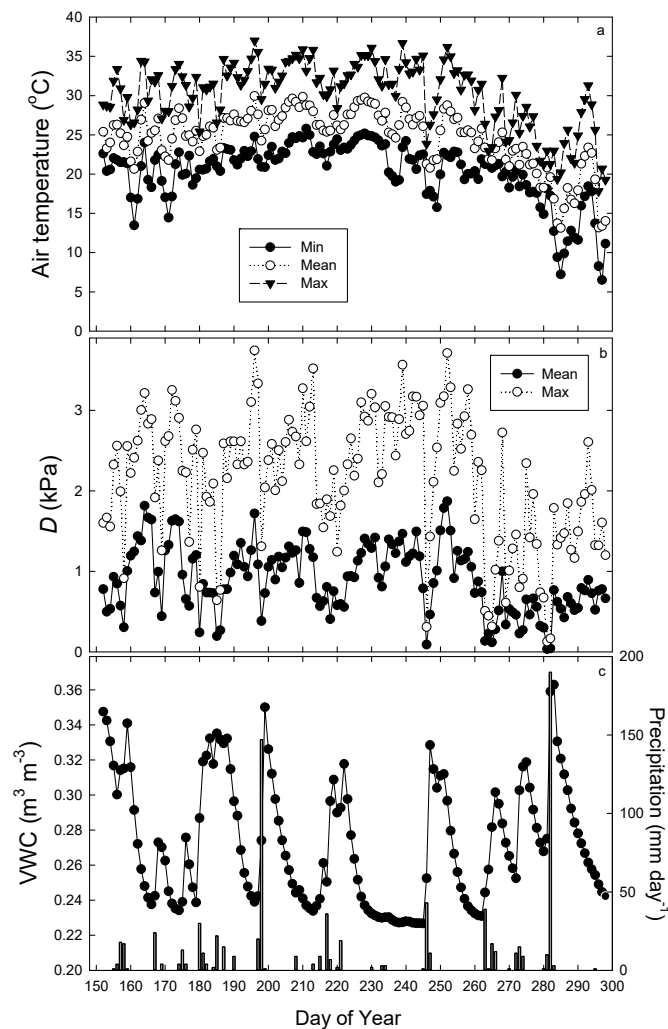
## 2.8. Statistical Analysis

Analysis of variance and covariance were used to analyze the effect of Clone or Group (H, I, L) (separate models) on stem biomass growth, GE,  $E_{t,\text{tree}}$ ,  $E_l$ ,  $G_s$ ,  $G_p$ , WUE<sub>p</sub>, and  $\Delta^{13}\text{C}$ . One tree (DD224), had a faulty sap flux probe and was dropped from analyses involving sap flux data. Time series measurements were analyzed by repeated measures ANOVA (PROC MIXED, SAS Inc., Cary, NC). A first-order autoregressive covariance structure (AR(1)) was selected based on AIC fit statistics. Block was considered a random factor and, depending on the model, Clone or Group, was a fixed factor. For response variables, treatments differences in LSMEANs were evaluated with Tukey's test. Where appropriate, initial DBH or stem biomass was used as a covariate in the ANCOVA to compensate for differences in tree size on traits. Linear regression was used to examine the relationship between GE and WUE<sub>p</sub> and RGR and the sensitivity of  $G_s$  to  $D$ . Group or Taxon (DD, DM, TD) effects on the slope and intercept were examined using full and reduced models [63] and treatment effects were tested using contrasts and a Bonferroni corrected alpha level.

### 3. Results

#### 3.1. Environmental Conditions

Mean daily temperature was 24.6 °C, with a maximum and minimum temperature of 36.9 °C and 6.5 °C, respectively (Figure 1). Temperatures over the 146 day study period (DOY 152–298) were relatively constant, until after DOY 260, when temperatures began to decrease. Mean daily  $D$  ranged from near 0 kPa to 1.8 kPa; however, maximum daily  $D$  often exceeded 3 kPa during the summer months (Figure 1b). Soil VWC averaged 0.27 m<sup>3</sup> m<sup>-3</sup> and ranged from 0.22 to 0.40 m<sup>3</sup> m<sup>-3</sup> (Figure 1c). Precipitation over the study period was 810 mm, and rainfall events exceeding 10 mm day<sup>-1</sup> occurred every 7–14 days, except for a dry period between DOY 222 and 245, that received only 8 mm of rain (Figure 1c).



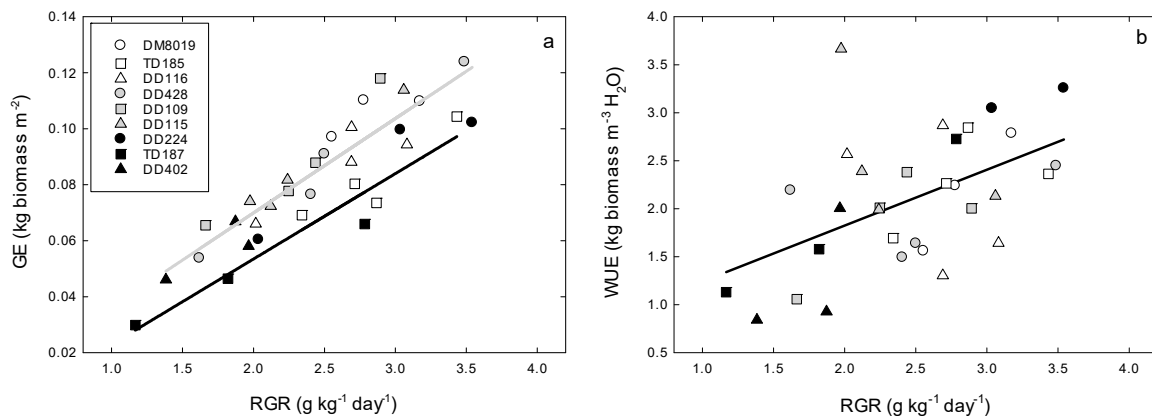
**Figure 1.** Mean, minimum, and maximum daily air temperature (a), mean and maximum daily vapor pressure deficit ( $D$ , kPa) (b), and daily mean volumetric water content (VWC) and precipitation (c) during the study period.

#### 3.2. Growth

Average DBH at the beginning of the study ranged from 4.5 to 7.8 cm (Table 1). Group H had significantly greater mean values for stem ( $4.32 \pm 0.38$  kg tree<sup>-1</sup>), foliage ( $1.30 \pm 0.09$  kg tree<sup>-1</sup>), and root biomass ( $1.18 \pm 0.09$ ) than Group I (stem:  $2.94 \pm 0.36$ ; foliage:  $0.95 \pm 0.09$ ; root:  $0.85 \pm 0.08$  kg tree<sup>-1</sup>) and Group L (stem:  $2.13 \pm 0.45$ ; foliage:  $0.71 \pm 0.10$ ; root:  $0.63 \pm 0.10$  kg tree<sup>-1</sup>). There were significant

Clone and Group differences in structural traits. Group I had significantly lower SLA,  $A_1:A_s$ ,  $A_1:Root$  than Group H or Group L, due to greater SLA of the TD hybrids in Groups H (TD185) and L (TD187). Stem biomass increment over the study period was greater in larger clones; however, there was no significant Clone or Group effect on the relative growth rate (RGR,  $g\ kg^{-1}\ day^{-1}$ ).

There were significant clone differences in growth efficiency (GE = stem biomass growth/tree leaf area). Averaged across Groups, Group H and I had higher GE,  $0.090 \pm 0.006\ kg\ tree\ m^{-2}$  and  $0.086 \pm 0.006\ kg\ tree\ m^{-2}$ , respectively, than Group L ( $0.063 \pm 0.007\ kg\ tree\ m^{-2}$ ) ( $p = 0.031$ ). Growth efficiency increased with RGR (Figure 2a). There was no significant Group  $\times$  RGR interaction, although, at a similar RGR, TD clones had a significantly lower GE than the DM and DD clones.

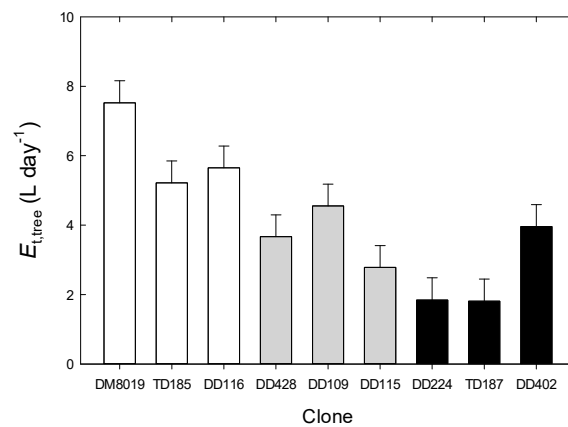


**Figure 2.** Relationships between (a) stem growth efficiency (GE) and (b) water use efficiency of production (WUEp) and average relative growth rate (RGR) of biomass growth over the 146 day study period in *P. deltoides* (DD), *P. trichocarpa x deltoides* (TD), and *P. deltoides x maximowiczii* (DM) clones. Open, light shaded, and dark shade symbols represent high (Group H), intermediate (Group I), and low (Group L) productivity genotypes, respectively. In panel a, the grey line is the regression for DM and DD clones and the black is for TD clones.

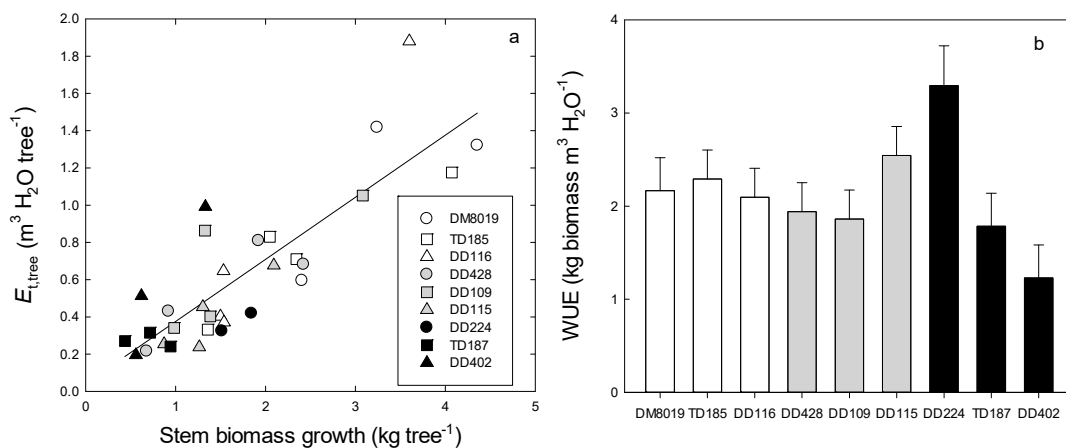
### 3.3. Tree Water Use and WUEp

Daily  $E_{t,tree}$  ranged from  $<1.0$  to  $>20.0\ L\ day^{-1}$  (Figure S1). Averaged over the study period, Group H had significantly greater  $E_{t,tree}$  ( $6.00 \pm 0.60\ L\ day^{-1}$ ) than Group I ( $3.43 \pm 0.60\ L\ day^{-1}$ ) or Group L ( $2.57 \pm 0.61\ L\ day^{-1}$ ) ( $p < 0.001$ ) (Figure 3). Total  $E_{t,tree}$  over the study period was strongly correlated with stem biomass growth (Figure 4a), although there were no significant Group effects on this relationship. Tree WUEp, calculated by dividing stem biomass growth with total  $E_{t,tree}$ , increased with RGR (Figure 2b). There were significant clone differences in WUEp ( $p = 0.031$ ), however, there were no discernible patterns among Groups ( $p = 0.746$ ) (Figure 4b). Clones with the highest (Clone DD224) and lowest (Clone DD402) WUEp were in Group L. Interestingly, WUEp was negatively correlated with total  $E_{t,tree}$  ( $p = 0.003$ , Figure 5). While there was no significant Group effect on the slope of this relationship, Group H had a greater intercept than Group L ( $p = 0.032$ ). Accounting for differences in Group  $E_{t,tree}$ , WUEp was  $2.45 \pm 0.20$ ,  $2.03 \pm 0.18$ , and  $1.72 \pm 0.23\ kg\ m^{-3}\ H_2O$  for Group H, I, and L, respectively.





**Figure 3.** Daily tree water use ( $E_{t,tree}$ , L day<sup>-1</sup>) in *P. deltooides* (DD), *P. trichocarpa* × *deltooides* (TD), and *P. deltooides* × *maximowiczii* (DM) clones. Data are lsmeans and standard error. Open, light shaded, and dark shade bars represent high (Group H), intermediate (Group I), and low (Group L) productivity genotypes, respectively.



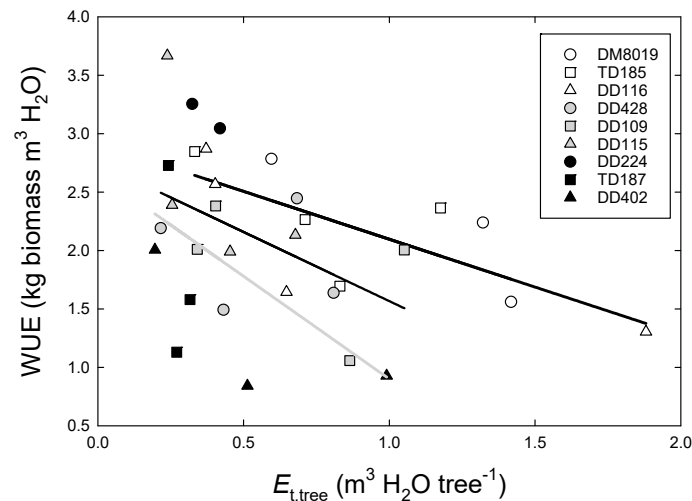
**Figure 4.** Relationship between total tree water use ( $E_{t,tree}$ , m<sup>3</sup> H<sub>2</sub>O tree<sup>-1</sup>) and stem biomass growth (a) and the mean (lsmeans ± SE) water use efficiency of production (WUEp) (b) in *P. deltooides* (DD), *P. trichocarpa* × *deltooides* (TD), and *P. deltooides* × *maximowiczii* (DM) clones. Open, light shaded, and dark shade bars represent high (Group H), intermediate (Group I), and low (Group L) productivity genotypes, respectively.

### 3.4. Canopy Stomatal Conductance and Hydraulic Conductance

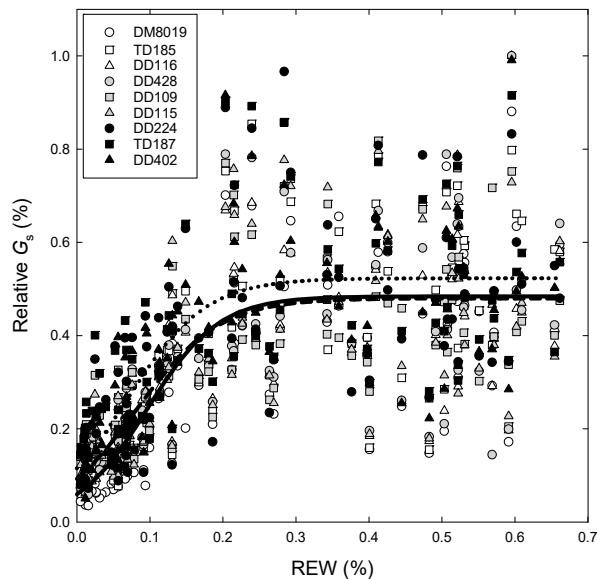
Because canopy leaf senescence began in mid to late September and compromised allometric estimates of tree leaf area, canopy stomatal conductance ( $G_s$ ) calculations were confined to the period between DOY 152 and 255, when tree canopies were still intact. Average daily  $G_s$  (PAR > 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) varied with Clones ( $p < 0.001$ ) and Groups ( $p < 0.001$ ). Mean  $G_s$  was  $29.01 \pm 1.07 \text{ mmol m}^{-2} \text{s}^{-1}$  in Group H (Figure S2a), compared to  $27.12 \pm 1.10$  and  $24.14 \pm 1.09 \text{ mmol m}^{-2} \text{s}^{-1}$  in Groups I and L, respectively (Figure S2b,c).

Canopy stomatal conductance decreased with REW (Figure 6). The relationship between relative  $G_s$  (relative  $G_s = G_s/G_{s,max}$ , where  $G_{s,max}$  is the maximum daily  $G_s$  measured during the study) and REW was best described using a three parameter logistic model:  $y = c/(1 + a \times \exp(-b \times \text{REW}))$ , where  $y = \text{relative } G_s(\%)$ . There was no significant effect of Clone or Group on the individual parameters ( $c = 0.493$ ,  $a = 4.754$ , and  $b = -18.7422$ ;  $R^2 = 0.54$ ). In general,  $G_s$  was not responsive to soil moisture when  $\text{REW} > 0.30$ , but began to decline at values between 0.25 and 0.30, and was linear when  $\text{REW} < 0.20$ . However, the REW at the inflection point ( $G_s = c/2$ ,  $\text{REW} = \ln(a)/b$ ), which describes the shape of the curve, was significantly higher in Group H (0.097) than in Group L (0.066) ( $p = 0.004$ ), and marginally higher than Group I (0.078) ( $p = 0.058$ ) (SE = 0.007), indicating that  $G_s$  began to decline

at a higher REW in larger trees. In addition, the TD clones had a significantly higher REW at the inflection point (0.11) than the DD and DM (0.072) (SE = 0.019,  $p = 0.005$ ) clones, indicating that the TD clones had greater sensitivity to declining REW (data not shown).



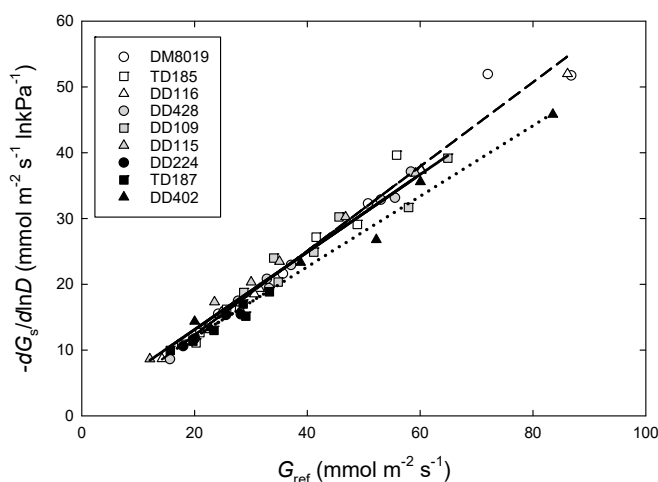
**Figure 5.** Relationship between water use efficiency of production (WUEp) and total tree water use ( $E_{t,tree}$ ,  $m^3 H_2O tree^{-1}$ ) over the 146 day study period in *P. deltooides* (DD), *P. trichocarpa* × *deltooides* (TD), and *P. deltooides* × *maximowiczii* (DM) clones. Open, light shaded, and dark shaded symbols, and heavy black, light black, and grey lines represent high (Group H), intermediate (Group I), and low (Group L) productivity genotypes, respectively.



**Figure 6.** The relationship between normalized canopy conductance ( $G_s$ , %), relative to maximum  $G_s$ , measured during a 102 day period (DOY 153–255), and relative extractable water (REW) in *P. deltooides* (DD), *P. trichocarpa* × *deltooides* (TD), and *P. deltooides* × *maximowiczii* (DM) clones. Open, light shaded, and dark shaded symbols represent high (Group H), intermediate (Group I), and low (Group L) productivity genotypes, respectively. Solid, dashed and dotted lines represent regression fits for the logistic model  $y = c/(1+a \times \exp(-b \times REW))$  for Group H, I, and L, respectively.

Average daily  $G_s$  (PAR > 800) was partitioned into dry (REW < 0.20) and wet (REW > 0.30) periods, to examine the sensitivity of  $G_s$  to  $D$  (Equation (2)). The model was fit to each tree, where the sample size was 969 and 654 observations per tree, for dry and wet periods, respectively. Slopes were

significant in all cases ( $p < 0.001$ ) and  $R^2$  ranged from 0.25 to 0.79. Soil moisture had a strong effect on  $G_{\text{ref}}$  ( $G_s$  at  $D = 1$  kPa) and the response of  $G_s$  to  $D$  ( $-dG_s/d\ln D$ ) (Table S1). Sensitivity to  $D$  increased under wet conditions. The relationship between  $-dG_s/d\ln D$  and  $G_{\text{ref}}$  was examined to determine if  $G_s$  sensitivity to  $D$  varied with  $G_{\text{ref}}$ . Averaged across Groups, there was a strong relationship between  $-dG_s/d\ln D$  and  $G_{\text{ref}}$  (slope = 0.63,  $R^2 = 0.96$ ). However, regression lines differed significantly when growth classes were assessed separately ( $p = 0.004$ ), Group L had a significantly lower slope (0.54) than Group H (0.66,  $p = 0.007$ ) and Group I (0.64,  $p = 0.002$ ) (Figure 7) indicative of a lower stomatal sensitivity to  $D$  in slower growing trees. In addition, the TD clones had a greater, although not significant, slope (0.70) than the DD and DM (0.62,  $p = 0.129$ ), possibly indicating greater stomatal sensitivity to  $D$  (data not shown).

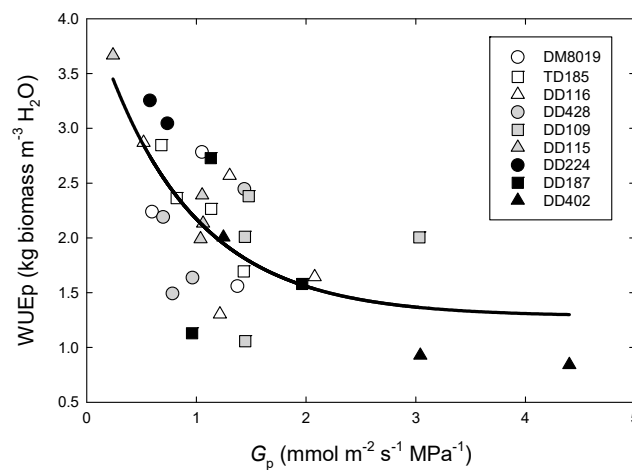


**Figure 7.** Relationship between the sensitivity of canopy stomatal conductance ( $G_s$ ) to vapor pressure deficit ( $D$ ) ( $-dG_s/d\ln D$ ) and  $G_s$  at  $D = 1$  kPa ( $G_{\text{ref}}$ ) at high PAR ( $\text{PAR} > 800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) in *P. deltooides* (DD), *P. trichocarpa*  $\times$  *deltooides* (TD), and *P. deltooides*  $\times$  *maximowiczii* (DM) clones. Open symbols and dashed line, light shaded symbols and solid line, and dark shaded symbols and dotted line represent high (Group H), intermediate (Group I), and low (Group L) productivity genotypes, respectively.

Whole-tree sapwood-specific ( $G_p$ ) hydraulic conductance was calculated for each tree on two days that differed in soil VWC. The first day (DOY 242) was near the end of a dry period, when soil VWC had been  $< 0.25 \text{ m}^3 \text{ m}^{-3}$  ( $\text{REW} < 0.14$ ) for the previous 15 days, and the second day (DOY 251) was after several precipitation events increased VWC  $> 0.30 \text{ m}^3 \text{ m}^{-3}$  ( $\text{REW} > 0.42$ ) (Figure 1c). Mean predawn leaf water potential ( $\Psi_{\text{pd}}$ ) was significantly lower on the dry day ( $-0.58 \pm 0.04$  MPa) than on the wet day ( $-0.29 \pm 0.04$  MPa), whereas midday leaf water potential ( $\Psi_{\text{md}}$ ) was lower on the wet day ( $-1.84 \pm 0.13$  MPa) than on the dry day ( $-1.52 \pm 0.13$  MPa). There were no significant Clone ( $p = 0.451$ , data not shown) or Group effects with REW for either  $\Psi_{\text{pd}}$  or  $\Psi_{\text{md}}$  (Table 2), however, there was a tendency for Group L to have a lower  $\Psi_{\text{diff}}$  ( $\Psi_{\text{diff}} = \Psi_{\text{pd}} - \Psi_{\text{md}}$ ;  $1.07 \pm 0.16$  MPa) than Group H ( $1.35 \pm 0.15$  MPa) or Group I ( $1.37 \pm 0.15$  MPa). Because of the lower  $\Psi_{\text{diff}}$ , smaller trees had higher  $G_p$  (Table 2). Group L had significantly greater  $G_p$  ( $1.66 \pm 0.22 \text{ mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ) than Group H ( $0.87 \pm 0.19 \text{ mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ,  $p = 0.026$ ) and I ( $0.97 \pm 0.18 \text{ mol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ,  $p = 0.052$ ). Soil REW had no effect on  $G_p$  (i.e., no Day effect). Tree WUE<sub>p</sub> decreased with increasing  $G_p$  (Figure 8,  $y = 2.858 \times e^{(-0.312X)}$ ,  $R^2 = 0.41$ ,  $p = 0.001$ ).

**Table 2.** Tree leaf water potential ( $\Psi$ ) and whole-plant hydraulic conductance ( $G_p$ ), measured under wet ( $REW > 0.30$ ) and dry ( $REW < 0.20$ ) soil conditions. Leaf water potential was measured at predawn ( $\Psi_{pd}$ ) and at midday ( $\Psi_{md}$ ) ( $\Psi_{diff} = \Psi_{pd} - \Psi_{md}$ ). Data are lsmeans (SE) for clones within Group with high (H), intermediate (I), and low (L) growth potential. Probability values are for Group and Day, and interaction effects.

Group	REW	$\Psi_{pd}$	$\Psi_{md}$	$\Psi_{diff}$	$G_p$
H	Dry	−0.51 (0.07)	−1.52 (0.17)	1.01 (0.19)	0.699 (0.269)
	Wet	−0.28 (0.07)	−1.96 (0.17)	1.69 (0.19)	1.040 (0.269)
I	Dry	−0.57 (0.07)	−1.71 (0.17)	1.13 (0.17)	0.788 (0.258)
	Wet	−0.29 (0.07)	−1.90 (0.17)	1.61 (0.17)	1.147 (0.258)
L	Dry	−0.650 (0.08)	−1.43 (0.18)	0.77 (0.19)	1.695 (0.316)
	Wet	−0.283 (0.08)	−1.65 (0.18)	1.37 (0.19)	1.620 (0.316)
<i>p</i> > <i>F</i>					
Group (Grp)		0.615	0.116	0.083	0.022
Day		<0.001	0.006	<0.001	0.370
Grp × Day		0.626	0.537	0.753	0.717



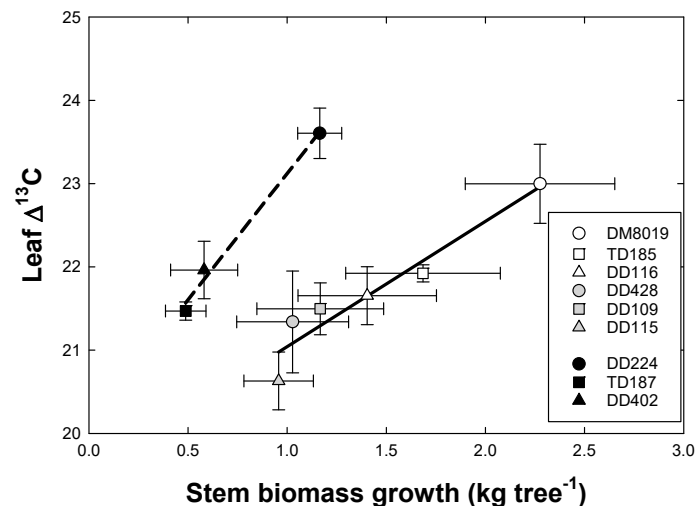
**Figure 8.** Water use efficiency of production (WUEp) as a function of whole-tree hydraulic conductance ( $G_p$ ) in *P. deltooides* (DD), *P. trichocarpa* × *deltooides* (TD), and *P. deltooides* × *maximowiczii* (DM) clones. Open symbols, light shaded symbols, and dark shaded symbols, represent high (Group H), intermediate (Group I), and low (Group L) productivity genotypes, respectively.

### 3.5. Stable Isotopes

There was a significant Clone and Group effect on leaf and wood  $\Delta^{13}C$  (Table 3). Group I had a significantly lower leaf  $\Delta^{13}C$  ( $21.16 \pm 0.34$ ) than Group H ( $22.10 \pm 0.34$ ,  $p = 0.045$ ) and Group L ( $22.26 \pm 0.34$ ,  $p = 0.011$ ). In contrast, Group H had a significant lower wood  $\Delta^{13}C$  ( $21.49 \pm 0.18$ ) than Group L ( $22.31 \pm 0.18$ ) ( $p = 0.003$ ). Leaf  $\Delta^{13}C$  was positively correlated with stem biomass growth and the relationship was significantly different between Groups (Figure 9) (note that plot averages are used as biomass growth, and  $\delta^{13}C$  measurements were made on different trees). Group L had a more positive  $\Delta^{13}C$  than Group I or H at a similar level of stem biomass growth. A similar, but less robust, relationship was observed between wood  $\Delta^{13}C$  and stem growth (data not shown). Lower values of  $\Delta^{13}C$  are associated with greater iWUE. There was no significant correlation between leaf ( $p = 0.259$ ) or wood ( $p = 0.320$ )  $\Delta^{13}C$  and WUEp (data not shown).

**Table 3.** Lsmeans (SE) and probability values for foliar and wood  $\Delta^{13}\text{C}$  for nine *Populus* clones. Genetic crosses are DD: *P. deltoides*  $\times$  *P. deltoides*, DM: *P. deltoides*  $\times$  *P. maximowiczii*, and TD: *P. trichocarpa*  $\times$  *P. deltoides*. Cells with no shading, light shading, or dark shading represent genotypes with high (H), intermediate (I), and low (L) growth potential, respectively.

Clone	Group	$\Delta^{13}\text{C}$ Foliage	$\Delta^{13}\text{C}$ Wood
DM8019	H	23.07 (0.41)	21.63 (0.22)
TD185	H	22.04 (0.37)	21.34 (0.19)
DD116	H	21.65 (0.37)	21.44 (0.19)
DD428	I	21.39 (0.37)	21.54 (0.19)
DD109	I	21.50 (0.37)	22.55 (0.19)
DD115	I	20.63 (0.37)	21.60 (0.19)
DD224	L	23.60 (0.37)	23.26 (0.19)
TD187	L	21.47 (0.37)	21.65 (0.19)
DD402	L	21.86 (0.37)	22.07 (0.19)
$p > F$			
Clone		<0.0001	<0.0001
Group		0.009	0.004



**Figure 9.** Relationship between leaf carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) and water use efficiency of production (WUEp) and stem biomass growth in *P. deltoides* (DD), *P. trichocarpa*  $\times$  *deltoides* (TD), and *P. deltoides*  $\times$  *maximowiczii* (DM) clones. Open, light shaded, and dark shaded symbols represent high (Group H), intermediate (Group I), and low (Group L) productivity genotypes, respectively. Each point is the mean of three or four or trees.

#### 4. Discussion

We found that growth efficiency (GE) and water use efficiency of production (WUEp) were positively correlated with growth. We further observed that, despite higher  $E_{t,\text{tree}}$ , larger clones had greater GE, and had greater WUEp and lower  $\Delta^{13}\text{C}$  (greater iWUE) than smaller clones. Tree GE (kg biomass  $\text{m}^{-2}$ ) increased with relative growth rate, and there was significant variation in GE related to tree size, where average GE for Group H was greater than for Group L. The robustness of the analyses of GE and WUEp depends, in part, on the degree to which allometric regressions accurately predict component biomass. We were unable to detect clone-specific differences in allometric relationships, mainly due to the small sample size ( $n =$  three or four per clone). However, the generalized equation for aboveground wood biomass was strong, lending confidence in estimates of wood biomass, biomass growth, and WUEp. In contrast, there was considerable variation in foliage biomass among harvested trees, possibly due to loss of foliage during harvesting. This led to a much less robust allometric relationship for foliage biomass, which leads to uncertainty in estimates of  $A_1$ ,  $A_1:A_s$ , and GE. Nevertheless, the positive relationship with

RGR suggests that larger, faster growing trees have greater GE. Other studies have observed strong correlations between stem growth and leaf area of several poplar species and hybrids [24,26,45,64–67]. For example, increased growth of *P. deltoides*, compared to *P. deltoides* × *P. nigra* hybrids, was attributed to a 32–120% increase in GE [68]. Total  $E_{t,tree}$  was strongly correlated with stem biomass growth, and the WUEp ranged between 1.2 and 3.3 kg m<sup>-3</sup> H<sub>2</sub>O. Tree WUEp increased with relative growth rate, indicating faster growing trees used water more efficiently. These results support our hypotheses that GE and WUEp are positively correlated with growth. The positive relationship between GE and WUEp and growth is linked to increased light and nutrient use efficiencies in larger trees [44] and a shift in carbon allocation to wood production, at the expense of belowground components [69–71].

The cause of variation in WUEp among Groups was less clear. Most of the variation in WUEp was observed in Group L, which had clones with the highest (DD224) and lowest (DD402) WUEp. Tree WUEp decreased with total tree water use (Figure 5), however, this relationship differed among Groups, such that, for a similar level of tree water use, larger clones (Group H) had a higher WUEp than Group I and L. A positive relationship between growth and WUEp has been reported for individual seedlings and trees, and at the stand-level. The work of Rasheed et al. [72] observed a strong positive relationship between seedling transpiration efficiency and biomass accumulation in *P. deltoides* × *P. nigra*. Growth was positively correlated with WUEp in six *Eucalyptus grandis* clones, but only under well-watered conditions [73]. The work of Forrester et al. [74] observed a strong relationship between  $E_t$  and stem growth in *Eucalyptus nitens*, wherein the slope of the relationship increased with increased  $E_t$ , indicating that larger trees with higher  $E_t$  had significantly greater WUEp. The work of Stape et al. [75] found that WUEp increased with increased water use in an irrigated *Eucalyptus grandis* × *urophylla* plantation. In *Eucalyptus saligna* plantations, Binkley et al. [76] reported that the largest 25% of trees accounted for half of stand water use and 60% of stand growth, reflecting greater WUE in larger trees. These studies, as well as this study, suffer from the confounding of genotype and tree, thus, lower WUE in slow growing trees may be due to inferior genetic growth potential. The work of Otto et al. [77] recognized this problem, and examined within clone variations of growth and WUE in *Eucalyptus grandis* × *urophylla* stands. They found that, across sites, dominant trees used water more efficiently than suppressed ones.

The relationship between WUE and growth, assessed by means of leaf and wood  $\Delta^{13}C$ , largely corroborated Clone and Group differences in growth-based WUEp. There was a strong positive correlation between leaf  $\Delta^{13}C$  and stem biomass growth, however, this relationship varied with Group (Figure 9 and Table 3), such that Group L had a greater leaf  $\Delta^{13}C$  (lower iWUE) for a given amount of stem growth than Groups H and I. Genotypic variation in  $\Delta^{13}C$  can be due to differences in photosynthesis ( $A$ ) or stomatal conductance ( $g_s$ ), or both; consequently, plant growth may be positively or negatively correlated with  $\Delta^{13}C$ , depending on how  $A$  and  $g_s$  affect the ratio of leaf internal to atmospheric ( $CO_2$ ) ( $C_i/C_a$ ) [23,78]. If  $A$  affects  $C_i/C_a$  more than  $g_s$ , then  $\Delta^{13}C$  should scale negatively to growth, and growth would be associated with high iWUE, whereas, if  $g_s$  has dominant control over  $C_i/C_a$ , then  $\Delta^{13}C$  should relate positively to growth, and fast growth would relate to low iWUE [79]. Our results suggest that, within Groups, differences in  $\Delta^{13}C$  were largely a function of  $g_s$ , but differences in  $\Delta^{13}C$  among Groups were less clear. Lower  $\Delta^{13}C$  in wood and foliage, after accounting for Group difference in stem growth, indicate that iWUE were largely sink-driven and the larger, more productive clones were more efficient in water use. This could occur if larger clones in Group H and I had greater photosynthetic capacity and higher  $A/g_s$  [23]. Several studies have reported a strong correlation between photosynthetic capacity and foliar nitrogen in *Populus* [65,80,81]. We observed a weak, but significant, negative relationship between  $\Delta^{13}C$  and leaf nitrogen content (mmol N m<sup>-2</sup>) ( $R^2 = 0.22$ ;  $p = 0.004$ ), suggesting that lower  $\Delta^{13}C$  were largely driven by  $A$  [82].

Variation in the relationship between  $\Delta^{13}C$  and growth has been reported for several tree species, families, and clones and including *Populus*. The work of Monclus et al. [30] observed, among 33 *P. deltoides* × *trichocarpa* genotypes, that  $\Delta^{13}C$  correlated negatively with growth, and that the most productive genotypes were the most water use efficient. A negative relationship was also observed in clones of

*Populus × euroamericana* [28,83] and in hybrid *P. deltoides × nigra* [72]. A similar negative relationship was observed for *Salix* species [84,85] and several coniferous species [86]. In contrast,  $\Delta^{13}\text{C}$  scaled positively with growth in *P. deltoides × P. nigra* [45], *P. davidiana* [25] and some *Eucalyptus* species [87,88], suggesting that high productivity is achieved at the expense of water use (i.e., low WUEi). Others have found a poor or no correlation between  $\Delta^{13}\text{C}$  and growth in Poplar sp. and hybrids [27,89,90].

While both WUEp and  $\Delta^{13}\text{C}$  indicated that the more productive clones used water more efficiently, there was no clear relationship between the two indices of WUE. Others have found a poor or non-existent relationship between tissue  $\Delta^{13}\text{C}$  and WUEp [21,41,73,91], although, a strong negative relationship between WUEp and  $\Delta^{13}\text{C}$  was observed in seedlings of *Larix occidentalis* [92] and *P. euroamericana* [28]. These contrasting results suggest that the relation between  $\Delta^{13}\text{C}$  and WUEp may change when scaling from leaf to whole plant. Clone differences in mesophyll conductance, tissue respiration, water loss through non-photosynthetic processes, carbon allocation and leaf area can affect the plant carbon and water balance independently of  $A$  and  $g_s$ , causing a decoupling of  $\Delta^{13}\text{C}$  and WUEp. Because of this, Seibt [93] concluded that  $\Delta^{13}\text{C}$  measurements might not be a useful predictor of WUEp. Clearly, a better understanding of the relationship between  $\Delta^{13}\text{C}$  and growth and WUEp is needed if  $\Delta^{13}\text{C}$  is to be a useful metric to estimate tree-level WUE.

Clone variation in WUEp and  $\Delta^{13}\text{C}$  was linked to increased stomatal sensitivity to  $D$  and REW and decreased whole-tree hydraulic conductance ( $G_p$ ). Canopy  $G_s$  was relatively insensitive to changes in soil moisture, until a threshold REW was reached, after which trees responded by closing stomata reducing  $E_t$ . The threshold appears to be between 20% and 25% REW (Figure 6), below which trees had tight control over water use. Clones in Groups H and I tended to reduce  $G_s$  sooner, as REW declined, than smaller clones in Group L, that is, less productive clones had a higher relative  $G_s$  at lower REW than more productive clones. The slower response to decreasing REW in Group L was reflected in a different stomatal sensitivity to  $D$ . Averaged across all Groups, there was a strong relationship between  $-dG_s/d\ln D$  and  $G_{ref}$  (slope = 0.63), which is close to the theoretical 0.6 predicted for isohydric plants that regulate leaf water potential above a species-specific threshold [58]. However, when analyzed separately, Group L had a significantly lower slope (0.54). Deviation from the theoretical  $0.6 \cdot G_{ref}$  could be due to Group L having less stomatal control over leaf  $\Psi$ . This fits with Group L being less sensitive to REW. Across genotypes, WUEp was negatively correlated with  $G_p$ , indicating a trade-off between water transport efficiency and WUEp (Figure 8). This finding is the opposite of our hypothesis, and contrary to the theory that that high whole-plant hydraulic is necessary for high productivity in forest trees [19]. The reduction in  $A_H$  in larger clones seems to be responsible for the decrease in  $G_p$ . The work of Fichot et al. [94] observed a negative relationship between whole-plant hydraulic conductance and relative growth rate in *P. deltoides × P. nigra* hybrids, possibly due to genotypic variation in xylem anatomy (vessel diameter and frequency) [95]. Trees display homeostatic adjustment of hydraulic properties in relation to growing conditions and climate, to avoid damaging leaf water potential [96]. This could include a reduction in  $A_l:A_s$ , increased  $G_p$ , or decreased  $G_s$ . We observed only small differences in  $A_l:A_s$  with tree size, thus, greater stomatal sensitivity to  $D$  was necessary to maintain leaf water potential in large trees. Increased stomatal sensitivity to  $D$ , with a decrease in  $G_p$ , is consistent with homeostasis of leaf water potential in isohydric plants [58].

There are other possible causes for variation in WUEp and  $\Delta^{13}\text{C}$  among Clones and Groups, related to differences in tree size and stand structure. The work of Binkley et al. [76] proposed that, following stand closure, competition among trees for resources intensifies, and that dominant trees would have better access to resources (light, nutrients, moisture), use more of those resources, and therefore have a higher resource use efficiency than suppressed trees. It has also been observed that the maintenance respiration cost relative to photosynthesis is greater in suppressed trees [97]. Consequently, the observed genotypic variation in GE and WUEp may simply be due to competition and stand structure.

The relationship between  $G_{ref}$  and  $-dG_s/d\ln D$  (Figure 7) assumes a strong coupling between the canopy and atmosphere [58]. Group H and I represented dominant and co-dominant trees,

whose canopies were likely well coupled with the atmosphere. In contrast, the canopies of suppressed trees in Group L may be less coupled with the atmosphere, creating a higher ratio between boundary layer conductance and  $g_s$  and less sensitivity to changes in  $D$  [98]. Suppressed trees also may have assimilated respired  $\text{CO}_2$  (depleted in  $^{13}\text{C}$ ) within the canopy. A high boundary layer resistance, and the use of respired  $\text{CO}_2$ , could have lowered  $\delta^{13}\text{C}$  (higher  $\Delta^{13}\text{C}$ ) [99]. Hydraulic architecture can have a strong effect on  $g_s$ ,  $C_i/C_a$ , and leaf  $\Delta^{13}\text{C}$  [100]. Increased hydraulic resistance, with an increased xylem flow-path length, in taller trees [101], and reduced water supply to the canopy, should decrease  $g_s$  relative to  $A$  and, therefore, result in lower  $\Delta^{13}\text{C}$  [100,102]. Our results showed that the larger clones in Group H and I had lower  $G_p$  and lower leaf and wood  $\Delta^{13}\text{C}$ ; however, the mean difference in height between Group H and I was small (1.3 m) and was not a significant covariate explaining Clone or Group variation in  $\Delta^{13}\text{C}$  or  $\text{WUE}_p$ . It is unclear if we would have observed similar  $\text{GE}$ ,  $\text{WUE}_p$ , and  $\Delta^{13}\text{C}$  if the clones were open-grown, where competition for light and soil resources is minimized. Nevertheless, these results highlight the importance of understanding the effect of tree size on physiological and hydraulic characteristics that determine water use and water use efficiency. A better understanding of tree-level physiology, and hydraulic characteristics and variation with tree size and growth rate, will facilitate scaling between small and large trees at the stand scale [103,104].

We observed a few differences in hydraulic traits and physiology that were related to the taxon of the clones. The TD clones (TD187 and TD185) had significantly greater SLA,  $A_1:A_s$ , and  $A_1:\text{Root}$ , and lower  $A_H$  (Table 1) than the DD or DM clones. These attributes contributed to a lower  $\text{GE}$  and an increased sensitivity to soil water deficits, decreasing relative  $G_s$  at a higher REW. Interestingly, when  $-dG_s/d\ln D$  was assessed as a function of  $G_{\text{ref}}$ , the TD clones had greater, although not significant, slope (0.70) than the DD and DM clones (0.62), suggesting greater stomatal sensitivity to  $D$ , although there were no significant differences in  $G_p$ . High SLA, along with increased  $G_s$  sensitivity to  $D$  and low  $G_p$ , would facilitate greater stomatal control of water loss [105]. The greater stomatal control of water loss of TD clones suggests that they may be more suitable for drier sites, although growth performance may be limited [5].

## 5. Conclusions

We found genotypic variability in the relationship between tree productivity,  $\text{GE}$ ,  $\text{WUE}_p$ , and  $\Delta^{13}\text{C}$ , among clones of *P. deltooides* and hybrids, when grown under field conditions. Larger clones generally had higher  $\text{GE}$  and  $E_{t,\text{tree}}$ , and used more water than smaller clones. While there was no clear relationship between  $\text{WUE}_p$  and  $\Delta^{13}\text{C}$ , both indices indicated that more productive clones used water more efficiently. The positive relationship between  $\text{WUE}_p$ , growth, and the lower leaf and wood  $\Delta^{13}\text{C}$  in faster growing clones suggest that  $\text{WUE}$  is controlled primarily by photosynthesis, rather than stomatal conductance. Our data reflect tree-level responses and do not reflect how stand-level responses may differ; however, tree-level physiological responses provide fundamental information about species-level and stand-level responses. Nevertheless, these results suggest the stand management activities and clone selection that focus on tree growth will likely result in increased  $\text{WUE}$ . For sites with seasonally variable soil moisture conditions, selecting clones with high productive capacity is probably a better strategy than selection based on  $\text{WUE}$ , although increased productivity may also increase drought risk. The focus should be on the tradeoff between production and risk of plantation failure from drought. Continued research of the varied stomatal regulation strategy for growth and  $\text{WUE}$  should prove beneficial.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1999-4907/10/11/993/s1>, Figure S1: Seasonal patterns of tree transpiration; Figure S2: Seasonal patterns of canopy stomatal conductance, Table S1: Parameter estimates for canopy stomatal conductance response to vapor pressure deficit ( $D$ ).

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