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Research paper

How does drought tolerance compare between two improved hybrids of balsam poplar and an unimproved native species?

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Poplars are one of the woody plants that are very sensitive to water stress, which may reduce the productivity of fast-growing plantations. Poplars can exhibit several drought tolerance strategies that may impact productivity differently. Trees from two improved hybrids, *Populus balsamifera* × *Populus trichocarpa* Torr. & Gray (clone B × T) and *P. balsamifera* × *Populus maximowiczii* A. Henry (clone B × M), having *P. balsamifera* L. as a parent and trees from native and unimproved *P. balsamifera* were subjected to a 1-month drying cycle in a growth chamber and then rewatered. The unimproved and native B clone maintained higher stomatal conductance (g_s) than the hybrids, and high photosynthetic activity and transpiration, even when soil water content was nearly zero. As a result, both instantaneous water use efficiency (WUE_i) and leaf carbon isotope composition ($\delta^{13}C$) indicated that this clone was less affected by drought than both hybrids at maximal drought stress. However, this clone shed its leaves when the drought threshold was exceeded, which implied a greater loss of productivity. The B × M hybrid showed a relatively conservative response to water stress, with the greatest decrease in transpiring versus absorbing surface (total leaf area to root biomass ratio). This clone was also the only one to develop new leaves after rewatering, and its total biomass production was not significantly decreased by drought. Among the two hybrids, clone B × T was the most vigorous, with the greatest transpiration (E_i) and net CO_2 assimilation (A) rates, allowing for high biomass production. However, it had a more risky strategy under drought conditions by keeping its stomata open and high E_i rates under moderate drought, resulting in a lower recovery rate after rewatering. The opposite drought response strategies of the two hybrids were reflected by clone B × T having lower WUE_i values than clone B × M at maximal drought, with a very low Ψ_{min} value of -3.2 MPa, despite closed stomata and stopped photosynthetic activity. Positive linear relationships between A and g_s for the three hybrids indicated strong stomatal control of photosynthesis. Moreover, the three poplar clones showed anisohydric behaviour for stomatal control and their use under long-term drought should be of interest, especially the B × M clone.

Keywords: anisohydric, growth, hydraulic conductance, leaf water potential, photosynthesis, productivity, stomatal conductance, transpiration, water use efficiency.

Introduction

Poplar (*Populus* spp.) species and their hybrids are increasingly established in fast-growing plantations around the world to meet the demand for industrial stemwood (Gordon 2001). Poplars are generally very productive species, due to their rapid and continuous leaf production, high leaf area index and high photosynthetic rates (Rhodenbaugh and Pallardy 1993).

Numerous improvement and hybridization programmes have resulted in the selection of very productive clones. Although balsam poplar (*Populus balsamifera*) is not especially valued by the forest industry due to its high resin content and dark-coloured heartwood, it is widely used in Canada as a parent to produce fast-growing hybrids (Dickmann 2001). Indeed, it is easily produced through vegetative propagation

(Riemenschneider et al. 2001) and, since it is native to boreal regions across North America, is used to provide cold hardiness to hybrids planted in boreal regions.

The natural distribution of poplars is usually associated with water availability (Rood et al. 2000), as they have high transpirational demand and are among the most sensitive woody plants to water stress (Marron et al. 2003). Consequently, drought stress is often the main cause of reduced productivity in plantations (Mazzoleni and Dickmann 1988, Ibrahim et al. 1997, Souch and Stephens 1998). Desirable clones for fast-growing plantations would thus combine increased productivity and drought-stress tolerance, especially in the climatic change context where precipitation patterns on a regional scale may become uncertain (Andalo et al. 2005).

The selection of drought-resistant and productive clones may not be simple to achieve, as variability may be quite important between clones of the same hybrids. Highly productive clones may have strong drought tolerance (Tschaplinski and Blake 1989, Chen et al. 1997), while others are more drought sensitive than less productive ones (Pallardy and Kozłowski 1981, Brignolas et al. 2000, Zhang et al. 2004). Poplars can exhibit several drought resistance strategies that may impact productivity differently: decreased leaf area, leaf abscission, enhanced root growth, increased water use efficiency (WUE), stomatal closure and osmotic adjustment, among others (Mazzoleni and Dickmann 1988, Roden et al. 1990, Blake et al. 1996, Monclus et al. 2006, Desrochers et al. 2007). In addition, the relationship between productivity and drought tolerance may not be generalized within a poplar species or hybrid, as trees from the same species have been found to exhibit different water conservation strategies depending on their provenance from wet or dry areas (Zhang et al. 2004).

In this study, trees from two improved hybrids having *P. balsamifera* L. as a parent and a native and unimproved *P. balsamifera* were subjected to a 1-month drying cycle in a growth chamber experiment. Both interspecific hybrids (*P. balsamifera* × *Populus trichocarpa* Torr. & Gray and *P. balsamifera* × *Populus maximowiczii* A. Henry) have been selected for northwestern Quebec, which was also the region where the unimproved clone originated from. In order to examine the relationship between drought tolerance and productivity, we evaluated the relationships between soil water content, growth, gas exchange, water potential and hydraulic conductance. Time-integrated WUE was also estimated from leaf carbon isotope composition ($\delta^{13}\text{C}$) (Farquhar et al. 1989) and compared with short-term WUE (WUE_i ; ratio of net carbon dioxide assimilation to transpiration). We examined differences between the drought responses of the three clones to study poplar drought resistance variability: Is an unimproved clone more resistant than two fast-growing hybrids? Do the hybrids show the same behaviour under drought conditions?

Material and methods

Plant material and growth conditions

One-year-old 5-cm branch cuttings of *P. balsamifera* L. (B) (clone PB9, provenance Amos, mesic habitat, Québec, Canada), *P. balsamifera* × *P. trichocarpa* Torr. & Gray (B × T) (clone 747210) and *P. balsamifera* × *P. maximowiczii* A. Henry (B × M) (clone 915005) were used. Both hybrids are recommended by the Ministère des Ressources Naturelles et de la Faune du Québec (MRNF) for plantation in the region where clone B was collected. Hybrid poplar cuttings were planted on 2 March 2007, and B cuttings were planted on 30 March 2007 in a peat-based substrate PRO-MIX® BX with *Bacillus subtilis* (Premier Horticulture Ltd, Dorval, Québec, Canada). Cuttings were placed in a greenhouse with a 16-h photoperiod and day/night temperatures of ~30/20 °C.

After 3 months, 30 cuttings of each clone were potted into 2.5-l pots in peat-based substrate PRO-MIX-HP substrate (Premier Horticulture Ltd, Dorval, Québec, Canada) and transferred into a controlled environment growth chamber (CONVIRON CG 108, Winnipeg, Manitoba, Canada) to reduce climatic fluctuations. The following conditions were set for the growth chamber 16-h photoperiod, day/night temperature 24/18 °C, day/night relative humidity (RH) 60/90% and photosynthetically active radiation at pot level 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Cuttings were fertilized bi-weekly with 15 ml of liquid fertilizer (N/P/K 20/20/20, 27% solution; Botanix, Boucherville, Québec, Canada).

Experimental design

The experimental design was a split-plot design with two fixed treatment factors: (i) two watering treatments (W: watered; D: droughted) as the main plot factor and (ii) three clones (B, B × T and B × M) as the subplot factor. Five trees were used for biomass measurements at the end of the experiment (destructive sampling) in each treatment combination of watering × clone, and replicated into two blocks ($n = 60$). Three or two trees were used for periodical water and gas exchange measurements in each treatment combination and replicated into two blocks (block 1: $n = 18$; block 2: $n = 12$).

Watering treatments

Cuttings were watered daily to field capacity until the beginning of the drought experiment. On 1 August 2007, the first day of the experiment, half the trees from each clone were selected for normal watering (W) treatment and the second half were submitted to a progressive drought (D) by reducing water supply. During all the experiment, soil volumetric water content (SVWC, the fraction of the total volume of soil sample that is occupied by the water contained in the soil, %) was measured twice daily (Field Scout TDR 100 with a 12-cm-long probe; Spectrum Technologies, Inc., Plainfield, IL, USA).

Trees in the W treatment were individually watered to maintain SVWC between 35 and 55%. In the D treatment, SVWC was ~40% before drought imposition, and was reduced by 5% each 3.5 days until it reached 10%. Soil volumetric water content was kept at 10% for 1 week. Then it was decreased to 5% on the 27th day of the experiment, and to 0% on the 31st day. The pots were rewatered to field capacity on the 35th day. Soil volumetric water content was measured twice daily and water supply was adjusted accordingly to meet the required drought level.

Water relations

Pre-dawn leaf water potential (Ψ_{wp} , MPa) and midday leaf water potential (Ψ_{mid} , MPa) were measured with a pressure chamber PMS Model 600 (PMS Instrument Company, Albany, OR, USA), on one leaf per tree, weekly when SVWC of D seedlings was >20% and bi-weekly when it decreased below 20%. Tree water loss was estimated the same day water potential was measured by weighing water loss of pots over a 3-h period. Soil evaporation was avoided by enclosing the pots in plastic bags. To estimate total tree transpiration rate (E , mmol m⁻² s⁻¹) over these 3 h, total leaf area (TLA, cm²) was estimated using a linear relationship between $w \times l$ (w , leaf maximum width (cm) perpendicular to the main vein; l , leaf maximum length (cm) from lamina tip to petiole insertion through the main vein) and individual leaf area (LA). Indeed, [Ceulemans et al. \(1993\)](#) have shown LA to be accurately estimated from non-destructive length and/or width measurements for poplars. The linear relationships ($P < 0.001$) were established using 10 W trees at the end of the experiment (309 leaves for clone B, 528 leaves for clone B $\times$ T and 1208 leaves for clone B $\times$ M) and were derived as follows:

$$\text{Clone B : LA} = 0.6792(w \times l), R^2 = 0.99$$

$$\text{Clone B} \times \text{T : LA} = 0.6687(w \times l), R^2 = 0.99$$

$$\text{Clone B} \times \text{M : LA} = 0.6846(w \times l), R^2 = 0.99$$

Root-to-leaf specific hydraulic conductance (KSL, mmol m⁻² s⁻¹ MPa⁻¹) was calculated as

$$\text{KSL} = \frac{E}{\Psi_{wp} - \Psi_{mid}}$$

Leaf gas exchange measurements

Net CO₂ assimilation (A , μmol m⁻² s⁻¹), stomatal conductance to water vapour (g_s , mmol m⁻² s⁻¹), instantaneous transpiration (E_i , mmol m⁻² s⁻¹) and internal CO₂ concentration (c_i) were measured on three trees per clone per watering treatment twice a week in the morning (09:00 am to 01:00 pm). Measurements were performed using a CIRAS-2 portable

infrared gas analyser equipped with a PLC6 broadleaf chamber illuminated by halogen lamps (photosynthetic photon flux density (PPFD) of 1400 μmol m⁻² s⁻¹ at leaf level) (PP Systems Inc., Amesbury, MA, USA). Measurements were made on one leaf per tree at 24 °C (ambient temperature), RH = 60% (ambient RH), air CO₂ concentration = 360 ppm, flow = 295 ml min⁻¹. The instantaneous water use efficiency (WUE_i , μmol CO₂ μmol H₂O⁻¹) was determined as instantaneous leaf transpirational WUE, or transpiration efficiency A/E_i ([Farquhar et al. 1989](#)).

Growth and biomass assessments

Stem height and basal diameter, number of leaves (NL) and TLA were measured before the drought treatment started and at the end of the experiment, after which trees were harvested and separated into roots, stems and leaves. Leaf area was measured prior to drying with the LI-3100C leaf area meter (LiCor, Lincoln, NE, USA). The different plant parts were then oven-dried at 70 °C and weighed. Biomass partitioning among the plant components was assessed by determining the root mass ratio (root dry mass/whole seedling dry biomass, RMR, g g⁻¹), the stem mass ratio (shoot dry mass/whole seedling dry biomass, SMR, g g⁻¹), the leaf mass ratio (leaf dry mass/whole seedling dry biomass, LMR, g g⁻¹) and the TLA to the root biomass ratio (cm² g⁻¹). Specific leaf area (SLA, TLA/total leaf dry mass, cm² mg⁻¹) and leaf area ratio (LAR, TLA/whole seedling dry biomass, cm² g⁻¹) were also calculated.

Carbon isotope ratio

The leaves that grew after the drought treatment was initiated were oven-dried at 70 °C and finely ground with a Brinkmann MM2 ball grinder (Brinkmann Instruments Ltd, Mississauga, ON, Canada). Isotope ratios (¹³C/¹²C) were determined using a Costech ECS 4010 Elemental Combustion System (Costech Analytical Technologies, Inc., Valencia, CA, USA) coupled to a continuous flow Finnigan Delta Plus Advantage IRMS (ThermoFinnigan, Bremen, Germany). The relative abundance of ¹³C in seedling leaves was expressed in terms of carbon isotope composition ($\delta^{13}\text{C}$), according to the following relationship:

$$\delta^{13}\text{C} = \frac{R_l - R_s}{R_s} \times 1000$$

where R_l and R_s refer to the ¹³C/¹²C ratio in the leaf sample and in the standards, respectively. BMO, CS and NBS 1575N were used as calibrating standards and red clover as working standard, with carbon isotope compositions of -23.91, -12.5, -26.3 and -27.42‰ relative to Pee Dee Belemnite, respectively.

Statistical analysis

For growth and biomass traits, data collected at the beginning and at the end of the experiment were submitted to two-way analyses of covariance (PROC GLM SAS Institute Inc., 2008).

Initial height, diameter and TLA were used as covariates for biomass assessment in order to compensate for size differences between clones at the beginning of the experiment. For water relations and gas exchange parameters, data collected at each date (and corresponding SVWC, in the D treatment) were also submitted to two-way analysis (treatment, clone) of covariance (PROC GLM). Estimated transpiration over 3 h (E), specific hydraulic conductance (KSL) and TLA were analysed using initial TLA as covariable, as these parameters could be influenced by differences in TLA among clones at the beginning of the experiment. All tested factors were fixed and the factor block was random. When effects were significant for a given trait, least-square means were estimated (LS MEANS statement) and Tukey tests were conducted at the 0.05 significance level. Pearson's correlations and regression lines were calculated between water relations and gas exchange parameters (PROC CORR and REG, SAS Institute, Inc., 2008). Slopes were compared according to Zar's (2010) method.

Results

Growth and biomass production

Trees within a clone had similar height, basal diameter and leaf number in the two watering treatments (D and W) at the beginning of the experiment. Clone B was generally less productive (height, diameter, LAR, root biomass and TLA) than both hybrids, under wet and dry conditions. Trees from the B × T hybrid showed superior growth potential in watered conditions with the greatest diameter ($P < 0.001$) increment (Table 1). The D treatment significantly decreased the diameter increment of the B × T hybrid, but not that of the other clones (Table 1).

Both hybrids had greater TLA than clone B under the W treatment, but under the D treatment at maximal drought, TLA of all the clones was similar (Table 2, Figure 1i). The hybrids had two different leaf development patterns to reach greater TLA: the large-leaved B × T clone had a mean individual LA 2–3 times greater than the other clones, while the small-leaved B × M hybrid had 2–4 times more leaves than the other clones (results not shown). The droughted B × M clone had more leaves at the end of the experiment than at the beginning, while the B × T clone had similar NL and the droughted B clone had fewer leaves (–50%) (results not shown). Clone B × T had the greatest root biomass under wet conditions ($P < 0.02$) (Figure 1ii). Under watered conditions, clone B × M had the greatest TLA to

root biomass ratio compared with the other clones (Figure 1iii). Under drought, this ratio decreased for all clones, the B × M clone being the most affected as there was no more difference between it and the other clones (Figure 1iii).

Water relations and leaf gas exchange

Ψ_{wp} , Ψ_{min} and KSL decreased in droughted trees compared with watered trees when SVWC was <20% and until SVWC reached 0% (Table 2, Figure 2i–iii). After rewatering, droughted trees from clone B had the highest Ψ_{min} values, whereas Ψ_{min} values of clone B × T remained the lowest (Figure 2ii). Similarly, A and g_s remained clearly lower for clone B × T submitted to the D treatment, showing lower recovering capacity compared with the other clones (Table 2, Figure 2iv and v).

When drought stress was low, clone B × T had the highest instantaneous transpiration rate (E_i), g_s and the highest A among clones (Table 2, Figure 2iv–vi). At maximal drought stress (SVWC = 0%), A , E_i and g_s were almost zero for hybrid trees in the D treatment, while clone B values decreased but stayed clearly positive compared with hybrids (Figure 2iv–vi). A and g_s , and E_i and g_s were strongly linearly related (Figure 3i and ii). The slopes of the lines were significantly different between the clones for both relations: $\ln A = f(\ln g_s)$, $F = 9.8$, and $\ln E_i = f(\ln g_s)$, $F = 4.5$ ($F_{0.05, [2, 72]} = 3.13$). Clone B × M had the highest slope for $A(g_s)$ and the lowest for $E_i(g_s)$, while it was the opposite for the B × T clone. We excluded three B × T values of A at maximal drought because they were negative, showing respiration rates rather than photosynthetic activity. The regression analysis between E_i and Ψ_{min} or Ψ_{wp} did not indicate any significant relationship (data not shown).

At maximal drought (SVWC = 0%, Day 31), two opposite trends appeared among the D trees: WUE_i of droughted clone B trees remained high, while droughted B × T WUE_i fell to values lower than the other clones (Table 2, Figure 4i). At the end of the experiment, $\delta^{13}C$ of droughted trees was greater than that of watered trees (Figure 4ii, $P < 0.0001$). Clone B had lower $\delta^{13}C$ values than the hybrids regardless of the treatment (Figure 4ii, $P < 0.0001$). Ψ_{min} reached the lowest values in the B × T hybrid at maximal drought (Day 31), with –3.2 MPa (Figure 4iii).

Discussion

The three studied clones reacted differently when submitted to drought. Clone B kept opened stomata, high water potentials

Q14 Table 1. Height and diameter increment (as a percentage of initial height and initial diameter) between the beginning and the end of the experiment for each clone (B, B × M and B × T) and watering treatments (W = watered and D = droughted). Mean (SE) ($n \geq 10$). Means that do not differ at the 0.05 level are noted with the same letter ($a < b < c < d$).

	B W	B D	B × M W	B × M D	B × T W	B × T D
Height increment (%)	182 (39) ^{bc}	101 (22) ^a	145 (9) ^{abc}	89 (7) ^a	215 (13) ^c	156 (14) ^{abc}
Diameter increment (%)	32 (9) ^a	31 (7) ^a	46 (7) ^a	35 (6) ^a	83 (9) ^b	46 (9) ^a

and active transpiration, even at maximal drought stress (SVWC = 0%), and massively shed its leaves when its drought threshold tolerance was exceeded. As a result, both WUE_i and $\delta^{13}C$ indicated that clone B was less affected by drought than either hybrid at maximal drought stress. Clone B $\times$ M had a more conservative strategy, by rapidly reducing its TLA, thus decreasing its transpiring versus absorbing surface (TLA to root biomass ratio). Finally, clone B $\times$ T had a more risky strategy by keeping high A , g_s and E_i rates under drought conditions, which resulted in a lower recovery rate after rewatering. These opposite strategies resulted in clone B $\times$ T having a lower WUE_i at maximal drought compared with clone B $\times$ M, reaching a very low Ψ_{min} value of -3.2 MPa. These differences in response to drought between the three clones highlight poplar variability in drought strategies.

Anisohydric behaviour (Tardieu and Simonneau 1998) was observed for the three clones, with a decline in Ψ_{min} as soil Ψ (measured by Ψ_{wp}) declined, resulting in a lower Ψ_{min} in droughted trees than in watered trees. All clones kept their stomata

opened until there was no more water in the soil. However, Tardieu and Simonneau (1998) found *Populus euramericana* (cv. I-214) to have isohydric behaviour, which indicated some versatility of stomatal control among poplar species and clones. This is in agreement with the considerable variation found in the poplar genus for drought responses (Hamanishi et al. 2010). However, this anisohydric behaviour does not seem specific to balsam poplar as another clone has been reported to slightly reduce midday xylem Ψ even when drought reached a sufficient level to induce leaf senescence (Amlin and Rood 2003). The anisohydric behaviour of our clones might be due to provenance from mesic habitats (Bassman and Zwier 1991).

Root to leaf hydraulic conductance of all clones decreased when drought stress was still mild (SVWC = 20%), suggesting early cavitation, but did not lead to a simultaneous decrease in stomatal conductance. Therefore, hydraulic control of g_s may be weak. Positive linear relationships between A and g_s imply strong stomatal control of photosynthesis for all clones, due to CO_2 restriction. At maximal drought stress, droughted trees

Table 2. Clone and treatment statistical effect on pre-dawn leaf water potential (Ψ_{wp}), minimum leaf water potential (Ψ_{min}), total leaf area (TLA), individual leaf area (LA), number of leaves (NL), transpiration rate (E), soil-to-leaf specific hydraulic conductance (KSL), net CO_2 assimilation (A , $\mu mol m^{-2} s^{-1}$), instantaneous transpiration rate (E_i , $mmol m^{-2} s^{-1}$), stomatal conductance (g_s), internal CO_2 concentration (c_i) and water use efficiency (WUE_i) for each corresponding SVWC applied in drought treatment.

Date	SVWC in D treatment (%)	Factor	Ψ_{wp}	Ψ_{min}	TLA	LA	NL	E	KSL	A	E_i	g_s	c_i	WUE_i
Day 0	45	Treatment	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		Clone	***	***	***	***	***	NS	***	NS	NS	NS	NS	NS
		Treatment $\times$ clone	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Day 7	30	Treatment	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
		Clone	***	**	***	***	***	***	NS	NS	NS	NS	NS	NS
		Treatment $\times$ clone	NS	NS	NS	NS	NS	NS	NS	NS	*	**	NS	NS
Day 14	20	Treatment	***	**	NS	NS	NS	*	*	NS	NS	NS	NS	NS
		Clone	***	NS	NS	***	***	***	NS	**	NS	NS	NS	NS
		Treatment $\times$ clone	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS
Day 17	15	Treatment	*	*				*	*	*	*	*	NS	NS
		Clone	NS	NS				***	**	*	NS	*	***	**
		Treatment $\times$ clone	NS	NS				NS	NS	NS	NS	NS	NS	NS
Day 21	10	Treatment	**	**	**	*	NS	***	***	***	***	***	*	NS
		Clone	NS	NS	***	***	***	***	NS	*	NS	NS	*	**
		Treatment $\times$ clone	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS
Day 24	10	Treatment	*	***				***	***	***	***	***	**	NS
		Clone	NS	NS				***	NS	NS	NS	NS	NS	NS
		Treatment $\times$ clone	NS	NS				NS	NS	*	NS	NS	NS	NS
Day 28	5	Treatment	***	***	***	NS	**	***	***	***	***	***	***	***
		Clone	NS	NS	***	***	***	***	NS	NS	NS	NS	*	NS
		Treatment $\times$ clone	NS	NS	***	NS	***	NS	NS	NS	NS	NS	NS	NS
Day 31	0	Treatment	***	***				***	***	***	***	***	NS	*
		Clone	***	***				***	NS	NS	NS	NS	***	***
		Treatment $\times$ clone	***	***				***	NS	NS	***	NS	***	***
Day 35	45	Treatment	NS	NS	NS	***	***	NS	NS	***	***	***	***	*
		Clone	NS	*	NS	***	***	*	NS	*	*	**	***	NS
		Treatment $\times$ clone	NS	NS	***	***	***	NS	NS	***	*	*	*	NS

Note: NS: non-significant.

* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$.

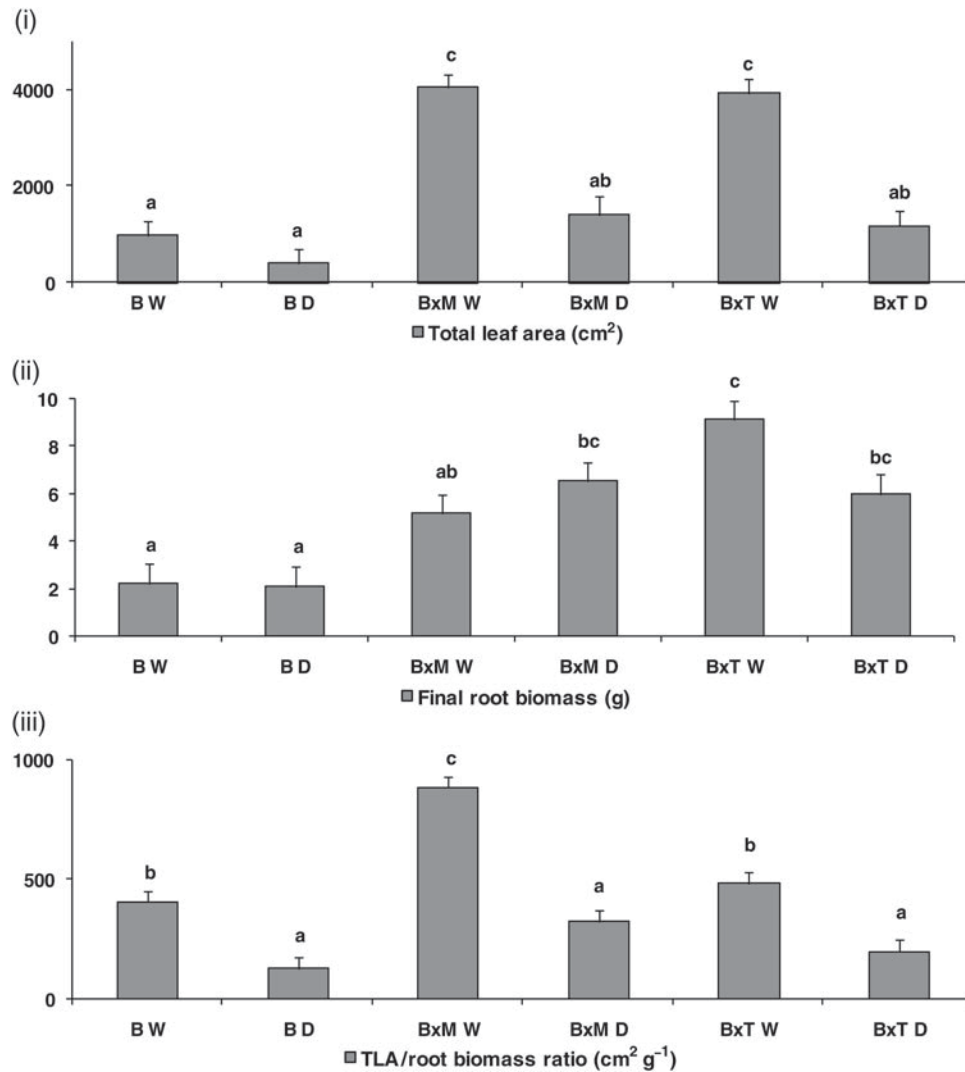


Figure 1. TLA (i), final root biomass (ii) and ratio of TLA to root biomass (iii) for each clone (B, B × M and B × T) and water treatment (W = watered and D = droughted) at the end of the experiment (Day 35). Mean ($n \geq 10$). Bars denote SE. Means that do not differ at the 0.05 level are noted with the same letter ($a < b < c$).

from clone B kept their stomata open (positive g_s), while droughted hybrids closed them, restricting CO_2 entry into leaves and increasing their $\delta^{13}\text{C}$. It is likely that the stomatal behaviour of clone B also led to a higher water deficit in its leaves, causing a massive shedding of leaves. Stomatal closure is reflected in greater $\delta^{13}\text{C}$ values, because intercellular spaces beneath closed stomata become increasingly enriched in $^{13}\text{CO}_2$, resulting in greater levels of its fixation (Farquhar et al. 1989). Here, $\delta^{13}\text{C}$ effectively seemed clearly linked to stomatal conductance. Trees from droughted clone B also had greater WUE_i values than droughted hybrids, because open stomata allowed photosynthetic activity while both hybrids had stopped it. Thus, in our experiment, both WUE_i and $\delta^{13}\text{C}$ indicators were well related. However, at maximal drought, WUE_i succeeded in reflecting the different strategies of the two hybrids while $\delta^{13}\text{C}$ values did not. Consequently, WUE_i seemed like a more accurate

indicator for assessing poplar water use efficiency under drought conditions.

The three clones had different slopes for the $A(g_s)$ relation, which might reflect different photosynthesis efficiencies (carboxylation rates) and/or CO_2 transfer conductance in mesophyll cells (g_i) (Ethier et al. 2006). The B × T clone had the highest net CO_2 assimilation rate among clones when drought increased (lower slope), which is in accordance with its more anisohydric behaviour and should confer it lower C starvation under long-term drought (McDowell et al. 2008). The three clones also had different slopes for the $E_i(g_s)$ relation, probably reflecting different cuticular resistances to water vapour of leaf epidermal surfaces or different abilities of mesophyll cell walls to evaporate water into intercellular spaces (Pallardy 2008). The B × M clone showed the highest instantaneous transpiration rate among clones when drought increased (lower slope), which may come

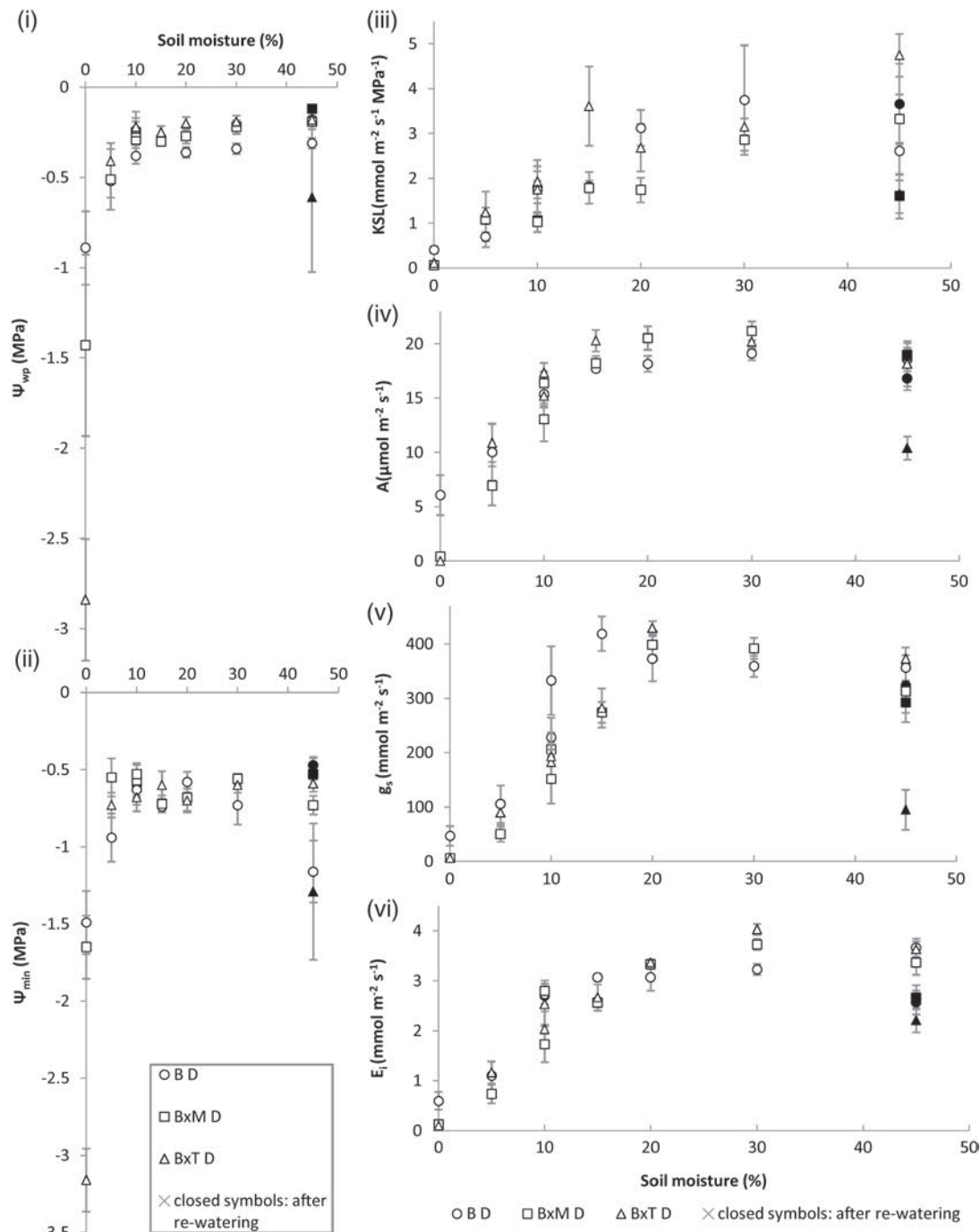


Figure 2. Evolution of pre-dawn leaf water potential (Ψ_{wp}) (i), midday leaf water potential (Ψ_{mid}) (ii), soil to leaf hydraulic conductance (KSL) (iii), net CO_2 assimilation (A) (iv), stomatal conductance (g_s) (v) and instantaneous transpiration (E_i) (vi) along the soil moisture gradient (% of SVWC) for each droughted clone (B D, B x M D and B x T D). Closed symbols indicate the parameters' rates after re-watering. Mean ($n = 3$). Bars denote SE.

from its ability to increase its root biomass and associated water uptake under drought compared with the other clones.

The hybrids were more productive than the pure B clone, with higher TLA, LAR and leaf number, and consequently produced more total biomass. Indeed, TLA is generally closely related to total biomass production and can be considered a determinant of productivity in poplars (Marron et al. 2005). It is also well known that the average volume production of hybrid

poplars can be two to three times greater than the average production of the parental species (Rodén et al. 1990). In addition to lower productive attributes (lower TLA and A), leaf shedding when drought became more severe probably limited the growth potential of clone B after rewatering by decreasing its LA and thus its global photosynthetic capacity (Mazzoleni and Dickmann 1988). However, this strategy of LA (transpiring surface) reduction seemed efficient for recovery after drought,

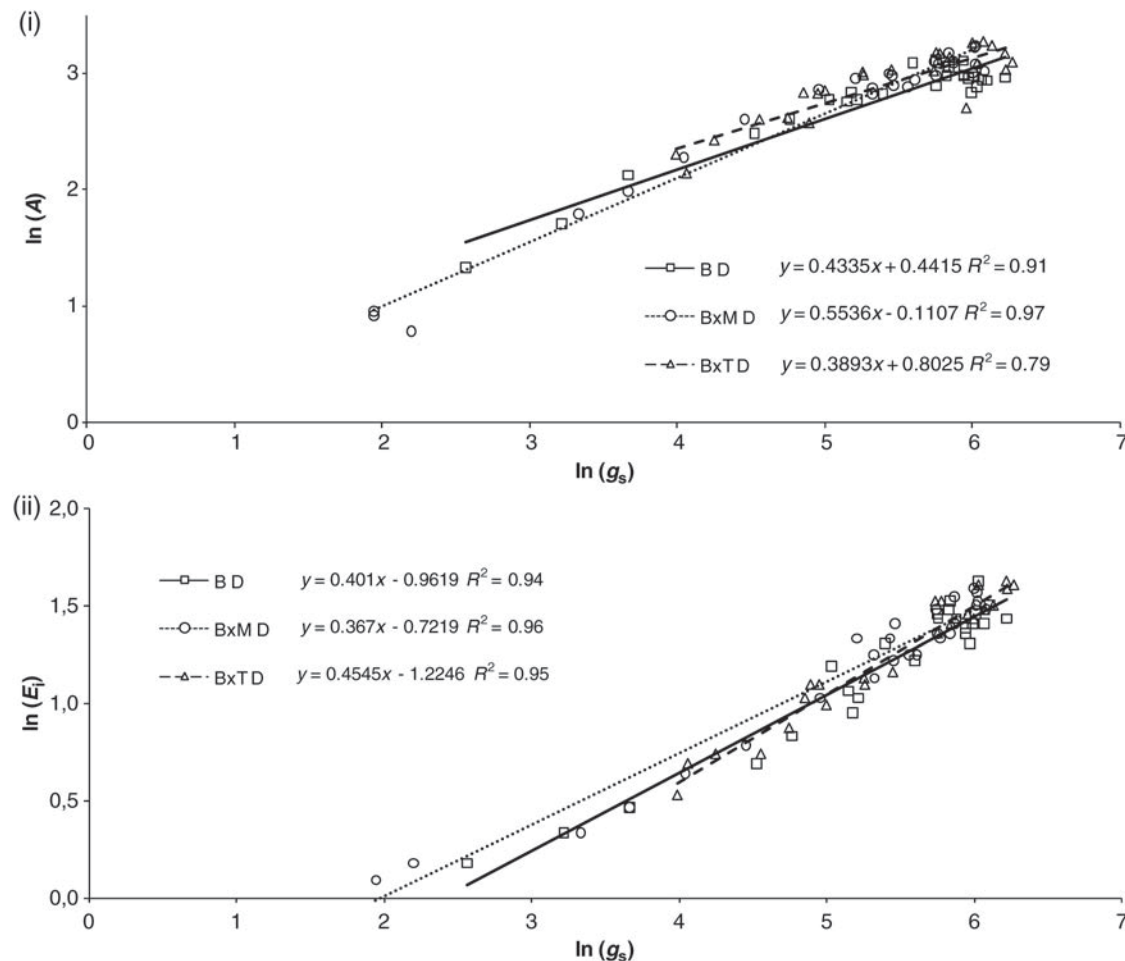


Figure 3. Comparison of the linear relationships $A(g_s)$ (i) and $E_i(g_s)$ (ii) for the three clones that were subjected to the water stress treatment ($n = 80$).

as this clone reached the highest $\Psi_{\min}$ after rewatering. Therefore, this clone may not achieve high productivity either in well-watered or droughted conditions, but it should survive severe drought events.

Among the two hybrids, clone B $\times$ T was more productive than clone B $\times$ M under watered conditions, with greater A values, height and diameter growth, and root biomass. These results are similar to those of Barigah et al. (1994), who reported that the best performing hybrid poplar clones in terms of biomass production had the highest net photosynthetic rates. In our experiment, we found mean values for A of between 17 and 23 $\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for well-watered trees, which is quite high compared with other studies (Tschaplinski and Blake 1989: 9–16 $\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$; Ibrahim et al. 1997: 3–11 $\mu\text{molCO}_2 \text{ m}^{-2} \text{ s}^{-1}$). All these attributes are characteristics of more vigorous clones (Tschaplinski and Blake 1989). However, clone B $\times$ T also showed the least conservative strategy under the drought treatment with highest E_i among clones. While trees from clones B and B $\times$ M showed a decrease in g_s and E_i as soon as SVWC decreased to 30%, these parameters of clone B $\times$ T did not respond to water depletion at this stage.

This behaviour may be inherited from its *P. trichocarpa* parent, as it is adapted to climatic regions dominated by moist Pacific Ocean air (Dickmann 2001). On the other hand, it also had the highest sensitivity of transpiration control by stomatal conductance, with the highest slope for the line $E_i(g_s)$. And its low transpiring to water absorbing surface (low TLA/root biomass ratio) might compensate for its high E_i rate. However, it reached the lowest Ψ values of -3.2 MPa at SVWC = 0%, despite closed stomata and absence of photosynthetic activity. At water potentials lower than -2 MPa, it is likely that severe vessel embolism occurred (Taiz and Zeiger 2006). This could explain why this clone had the lowest recovery for $\Psi_{\min}$, A , E_i and g_s after rewatering, affecting its global photosynthetic capacity and productivity. In conclusion, despite the very high productivity of this clone, its lack of conservative pattern in response to drought caused hydraulic failure. But according to McDowell et al. (2008), it should be the more resistant to long-term drought as it is the most anisohydric of our clones and thus has the highest ability to avoid C starvation under drought not too intense to cause hydraulic failure. However, its extra photosynthate production was not used to increase root

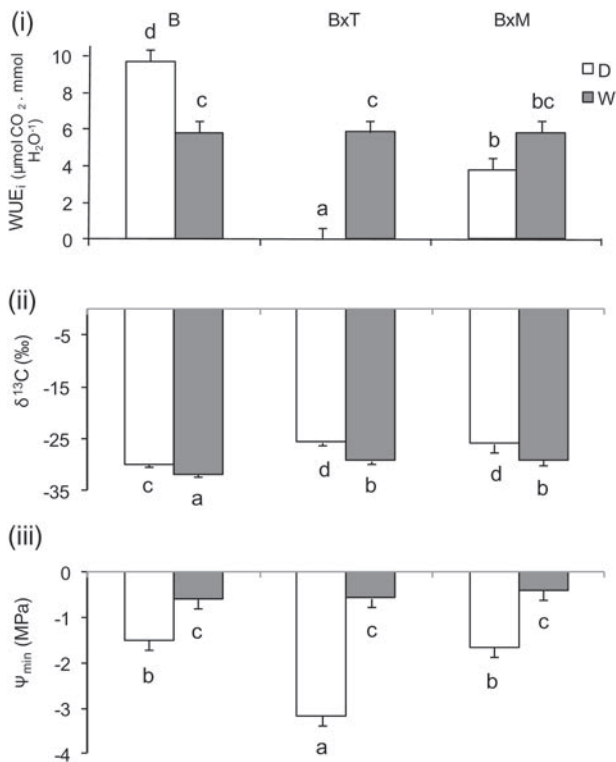


Figure 4. Leaf instantaneous water use efficiency (WUE_i) (i), leaf carbon isotopic composition (δ¹³C) (ii) and minimal leaf water potential (Ψ_{min}) (iii) for each clone (B, B × M and B × T) and watering treatment (W = watered and D = droughted) at maximal stress level (Day 31). Mean ($n \geq 3$). Bars denote SE. Means that do not differ at the 0.05 level are noted with the same letter (a < b < c < d).

biomass (as root biomass decreased under drought) and associated access to additional water resources and should limit its performance under long-term drought (Maseda and Fernandez 2006).

The best performing clone for both productivity and drought adaptation was the B × M clone. The drought treatment did not significantly decrease its total biomass production (26 versus 38 g, $P = 0.31$). Its decrease in the TLA to root biomass ratio in response to drought stress is in agreement with the functional equilibrium model, which states that plants respond to a decrease in below-ground resources with an increased allocation to roots and a decreased allocation to aerial biomass, especially in leaves (Poorter and Nagel 2000). After rewatering, leaf water potentials returned to pre-drought values for this clone, showing good recovery after cavitation despite a low root to leaf specific water conductance (Figure 2). These drought adaptive abilities may come from the *P. maximowiczii* parent of this clone, which is reportedly a drought-hardy species (Blake et al. 1984).

The quick recovery in A for this clone after rewatering (Figure 2iv) suggests that the integrity of the photosynthetic apparatus was maintained, a characteristic of drought resistance, and confirms that the CO₂ limitation through stomatal closure was a

major constraint to A (Liu and Dickmann 1993). Indeed, this clone was the most sensitive for photosynthesis control by stomatal conductance (highest slope for the $A(g_s)$ line). This clone was also the only one to make new leaves after rewatering, allowing new photosynthetic organs to develop and productivity to further increase. As a consequence, this anisohydric clone combined productive and drought-tolerant abilities and would be of prime interest in zones prone to drought events, as well as in well-watered zones.

Ridolfi and Dreyer (1997) found a drought threshold of -0.6 MPa for *Populus robusta*. In our study, we found a similar and sharp decrease in A , g_s and E_i for both hybrids when pre-dawn leaf water potential dropped below -0.5 MPa. At SVWC = 0%, A rates were approaching zero and leaf water potentials were lower than -1.5 MPa for both hybrids, a threshold frequently reported for photosynthesis to stop in poplars (Rhodenbaugh and Pallardy 1993).

Conclusion

Maximum biomass production is essential to profitability of fast-growing plantations. Facing the occurrence of drought, desirable trees should (i) continue to grow despite a mild lack of water and (ii) maintain the integrity of their photosynthetic apparatus in case of severe drought to be able to resume growth when water is once again available. The leaf abscission strategy of the unimproved and native B clone used in this experiment when more severe drought occurred implied a greater loss of productivity after rewatering. The two fast-growing improved hybrids had different strategies in response to drought: the very productive and large-leaved B × T hybrid showed the most anisohydric behaviour, and its use under long-term drought should be of interest. However, the B × M clone should be preferred because it showed better ability to withstand severe drought events with its rather conservative strategy for water in addition to its anisohydric properties.

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