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## **Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes**

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## Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes

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### Key words :

Water use efficiency  
Transpiration efficiency  
Nocturnal transpiration  
Water deficit  
Poplar  
Intraspecific diversity

## Abbreviations :

A: net CO<sub>2</sub> assimilation rate,  
A<sub>mean</sub>: mean of net CO<sub>2</sub> assimilation rate measured *in situ*,  
A<sub>sat</sub>: net CO<sub>2</sub> assimilation rate measured under light-saturated conditions,  
C<sub>i</sub>: CO<sub>2</sub> internal concentration,  
Cumult: cumulated water loss,  
DMincr: total dry mass increment,  
DTR: diurnal transpiration rate,  
δ<sup>13</sup>C: carbon isotope composition,  
FinalH: final stem height,  
FinalD: final stem diameter,  
g: stomatal conductance to water vapour,  
g<sub>m</sub>: mesophyll conductance for CO<sub>2</sub>,  
g<sub>mean</sub>: stomatal conductance to water vapour measured *in situ*,  
g<sub>sat</sub>: stomatal conductance to water vapour measured under light-saturated conditions,  
J<sub>max</sub>: maximum photosynthetic electron flux,  
LA: total leaf area,  
LeafDM: leaf dry mass,  
LeafF: leaf fraction,  
Φ<sub>w</sub>: proportion of unproductive water loss to productive water loss,  
NTR: nocturnal transpiration rate,  
RootF: root fraction,  
StemF: stem fraction,  
TE: whole plant transpiration efficiency,  
TotalDM: total dry mass,  
TR: daily transpiration rate,  
V<sub>Cmax</sub>: maximum CO<sub>2</sub> carboxylation rate,  
WUE: water use efficiency,  
W<sub>i</sub>: leaf intrinsic water use efficiency  
W<sub>isat</sub>: leaf intrinsic water use efficiency measured under light-saturated conditions,  
W<sub>imean</sub>: mean leaf intrinsic water use efficiency measured *in situ*.

## 1 Abstract

2  
3 Poplar plantations, widely used for the production of woody biomass, might be at high risk from the  
4 climate change-induced increase in the frequency of drought periods. Therefore, selecting improved  
5 genotypes, which are highly productive but with a high water use efficiency (WUE), is becoming a  
6 major target. The use of automated weighing systems in controlled environments facilitates the  
7 estimation of cumulated water loss and whole plant transpiration efficiency (TE). Differences in TE and  
8 leaf level intrinsic WUE as well as the contribution of underlying ecophysiological traits were  
9 determined in three contrasting *P. nigra* genotypes. Strong differences in TE among the selected  
10 genotypes were congruent with differences in leaf level intrinsic WUE. Our data show that a high total  
11 leaf area was overcompensated by a low per leaf area transpiration rate, leading to higher TE in highly  
12 productive genotypes originating from cool locations. Nocturnal water loss was relatively low but  
13 contributed to variations in TE among genotypes. In response to drought, leaf level WUE increased  
14 but not TE, suggesting that carbon losses due to whole plant respiration could offset the drought-  
15 induced increase in intrinsic WUE.

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## 18 Highlights

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- The Spanish genotype was less water use efficient than northern genotypes
- Low WUE was linked to a high transpiration rate and a large root system
- A trade-off appeared between total leaf surface and per surface transpiration rate
- Intrinsic WUE was increased by drought but not transpiration efficiency

## 29 1. Introduction

30

31 To limit the increasing global temperature, there is an urgent need to reduce greenhouse gas  
32 emissions coming from fossil fuels. Biofuels which come from dedicated crops and tree plantations  
33 can contribute to meet this target (Sannigrahi et al., 2010) and poplar plantations are widely used for  
34 the production of woody biomass (Navarro et al., 2018). On the other hand, future climate change is  
35 projected to reduce the productivity of plantation forestry in the coming decades through changes in  
36 net primary production (Hanson and Weltzin, 2000). Moreover Domec et al. (2015) suggested that  
37 intensively managed plantations are more drought-sensitive than natural forests. Considering this, as  
38 well as the high vulnerability of poplars to drought-induced cavitation (Fichot et al., 2015), poplar  
39 plantations might be at high risk from the climate change-induced increase in the frequency of  
40 droughts. To meet the worldwide increasing demand of wood biomass in the context of climate  
41 change, selecting improved tree genotypes, which are highly productive but with a high water use  
42 efficiency (WUE), is becoming a major target.

43 At the whole plant level, WUE is called transpiration efficiency (TE) and is defined as the ratio between  
44 the biomass accumulated and the water transpired over a defined period of time. At the leaf level,  
45 WUE is reflected by intrinsic WUE ( $W_i$ ), the ratio between net  $\text{CO}_2$  assimilation rate ( $A$ ) and stomatal  
46 conductance of water vapour ( $g$ ). For any one plant, the relationship between  $A$  and  $g$  is curvilinear,  
47 approaching asymptotically a maximum  $A$  when stomata are fully open. Under optimal watering  
48 conditions, stomata are often more open than required to achieve a maximum  $A$  under the given  
49 atmospheric conditions resulting in "luxurious" water consumption. During an increasing soil water  
50 deficit, this results in stomatal closure affecting  $A$  less than proportionally, thereby increasing  $W_i$  (see  
51 for example Suppl Fig 2 of Marguerit et al., 2014). In the case of large-scale screening of poplar  
52 genotypes for WUE (Kruse et al., 2012; Viger et al., 2013), an indirect estimation of  $W_i$  is often used  
53 by measuring the carbon stable isotope composition ( $\delta^{13}\text{C}$ ) of organic material such as leaf, wood or  
54 extracted cellulose (Bussotti et al., 2015; Farquhar et al., 1982). However, even if  $\delta^{13}\text{C}$  is measured on  
55 wood or extracted cellulose, it still represents a spatio-temporal assimilation-weighted integration of  
56 leaf level processes during daytime ( $A$  and  $g$ ). Therefore,  $\delta^{13}\text{C}$  does not include processes in other  
57 plant parts and those occurring during the night, which can contribute to variations in biomass  
58 accumulation and water loss, and thus TE. These processes relate to respiration of the whole plant  
59 during day and night (except leaves during the daytime as this is included in net  $\text{CO}_2$  assimilation),  
60 water losses from plant organs other than leaves and also water losses from leaves during the night  
61 (Cernusak et al., 2007). Thus, choosing water efficient genotypes for tree plantations on the base of  
62 the whole plant transpiration efficiency could be more judicious than on the more widely used leaf level  
63 estimates ( $\delta^{13}\text{C}$ ,  $W_i$ ). However, the estimation of TE in adult trees in the field is challenging, because  
64 of the difficulties of estimating both the biomass increase, especially that of the root system, and the  
65 water use of a whole tree over long time periods. TE of a single tree can be estimated by an allometric  
66 estimation of aboveground biomass increase and direct sap flow measurements (Navarro et al., 2018).  
67 However, the root biomass increase is ignored and such measurements are not feasible on a large  
68 number of individuals. Biomass increments of potted plants can be more easily assessed and the use

69 of automated weighing systems facilitates the estimation of cumulated water use in controlled  
70 environments. Such systems are either based on multiple balances (Cirelli et al., 2012) or robotic  
71 systems (Buré et al., 2016; Granier et al., 2006) and allow many plants to be weighed at a high  
72 frequency, thus both controlling soil humidity and quantifying water loss. This in turn allows an  
73 accurate estimation of TE and underlying traits as well as comparisons with  $\delta^{13}\text{C}$  or  $W_i$ .

74 Commercial poplar genotypes have been selected primarily for high productivity or resistance to foliar  
75 rust but not for high WUE (Monclus et al., 2006). The lack of correlation between productivity and  $W_i$   
76 across 29 *Populus x canadensis* hybrids suggested that it would be possible to select genotypes  
77 which combine high productivity and high WUE (Monclus et al., 2005). Conversely, a negative  
78 relationship between TE and productivity was found in the Asian species *P. davidiana* (Zhang et al.,  
79 2004), questioning the independence between productivity and WUE. European black poplar (*P. nigra*,  
80 L.) is a key pioneer tree species, essential for the dynamics of riparian habitats and for soil  
81 stabilisation. Further, it has an economic value as a parent pool for genetic breeding of *P. x*  
82 *canadensis* cultivars (Chamaillard et al., 2011; Sow et al., 2018). *P. nigra* has a wide natural  
83 distribution with populations growing in different climatic conditions across Europe and showing  
84 significant genetic differentiation as well as phenotypic variation in growth rate, plant architecture and  
85 leaf size (DeWoody et al., 2015; Viger et al., 2016).

86 To improve our understanding of the determinants of TE and their responses to drought, we  
87 determined TE in three contrasting *P. nigra* genotypes, which originate from different regions and  
88 which strongly differ in terms of growth and leaf morphology (DeWoody et al., 2015; Wildhagen et al.,  
89 2018). Here, we analysed underlying ecophysiological traits as well as leaf level estimators of WUE.  
90 Our first aim was to investigate which traits explained differences in TE among genotypes under  
91 optimal watering conditions. Here we test the following hypotheses i) The differences in TE among  
92 genotypes are driven by transpiration rate rather than by biomass accumulation rate ii) The leaf level  
93 WUE is a main driver of whole plant TE iii) Unproductive water losses may decouple whole plant TE  
94 from leaf level WUE. A second aim was to determine if TE would be changed differently among  
95 genotypes in response to drought, and which underlying traits would drive this acclimation.

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## 99 2. Material and methods

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### 101 2.1. Plant material and growth conditions

102 Three genotypes of *Populus nigra* L., originating from individual trees of natural populations in France  
103 (Drôme 6; FR-6), Italy (La Zelata; IT1) and Spain (Ebro 2; SP-2) (DeWoody et al., 2015) and showing  
104 different leaf morphology were studied in controlled conditions (Fig. 1). Mean temperatures and  
105 precipitations of the three locations are provided in Supp Table 1. Growth, gas exchange and TE were  
106 measured on a subset of plants grown as part of the experiment described by Wildhagen et al. (2018),  
107 with six replicates per genotype x treatment. Briefly, woody cuttings were obtained from clonal  
108 propagation and were planted in 10 l plastic pots filled with a 1:1 (v/v) mixture of peat and sand,  
109 amended with a slow release fertiliser (4 g l<sup>-1</sup> of Nutricote T100, 13:13:13 NPK and micronutrients;

110 FERTIL S.A.S, Boulogne Billancourt, France) and  $1 \text{ g l}^{-1} \text{ CaMg}(\text{CO}_3)_2$ . Plants were grown in two  
111 compartments of a glasshouse located at Champenoux, France ( $48^\circ 45' 09.3'' \text{N}$ ,  $6^\circ 20' 27.6'' \text{E}$ ), under  
112 natural light conditions with daily maxima of irradiance ranging from 150 to  $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$   
113 photosynthetically active radiation (PAR, Fig. 2). Environmental conditions in the greenhouse were  
114 affected by weather conditions, but the temperature was maintained between 15 and  $26^\circ \text{C}$  (Fig. 2).  
115 After planting, plants were watered 2–4 times a day –according to plant size and weather conditions–  
116 to 85% of field capacity with an automated weighing and watering system (Buré et al., 2016). The  
117 position of plants in the greenhouse was rotated at each weighing event.

118

## 119 *2.2. Control of water deficit*

120 After six weeks of growth, plants of each genotype were randomly assigned to either a control or a  
121 drought treatment for five weeks (day 0 was 21 May 2013). Control plants were watered to 85% soil  
122 relative extractable water content ( $\text{REW}_{\text{soil}}$ ) by the automated system for the whole experiment.  
123  $\text{REW}_{\text{soil}}$  of control plants oscillated between 65 and 85% (data not shown). For drought-treated plants,  
124  $\text{REW}_{\text{soil}}$  was progressively decreased to reach 20% in two weeks and then maintained at this target  
125 level for the following three weeks (Fig. 2).

126 The control of the available soil water content (SWC) was based on a calibration between volumetric  
127 SWC measured by Time Domain Reflectometry (Trime Pico-32, IMKO) and pot weight. Target weights  
128 were defined individually for each pot, and were updated every day during the first two weeks to  
129 control the SWC decrease (Fig. 2) and were corrected for plant biomass increment using allometric  
130 relationships once a week. Each plant was thus submitted to the same stress level, irrespective of  
131 plant size and water consumption. Available water was expressed as soil relative extractable water  
132 content ( $\text{REW}_{\text{soil}}$ ), which is defined as:

$$133 \quad \text{REW}_{\text{soil}} = \left( \frac{\text{SWC} - \text{SWC}_{\text{wiltingpoint}}}{\text{SWC}_{\text{fieldcapacity}} - \text{SWC}_{\text{wiltingpoint}}} \right) \times 100\%,$$

134 with SWC at wilting point = 3%; SWC at field capacity = 32%.

135

## 136 *2.3. Growth, gas exchange, transpiration rate and transpiration efficiency*

### 137 *Height and diameter*

138 Plant height was measured from the soil surface to the shoot apex twice per week. The stem base  
139 was photographed with a ruler attached to the stem for scale calibration, twice per week. Stem  
140 diameter was measured from picture analysis with ImageJ (Schneider et al, 2012).

### 141 *Total leaf area*

142 For each genotype, a relationship between leaf area and maximal leaf width was built from a sample  
143 of approximately 80 leaves taken from the full range of leaf sizes. Regression coefficients were over  
144 0.98 for each of the three genotypes. The width of all leaves of each plant was measured once a week  
145 and converted to area using the established relationships. Individual leaf areas were summed to  
146 calculate the total leaf area (LA) of each plant. Spline adjustment (interspline function, R) was used to  
147 estimate LA for dates in between days of measurement.

### 148 *Dry biomass*



149 At the end of the experiment, all plants were harvested. For each plant, the cutting, stem, roots and  
150 leaves were separated, dried at 70 °C for 48 h and weighed. Growth allocation was estimated through  
151 the calculation of root, leaf and stem biomass fractions (root biomass, leaf biomass and stem biomass  
152 over total biomass, respectively).

153

#### 154 *Gas exchange and intrinsic water use efficiency*

155 Gas exchange was measured *in situ* in the greenhouse. Net CO<sub>2</sub> assimilation (A) and stomatal  
156 conductance to water vapour (g) were measured using two inter-calibrated portable photosynthesis  
157 systems LI-COR 6200 (LI-COR® Inc, Lincoln, NE, USA). Measurements were performed on the  
158 youngest fully expanded mature leaves at the beginning of the experiment, corresponding to the 8th–  
159 10th leaf down from the first apical leaf, between 11:00–12:00 twice a week over the five week-  
160 experiment. Intrinsic water use efficiency at the leaf level (Wi) was calculated as the ratio of A/g. A, g  
161 and Wi were averaged over the five last measurement days corresponding to the steady drought  
162 period during the three last weeks (A<sub>mean</sub>, g<sub>mean</sub> and Wi<sub>mean</sub>, respectively) and these means were used  
163 for the ANOVA (Tables 1 and 2).

164 We also estimated the photosynthetic capacity by measuring gas exchange under light-saturated  
165 conditions, with calibrated Li-6400 XT portable gas analyzers (LI-COR® Inc, Lincoln, NE, USA) 4-6  
166 days before the harvest. CO<sub>2</sub> concentration was 400 μmol mol<sup>-1</sup>, light intensity (PAR) was 1500  
167 μmol m<sup>-2</sup> s<sup>-1</sup> and block temperature was 25 °C. All measurements were performed in the corridor next  
168 to the greenhouse compartments, on the same leaf used for *in situ* gas exchange. For each plant, the  
169 same procedure was followed. We waited for stomatal conductance to reach a steady state (typically  
170 after 20–30 min), then the A-C<sub>i</sub> (C<sub>i</sub>: CO<sub>2</sub> internal concentration) curve was performed by changing the  
171 [CO<sub>2</sub>] entering the leaf chamber with the following steps: 400, 300, 250, 200, 150, 100, 50, 400, 400,  
172 500, 600, 700, 800, 1000, 1200 and 1500 μmol mol<sup>-1</sup>, typically with 2–3 min between each step.  
173 Maximum carboxylation rate (V<sub>Cmax</sub>), maximum electron transport rate (J<sub>max</sub>) and mesophyll  
174 conductance (g<sub>m</sub>) were estimated with the method by Ethier and Livingston (2004) that fits A-C<sub>i</sub> curves  
175 with a non-rectangular hyperbola version of Farquhar's biochemical model of leaf photosynthesis  
176 (Farquhar et al., 1980). This is based on the hypothesis that g<sub>m</sub> reduces the curvature of the Rubisco-  
177 limited portion of an A-C<sub>i</sub> response curve. The Rubisco kinetic traits and specificity for CO<sub>2</sub>/O<sub>2</sub> were  
178 characterized *in vitro* as described previously (Galmes et al., 2014). The values of the Rubisco  
179 Michaelis-Menten constants for CO<sub>2</sub> (K<sub>c</sub>), and O<sub>2</sub> (K<sub>o</sub>) and the chloroplast CO<sub>2</sub> compensation point (Γ\*)  
180 were obtained at 15, 25 and 35 °C and adjusted to the measured temperature using the Arrhenius  
181 function (see details on Rubisco kinetic traits and specificity for CO<sub>2</sub>/O<sub>2</sub> in the supplementary material  
182 and methods).

183

#### 184 *Transpiration rates*

185 Daily transpiration rate (TR) was calculated on a daily basis as the ratio between the water loss over  
186 24 h and LA on that day, and then averaged over the whole experimental period. Days 17, 18, 26, 27,  
187 28, 29 were used to calculate a mean diurnal transpiration rate (DTR) and a mean nocturnal  
188 transpiration rate (NTR), using the ratio between water loss during the 05:00–22:00 period and the

189 following 22:00–05:00 period, respectively, and LA. The 22:00–05:00 was chosen as a period of full  
190 darkness (astronomic sunset to sunrise). The proportion of unproductive water loss to productive  
191 water loss  $\Phi_w$  (Farquhar et al., 1989) was estimated as  $\Phi_w = \text{NTR} \cdot 9 / (\text{DTR} \cdot 15)$  as the unproductive time  
192 (civil sunset to sunrise) was approximately 9 h during the experiment.

193

#### 194 *Transpiration efficiency*

195 Transpiration efficiency (TE) was calculated as the ratio between the biomass gain (final total dry  
196 biomass – mean initial total dry biomass) and the cumulative water loss over the experiment period.  
197 For each genotype, the mean initial total dry biomass was estimated on a separate set of four plants  
198 harvested at day 0 (4.2 g, 6.2 g and 4.0 g for the French, Italian and Spanish genotypes, respectively)

199

#### 200 *$\delta^{13}\text{C}$ determination*

201 The first leaf that had completely developed during the drought stress (mature at the harvest time) was  
202 harvested for carbon isotope analysis; dried for 48 h in an oven at 70 °C and ground into a fine  
203 powder. Subsamples of 1 mg  $\pm$  0.1 mg were weighed into tin capsules. The carbon isotopic  
204 composition was measured with a coupled isotope ratio mass spectrometer (Thermo-Finnigan; Delta  
205 S, Bremen, Germany).  $\delta^{13}\text{C}$  was calculated according to the international standard (Vienna Pee Dee  
206 Belemnite, VPDB) using the following equation:  $\delta^{13}\text{C} = (\text{Rs} - \text{Rstd}) / \text{Rstd} \times 1000$ , where Rs and Rstd  
207 are the isotopic ratios  $^{13}\text{C}/^{12}\text{C}$  of the sample and the standard, respectively. The precision of  
208 spectrometric analysis (standard deviation of  $\delta^{13}\text{C}$ ) was assessed with a calibrated, internal laboratory  
209 reference material with a matrix close to the measured samples (oak leaves, n = 16, SD = 0.05 ‰).

210

#### 211 *2.4. Statistical analyses*

212 All statistical analyses were performed with R (R Core Team, 2018). All data-sets were tested for  
213 outliers using the generalized ESD test (Extreme Studentized Deviate, Rosner and Bernard, 1983).  
214 Only outliers for which evidence for analytical errors were found were actually removed from the  
215 analyses.

216 A two-way ANOVA model with interaction was run for traits in Tables 1 and 2, using genotype and  
217 treatment as factors and type III sum of squares (Anova function of the car library). As a large number  
218 of variables were tested, the model significance was adjusted using False Discovery Rate (p-adjust  
219 function with the "fdr" option). Significant differences among factor levels were computed using  
220 Tukey's Highest Significant Difference test (HSD.test function of the agricolae package). Normality of  
221 the residuals was tested using Shapiro-Wilk test (shapiro.test function). Variables that showed a  
222 Shapiro-Wilk test with  $p < 0.05$  were transformed using the boxCox function (car package). Then the  
223 above described ANOVA was run again for all transformed variables and the significance levels were  
224 compared with those of untransformed variables. Only one result changed, the interaction for  $W_{i\text{mean}}$   
225 became significant (0.036 for transformed versus 0.060 for untransformed), therefore we presented  
226 the results of untransformed variables. The correlation analysis was conducted with the cor function  
227 using the Pearson method and the matrix was ordered according to the first principal component axis.

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### 3. Results

#### 3.1. Genotype differences

We tested the influence of genotype and drought on poplar traits by two-way ANOVA. We did not find significant genotype x drought interactions for 26 out of 28 variables tested (Table 1). Therefore, differences between genotypes are presented based on overall means. After 11 weeks of growth, the development of the three genotypes differed significantly. The Spanish genotype was much smaller in height, stem diameter and biomass than the other two genotypes (Table 1). This difference in height was the result of a smaller growth rate of the Spanish genotype ( $2.5 \text{ cm day}^{-1}$ ) compared to those of the French and Italian genotypes ( $3\text{--}3.5 \text{ cm day}^{-1}$ ) (Fig supp 1). The differences in stem diameter growth rates between genotypes were smaller than those of height growth rates (Fig supp 1). In addition, the Spanish genotype had many branches (more than the French whereas the Italian had none, data not shown) and many leaves, but it showed the smallest total leaf area (LA) due to much smaller leaves (Table 1, Fig 1, Fig supp 2). The relative allocation of growth to the roots was another important difference between genotypes: the root fraction (RootF) of the Spanish genotype was higher than that of the French genotype, which was higher than that of the Italian genotype (Table 1).

These differences in growth were accompanied by differences in ecophysiological traits. The Italian genotype had the lowest daily transpiration rate (TR,  $1.70 \text{ kg m}^{-2} \text{ day}^{-1}$ ), diurnal transpiration rate (DTR,  $126 \text{ g m}^{-2} \text{ h}^{-1}$ ) and nocturnal transpiration rate (NTR,  $3.9 \text{ g m}^{-2} \text{ h}^{-1}$ ) and also the lowest proportion of unproductive water loss to productive water loss ( $\Phi_w$ , 1.9 %) (Fig. 3, Table 1). The Spanish genotype showed a very high TR ( $2.66 \text{ kg m}^{-2} \text{ day}^{-1}$ ) and DTR ( $193 \text{ g m}^{-2} \text{ h}^{-1}$ ), in accordance with a significantly higher stomatal conductance ( $g_{\text{mean}}$ ,  $0.88 \text{ mol m}^{-2} \text{ s}^{-1}$ ), and a very high NTR ( $13.5 \text{ g m}^{-2} \text{ h}^{-1}$ ) and  $\Phi_w$  (4.2 %) (Fig. 3, Table 1). However, the Spanish genotype had a very small LA, resulting in a significantly lower cumulative water loss over the experiment (CumulT) than those of the two other genotypes (Table 1).

Traits related to gas exchange measured in optimal conditions ( $V_{C_{\text{max}}}$ ,  $J_{\text{max}}$ ,  $A_{\text{sat}}$ ,  $g_{\text{sat}}$ ,  $g_m$ ,  $C_i$ ,  $W_{i_{\text{sat}}}$ ) or *in situ* ( $A_{\text{mean}}$ ,  $g_{\text{mean}}$ ,  $W_{i_{\text{mean}}}$ ) were similar in the French and the Italian genotypes (Table 1). The Spanish genotype showed higher  $g_m$ ,  $g_{\text{sat}}$ ,  $g_{\text{mean}}$  and  $A_{\text{mean}}$  (and a tendency for higher  $A_{\text{sat}}$ ) compared to the two other genotypes (Table 1).

The three genotypes differed significantly in whole plant transpiration efficiency (TE), which was corroborated by the integrated leaf level intrinsic WUE as estimated by  $\delta^{13}\text{C}$  (Table 1). The Italian genotype had a higher TE and  $\delta^{13}\text{C}$  than the French, which had a much higher TE and  $\delta^{13}\text{C}$  than the Spanish. There were no significant differences among genotypes in instantaneous WUE ( $W_{i_{\text{sat}}}$  and  $W_{i_{\text{mean}}}$ ), but the trait values showed a similar gradient as for TE across genotypes, confirming that the Spanish genotype had the lowest WUE.

#### 3.2 Drought effect

The drought stress was applied for five weeks by reducing soil REW to 20%. Stress level was moderate so that drought-exposed trees still grew but at a reduced rate (Table 1, Table 2, Fig Supp 1). Drought significantly reduced the growth rate in height of the French genotype as early as day 8, while

270 this reduction in growth rate occurred later for the Spanish and the Italian genotypes (at day 11 and  
271 15, respectively; Supp Fig 1). Stem diameter growth was also reduced but it seemed less sensitive  
272 than stem height growth in the French and Italian genotypes (-30 % for diameter growth rate versus -  
273 40% for height growth rate) and more sensitive for the Spanish genotype (-40% versus -30%) (Supp  
274 Fig 1). For all genotypes, the decrease of stem diameter growth became significant from day 15.  
275 Although only few significant genotype x environment interactions were detected in the ANOVA, post-  
276 hoc Tukey's HSD tests suggested some species-specific drought responses. The total dry mass  
277 tended to be less reduced under drought in the Italian genotype (-20%), compared to that of the  
278 Spanish and French (-34 and -38%) (Table 2). Growth allocation was also differentially affected by  
279 drought among genotypes. In particular, the Italian genotype maintained allocation to roots during  
280 drought so that its root dry mass was not affected and its RootF increased (Table 2). The leaf fraction  
281 of the French genotype was reduced but not its RootF, whereas allocation was not changed in the  
282 Spanish genotype (Table 2). Drought reduced LA in all three genotypes, but the effect was most  
283 pronounced in the French genotype (Table 1, Supp Fig 2). Drought also reduced the total leaf number  
284 of the Spanish and French genotypes (Supp Fig 2). In the Italian genotype, drought reduced LA but  
285 not the number of leaves, indicating that leaf growth rate was more sensitive than leaf production rate  
286 by the meristem (Supp Fig 2).

287 The moderate drought level applied here did not significantly affect the following leaf traits:  
288 photosynthetic capacity ( $V_{cmax}$ ,  $J_{max}$ ), mesophyll conductance to  $CO_2$  ( $g_m$ ) and net  $CO_2$  assimilation  
289 rate ( $A_{sat}$  and  $A_{mean}$ ). By contrast, stomatal conductance decreased under drought as compared to  
290 well-watered conditions ( $g_{sat}$  and  $g_{mean}$ ) (Table 1). TR, DTR, NTR and  $\Phi_w$  ( $p=0.054$ ) were also strongly  
291 decreased. Consequently, the cumulative water loss (CumulT) was lowered under drought. The  
292 estimates of intrinsic water use efficiency ( $W_{i,sat}$ ,  $W_{i,mean}$ ,  $\delta^{13}C$ ) indicated a significant increase of WUE  
293 at the leaf level by 35%. By contrast, TE did not respond to drought as the biomass accumulation  
294 (DMincr) and CumulT were similarly affected within each genotype (Table 1, Table 2). However,  
295 DMincr and CumulT were more reduced by drought in the French and the Spanish genotypes  
296 (approximately -40%) compared to the Italian genotype (-22%).

### 297 298 3.3 Correlations

299 A correlation analysis based on individual data highlighted, in both well-watered and drought  
300 conditions, that TE strongly correlated with  $\delta^{13}C$  ( $R = 0.88$  and  $0.90$  for control and drought,  
301 respectively, Fig. 4, Fig. 5A), but the relationship was weaker with  $W_{i,sat}$  ( $R = 0.26$  and  $0.62$ ) and  $W_{i,mean}$   
302 ( $R = 0.42$  and  $0.22$ ) (Fig. 4). TE correlated more strongly with DMincr ( $R = 0.83$  and  $0.94$ ) than with  
303 CumulT ( $R = 0.56$  and  $0.83$ ) (Fig. 4). TE was also positively correlated with dry mass accumulation  
304 rate (DMinc/36 days) and was related negatively with the transpiration rates (daily, diurnal and  
305 nocturnal), and with  $g_{mean}$  to a lesser extent (Fig 4, 5B and 5C). Traits related to photosynthetic  
306 capacity and assimilation rate were weakly related to TE and DMincr under control conditions, but they  
307 were slightly negatively related under drought. It is also noticeable that transpiration rates (TR, DTR  
308 and NTR) were highly negatively correlated to traits related to LA and overall biomass accumulation,  
309 and positively correlated to the relative investment in roots (RootF) and to  $g_{mean}$  (Fig 4).  $\Phi_w$  and NTR  
310 were positively correlated to TR and negatively to TE (Fig 4 and 5D).

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#### 4. Discussion

##### ***Transpiration efficiency differs strongly among genotypes***

Transpiration efficiency (TE) is a long term whole plant measure of WUE, estimated as the ratio between biomass accumulation and water loss over time. TE variations can originate from different processes, daytime leaf processes such carbon assimilation rate and stomatal conductance but also from unproductive water losses such as nocturnal transpiration, and from carbon losses such as respiration of non-photosynthetic organs. In this study, we measured TE and traits related to TE in three contrasting *P. nigra* genotypes. We found a strong genotype effect for TE, where the Italian genotype showed the highest value (5.2 g kg<sup>-1</sup>) and the Spanish genotype the lowest value (3.3 g kg<sup>-1</sup>). This TE range was similar to that found in the same French and Italian genotypes in an earlier study (4.9 and 5.4 g kg<sup>-1</sup>, respectively; Durand et al., 2019) and in other *P. nigra* genotypes grown under high vapour pressure deficit (3.1 to 5.9 g kg<sup>-1</sup>) (Rasheed et al., 2015).

##### ***Differences in transpiration rate and in the proportion of unproductive water loss explain the genotypic differences in TE***

The genotypic differences in TE were corroborated by the integrated measure of leaf level intrinsic WUE ( $\delta^{13}\text{C}$ ), and by instantaneous measurements ( $W_{\text{isat}}$ ,  $W_{\text{imean}}$ ) although differences were not significant. Guet et al. (2015) tested genotypes from geographically close populations and also found higher WUE ( $\delta^{13}\text{C}$ ) for Italian genotypes compared to French genotypes grown in a plantation with fertile soil and wet conditions. By contrast, Viger et al. (2016) compared *P. nigra* genotypes coming from geographically close French, Italian and Spanish populations in a greenhouse experiment and did not find similar differences of carbon isotope discrimination among these populations.

The strong correlation between TE and  $\delta^{13}\text{C}$ , indicates that a significant part of the differences in TE among plants were driven by leaf level processes. Similarly strong correlations were found for *P. nigra* by Rasheed et al. (2015) and for *P. deltoides x nigra* crosses by Guo et al. (2011) and Rasheed et al. (2013). The weak difference in photosynthetic traits that we observed did not explain the genotypic differences in TE whereas the higher stomatal conductance of the Spanish genotype could clearly explain its low TE. Also all three transpiration rates, TR (day scale), DTR (diurnal) and NTR (nocturnal), correlated strongly and negatively with TE, indicating that the water efficient genotypes were transpiring less per leaf area, during the day as well as during the night. The French and Spanish genotypes with high NTR showed more negative values of predawn leaf water potential under control conditions (Supp Table 2), suggesting that the equilibration of the water potential between plant and soil was less complete for these genotypes than for the Italian one, which could be due to high NTR. The nocturnal transpiration represents an unproductive water loss (Farquhar et al., 1989) and therefore, in theory, impacts TE independently from leaf level WUE (Cernusak et al., 2007). Interestingly, the proportion of unproductive water loss to productive water loss ( $\Phi_w$ ) also correlated strongly and negatively with TE. Indeed the Italian genotype transpired proportionally less during the



351 night than the other tested genotypes, and showed the highest TE. In our *P. nigra* experiment,  $\Phi_w$   
352 ranged from 1.9% to 4.2%, which is a similar range to estimations for tropical tree species (1.2% to  
353 5.2%; Cernusak et al., 2009) or for another plantation tree species such as *Eucalyptus grandis* (5%,  
354 Benyon, 1999). Higher  $\Phi_w$  (9 to 30%) were found for other poplar species (Cirelli et al., 2016; Rohula  
355 et al., 2014), indicating that nocturnal transpiration was relatively low in *P. nigra*, and that a gain in TE  
356 due to reduced  $\Phi_w$  would be small. However, it may be that introgressing *P. nigra* into other *Populus*  
357 species could reduce nocturnal water losses and increase TE. Differences in  $\Phi_w$  were more closely  
358 related to differences in NTR than in DTR, suggesting that stomatal regulation during the night was  
359 partly independent from daytime regulation. Maintaining a significant nocturnal transpiration might  
360 enhance nutrient acquisition (Kupper et al., 2012), prevent a build-up of CO<sub>2</sub> within the leaves (Marks  
361 and Lechowicz, 2007), or facilitate a fast increase of net photosynthesis during early morning (Dawson  
362 et al., 2007).

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### 364 **Genotypic differences in TE are related to origin and biomass allocation**

365 The three studied genotypes were chosen as representative of contrasting populations in terms of  
366 individual leaf size and of location in Europe: the Italian genotype had the largest leaves and the  
367 Spanish genotype the smallest leaves and a smaller LA than the two other genotypes. The observed  
368 strong correlation between TE and DM<sub>incr</sub> was mainly due to the smaller plant size and the lower TE  
369 of the Spanish genotype. The observed difference in plant size is in accordance with Viger (2011),  
370 who showed higher growth and larger leaves for *P. nigra* from central Europe with relatively wet  
371 climate (such as the Italian genotype) compared to trees from regions with hot and dry Mediterranean  
372 summers (such as the Spanish genotype). Also in hybrid poplars, individual leaf area was a good  
373 predictor of growth rate and productivity (Marron et al., 2007). Our data suggest that the genotypes  
374 with a high growth rate, a high individual leaf area and a high total LA showed a much lower per leaf  
375 area transpiration rate, reducing total water loss, and resulting in a higher TE.

376 The variation of TE, TR and growth rate among the genotypes appears coherent with the climatic  
377 gradient across their region of provenance. A high transpiration rate is expected to lead to a strong  
378 leaf cooling effect, which could be advantageous for plants growing in hot climates and having access  
379 to water. The constitutively higher investment into roots as compared to leaves by the Spanish  
380 genotype (higher RootF) is consistent with a higher TR and thus supports the hypothesis of a higher  
381 water flow requirement due to a hotter and drier climate. Overall, the differences in biomass allocation  
382 among the genotypes resulted in the observed strong negative correlation between TE and RootF.  
383 Similarly, it was shown for different provenances of *Castanea sativa*, that ecotypes from regions with  
384 low precipitation and higher mean temperature had lower intrinsic WUE (Lauteri et al., 1997) and a  
385 deeper rooting pattern (personal comm. M. Lauteri). Other *C. sativa* populations from drought prone  
386 sites also showed lower intrinsic WUE, and lower growth and total biomass (Lauteri et al., 2004; Pliura  
387 and Eriksson, 2002). Also a maritime pine ecotype originating from a dry and hot location in Morocco  
388 (Tamjoute) had a lower growth rate and a lower intrinsic WUE than two ecotypes from wet and cooler  
389 locations in France (Landes, Porto-Vecchio) (Guehl et al., 1995). Overall, the leaf cooling effect of  
390 transpiration and the high carbon allocation to the root system might be an adaptive strategy resulting

391 in lower TE for ecotypes from hot environments where deep water is available. Using neutral markers  
392 and phenotypic measurements, DeWoody et al. (2015) showed that isolation by distance played a  
393 major role in the differentiation among the western European *P. nigra* populations. In addition, they  
394 showed that adaptive differentiation also occurred for small-leaf populations from the Mediterranean  
395 area, supporting the idea that the Spanish specificities could result from local adaptation.

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### 397 ***Drought increased intrinsic WUE but not TE***

398 Globally, the three genotypes responded similarly to drought and we found only two significant  
399 genotype  $\times$  drought interactions in the statistical model ( $\delta^{13}\text{C}$  and NTR). As expected, biomass of all  
400 compartments decreased, or tended to decrease, under drought. Growth was clearly more sensitive  
401 than assimilation rate. Consequently, growth limitation was independent from carbon supply, as  
402 already found in other poplar species (Bogeat-Triboulot et al., 2007; Cohen et al., 2010). However,  
403 growth allocation showed some genotype specific patterns. The French genotype decreased mainly  
404 the leaf fraction, as found in a previous study (Durand et al., 2019). The Italian genotype, which  
405 showed the least reduction in biomass under drought, decreased mainly the stem fraction. These  
406 responses differ from those recorded in the same genotype in previous experiments (Durand et al.,  
407 2019; Viger et al., 2016), suggesting a strong plasticity of biomass allocation.

408 The drought-induced increase in leaf level WUE ( $W_{i\text{mean}}$ ,  $\delta^{13}\text{C}$ ) was most likely due to a decrease in  
409 stomatal conductance, mainly observable in the *in situ* measurements, whereas neither photosynthetic  
410 capacity nor assimilation rates were significantly changed. Increased  $\delta^{13}\text{C}$  and thus increased intrinsic  
411 WUE under drought due to stomatal closure is a classical response in plants showing luxurious water  
412 consumption in well watered conditions, as shown for different poplar species (Monclus et al., 2006;  
413 Viger et al., 2016). In addition, changes in intrinsic WUE seemed genotype dependent: genotype  $\times$   
414 drought interaction was significant for  $\delta^{13}\text{C}$  and almost significant for  $W_{i\text{mean}}$  ( $p_{\text{int}} = 0.060$ ). The French  
415 genotype showed a significant and greater increase in leaf level WUE, linked to a relatively stronger  
416 reduction in  $g_{\text{mean}}$  than in the two other genotypes. These results suggest differences in stomatal  
417 regulation among these genotypes, as already found in a shorter drought experiment (Durand et al.,  
418 2019).

419 Surprisingly, TE was not increased under drought in any genotype, indicating that there were other  
420 factors apart from leaf level processes, which negated the effects of improved intrinsic WUE on TE.  
421 One such process could be the nocturnal water loss, which decouples leaf level from whole plant  
422 water use efficiency (Cernusak et al., 2007). However the observed reduction in NTR and  $\Phi_w$  under  
423 drought should have had a positive effect on TE and did therefore not offset the increased leaf intrinsic  
424 WUE. Another factor decoupling whole plant from leaf level WUE could be carbon losses other than  
425 day respiration by leaves (Cernusak et al., 2007). An increased whole plant respiration under drought  
426 would decrease TE and therefore would offset the increase of leaf intrinsic WUE. Published effects of  
427 water deficit on respiration are not consistent, from inhibition to stimulation (Brito et al., 2018; Flexas et  
428 al., 2005) and here leaf respiration after 30 minutes in darkness was not affected by drought (P-value  
429 = 0.41; data not shown). However whole plant carbon losses might depend also on leaf fraction. The  
430 observed decrease in leaf fraction under drought, which was stronger in the French genotype, implies

431 an increase in the fraction of only respiring organs, which should in turn increase the whole plant  
432 respiration and contribute to offset the drought-induced increase of intrinsic WUE. This hypothesis is  
433 also congruent with the highest TE of the Italian genotype, which had the highest LA and should  
434 therefore have lower carbon losses by whole plant respiration relative to its size.

435

#### 436 **Conclusions**

437 Strong differences in TE among the selected genotypes were congruent with differences in WUE at  
438 the leaf level. Our data suggest that a high total leaf area is offset by a low per leaf area transpiration  
439 rate, leading to higher TE in highly productive genotypes from cool locations. Nocturnal water loss  
440 contributes to variations in TE but are relatively low in *P. nigra*, reducing the possibility to improve TE  
441 in this species by selecting genotypes with low  $\Phi_w$ . However,  $\Phi_w$  has been shown to be much higher  
442 for other poplar species, and introgression of black poplar might provide a gain in nocturnal water  
443 losses. Our data also suggest that carbon losses due to whole plant respiration might contribute to the  
444 TE differences among genotypes and could offset the drought-induced increase in intrinsic WUE.  
445 Future studies should include measurements of respiratory carbon losses of different plant organs.

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#### 448 **Conflict of interest statement**

449 The authors have no conflicts of interest to declare.

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#### 451 **Author contributions**

452 MBBT, OB, HW, CD, DLT, IH, HKS, JF, AP, and GT conceived the original research plans. MBBT,  
453 CB, CD, JF, HW, PAC, TG, DLT, OB, HKS, AM and JG performed the greenhouse experiment and the  
454 analytical measurements. MBBT, HW, HKS, CD, IH, DLT, MD, AM, JG and OB analysed the data;  
455 MBBT and OB wrote the article with contributions of all authors. All authors approved the final version  
456 of the manuscript.

457

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### 631 **Figure legends:**

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633 **Figure 1:** A) general view of the plants in the greenhouse on day seven. B, C and D) Pictures of  
634 typical plants of the Italian, French and Spanish genotypes, respectively, on day 21. Mean individual  
635 leaf area was calculated as the ratio between total leaf area and leaf number on day 28 (mean  $\pm$  s.e.,  
636 n=6).

637

638 **Figure 2:** A. Minimum and maximum temperature in the greenhouse (dotted black and plain black  
639 lines, respectively), mean PAR radiation over 8:00 to 19:00 (red line) and mean soil relative  
640 extractable water in the drought-subjected plants (blue line) over the 5 week-experiment.

641

642 **Figure 3:** Daily transpiration rate of the three genotypes under well-watered conditions (black circle)  
643 and under drought (white circle) over the 5 week-experiment. Mean PAR radiation over 8:00 to 19:00  
644 (red dotted line). Mean  $\pm$  s.e, n=6.

645

646 **Figure 4:** Correlations between traits in control plants (upper part) and in drought-subjected plants  
647 (lower parts) (n=18 for each subplot). Only significant correlations were displayed (P-value<0.05).

648  $A_{\text{mean}}$ : mean of net CO<sub>2</sub> assimilation rate measured *in situ*,  $A_{\text{sat}}$ : net CO<sub>2</sub> assimilation rate measured  
649 under light-saturated conditions,  $C_i$ : CO<sub>2</sub> internal concentration, CumulT: cumulated water loss,  
650 DMincr: total dry mass increment, DTR: diurnal transpiration rate,  $\delta^{13}\text{C}$ : carbon isotope composition,  
651 FinalH: final stem height, FinalD: final stem diameter,  $g_m$ : mesophyll conductance for CO<sub>2</sub>,  $g_{\text{mean}}$ :  
652 stomatal conductance to water vapour measured *in situ*,  $g_{\text{sat}}$ : stomatal conductance to water vapour  
653 measured under light-saturated conditions,  $J_{\text{max}}$ : maximum photosynthetic electron flux, LA: total leaf  
654 area, LeafF: leaf fraction,  $\Phi_w$ : proportion of unproductive water loss to productive water loss, NTR:  
655 nocturnal transpiration rate, RootF: root fraction, TE: whole plant transpiration efficiency, TotalDM:  
656 total dry mass, TR: daily transpiration rate,  $V_{\text{cmax}}$ : maximum CO<sub>2</sub> carboxylation rate,  $W_{\text{isat}}$ : leaf intrinsic  
657 water use efficiency measured under light-saturated conditions,  $W_{\text{imean}}$ : mean leaf intrinsic water use  
658 efficiency measured *in situ*.

659

660 **Figure 5:** Correlation between whole plant transpiration efficiency (TE) and A) carbon isotope  
661 composition ( $\delta^{13}\text{C}$ ), B) daily transpiration rate (TR), C) biomass increment (DMincr) and D) proportion  
662 of unproductive water loss to productive water loss ( $\Phi_w$ ). Each point corresponds to a plant. The blue,  
663 green and red symbols denote the French, the Italian and the Spanish genotypes, respectively.  
664 Closed and open symbols denotes control and drought treatments, respectively. Internal whiskers  
665 represent s.e., external whiskers represent 95% confidence interval.

666  
667  
668 **Table1: Results of Two-way ANOVA of different traits.** Significance and adjusted correlation  
669 coefficient of the model, significance of the factors (genotype and drought) and of the interaction.  
670 Marginal mean  $\pm$  s.e. are given for the three genotypes and for the treatments. Different letters denote  
671 significant differences between groups according to Tukey post-hoc tests.

672  $A_{\text{mean}}$ : mean of net  $\text{CO}_2$  assimilation rate measured *in situ*,  $A_{\text{sat}}$ : net  $\text{CO}_2$  assimilation rate measured  
673 under light-saturated conditions,  $C_i$ :  $\text{CO}_2$  internal concentration, CumulT: cumulated water loss,  
674 DMincr: total dry mass increment, DTR: diurnal transpiration rate,  $\delta^{13}\text{C}$ : carbon isotope composition,  
675 FinalH: final stem height, FinalD: final stem diameter,  $g_m$ : mesophyll conductance for  $\text{CO}_2$ ,  $g_{\text{mean}}$ :  
676 stomatal conductance to water vapour measured *in situ*,  $g_{\text{sat}}$ : stomatal conductance to water vapour  
677 measured under light-saturated conditions,  $J_{\text{max}}$ : maximum photosynthetic electron flux, LA: total leaf  
678 area, LeafF: leaf fraction,  $\Phi_w$ : proportion of unproductive water loss to productive water loss, NTR:  
679 nocturnal transpiration rate, RootF: root fraction, TE: whole plant transpiration efficiency, TotalDM:  
680 total dry mass, TR: daily transpiration rate,  $V_{c\text{max}}$ : maximum  $\text{CO}_2$  carboxylation rate,  $W_{i\text{sat}}$ : leaf intrinsic  
681 water use efficiency measured under light-saturated conditions,  $W_{i\text{mean}}$ : mean leaf intrinsic water use  
682 efficiency measured *in situ*.

683  
684 **Table 2: Complement of Table 1.** Mean  $\pm$  s.e. of different traits within each genotype x treatment  
685 group (n=4 - 6). Different letters denote significant difference between groups according to Tukey post-  
686 hoc tests. Acronyms are identical to those in Table 1.

## 691 **Supplementary material**

692  
693 **Supplementary material and methods:** Rubisco kinetic traits and specificity for  $\text{CO}_2/\text{O}_2$   
694 characterisation

695 **Supplementary Table 1:** Climatic data at the locations of the three populations

696 **Supplementary Table 2:** Predawn leaf water potential (MPa) of the three poplar genotypes

697 **Supplementary Figure 1:** Growth rate in height and in stem diameter of the three genotypes over the  
698 5-week experiment.

699 **Supplementary Figure 2:** Leaf number and total leaf surface area of the three genotypes over the 5-  
700 week experiment.

701 **Supplementary Figure 3:** Net CO<sub>2</sub> assimilation rate, stomatal conductance and intrinsic water use  
702 efficiency over the 5-week experiment.

703



Table 1

		model		Factors												
		model	R2	Genot.	Drought	G x D	French		Italian		Spanish		Control		Drought	
TE	g kg <sup>-1</sup>	***	0.85	***	n.s.	n.s.	<b>4.6 ± 0.1</b>	b	<b>5.2 ± 0.1</b>	a	<b>3.3 ± 0.1</b>	c	<b>4.4 ± 0.2</b>	a	<b>4.4 ± 0.2</b>	a
δ <sup>13</sup> C	‰	***	0.85	***	*	*	<b>-31.2 ± 0.2</b>	b	<b>-29.6 ± 0.2</b>	a	<b>-32.8 ± 0.1</b>	c	<b>-31.5 ± 0.3</b>	b	<b>-30.9 ± 0.4</b>	a
Wi <sub>sat</sub>	μmol mol <sup>-1</sup>	*	0.24	n.s.	**	n.s.	<b>66.3 ± 5.9</b>	a	<b>75.8 ± 7.8</b>	a	<b>56.0 ± 6.7</b>	a	<b>55.8 ± 5.0</b>	b	<b>76.3 ± 5.3</b>	a
Wi <sub>mean</sub>	μmol mol <sup>-1</sup>	n.s.	0.15	n.s.	**	n.s.	<b>32.2 ± 4.4</b>	a	<b>33.2 ± 2.4</b>	a	<b>28.7 ± 2.2</b>	a	<b>26.7 ± 1.5</b>	b	<b>36.0 ± 3.0</b>	a
CumULT	kg	***	0.61	***	***	n.s.	<b>12.9 ± 1.3</b>	a	<b>12.2 ± 0.7</b>	a	<b>8.5 ± 1.0</b>	b	<b>13.5 ± 0.8</b>	a	<b>8.9 ± 0.6</b>	b
TR	kg m <sup>-2</sup> day <sup>-1</sup>	***	0.91	***	***	n.s.	<b>1.96 ± 0.07</b>	b	<b>1.70 ± 0.03</b>	c	<b>2.66 ± 0.07</b>	a	<b>2.25 ± 0.11</b>	a	<b>1.92 ± 0.09</b>	b
DTR	g m <sup>-2</sup> h <sup>-1</sup>	***	0.87	***	***	n.s.	<b>148 ± 7</b>	b	<b>126 ± 5</b>	c	<b>193 ± 8</b>	a	<b>175 ± 8</b>	a	<b>136 ± 7</b>	b
NTR	g m <sup>-2</sup> h <sup>-1</sup>	***	0.88	***	***	**	<b>8.9 ± 0.7</b>	b	<b>3.9 ± 0.2</b>	c	<b>13.4 ± 1.0</b>	a	<b>10.4 ± 1.2</b>	a	<b>7.0 ± 0.8</b>	b
Φ <sub>w</sub>	%	***	0.75	***	n.s.	n.s.	<b>3.6 ± 0.2</b>	a	<b>1.9 ± 0.1</b>	b	<b>4.2 ± 0.2</b>	a	<b>3.4 ± 0.3</b>	a	<b>3.0 ± 0.2</b>	a
FinalH	m	***	0.75	***	***	n.s.	<b>1.46 ± 0.06</b>	a	<b>1.24 ± 0.04</b>	b	<b>1.03 ± 0.03</b>	c	<b>1.36 ± 0.06</b>	a	<b>1.14 ± 0.04</b>	b
FinalD	mm	***	0.72	***	***	n.s.	<b>11.4 ± 0.3</b>	a	<b>10.4 ± 0.3</b>	b	<b>8.4 ± 0.6</b>	c	<b>11.0 ± 0.4</b>	a	<b>9.2 ± 0.4</b>	b
TotalDM	g	***	0.77	***	***	n.s.	<b>73.5 ± 6.0</b>	a	<b>79.2 ± 4.1</b>	a	<b>34.9 ± 4.2</b>	b	<b>73.1 ± 6.0</b>	a	<b>53.0 ± 5.2</b>	b
DMincr	g	***	0.77	***	***	n.s.	<b>69.3 ± 5.9</b>	a	<b>73.0 ± 3.9</b>	a	<b>30.8 ± 4.2</b>	b	<b>68.2 ± 5.8</b>	a	<b>48.1 ± 5.1</b>	b
LA	m <sup>2</sup>	***	0.63	***	***	n.s.	<b>0.32 ± 0.03</b>	a	<b>0.31 ± 0.02</b>	a	<b>0.20 ± 0.03</b>	b	<b>0.34 ± 0.02</b>	a	<b>0.22 ± 0.02</b>	b
LeafF	g g <sup>-1</sup>	***	0.67	***	**	n.s.	<b>0.347 ± 0.009</b>	c	<b>0.422 ± 0.006</b>	a	<b>0.393 ± 0.006</b>	b	<b>0.398 ± 0.007</b>	a	<b>0.376 ± 0.011</b>	b
RootF	g g <sup>-1</sup>	***	0.68	***	*	n.s.	<b>0.143 ± 0.004</b>	b	<b>0.129 ± 0.004</b>	c	<b>0.175 ± 0.004</b>	a	<b>0.144 ± 0.006</b>	a	<b>0.152 ± 0.005</b>	a
V <sub>Cmax</sub>	μmol m <sup>-2</sup> s <sup>-1</sup>	n.s.	-0.06	n.s.	n.s.	n.s.	<b>170 ± 11</b>	a	<b>154 ± 12</b>	a	<b>171 ± 11</b>	a	<b>166 ± 10</b>	a	<b>164 ± 9</b>	a
J <sub>max</sub>	μmol m <sup>-2</sup> s <sup>-1</sup>	n.s.	0.04	n.s.	n.s.	n.s.	<b>171 ± 8</b>	a	<b>176 ± 5</b>	a	<b>184 ± 9</b>	a	<b>170 ± 6</b>	a	<b>183 ± 5</b>	a
g <sub>m</sub>	mol m <sup>-2</sup> s <sup>-1</sup>	***	0.46	***	n.s.	n.s.	<b>0.42 ± 0.06</b>	b	<b>0.37 ± 0.07</b>	b	<b>0.90 ± 0.11</b>	a	<b>0.49 ± 0.07</b>	a	<b>0.58 ± 0.10</b>	a
A <sub>sat</sub>	μmol m <sup>-2</sup> s <sup>-1</sup>	n.s.	0.08	n.s.	n.s.	n.s.	<b>21.1 ± 0.6</b>	a	<b>19.5 ± 1.6</b>	a	<b>24.3 ± 1.8</b>	a	<b>21.7 ± 1.1</b>	a	<b>21.6 ± 1.4</b>	a
g <sub>sat</sub>	mol m <sup>-2</sup> s <sup>-1</sup>	*	0.27	*	*	n.s.	<b>0.35 ± 0.03</b>	b	<b>0.31 ± 0.05</b>	b	<b>0.49 ± 0.06</b>	a	<b>0.44 ± 0.04</b>	a	<b>0.33 ± 0.04</b>	b
C <sub>i</sub>	μmol mol <sup>-1</sup>	*	0.23	n.s.	**	n.s.	<b>272 ± 9</b>	a	<b>259 ± 12</b>	a	<b>287 ± 11</b>	a	<b>288 ± 8</b>	a	<b>256 ± 8</b>	b
A <sub>mean</sub>	μmol m <sup>-2</sup> s <sup>-1</sup>	*	0.21	**	n.s.	n.s.	<b>18.2 ± 1.4</b>	b	<b>16.1 ± 0.9</b>	b	<b>24.4 ± 2.5</b>	a	<b>20.1 ± 1.7</b>	a	<b>19.1 ± 1.5</b>	a
g <sub>mean</sub>	mol m <sup>-2</sup> s <sup>-1</sup>	***	0.47	***	**	n.s.	<b>0.68 ± 0.06</b>	b	<b>0.53 ± 0.03</b>	b	<b>0.88 ± 0.07</b>	a	<b>0.79 ± 0.05</b>	a	<b>0.60 ± 0.05</b>	b

Comment citer ce document :

Bogeat-Triboulot, M.-B., Buré, C., Gerardin, T., Chuste, P.-A., Le Thiec, D., Hummel, I., Durand, M., Wildhagen, H., Douthe, C., Molins, A., Galmés, J., Smith, H., Flexas, J., Polle, A., Taylor, G., Brendel, O. (2019). Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes.

Table 2 :

		French control		French drought		Italian control		Italian drought		Spanish control		Spanish drought	
TE	g kg <sup>-1</sup>	<b>4.61 ±</b>	0.07 b	<b>4.64 ±</b>	0.20 ab	<b>5.25 ±</b>	0.13 a	<b>5.23 ±</b>	0.11 a	<b>3.47 ±</b>	0.14 c	<b>3.07 ±</b>	0.17 c
δ <sup>13</sup> C	‰	<b>-31.84 ±</b>	0.07 c	<b>-30.54 ±</b>	0.19 b	<b>-29.74 ±</b>	0.34 ab	<b>-29.37 ±</b>	0.29 a	<b>-32.78 ±</b>	0.22 cd	<b>-32.89 ±</b>	0.22 d
Wi <sub>sat</sub>	μmol mol <sup>-1</sup>	<b>54.6 ±</b>	7.5 b	<b>77.9 ±</b>	6.4 ab	<b>59.8 ±</b>	7.9 ab	<b>91.7 ±</b>	9.1 a	<b>53.6 ±</b>	11.2 b	<b>58.9 ±</b>	7.1 ab
Wi <sub>mean</sub>	μmol mol <sup>-1</sup>	<b>21.9 ±</b>	1.4 b	<b>42.4 ±</b>	6.6 a	<b>31.6 ±</b>	2.8 ab	<b>34.9 ±</b>	4.1 ab	<b>26.5 ±</b>	1.7 ab	<b>30.8 ±</b>	4.1 ab
CumulT	kg	<b>16.4 ±</b>	1.0 a	<b>9.4 ±</b>	1.1 c	<b>13.8 ±</b>	0.9 ab	<b>10.6 ±</b>	0.6 bc	<b>10.3 ±</b>	1.3 bc	<b>6.3 ±</b>	0.6 c
TR	kg m <sup>-2</sup> day <sup>-1</sup>	<b>2.13 ±</b>	0.06 c	<b>1.80 ±</b>	0.08 d	<b>1.80 ±</b>	0.02 d	<b>1.60 ±</b>	0.03 d	<b>2.82 ±</b>	0.07 a	<b>2.46 ±</b>	0.05 b
DTR	g m <sup>-2</sup> h <sup>-1</sup>	<b>166.0 ±</b>	6.0 b	<b>129.5 ±</b>	7.2 cd	<b>141.5 ±</b>	2.2 c	<b>109.7 ±</b>	4.5 d	<b>216.7 ±</b>	5.8 a	<b>168.5 ±</b>	4.7 b
NTR	g m <sup>-2</sup> h <sup>-1</sup>	<b>10.9 ±</b>	0.3 b	<b>6.9 ±</b>	0.5 c	<b>4.4 ±</b>	0.3 cd	<b>3.4 ±</b>	0.2 d	<b>16.0 ±</b>	1.1 a	<b>10.8 ±</b>	0.9 b
Φ <sub>w</sub>	%	<b>4.0 ±</b>	0.2 ab	<b>3.3 ±</b>	0.3 b	<b>1.9 ±</b>	0.1 c	<b>1.9 ±</b>	0.1 c	<b>4.5 ±</b>	0.3 a	<b>3.9 ±</b>	0.3 ab
FinalH	m	<b>1.63 ±</b>	0.04 a	<b>1.29 ±</b>	0.07 bc	<b>1.35 ±</b>	0.05 b	<b>1.13 ±</b>	0.03 cd	<b>1.09 ±</b>	0.03 cd	<b>0.97 ±</b>	0.05 d
FinalD	mm	<b>12.2 ±</b>	0.2 a	<b>10.6 ±</b>	0.3 ab	<b>11.1 ±</b>	0.4 ab	<b>9.7 ±</b>	0.3 b	<b>9.6 ±</b>	0.7 b	<b>7.1 ±</b>	0.3 c
TotalDM	g	<b>88.6 ±</b>	4.9 a	<b>58.3 ±</b>	6.3 bc	<b>88.1 ±</b>	5.5 a	<b>70.4 ±</b>	3.4 ab	<b>42.5 ±</b>	5.7 cd	<b>25.7 ±</b>	3.0 d
DMincr	g	<b>84.4 ±</b>	4.7 a	<b>54.2 ±</b>	6.2 bc	<b>81.7 ±</b>	5.1 a	<b>64.3 ±</b>	3.1 ab	<b>38.6 ±</b>	5.6 cd	<b>21.5 ±</b>	2.8 d
LA	m <sup>2</sup>	<b>0.41 ±</b>	0.03 a	<b>0.23 ±</b>	0.03 cd	<b>0.36 ±</b>	0.02 ab	<b>0.27 ±</b>	0.01 bc	<b>0.25 ±</b>	0.04 cd	<b>0.14 ±</b>	0.02 d
LeafF	g g <sup>-1</sup>	<b>0.369 ±</b>	0.008 b	<b>0.325 ±</b>	0.012 c	<b>0.426 ±</b>	0.010 a	<b>0.417 ±</b>	0.007 a	<b>0.397 ±</b>	0.007 ab	<b>0.388 ±</b>	0.011 ab
RootF	g g <sup>-1</sup>	<b>0.140 ±</b>	0.006 bc	<b>0.146 ±</b>	0.007 b	<b>0.117 ±</b>	0.002 c	<b>0.141 ±</b>	0.004 b	<b>0.174 ±</b>	0.006 a	<b>0.176 ±</b>	0.005 a
V <sub>Cmax</sub>	μmol m <sup>-2</sup> s <sup>-1</sup>	<b>164 ±</b>	22 a	<b>176 ±</b>	9 a	<b>171 ±</b>	17 a	<b>137 ±</b>	16 a	<b>164 ±</b>	17 a	<b>179 ±</b>	16 a
J <sub>max</sub>	μmol m <sup>-2</sup> s <sup>-1</sup>	<b>163 ±</b>	13 a	<b>179 ±</b>	8 a	<b>173 ±</b>	8 a	<b>179 ±</b>	8 a	<b>175 ±</b>	12 a	<b>195 ±</b>	12 a
g <sub>m</sub>	mol m <sup>-2</sup> s <sup>-1</sup>	<b>0.36 ±</b>	0.08 b	<b>0.48 ±</b>	0.08 b	<b>0.46 ±</b>	0.11 b	<b>0.26 ±</b>	0.06 b	<b>0.74 ±</b>	0.17 ab	<b>1.07 ±</b>	0.08 a
A <sub>sat</sub>	μmol m <sup>-2</sup> s <sup>-1</sup>	<b>20.7 ±</b>	1.0 ab	<b>21.5 ±</b>	0.8 ab	<b>22.0 ±</b>	1.0 ab	<b>17.1 ±</b>	2.8 b	<b>22.6 ±</b>	2.8 ab	<b>26.2 ±</b>	1.8 a
g <sub>sat</sub>	mol m <sup>-2</sup> s <sup>-1</sup>	<b>0.41 ±</b>	0.04 ab	<b>0.29 ±</b>	0.03 ab	<b>0.41 ±</b>	0.05 ab	<b>0.21 ±</b>	0.05 b	<b>0.49 ±</b>	0.09 a	<b>0.50 ±</b>	0.07 a
C <sub>i</sub>	μmol mol <sup>-1</sup>	<b>291 ±</b>	12 a	<b>253 ±</b>	10 a	<b>282 ±</b>	13 a	<b>235 ±</b>	13 a	<b>291 ±</b>	18 a	<b>282 ±</b>	11 a
A <sub>mean</sub>	μmol m <sup>-2</sup> s <sup>-1</sup>	<b>17.3 ±</b>	1.1 ab	<b>19.2 ±</b>	2.7 ab	<b>17.7 ±</b>	0.6 ab	<b>14.5 ±</b>	1.5 b	<b>25.2 ±</b>	4.6 a	<b>23.6 ±</b>	2.4 ab
g <sub>mean</sub>	mol m <sup>-2</sup> s <sup>-1</sup>	<b>0.83 ±</b>	0.05 ab	<b>0.54 ±</b>	0.05 bc	<b>0.61 ±</b>	0.02 bc	<b>0.45 ±</b>	0.04 c	<b>0.92 ±</b>	0.11 a	<b>0.83 ±</b>	0.10 ab

Comment citer ce document :

Bogeat-Triboulot, M.-B., Buré, C., Gerardin, T., Chuste, P.-A., Le Thiec, D., Hummel, I., Durand, M., Wildhagen, H., Douthe, C., Molins, A., Galmés, J., Smith, H., Flexas, J., Polle, A., Taylor, G., Brendel, O. (2019). Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes.





**Figure 1**

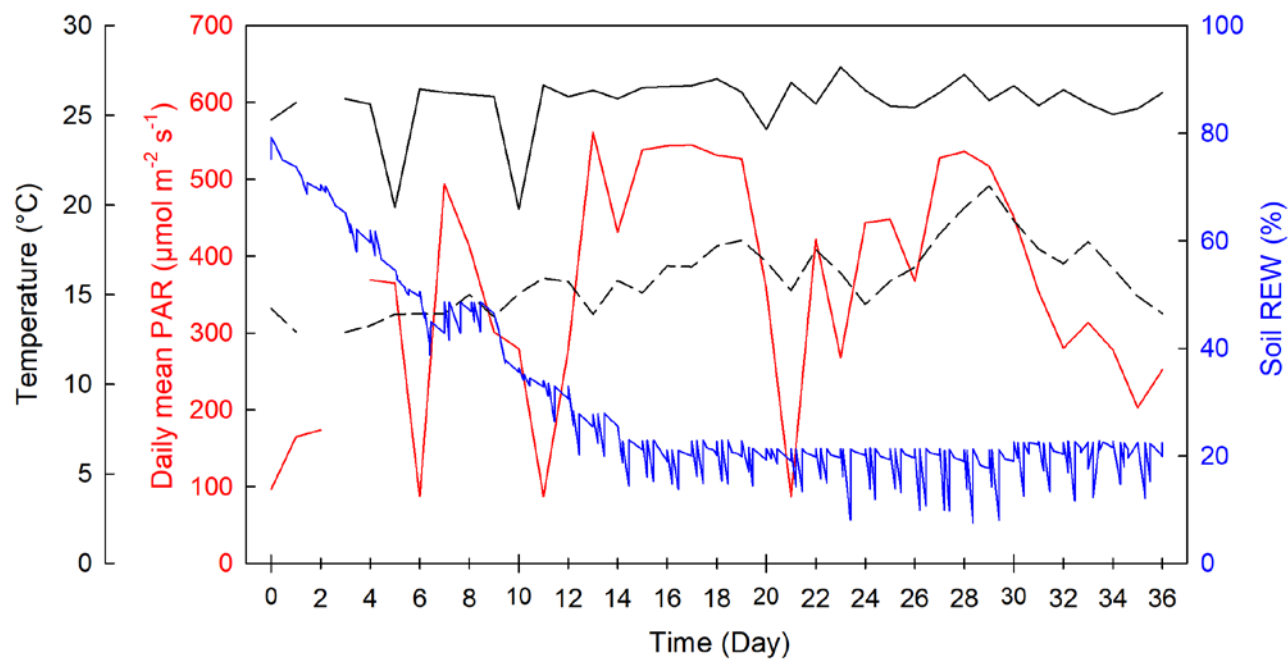


Figure 2

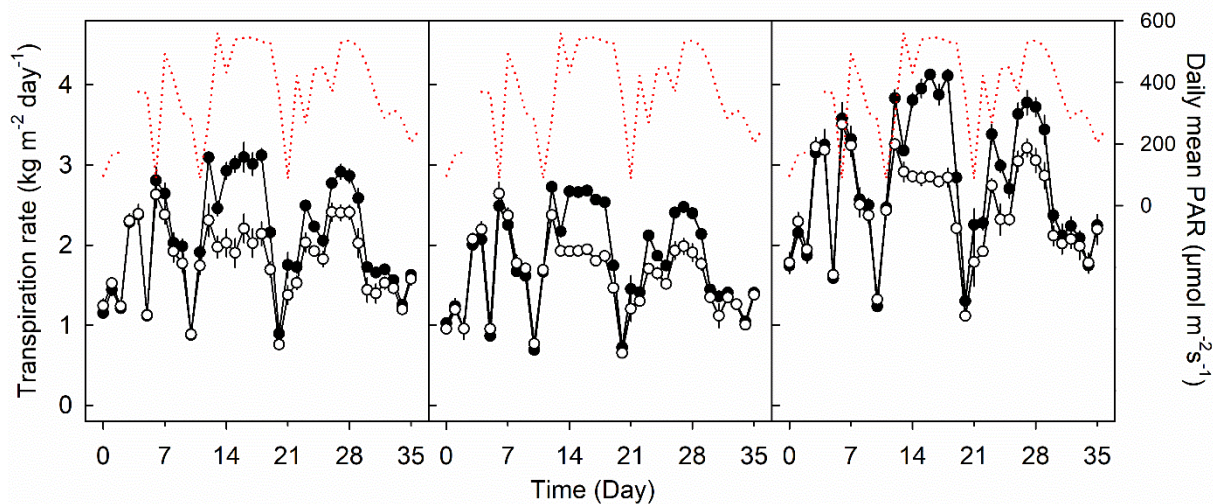
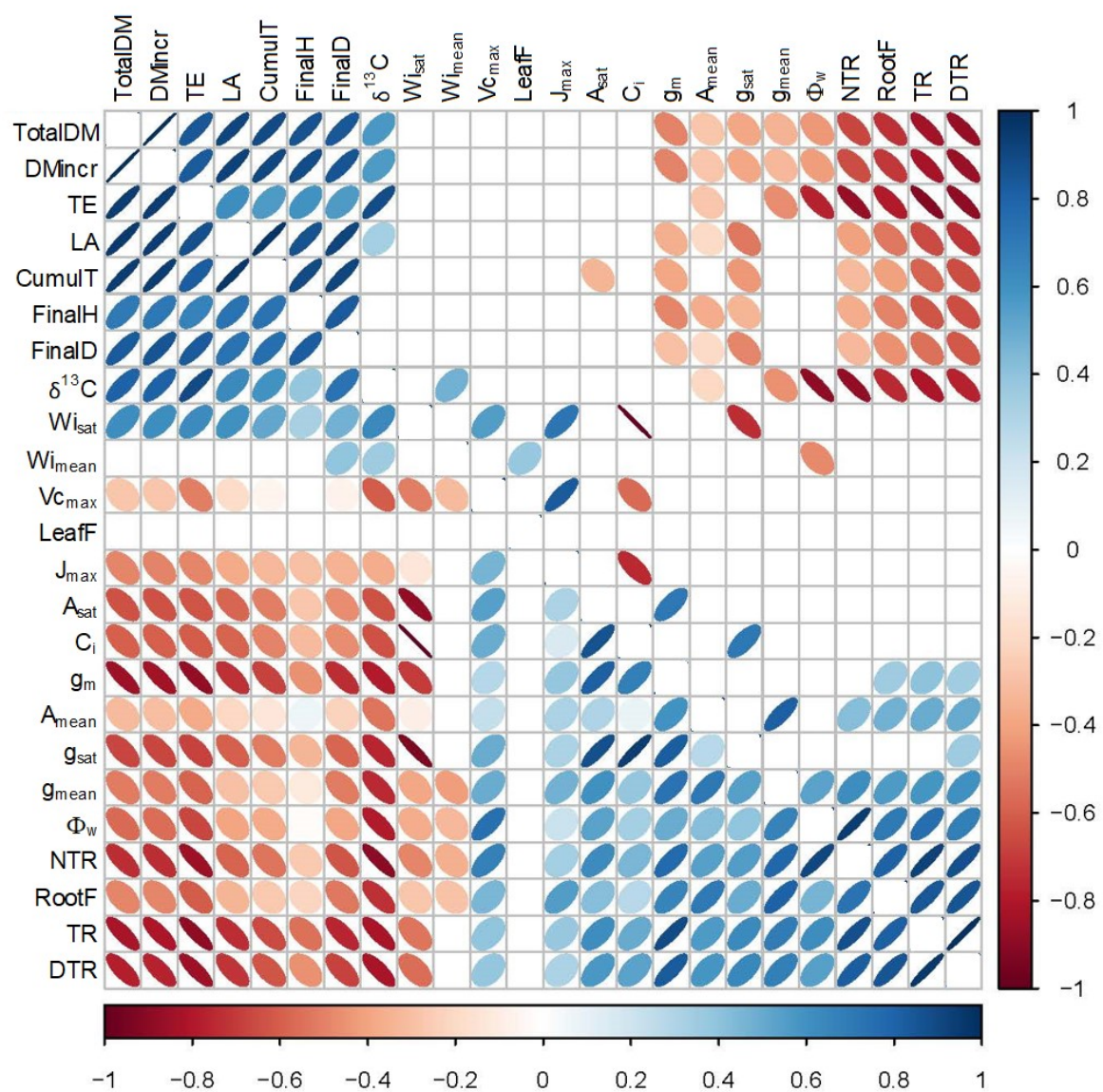


Figure 3





**Figure 4**

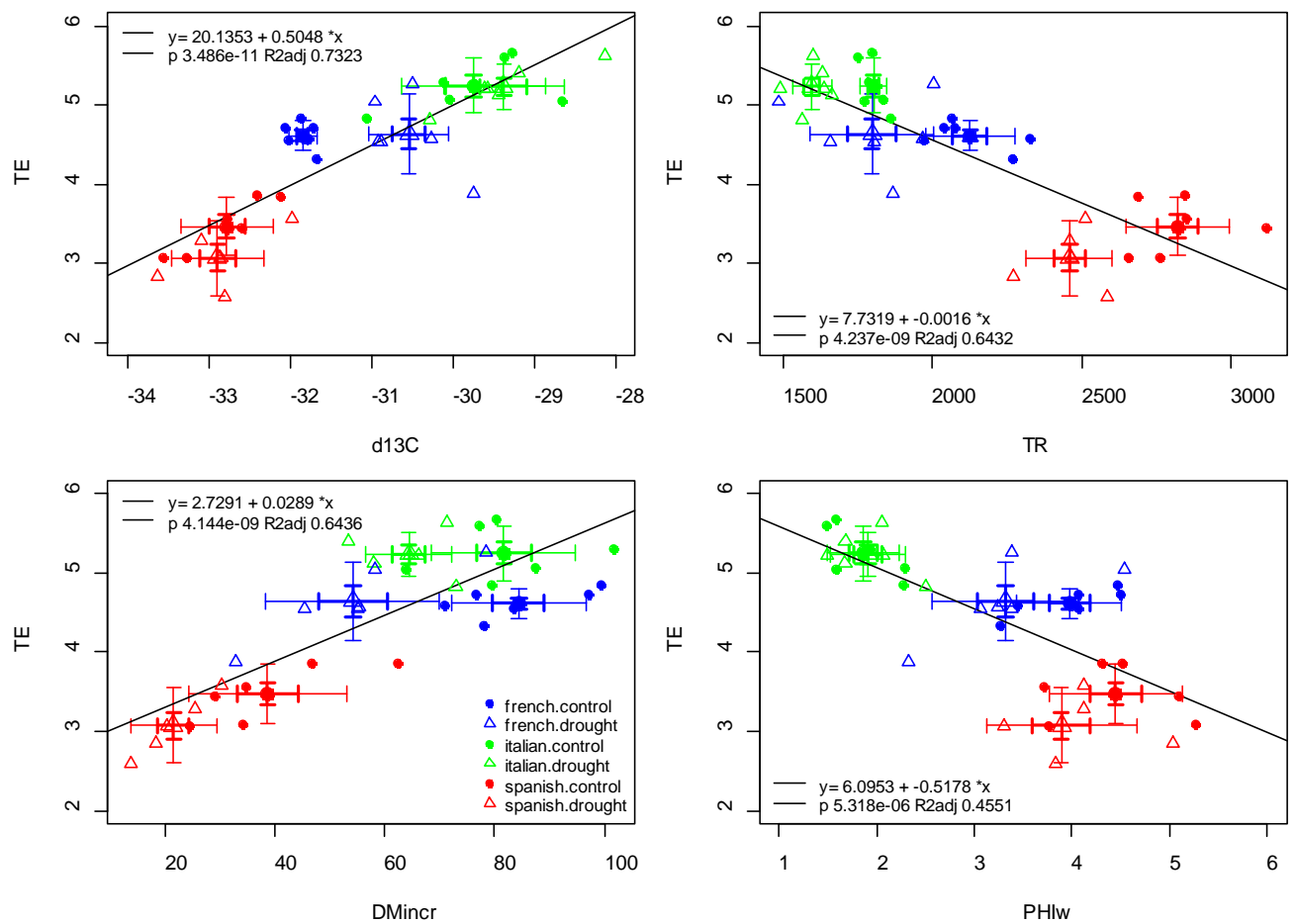


Figure 5

# Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes

Bogeat-Triboulot MB et al, 2019

<https://doi.org/10.1016/j.envexpbot.2019.05.021>

## Supplementary data

### Supplementary Material and Methods: Rubisco characterisation

#### 1. Sequence of the Rubisco large subunit gene (*rbcL*)

Total genomic DNA was isolated from leaf sample of the three genotypes independently and purified using the DNeasy™ Plant Minikit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The primers used for amplification and sequencing are listed in Table 1.

Primer	Sequence	Reference	Amplification	Sequencing
esp_2_F	ATGAGTTGTAGGGAGGGAC	Hermida <i>et al.</i> 2016	x	
414_R	CAAATCCTCCAGACGTAGAGC	Chen <i>et al.</i> 1998		x
991_R	CGGTACCAGCGTGAATATGAT	Chen <i>et al.</i> 1998		x
1494_R	GATTGGGCCGAGTTTAATTAC	Chen <i>et al.</i> 1998	x	x

Table 1: List of the primers used for amplification and sequencing.

PCR reactions were performed in 50 µl using EmeraldAmp GT PCR Master Mix (Takara, Shiga, Japan). PCR program for amplifications comprised initial cycle at 94°C for 2 min, 55°C for 30 s, 72°C for 4 min, followed by 30 cycles of 94°C for 30 s 56°C for 45 s and 72°C for 1 min, and a final elongation at 72°C for 5 min. Amplifications were carried out on a 96-well SensoQuest labcycler (Progen Scientific Ltd., South Yorkshire, UK). The PCR products were separated on 2% agarose gels and purified using Illustra GFX PCR DNA and Gel band Purification kit (GE Healthcare Life Science, Buckinghamshire, England). The amplified PCR products were cloned using the TOPO TA cloning kit (Invitrogen, USA) following manufacturer's instructions, and sequenced with an ABI 3100 Genetic analyzer using the ABI BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California). Sequence chromatograms were checked and manually corrected,

and the contigs were assembled and aligned using MEGA 5.0 (Tamura *et al.*, 2011). The sequences were submitted to Genbank with the accession number: Populus\_nigra\_Drome\_6.sqn Populus\_nigra\_Drome\_6 MK757467.

At the genomic level, a few DNA mutations were found in *rbcL* of the three genotypes but all of them were synonymous and provided an identical amino acid sequence. Therefore, the functional characterisation of Rubisco catalytic traits was performed only in the French genotype.

## 2. Rubisco kinetics and specificity for CO<sub>2</sub>/O<sub>2</sub> characterization

Fresh leaf tissue of the French genotype was sampled in full sunlight, immediately frozen in liquid nitrogen and 0.4-0.5 g was ground in a mortar with 2 ml of ice-cold extraction buffer containing 100 mM Bicine (pH 8.2), 6% (w/v) PEG 4000, 2 mM MgCl<sub>2</sub>, 0.1 mM EDTA, 1 mM benzamidine, 1 mM aminocaproic acid, 50 mM 2-mercaptoethanol, 10 mM DTT, 2 μM pepstain A, 10 μM E64, 10 μM chymostatin, 2 mM PMSF and 2.5% (w/v) PVPP. The homogenate was clarified by centrifugation at 13000 × g during 4 min at 4 °C. Then, 1 mL of the supernatant was applied to a Sephadex PD-10 column (GE Healthcare, UK) pre-equilibrated with desalt buffer, containing 100 mM Bicine (pH 8.2), 20 mM MgCl<sub>2</sub>, 10 mM DTT, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 0.5 mM EDTA, 1 mM benzamidine, 1 mM aminocaproic acid and 10 mM NaHCO<sub>3</sub>. The protein peak (in 1 ml) was supplemented with protease inhibitors (4 μM pepstain A, 20 μM E64 and 20 μM chymostatin) and 250 μL of this mixture were supplemented with sufficient carrier-free NaH<sup>14</sup>CO<sub>3</sub> to adjust the specific radioactivity to 3.7 × 10<sup>10</sup> Bq mol<sup>-1</sup>. The remaining extract volume was frozen immediately in liquid nitrogen to measure the Rubisco active site concentration.

Rates of Rubisco <sup>14</sup>CO<sub>2</sub>-fixation using the activated protein extract were measured at 15, 25 and 35°C, each at two concentrations of O<sub>2</sub> (0 and 21% v/v). In all the cases, nine different concentrations of <sup>14</sup>CO<sub>2</sub> were used (0 to 93 μM, each with a specific radioactivity of 3.7 × 10<sup>10</sup> Bq mol<sup>-1</sup>), as described previously (Galmés *et al.*, 2014). Measurements were performed in 7 ml septum capped scintillation vials, containing reaction buffer (yielding final concentrations of 110 mM Bicine-NaOH pH 8.0, 22 mM MgCl<sub>2</sub>, 0.4 mM RuBP and about 100 W-A units of carbonic anhydrase), and equilibrated either with nitrogen (N<sub>2</sub>) or a mixture of O<sub>2</sub> and N<sub>2</sub> (21:79). Assays (1.0 ml total volume) were started by the prompt addition of 10 μL of activated leaf extract, and quenched after 1 min by the addition of 0.2 ml of 10 M formic acid. Acid-stable <sup>14</sup>C was determined by liquid scintillation counting, following removal of acid-labile <sup>14</sup>C by evaporation. The Michaelis-Menten constant for CO<sub>2</sub> (*K<sub>c</sub>*) was determined from the fitted data as described by Bird *et al.* (1982). Replicate measurements (*n* = 4-5) were made using independent protein preparations from different individuals. For each sample, the maximum rate of carboxylation (*K<sub>cat</sub>*<sup>c</sup>) was extrapolated from the corresponding *V<sub>max</sub>* value after allowance was made for the Rubisco active site concentration, as determined by [<sup>14</sup>C]CPBP binding (Yokota & Calvin, 1985).

The Rubisco specificity for CO<sub>2</sub>/O<sub>2</sub> (*S<sub>c/o</sub>*) was also measured at 15, 25 and 35°C (*n* = 7-8) using purified leaf extracts obtained as in Galmés *et al.* (2006) and the oxygen electrode method described by Parry, Keys & Gutteridge (1989), using a DW1 oxygen electrode (Hansatech, Kings Lynn., UK). Reaction mixtures contained (final concentrations) 100 mM Bicine-NaOH (pH 8.2), 10 mM MgCl<sub>2</sub>, 0.15

mg mL<sup>-1</sup> carbonic anhydrase, 2 mM NaH<sup>14</sup>CO<sub>3</sub> (18.5 kBq mol<sup>-1</sup>), 20 μL activated Rubisco from purified extracts and 2.5 μM RuBP. The basic buffer was pre-equilibrated with CO<sub>2</sub>-free air at the temperature of measurement. RuBP oxygenation was calculated from the oxygen consumption and carboxylation from the amount of <sup>14</sup>C incorporated into PGA when all the RuBP had been consumed.

The Rubisco kinetics at three different temperature, with the Michaelis-Menten constant ( $K_M$ ) for CO<sub>2</sub> ( $K_c$ ), the Michaelis-Menten constant for CO<sub>2</sub> measured under 21% O<sub>2</sub> conditions ( $K_c^{air}$ ), the maximum rate of carboxylation ( $k_{cat}^c$ ), the specificity for CO<sub>2</sub>/O<sub>2</sub> ( $S_{c/o}$ ) and the CO<sub>2</sub> compensation point in absence of dark respiration ( $\Gamma^*$ ) are presented in Table 2.

Temperature	15°C		25°C		35°C	
	Mean	SE	Mean	SE	Mean	SE
$K_c$ (μM)	4.96	0.59	9.38	0.60	15.46	0.61
$K_c^{air}$ (μM)	6.64	0.45	13.60	0.76	24.64	1.85
$k_{cat}^c$ (s <sup>-1</sup> )	1.35	0.13	2.17	0.06	3.69	0.18
$k_{cat}^c/K_c$ (μM <sup>-1</sup> s <sup>-1</sup> )	0.30	0.04	0.23	0.02	0.23	0.02
$S_{c/o}$ (mol mol <sup>-1</sup> )	122.10	1.86	81.24	2.86	68.08	2.15
$K_c$ (μmol mol <sup>-1</sup> air)	110	13.04	275.75	17.60	590.25	23.18
$K_c^{air}$ (μmol mol <sup>-1</sup> air)	147.80	9.77	400	22.36	941.25	70.79
$K_o$ (μM)	414.4	92.1	615.7	60.5	301.3	17.5
$\Gamma^{**}$ (μmol mol <sup>-1</sup> )	30.76	0.48	50.52	1.92	64.97	2.09

Table 2: Rubisco kinetic parameters measured at three different temperature, with the Michaelis-Menten constant for CO<sub>2</sub> ( $K_c$ ) and O<sub>2</sub> ( $K_o$ ), the Michaelis-Menten constant for CO<sub>2</sub> measured under 21% O<sub>2</sub> conditions ( $K_c^{air}$ ), the maximum rate of carboxylation ( $k_{cat}^c$ ), the specificity for CO<sub>2</sub>/O<sub>2</sub> ( $S_{c/o}$ ) and the chloroplast CO<sub>2</sub> compensation point ( $\Gamma^*$ ).

The parameters of the Arrhenius function (C, the scaling factor and  $\Delta H_a$ , the activation energy), which describe the temperature dependence, were calculated for each Rubisco kinetic parameter (Table 3).

Parameter	C		$\Delta H_a$ (KJ mol <sup>-1</sup> )	
	Mean	SE	Mean	SE
$K_c$ (μM)	18.45	0.80	40.25	2.06
$K_c^{air}$ (μM)	21.97	1.56	48.09	3.82
$k_{cat}^c$ (s <sup>-1</sup> )	18.54	1.70	44.14	4.33
$S_{c/o}$ (mol mol <sup>-1</sup> )	-4.67	0.76	-22.64	1.85
$K_c$ (μmol mol <sup>-1</sup> air)	29.45	0.96	59.10	2.47
$K_c^{air}$ (μmol mol <sup>-1</sup> air)	33.11	1.94	53.95	9.58
$\Gamma^*$ (μmol mol <sup>-1</sup> )	13.77	0.48	24.60	1.63

Table 3: Parameters of the Arrhenius function for each Rubisco kinetic parameter

### 3. References:

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### Supplementary Table 1:

Climatic data at the location of the three populations from which the genotypes come from (from Dewoody et al, 2015).

Country	Population	Latitude	Longitude	Average annual temperature (°C)	Maximum temperature of warmest month (°C)	Minimum temperature of coolest month (°C)	Average annual precipitation (mm)	Precipitation of wettest month (mm)	Precipitation of driest month (mm)
France	Drôme 6	44.75	4.92	12.4	28.1	0.0	840	95	41
Italy	La Zelata	45.26	8.98	13.0	29.0	-1.0	982	122	55
Spain	Ebro 2	41.58	-1.00	13.7	29.5	1.3	365	53	17

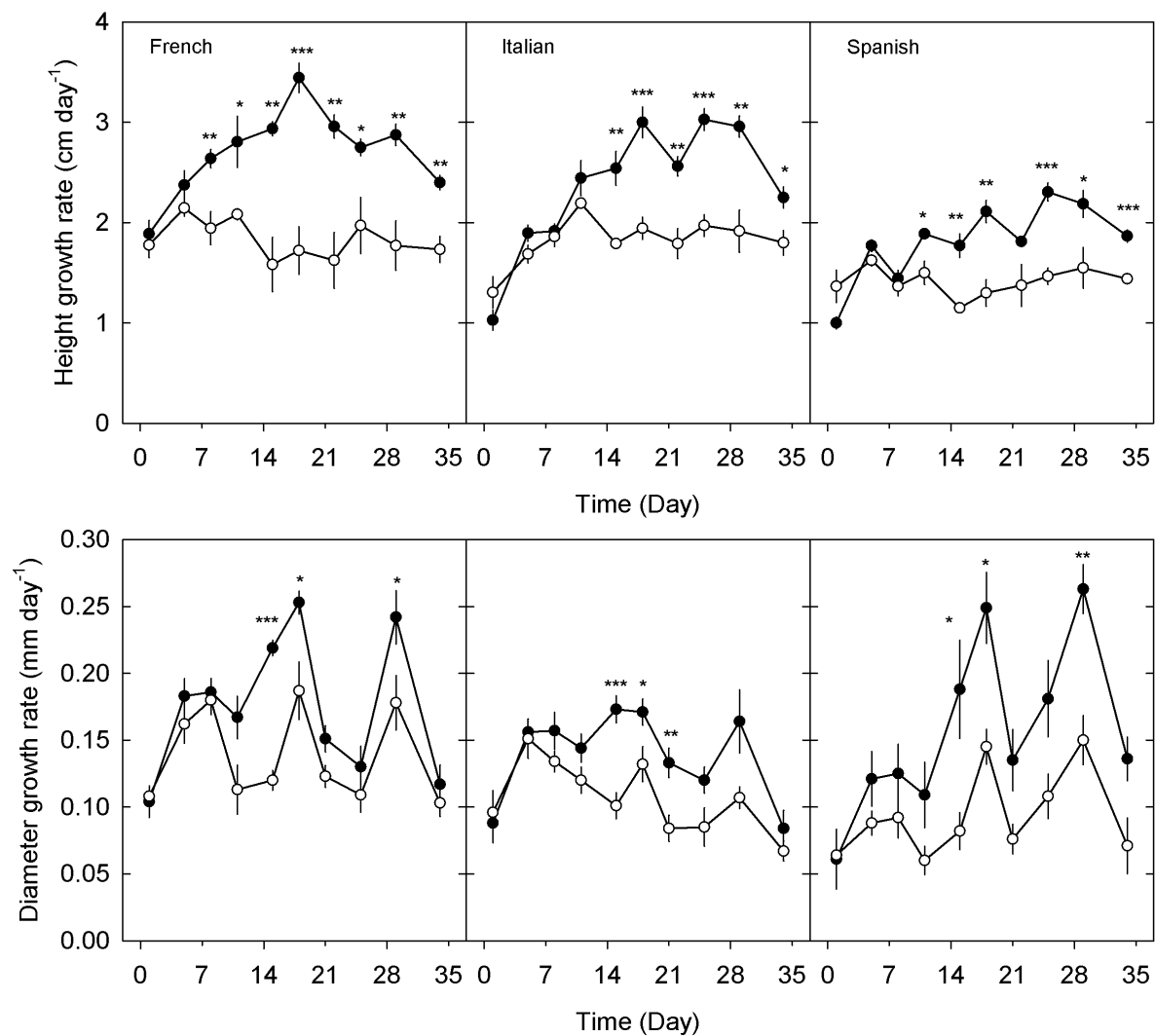
DeWoody, J., Trewin, H., Taylor, G., 2015. Genetic and morphological differentiation in *Populus nigra* L.: isolation by colonization or isolation by adaptation? *Mol. Ecol.* 24, 2641-2655.

### Supplementary Table 2:

Predawn leaf water potential (MPa) of the three poplar genotypes measured on day 14 with a Scholander chamber in another batch of plants of the same experiment (Wildhagen et al, 2018). Mean  $\pm$  s.e., n=4 – 6.

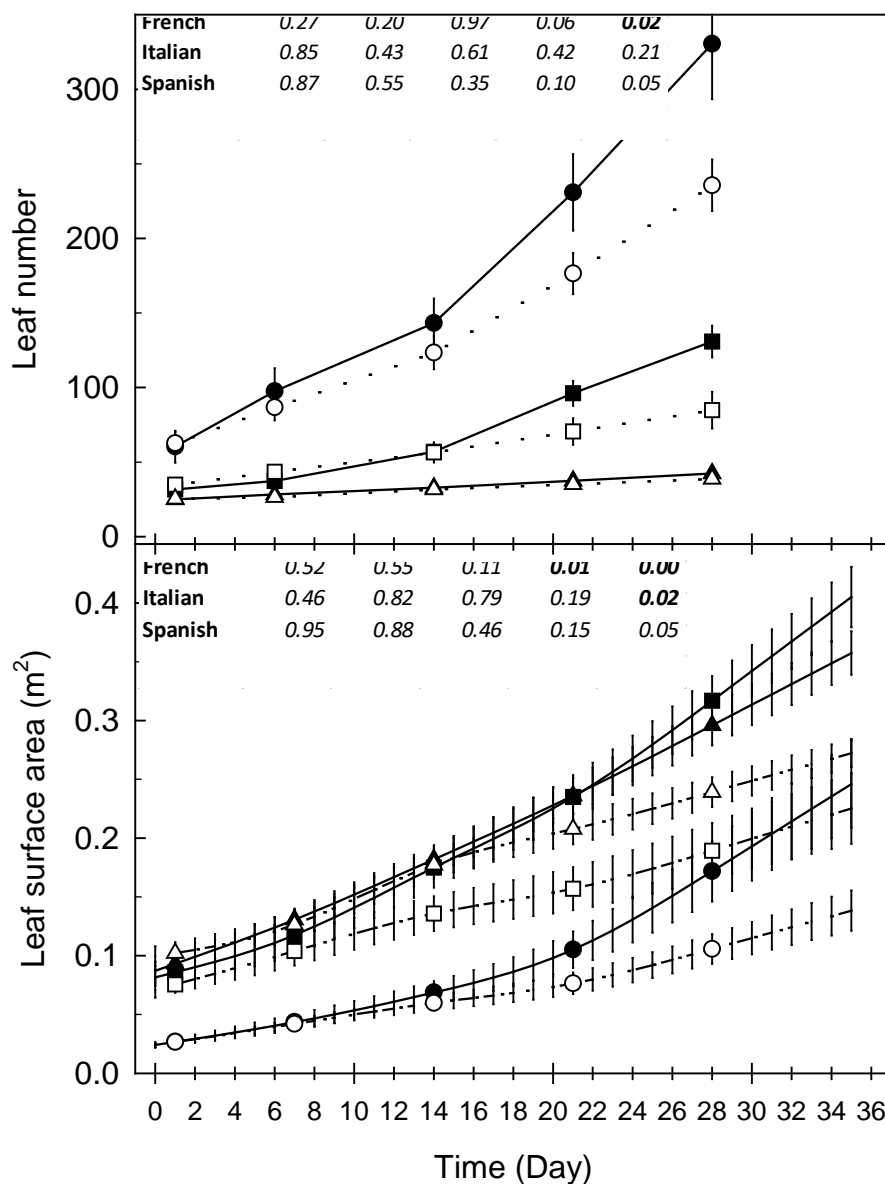
	French	Italian	Spanish
control	-0.23 $\pm$ 0.01	-0.08 $\pm$ 0.02	-0.22 $\pm$ 0.04
drought	-0.29 $\pm$ 0.01	-0.26 $\pm$ 0.02	-0.20 $\pm$ 0.04

Wildhagen, H, Paul, S, Allwright, M, Smith, HK, Malinowska, M, Schnabel, SK, Paulo, MJ, Cattonaro, F, Vendramin, V, Scalabrin, S, et al. 2018 Genes and gene clusters related to genotype and drought-induced variation in saccharification potential, lignin content and wood anatomical traits in *Populus nigra*. *Tree Physiol.* 38, 320-339. DOI : 10.1093/treephys/tpx054



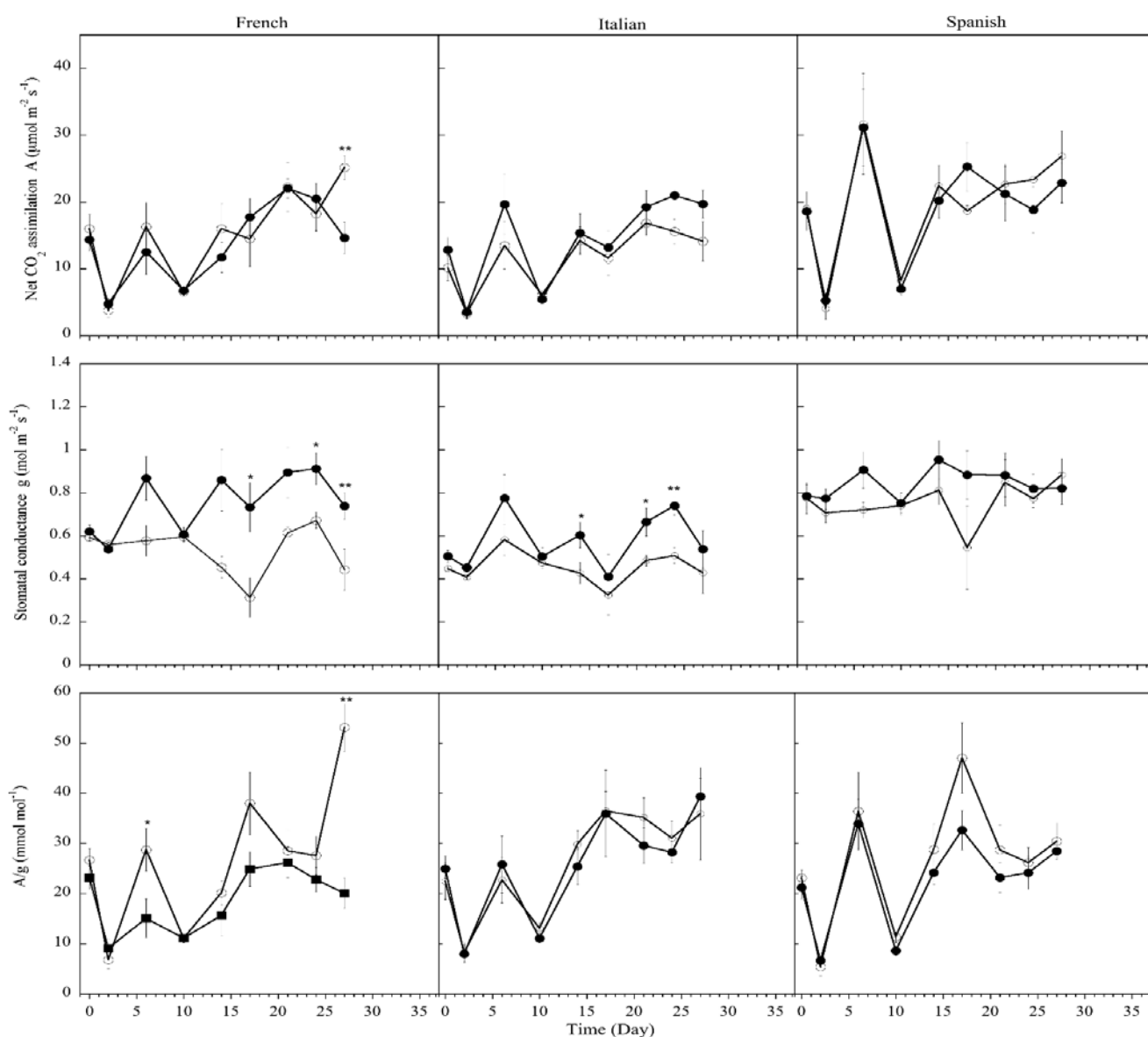
### Supplementary Figure 1 :

Growth rate in height and in stem diameter of the three genotypes over the 5-week experiment. Mean  $\pm$  s.e. (n=6). Closed and open symbols denote control and drought treatments, respectively. Symbols \*\*\*, \*\*, \* denote significance level (<0.001, 0.01 and 0.05, respectively).



**Supplementary Figure 2:**

Leaf number and total leaf area of the three genotypes over the 5-week experiment. Mean  $\pm$  s.e. (n=6). Closed and open symbols denotes control and drought treatments, respectively. Squares, triangles and circles denote the French, the Italian and the Spanish genotypes, respectively. Closed and open symbols denote control and drought treatments, respectively. Total leaf area was measured at five time points (symbols) and estimated at the other dates from the adjustment of an interspline function (R). P-values of t-test between control and drought treatment within each genotype are given.



### Supplementary Figure 3

Net CO<sub>2</sub> assimilation rate, stomatal conductance and intrinsic water use efficiency over the 5-week experiment. Closed and open symbols denote control and drought treatments, respectively. Symbols \*\*\*, \*\*, \* denote significance level (>0.001, 0.01 and 0.05, respectively).