



# Plastic response of four maritime pine (*Pinus pinaster* Aiton) families to controlled soil water deficit

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## Abstract

- **Key message** Separating the internal (ontogenetic) and external (environmental) components of maritime pine development during controlled soil water deficit helps to highlight the plastic response. The adjusted measurements reveal significant differences between families for their plastic response for several physiology and growth traits.
- **Context** Soil water deficit is and will be a growing problem in some regions. *Pinus pinaster* Ait. is a species of commercial interest and is recognized as a drought-avoiding species. It is thus of interest to evaluate the adaptation potential of *P. pinaster* to soil water deficit.
- **Aims** This paper aims to estimate the plastic response to the variation of water availability at the family level (half-sibs).

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**Contribution of the co-authors** Muriel Feinard-Duranceau was in charge of the corresponding task in the Region Centre Val de Loire Xylome project, directed the measurements, supervised the Master Student Alexane Berthier, participated to the data analysis, and wrote the article with Philippe Rozenberg.

Alexane Berthier was the Master student participating to the project.

Cécile Vincent-Barbaroux participated to the design of the experiment and to the measurements, participated to the supervision of the Master student Alexane Berthier, and corrected the successive versions of the article.

Sara Marin was in charge of the experiment design and management, participated to the measurements and to the data collection, and corrected the article.

Francisco-José Lario selected and provided the plant material, discussed the experiment and the results, and corrected the article.

Philippe Rozenberg was the designer and the coordinator of the Region Centre Xylome project, co-supervised the Master student Alexane Berthier, analyzed the data, and wrote the article with Muriel Feinard-Duranceau.

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- **Methods** Two-year-old *P. pinaster* cuttings from four families were submitted during 6 weeks to two contrasting watering regimes. The experiment started in April 2011 shortly after sprouting. The photosynthesis and stomatal conductance to water vapor were measured on 1-year-old needles. Intrinsic water-use efficiency was calculated as the ratio of photosynthesis to stomatal conductance. Radial growth, length of terminal shoot, and total height were also measured. The ontogenetic component of tree development was estimated on the well-watered trees for all the traits. Then, this development effect was eliminated from the data collected on the trees submitted to the soil water deficit in order to keep only the effect of this soil water deficit.
- **Results** After 6 weeks of reduced watering, the value of all adjusted traits decreased. An *average plastic response* to the variation of water availability was found to be significant and variable at the family level for the six adjusted variables.
- **Conclusion** These results suggest that there is genetic variation of phenotypic plasticity to drought in *P. pinaster* for several traits, including stomatal conductance, which appears to be a promising variable for future selection for resistance to drought.

**Keywords** Adaptation · Ontogenetic · CO<sub>2</sub> assimilation · Stomatal conductance · Water-use efficiency · Growth

## 1 Introduction

With climate warming, plants face more frequently longer periods of increased water demand and decreased water availability. In this context, a better understanding of the processes involved in tree response to water availability is required (Pachauri et al. 2015). Maritime pine (*Pinus pinaster* Ait.) is recognized as a drought-avoiding species (Granier and Loustau 1994; Picon et al. 1996). Such species are often isohydric plants (Tardieu 1998), with a high stomatal sensitivity to soil water deficit. In response to mild water stress, these plants close their stomata to regulate water flux, and reduce transpiration and soil water uptake. This is followed by a reduction of stomatal conductance to water vapor (Schulze et al. 1987; Picon et al. 1996; Fernández et al. 1999; Fernández et al. 2000; Flexas 2002; Sánchez-Gómez et al. 2010) and of predawn leaf water potential (Aussenac and Granier 1978; Fernández et al. 2000; Fernández et al. 1999; Picon-Cochard and Guehl 1999). A restriction of the CO<sub>2</sub> transfers from the atmosphere to the chloroplast leads to decreased photosynthesis (Chaves 1991; Cornic 2000; Farquhar and Sharkey 1982; Lawlor and Cornic 2002).

Selecting fast growing water-saving genotypes is an important objective for *P. pinaster* breeders (de la Mata et al. de la Mata et al. 2014, 2012; Guehl et al. 1994; Plomion et al. 2016). Water-use efficiency (WUE) is an integrative trait related to plant water economy. WUE is defined at the whole-plant level as the ratio between biomass production and cumulative water losses by transpiration. While WUE is difficult to measure at the whole plant level, intrinsic water-use efficiency ( $W_i$ ), defined as the ratio between net CO<sub>2</sub> assimilation and stomatal conductance to water vapor, is easily measured at the leaf level. QTL were detected for  $W_i$  in maritime pine in different water regimes by Brendel et al. (2002) and de Miguel et al. (2014). According to Marguerit et al. (2014), the lack of genetic link between WUE and growth allows simultaneous improvement of both traits. Our objective in this article is to contribute to the evaluation of the adaptation potential of

*P. pinaster* to water stress. The adaptation potential depends, on one hand, on the genetic determinism (a long-term response at population level) and, on the other hand, on the phenotypic plasticity (a shorter-term response at individual level) of adaptive traits (Schlichting 1986). According to DeWitt and Scheiner (2004), the phenotypic plasticity is the ability of a genotype to change its phenotype in response to changes in the environment. This variation can be described as a norm of reaction (Ghalambor et al. 2007). Environment varies in space and time; hence, phenotypic plasticity can be considered both against space-related and time-related environmental variation (Scheiner 2013). Space-related variation is especially relevant for multi-trial common garden experiments and for controlled-conditions experiments with genetic entities and treatments. In consequence, phenotypic plasticity is estimated either with vegetative copies of a genotype distributed across spatially distinct environments, or with a single genotype observed across time-related environmental variation. Each dimension corresponds to a type of plastic response. In this article, we estimate the space-related phenotypic plasticity between the treatments of a controlled-condition experiment: we call it *between-treatment* plasticity. We call the effect of temporal environmental changes *time-related* plasticity. The genetic entities in our study are half-sib families where the individuals are genetically related genotypes. On rigorous application of the phenotypic plasticity definition of DeWitt and Scheiner (2004), we called *average plastic response* the plasticity studied at the general level and *family plastic response* the plasticity studied with norms of reaction at the family level. The *average plastic response* is the phenotypic response considering the pooled sample, while the *family plastic response* is the average phenotypic response of each family. Significant genotype  $\times$  treatment and genotype  $\times$  site interactions are evidences of significant genetic variation for space-related (between-treatment, between-site) phenotypic plasticity.

In the literature, the phenotypic plasticity of *P. pinaster* to water stress was studied for different types of genetic

entities and traits. At the population level, *P. pinaster* presented significant phenotypic changes with water stress on biomass allocation (Aranda et al. 2010; Chambel et al. 2007) and carbon isotope discrimination (as a proxy of  $W_i$ ) (Aranda et al. 2010; Corcuera et al. 2012). This plasticity was also observed on the molecular machinery involved in wood formation (Paiva et al. 2008) and for hydraulic traits such as xylem specific conductivity (Corcuera et al. 2011) and cavitation resistance (Corcuera et al. 2011; Lamy et al. 2014).

In some studies, no genetic difference was observed between populations of *P. pinaster* in their phenotypic plasticity. It was the case for plasticity to water stress (population  $\times$  water stress), biomass allocation (Aranda et al. 2010; de la Mata et al. 2014; Sánchez-Gómez et al. 2010), height growth (Chambel et al. 2007; Corcuera et al. 2010), relative height growth rate (Sánchez-Gómez et al. 2010), and cavitation resistance (Lamy et al. 2014). But genetic variation of plasticity were found in other studies for biomass-related variables (Chambel et al. 2007), height growth (de la Mata et al. 2014; Lamy et al. 2014), cavitation resistance (Corcuera et al. 2011), and carbon isotope discrimination (Aranda et al. 2010; Corcuera et al. 2012, 2010).

Few studies were conducted at the family level on plastic response to soil water deficit for early growth and physiological traits. Significant differences were observed between *P. pinaster* families for growth (Corcuera et al. 2010; Fernández et al. 2006), biomass and  $W_i$  based on gas exchange measurements (Fernández et al. 2006), and  $\delta^{13}\text{C}$  (Corcuera et al. 2010). An average plastic response to drought was found at the family level for height (Corcuera et al. 2010; Fernández et al. 2006) and for  $\delta^{13}\text{C}$  by Corcuera et al. (2010). Nevertheless, no significant family  $\times$  treatment interaction was found for  $\delta^{13}\text{C}$  and gas exchange ( $A$ ,  $g_s$ ,  $W_i$ ) by Fernández et al. (2006). At the clone level, a plastic response to drought was detected for *P. pinaster* for  $g_s$  and  $W_i$  (de Miguel et al. 2012). Hence, according to the sample, the phenotypic trait, and the environmental variable, *P. pinaster* plastic response to water stress was not always found to be genetically variable.

Our aim is to contribute to the study of the family variation of the early-growing season phenotypic plasticity of young *P. pinaster* trees submitted to variable watering regimes in controlled conditions for several traits, including  $\text{CO}_2$  assimilation, stomatal conductance, and growth.

During the early growing season, the ontogenetic development follows and partly overlaps the recovery of photosynthesis from winter inhibition. These internal effects coincide and are confused with the response to environmental variation: according to the trait, such response can be underestimated or overestimated (Maseda and Fernández 2006; Egea et al. 2011). Ignoring them may lead to biased results and misinterpretation. This

could explain part of the diverging published results. In this article, we separated the internal ontogeny and recovery effects from the response to environmental variation. We studied the variation with time and between families of  $\text{CO}_2$  assimilation ( $A$ ), stomatal conductance ( $g_s$ ),  $W_i$  and of the growth traits (total height (*height*), length of terminal shoot (*shoot*), and diameter at root collar (*diam*) between well-watered and water-stressed *P. pinaster*. Then, we estimated the phenotypic plasticity (*average* and *family plastic response*) of the same traits to between-treatment and time-related variation of soil water deficit. Taking into account the combined recovery and ontogenetic effects as one, single internal effect was a key step in investigating the between-family variation for phenotypic plasticity.

## 2 Materials and methods

### 2.1 Experimental design, plant material, and water stress application

The experiment was conducted in 2011 in an automatically ventilated greenhouse for overheat control at INRA Val de Loire, Orléans, France. The plant material were *P. pinaster* cuttings of October 2009 coming from a seed orchard from Monfero, Galicia, Spain, potted in early February 2011 in 10-L cylindrical pots filled with compost (Orga Agrumes et Rosiers de Fertil'Aquitaine®). Each family of 16 trees was produced at TRAGSA, Maceda, Spain, by vegetative propagation of half-sibs. In the standard procedure, TRAGSA mixes the propagated half-sibs of each family, retaining their family identity but not their individual clonal identity. Hence, each family is a mixture of genetically related (half-sibs) clones of unknown identity. The experimental design is formed of two complete replicates with eight randomly distributed families, corresponding to two treatments. After 2 months at field capacity, the trees were subjected to two watering treatments during 6 weeks. At the beginning of the experiment, from April 4 to 8, the trees in both treatments were watered to field capacity every 2 days. The water status of all pots was adjusted daily by weighing 10 randomly chosen pots. The pots were supplemented with mineral elements ( $1 \text{ g l}^{-1}$  of 18:12:18 NPK) using hydro soluble fertilizer. Fertilization was applied until the beginning of the water stress period then was stopped in both treatments. Then, on April 8, watering was stopped on the water-stressed (WS) treatment. Until the end of the experiment (May 20), half of the trees were well-watered (WW treatment) and maintained to field capacity, while the other half was water-stressed (WS treatment) till 40% of field capacity by stopping watering. The amount of water to

maintain the 40% water status (since May 9) was adjusted by daily weighing of 10 randomly chosen pots. Volumetric soil water content (SWC; %) was measured each day for each of the 64 trees during all the experiment with a soil moisture sensor (ThetaProbe, type ML2x, Delta T, Cambridge, UK). The water-stressed trees were at 60% of field capacity on April 18 and reached 40% of field capacity on May 9. We maintained a 40% field capacity until May 20. Then, the response to water stress was measured on 32 individuals per treatment and 16 individuals per family (four families).

The greenhouse evaporative cooling automatically regulated the temperature and humidity during the experiment period, in order to avoid heat peaks. Average day/night air temperature and hygrometry in the greenhouse were 17.9/10.8 °C and 65.3/86.5%, respectively, during the experiment and were found to be stable during the 6-week time lag of the experiment (variation of air temperature and hygrometry in the greenhouse during the experiment shown in Fig. 3 in the supplementary data).

## 2.2 Water relations

In addition to volumetric SWC (%), needle predawn water potential ( $\Psi_b$ , -MPa) was measured with a Scholander pressure chamber (Scholander et al. 1965) at the end of week 6 on the well-watered ( $n = 4$ ) and water-stressed trees ( $n = 8$ ).

## 2.3 Growth traits

The total height (*height*, cm), diameter at root collar (5 cm, *diam*, cm), and length of terminal shoot (*shoot*, cm, issued from the annual bud) from each cutting were measured each week during the experiment at a 1-mm precision.

## 2.4 Gas exchange measurements

Net CO<sub>2</sub> assimilation rate ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and stomatal conductance to water vapor ( $g_s$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) were measured using a LI-6400 XT portable gas exchange system (Li-Cor Biosciences Inc., Lincoln, NE, USA, standard  $2 \times 3$ -cm clear top chamber). The measurements were performed on three 1-year-old leaf fascicles (two needles per pseudophylls) placed across the width of the chamber. Needle length ( $l$ ) and diameter ( $d$ ) were measured in order to estimate the total external photosynthetic surface, calculated as  $(1 + \pi/2)ld \times 6$  needles  $\times 1/2$ , the plane surface of one needle and the semicylindrical surface of the other needle of a fascicle being illuminated with the LED light source (6400-02 LED). The carbon dioxide assimilation rate was related to this total external needle surface. The measurements were completed with the LED light source set at  $1200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , which

corresponds to a saturated photosynthetic photon flux density (PPFD) for *P. pinaster*, a constant flow rate of  $500 \mu\text{mol s}^{-1}$ , a leaf vapor pressure deficit of  $1.33 \pm 0.18 \text{ kPa}$ , and a reference CO<sub>2</sub> concentration of  $400 \mu\text{mol mol}^{-1}$ . The value of  $1200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  was determined in preliminary measurements. We measured light-saturated net CO<sub>2</sub> assimilation rate and stomatal conductance at ambient CO<sub>2</sub> concentration at steady state conditions. The measurements were performed each day on weeks 0, 2, 5, and 6 after the beginning of the application of the stress on 16 randomly chosen pine trees so that all the 64 pines were characterized in 4 days per week. Three repeated measurements were averaged per plant (eight trees per family, four families per treatment, and two treatments).

## 2.5 Water-use efficiency

Intrinsic water-use efficiency ( $W_i$ ,  $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ) was calculated from gas exchange measurements on individual plants as the ratio of  $A$  to  $g_s$ .

## 2.6 Data analysis

The data was analyzed using the R software (version 2.8.0, R Development Core Team 2008). The data was found to meet the assumptions of homoscedasticity and of normal distribution of the residuals. The statistical tests were considered significant at  $P \leq 0.05$ .

The genetic variation and the effect of time on the study traits were analyzed in the WW treatment using the following model:

$$X_{ij} = \mu + \text{week}_i + F_j + (\text{week}_i \times F_j) + \varepsilon_{ij} \quad (1)$$

where  $X_{ij}$  is the value of the trait,  $\mu$  is the general mean, *week* is the time effect,  $F$  is the family effect, (*week*  $\times F$ ) is their corresponding interactions, and  $\varepsilon_{ij}$  is the residual. We observed that during the 6 weeks of the experiment, the air temperature and the air humidity did not vary significantly in the greenhouse (Fig. 3 in supplementary material). This assumption is consistent with our measurements of water content in the tree containers of the WW treatment. In this case, the week effect estimated on the WW trees measures the combined recovery and ontogenetic effect, consequence of tree development on the variables (called ontogenetic effect in the following).

Then, the data measured on the WS treatment was adjusted of this ontogenetic effect.

$$X_{ij} = \mu + \text{week}_i + \varepsilon_{ij} \quad (2)$$

was used to estimate the week effect on the dataset of the WW treatment.

$$X_{ij}^{-week_i} = \mu + \varepsilon_{ij} \quad (3)$$

was used to adjust of the week effect on the dataset of the WS treatment.

The between-treatment plastic response between both treatments was studied using the following model of ANOVA on the pooled adjusted data of week 0 and 2 on the one hand and of weeks 5 and 6 on the other hand (weeks during which the difference between the treatments for SWC was the highest)

$$X_{ij} = \mu + SWC + F_i + (SWC \times F_i) + \varepsilon_{ij} \quad (4)$$

where  $X_{ij}$  is the value of the trait,  $\mu$  is the general mean, SWC is the soil water content measured in each pot of each tree,  $F_i$  is the family effect,  $SWC \times F_i$  is the interaction between these two factors, and  $\varepsilon_{ij}$  is the residual. In this case, the linear relationship estimated by the SWC effect corresponds to the *average plastic response*, while the  $SWC \times F_i$  interaction estimates the family effect on this relationship and thus the *family plastic response*.

Finally, the time-related plastic response was studied using Eq. (4) applied to a different dataset: the adjusted data of the WS treatment of weeks 0 to 6. In this way, we test the effect of the temporal variation of SWC on the value of the traits. Different transformations of the SWC variable were tested to meet the assumptions of the linear model.

The general relationship between SWC and the variable  $X_{ij}$  describes the temporal *average plastic response* of *P. pinaster* when SWC decreases with time in the stressed treatment. The ( $SWC \times F_i$ ) interaction tests the differences between the four families for this time-related plastic response.

## 3 Results

### 3.1 Variation with time of the physiological and growth traits in the WW and WS treatments and estimation of an ontogenetic effect

Figure 1 and Table 1 show that in the WW treatment, the SWC in the pots is stable with time (average 26.6%) while it decreases in the WS treatment from 27.88 to 12.08% from week 0 to week 6. In the WW treatment, the raw values of  $A$ ,  $gs$ ,  $diam$ ,  $shoot$ , and  $height$  increase from week 0 to week 5, then levels off (Table 1). Needle predawn water potential ( $\Psi_b$ ) is almost zero for the WW trees ( $n = 4$ ) on week 6. Indeed, for all the WW data (Table 2a), we find a significant week (time) effect for  $A$ ,  $gs$ ,  $Wi$ ,  $diam$ ,  $shoot$ , and  $height$  with no significant week  $\times$  family interaction (week  $\times F$ ), meaning that the week effect is the same for all the families. Using the daily weather data automatically collected by the greenhouse meteorological station, we find that there was neither significant temperature

nor relative air humidity variation during the 6-week time lag of the experiment (results shown in Fig. 3 in the supplementary data). Thus, we attribute the phenotypic variation observed in the WW trees to the ontogenetic, developmental effect. We used this ontogenetic effect estimated on the WW trees to adjust the data collected on the WS trees. The unadjusted and adjusted data ( $A$ ,  $gs$ ,  $Wi$ ,  $diam$ ,  $shoot$ , and  $height$ ) are shown in Table 1. The WW and WS trees have different variation patterns: the adjusted WS data shows a significant decrease of  $A$ ,  $gs$ ,  $diam$ ,  $shoot$ , and  $height$  and a significant increase of  $Wi$  when SWC lowers (SWC is approximately 12% while  $\Psi_b$  of the WS trees was  $-0.54 \text{ MPa} \pm 0.12$  on week 6). The time-related SWC effect is significant on all the adjusted WS variables (Table 2d).

### 3.2 Variation between the four families for the physiological and growth traits

There is a significant family effect for  $A$ ,  $gs$ ,  $diam$ ,  $shoot$ , and  $height$  in the WW treatment (Table 2a). There is also a significant family effect for  $Wi$  estimated in well-watered conditions for the WW and WS trees on the weeks 0–2 (Table 2b,  $F$ ).

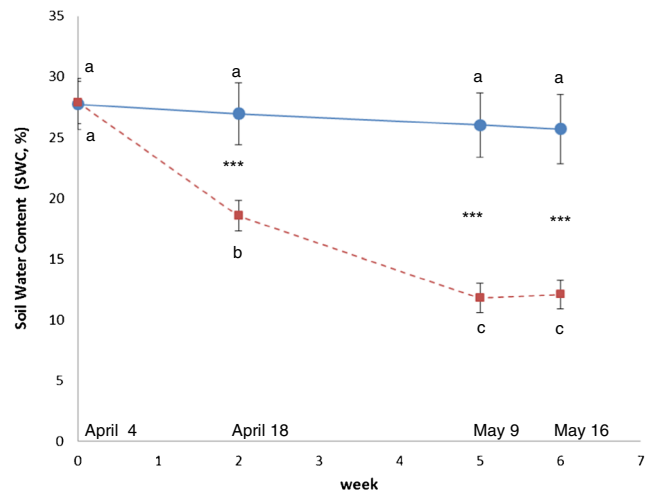
### 3.3 Average and family plastic response to the spatial and temporal variations of water availability

At the beginning of the experiment (Table 2b), there is no effect of SWC on  $A$ ,  $gs$ ,  $Wi$ ,  $diam$ , and  $height$  for the 64 trees in both treatments. Conversely, at the end of the 6-week experiment, there is a significant SWC effect (Table 2c) on all the traits: in other words, there is a significant between-treatment phenotypic relationship between SWC and all the variables at the tree level in the pooled WW and WS treatments during weeks 5 and 6. This relationship measures the average between-treatment plastic response of the grouped families to the variation of water availability between both treatments. We note a significant family effect for  $gs$ ,  $Wi$ ,  $diam$ ,  $shoot$ , and  $height$  (Table 2c,  $F$ ) but no  $SWC \times$  family interaction (Table 2c). This means that there is no significant difference between the four families for their between-treatment plastic response to SWC variation.

The significant effect of SWC on adjusted  $A$ ,  $gs$ ,  $Wi$ ,  $diam$ ,  $shoot$ , and  $height$  for the 32 trees in the WS treatment during the 6 weeks of the experiment (Table 2d) reveals an average plastic response to the temporal variation of SWC. We found a significant family effect for  $gs$ ,  $shoot$ , and  $height$  and a significant  $SWC \times$  family interaction for  $gs$  and  $Wi$  (Table 2d,  $F$ ,  $SWC \times F$ ). This interaction corresponds to a significant family effect for the time-related plastic response to SWC for these two variables. Conversely, we found no significant  $SWC \times$  family interaction for  $A$  and the three growth traits.

The corresponding plots are shown in Fig. 2a–f for  $A$ ,  $gs$ ,  $Wi$ ,  $diam$ ,  $shoot$ , and  $height$ , respectively. The fitted linear

**Fig. 1** Volumetric soil water content (SWC; %) of the 64 trees during the 6 weeks of the experiment. Week 0 (April 4 to 8), week 2 (April 18 to 22), week 5 (May 9 to 13), week 6 (May 16 to 20). The well-watered trees are represented by circles and the water-stressed trees by squares. The level of significance of the SWC effect is indicated by asterisks: \*\*\* $P < 0.001$ , \*\* $0.001 \leq P < 0.01$ , \* $0.01 < P < 0.05$ . Different letters (*a*, *b*, and *c*) indicate significant differences ( $p = 0.05$  by Student's *t* test) between weeks



relationships are family norms of reaction that measure their time-related plastic response (Fig. 2). We could fit significant family norms of reaction for one to three of the four families, according to the trait, for *A*, *gs*, *Wi*, *diam*, *shoot*, and *height*. For the range of variation of SWC measured in the experiment, these norms of reaction are linear models. These family norms of reaction measure the time-related family plasticity to SWC variation in the WS treatment: there is a significant family variation for these norms of reaction for *gs* and *Wi* (Fig. 2b, c).

### 3.4 Correlations between variables

Table 3 shows the correlations between the physiological and growth variables. As expected, *A* and *gs* are positively correlated. *Wi* is highly negatively correlated with *gs* in the WW and WS conditions. The growth variables positively correlate all together in all cases and are positively correlated with *Wi* in the WS conditions.

## 4 Discussion

### 4.1 Variation in the WW and WS treatments and estimation of an ontogenetic effect

The photosynthesis increase observed during the first 2 weeks after bud flush on 1-year-old *P. pinaster* needles in the well-watered treatment (WW, weeks 0 and 2; Table 1) is in accordance with other results on 1-year-old *Pinus sylvestris*, *Picea abies*, and *Pinus contorta* needles (Strand et al. 2002; Strand and Lundmark 1995). The significant week effect observed on all the physiological traits in the well-watered treatment (WW; Table 2a) is due to both the early spring recovery and the ontogenetic development effect (Slaney 2006). The ontogenetic development is observed on growth traits (Table 1) in the WW conditions as in other studies (Chambel et al. 2007;

Fernández et al. 2006). It follows photosynthesis recovery with a gradual transition of unknown duration: we call their combination the ontogenetic effect. This seasonal change in *A* was also observed in spring on other species like *Quercus douglasii* (Xu and Baldocchi 2003). As them, we observe a similar pattern for *gs* and *A* that increase then level off (Table 1; weeks 5 and 6, WW, measurements at saturated PAR).

The evaluation of the ontogenetic effect in the WW treatment gave us the possibility to separate the internal ontogenetic effect from the plastic response in the WS treatment. Few studies have tried to separate the role of ontogenetic and water stress on photosynthesis (Maseda and Fernández 2006; Egea et al. 2011). The effect of this separation is obvious in Table 1: the developmental effect dominates and masks the response to the SWC decrease of all the phenotypic variables. Thanks to this separation, their response to environmental variation becomes apparent, statistically significant, and consistent with what is expected. As an example, *Wi*, *diam*, and total *height* are not significantly different between WW and WS during weeks 5 and 6 (Table 1), whereas a significant SWC effect is detected on these traits when data is corrected from the ontogenetic effect (Table 2c and d; SWC).

The high positive correlation between *A* and *gs* (Table 3) shows, in accordance with the literature (Galmés et al. 2007; Lawlor and Cornic 2002; Xu and Baldocchi 2003), that the stomatal closure during drought induces a decrease of *A* (adjusted WS data during the weeks 5 and 6; Table 1) (Cornic 2000; Flexas 2002; Lawlor and Cornic 2002; Limousin et al. 2010; Picon et al. 1996; Xu and Baldocchi 2003). The restriction of the CO<sub>2</sub> diffusion through the closing stomata during mild water stress explains most of this decrease (Dietz and Heber 1983; Lawlor and Cornic 2002; Limousin et al. 2010). The same type of response was observed on *P. pinaster* families (de Miguel et al. 2012; Fernández et al. 2006) and on *P. pinaster*

**Table 1** For each date (week), mean values of soil water content (SWC; %), net CO<sub>2</sub> assimilation (A;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance to water vapor (gs;  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), intrinsic water-use efficiency ( $W_i = A/g_s$ ), diameter at 5 cm (*diam*; cm), length of terminal shoot (*shoot*; cm), and total height (*height*; cm) of the well-watered (WW) *P. pinaster* trees and the water-stressed (WS) *P. pinaster* trees for each measurement week

Date (week)	SWC (%)	A ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) Nad/Ad for WS	gs ( $\text{mmol m}^{-2} \text{ s}^{-1}$ ) Nad/Ad for WS	$W_i$ ( $\mu\text{mol CO}_2 \text{ mol H}_2\text{O}^{-1}$ ) Nad/Ad for WS	<i>diam</i> (cm) Nad/Ad	<i>shoot</i> (cm) Nad/Ad	<i>height</i> (cm) Nad/Ad
WW April 4	27.8 ± 2.1 a	13.7 ± 2.7 a	206.3 ± 74.3 a	71.3 ± 16.04 a	3.7 ± 1.6 a	3.4 ± 2.3 a	22.9 ± 5.4 a
WS Week 0	27.9 ± 1.8 a	12.6 ± 2.4 a/15.57	187.3 ± 74.1 a/254.2	73.4 ± 17.3 a/67.2	3.5 ± 0.4 a/4.1	2.4 ± 1.7 a/8.0	20.6 ± 3.5 a/26.0
WW April 18	27 ± 2.6 a	15.9 ± 4.2 b	254.4 ± 74.7 b	68.6 ± 16.2 a	4.1 ± 0.6 b	8.3 ± 5.2 b	27.7 ± 6.8 b
WS Week 2)	18.5 ± 1.3 b	14.3 ± 4.3 ab/15.054	208.2 ± 108.7 ab/227.0	75.9 ± 18.0 a/72.3	3.7 ± 0.4 b/4.0	6.2 ± 4.4 b/7.0	24.0 ± 5 b/24.6
WW May 9	26.0 ± 2.6 a	18.2 ± 2.7 c	315.0 ± 43.6 c	60.3 ± 12.2 b	4.6 ± 0.8 c	11.9 ± 7 c	30.7 ± 8.1 b
WS Week 5	11.8 ± 1.2 c	14.4 ± 3.4 b/13.299	167.5 ± 25.3 a/131.0	98.5 ± 43.9 b/96.3	4.1 ± 0.4 c/3.8	8.0 ± 5.4 b/5.4	25.4 ± 5.5 b/23.0
WW May 16	25.7 ± 2.9 a	18.707 ± 2.5 c	317.3 ± 68.6 c	60.1 ± 7.1 b	4.9 ± 0.8 c	12.706 ± 7.1 c	31.7 ± 8.3 b
WS Week 6	12.1 ± 1.2 c	15.8 ± 2.5 b/13.699	185.7 ± 62.8 a/141.7	89.4 ± 18.9 b/94.3	4.2 ± 0.5 c/3.6	8.6 ± 5.8 b/5.0	26.2 ± 5.9 b/22.7

Each data is the mean of 32 independent measurements ± standard deviation. For the WS trees, unadjusted (NAd) and adjusted (Ad, in bold) mean values are presented. The adjustment takes into account the ontogenetic effect discovered using the WW trees only. Different letters (a, b, and c) indicate significant differences ( $p = 0.05$  by Student's *t* test) between weeks for Nad data

provenances (Fernández et al. 2000; Picon-Cochard and Guehl 1999). The higher negative correlation between  $W_i$  and  $g_s$  than between  $W_i$  and  $A$  (Table 3) shows that the  $W_i$  increase with drought (Limousin et al. 2010) (Table 1; Ad data) is mainly explained by  $g_s$ . This is in accordance with previous works on *P. pinaster* (de Miguel et al. 2012), *Populus* (Monclus et al. 2006), and the fact that the reduction of stomatal aperture is one of the first protection reactions of isohydric plants like *Pinus* trees (Picon et al. 1996).

#### 4.2 Variation between the four families

The family variation in the WW treatment for  $A$  and  $g_s$  is in agreement with previous works on *P. pinaster* showing between-family variation of  $A$  (Fernández et al. 2006) and between-clone variation of  $g_s$  (de Miguel et al. 2012). Such significant effect was also observed between 25 populations of *Pseudotsuga menziesii* for  $A$ ,  $g_s$ ,  $W_i$ , and carbon isotopic discrimination (Zhang et al. 1993) and between five families of western larch trees for carbon isotopic discrimination and  $g_s$  (Zhang et al. 1994). Nevertheless, in other studies, no significant difference was found for  $A$  and  $g_s$  between five provenances of *P. pinaster* (Fernández et al. 2000) and between two varieties of *Pinus nigra* (Lebourgeois et al. 1998), neither for  $A$  and  $W_i$  between five families of western larch (Zhang et al. 1994). According to Fernández et al. (2000), limitations in the measurement equipment could explain that no genetic variation was found for gas exchange rate in some studies, while according to de Miguel et al. (2012), accurate measurement of gas exchange rates increases statistical power and allows to observe significant between-family difference. But the adjustment of the ontogenetic could be the main component of this improvement. We found a significant family effect for all the variables except  $W_i$  in the WW treatment during the 6 weeks of the experiment. When we pool the data of the WW and WS treatments for weeks 0–2 (no water stress), the family effect is significant for all variables except *diam* and *height*.

Our results demonstrate that there is significant variation between the four families. Of course, we are aware that the low number of families does not permit a robust quantitative estimate of the genetic variation. However, it proves that there is genetic variation for the studied traits.

#### 4.3 Average plastic response to the between-treatment and time-related variation of water availability

The adjustment of the ontogenetic effect gave us the opportunity of a double approach of plasticity: between-treatment (between the two treatments in the greenhouse) and time-related (along the 6 weeks of increased drought stress) related. We observed a significant average plastic response of

**Table 2** Proportion of the variance due to different factors: time (week), family ( $F$ ), time by family interaction (week  $\times F$ ), soil water content (SWC; %), and soil water content by family interaction (SWC  $\times F$ ) on all variables (net CO<sub>2</sub> assimilation ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance to water vapor ( $g_s$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), intrinsic water-use efficiency ( $W_i = A/g_s$ ), diameter at 5 cm ( $diam$ , cm), length of terminal shoot ( $shoot$ , cm), and total height ( $height$ , cm)) for (a) the 32

WW trees (all the WW data), (b) the 64 trees of the pooled WW and WS treatments adjusted from the ontogenetic effect for weeks 0–2 on one hand (WW and WS data for weeks 0–2 pooled), (c) for weeks 5–6 on the other hand (WW and WS data for weeks 5–6 pooled), and (d) the 32 WS trees adjusted from the ontogenetic effect discovered in the WW treatment (WS data for weeks 0 to 6)

Dataset	Factors	$A$	$g_s$	$W_i (=A/g_s)$	$diam$	$shoot$	$height$
(a) All WW data	Week	60.754***	43.698***	17.543***	55.991***	58.391***	30.694***
	$F$	7.974***	4.589**	1.851 NS	3.340*	8.373***	5.899***
	Week $\times F$	0.221 NS	0.695 NS	0.759 NS	0.631 NS	0.754 NS	0.533 NS
(b) WW and WS data for weeks 0–2 pooled (between-treatment)	SWC	0.622 NS	0.066 NS	0.695 NS	0.027 NS	5.700*	0.546 NS
	$F$	5.517**	5.113**	3.216*	1.926 NS	5.522**	2.363 NS
	SWC $\times F$	0.728 NS	0.523 NS	0.577 NS	0.674 NS	0.933 NS	0.717 NS
(c) WW and WS data for weeks 5–6 pooled (between-treatment)	SWC	49.682***	139.519***	114.333***	23.392***	6.206*	11.060**
	$F$	1.448 NS	2.763*	2.928*	3.976**	6.973***	4.158**
	SWC $\times F$	1.993 NS	1.122 NS	0.235 NS	0.905 NS	1.864 NS	1.629 NS
(d) WS data for weeks 0 to 6 (time-related)	SWC	10.721**	63.426***	61.442***	16.396***	7.425**	6.463*
	$F$	2.610 NS	2.760*	1.127 NS	2.556 NS	12.268***	4.577**
	SWC $\times F$	0.021 NS	2.906*	3.600*	0.140	2.423 NS	2.372 NS

For the WW and WS pooled data, SWC is the soil water content effect, here varying with space (between-treatment). For the WS data, SWC is the soil water content effect, here varying with time (time-related). The level of significance is indicated by asterisks: \*\*\* $P < 0.001$ , \*\* $0.001 \leq P < 0.01$ , \* $0.01 < P < 0.05$ , NS  $> 0.05$

*P. pinaster* to decreasing water availability for both approaches (between-treatment (Table 2c) and time-related (Table 2d)) with a significant effect of SWC on the six traits studied. A significant *between-treatment plastic response* for the same variables was often found in the form of a significant treatment effect in water stress experiments (Fernández et al. 2006; Aranda et al. 2010; Sánchez-Gómez et al. 2010; de la Mata et al. 2014; Chambel et al. 2007; Corcuera et al. 2010) or along a rainfall gradient (Martin-StPaul et al. 2013). However, to our knowledge, there is no published result about time-related plasticity for these variables in this type of water-stressed experiment for *P. pinaster* or other conifer species, mostly because the time-related plastic response is generally confounded with the ontogenetic development.

#### 4.4 Family effect for plastic response

We found no family effect for the between-treatment plastic response for any variable (no significant SWC  $\times$  family interaction). Genetic variation of plastic response against spatial environmental variation is often studied in the form of genotype  $\times$  environment interaction in drought-stress experiments. As we did, Fernández et al. (2006) found no significant family effect on between-treatment plasticity for water availability for  $g_s$ ,  $A$ , and  $W_i$ . Others (Aranda et al. 2010; Corcuera et al. 2010, 2012) found significant population or family effect for  $W_i$  using carbon isotope discrimination as a proxy. For growth

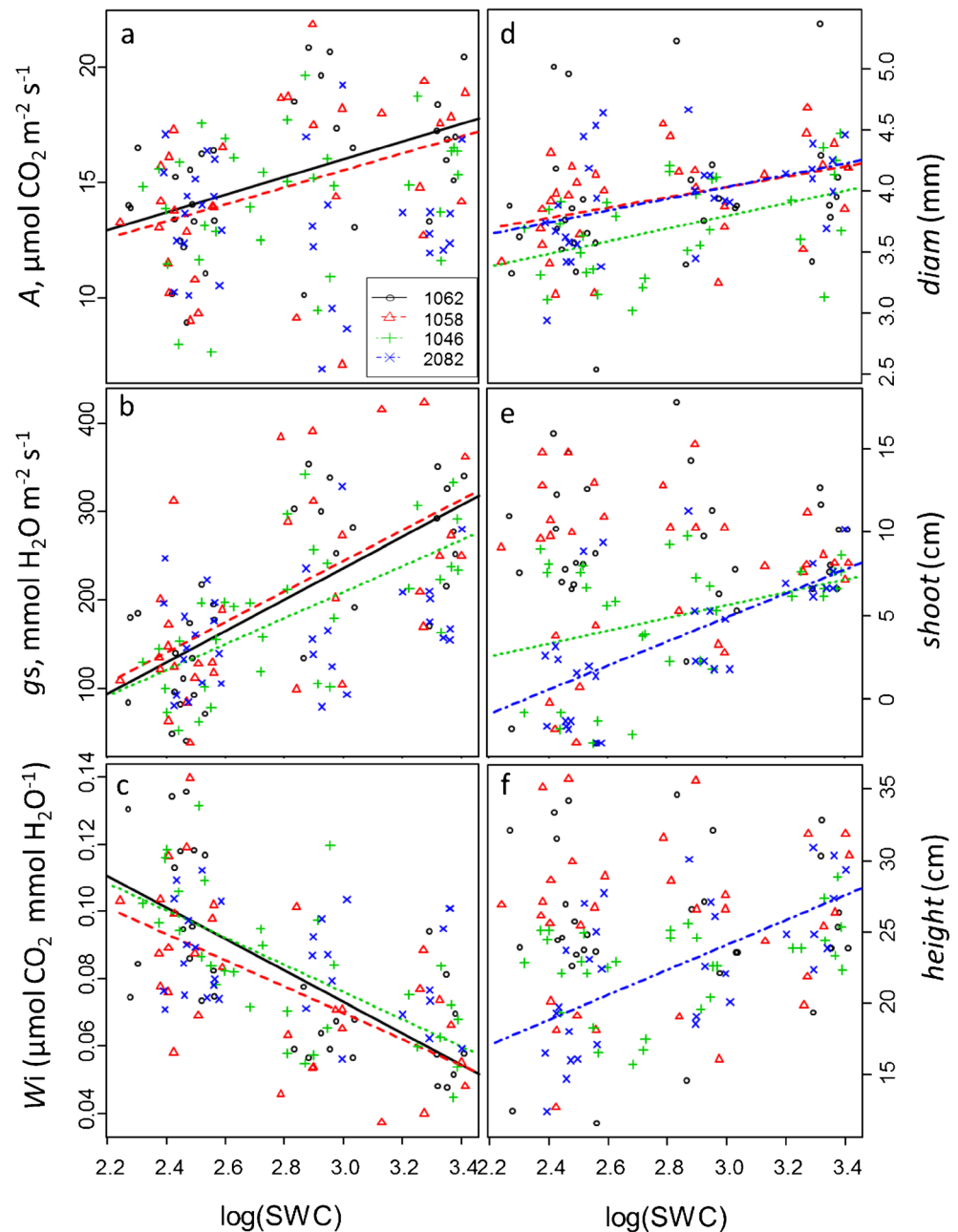
traits, the results are very diverse: significant (de la Mata et al. 2014) or non-significant (Corcuera et al. 2010; Chambel et al. 2007) population plastic response for height; similar behavior of populations in mesic and xeric sites for total height at age 1 after planting (Gaspar et al. 2013); and non-significant population plastic response for relative height and diameter growth rate (Sánchez-Gómez et al. 2010), but significant for diameter (Chambel et al. 2007). Finally, Fernández et al. (2006) found a significant family plastic effect for dry weight while Aranda et al. (2010) did not. Correia et al. (2008) found significant differences between *P. pinaster* populations for total height and carbon isotope discrimination used as a proxy of  $W_i$ .

While we found no family effect for the between-treatment plastic response for any variable, we found a significant family effect for the time-related plastic response for  $g_s$  and  $W_i$ . The adjustment of the ontogenetic effect allowed us to access to this time-related plasticity and contributed to the identification of this significant family effect. To our knowledge, this is the first time that this type of time-related plastic response to water availability is observed in *P. pinaster*.

The variety of results observed suggests that time-related and between-treatment plasticity are not alike. This is consistent with predictions using a theoretical model showing that variation in time and variation in space have different effects on plasticity (Scheiner 2013). Accurate comparison of between-treatment and time-related plasticity in fixed organisms like plants requires large-scale controlled condition



**Fig. 2** Plot of the family norms of reaction (family plastic response) for the net  $\text{CO}_2$  assimilation rate ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) (a), the stomatal conductance to water vapor ( $g_s$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) (b),  $W_i$  ( $=A/g_s$ ,  $\mu\text{mol CO}_2 \text{ mmol H}_2\text{O}^{-1}$ ) (c), the diameter at 5 cm ( $diam$ , cm) (d), the length of the terminal shoot ( $shoot$ , cm) (e), and the total height ( $height$ , cm) (f) as a function of the temporal variation of the log of the soil water content ( $\log(\text{SWC})$ ) in the WS treatment. Each family (1062, 1058, 1046, 2082) is represented by a geometric symbol (o,  $\Delta$ , +,  $\times$ ). Only the lines corresponding to the significant norms of reaction are drawn. Solid, dashed, and dotted lines represent the norm of reaction for families 1062, 1058, and 1046, respectively. The significant correlation coefficient ( $R$ ) for the pooled data are 0.27 for  $A$  ( $P=0.0019$ ), 0.56 for  $g_s$  ( $P<0.0001$ ),  $-0.56$  for  $W_i$  ( $P<0.0001$ ), 0.34 for  $diam$  ( $P<0.0001$ ), 0.21 for  $shoot$  ( $P=0.0185$ ), and 0.21 for  $height$  ( $P=0.0177$ )



experiments with a strict monitoring of ontogenetic effects. Our study suggests that such monitoring is possible.

In conclusion, we found variation of physiological and growth traits with water-stressed intensity in young *P. pinaster* trees studied few weeks after bud flush, along time as well as between treatments. We were able to quantify an ontogenetic effect for the six variables studied. We used this ontogenetic effect to better estimate the plastic response to water availability. We found a significant average plastic response to the spatial (between-treatment) and temporal (time-related) variation of water availability for the six variables. In the spatial

analysis, the trees of each family in the WW and WS treatments are different individuals of the same half-sib families: intra-family variation is added to between-treatment variation. In the temporal analysis, the same trees of each family in the WS treatment are observed at different times during the 6-week experiment. Therefore, the time-related plastic response is more precisely estimated than the between-treatment plastic response. This is probably why we found a significant family effect for the time-related plastic response for  $g_s$  and  $W_i$ , while this effect was not significant for the between-treatment plastic response. The observed plastic response to SWC is a

**Table 3** Linear correlations (Spearman's coefficients) computed for the well-watered (WW; below the diagonal line) and the water-stressed (WS; above the diagonal line) treatments on week 6 of the experiment between the six variables: net CO<sub>2</sub> assimilation (*A*, μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance to water vapor (*gs*, mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), intrinsic water-use efficiency (*W*<sub>i</sub> = *A*/*gs*), diameter at 5 cm (*diam*, cm), length of terminal shoot (*shoot*, cm), and total height (*height*, cm) with 32 trees in each treatment

	<i>A</i>	<i>gs</i>	<i>W</i> <sub>i</sub>	<i>diam</i>	<i>shoot</i>	<i>height</i>
<i>A</i>	–	0.76**	NS	NS	NS	NS
<i>gs</i>	0.83***	–	–0.82***	NS	NS	NS
<i>W</i> <sub>i</sub>	–0.41*	–0.80***	–	0.36*	0.63**	0.54**
<i>diam</i>	NS	NS	NS	–	NS	0.45**
<i>shoot</i>	NS	NS	NS	0.52**	–	0.84***
<i>height</i>	NS	NS	NS	0.64***	0.90***	–

The level of significance is indicated by asterisks: \*\*\* $P < 0.001$ , \*\* $0.001 \leq P < 0.01$ , \* $0.01 < P < 0.05$ , NS  $> 0.05$

convenient integrated measure of *P. pinaster* response to drought. Stomatal conductance (*gs*) and *shoot* are the only traits showing a significant family effect at all time in both treatments. Stomatal conductance was found to be more variable than *A* and more strongly correlated with *W*<sub>i</sub>. All this suggests that the family plastic response for *gs* could be a suitable integrated selection criterion for improving *W*<sub>i</sub> in this species. This result, if verified at early and adult age, with more trees and families for a precise estimation of genetic variation, could be used as a tool for early selection and prediction of future performance under water limitation conditions. At the same time, the growth traits were found to be mostly independent from the physiological traits. It suggests that it may be possible to minimize the negative impact of drought on growth when improving maritime pine drought tolerance.

**Data availability** Data generated or analyzed during this study are included in this published article and its supplementary information files.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Aranda I, Alía R, Ortega U, Dantas ÂK, Majada J (2010) Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations. *Tree Genet Genomes* 6:169–178. <https://doi.org/10.1007/s11295-009-0238-5>
- Aussenac G, Granier A (1978) Quelques résultats de cinétique journalière du potentiel de sève chez les arbres forestiers. *Ann Sci For* 35:19–32. <https://doi.org/10.1051/forest/19780102>
- Brendel O, Pot D, Plomion C, Rozenberg P, Guehl JM (2002) Genetic parameters and QTL analysis of delta C-13 and ring width in maritime pine. *Plant Cell Environ* 25:945–953
- Chambel MR, Climent J, Alía R (2007) Divergence among species and populations of Mediterranean pines in biomass allocation of seedlings grown under two watering regimes. *Ann For Sci* 64:87–97. <https://doi.org/10.1051/forest:2006092>
- Chaves MM (1991) Effects of water deficits on carbon assimilation. *J Exp Bot* 42:1–16. <https://doi.org/10.1093/jxb/42.1.1>
- Corcuera L, Gil-Pelegrin E, Notivol E (2010) Phenotypic plasticity in *Pinus pinaster* δ13C: environment modulates genetic variation. *Ann For Sci* 67:812–812. <https://doi.org/10.1051/forest/2010048>
- Corcuera L, Cochard H, Gil-Pelegrin E, Notivol E (2011) Phenotypic plasticity in Mesic populations of *Pinus pinaster* improves resistance to xylem embolism (P50) under severe drought. *Trees* 25:1033–1042. <https://doi.org/10.1007/s00468-011-0578-2>
- Corcuera L, Gil-Pelegrin E, Notivol E (2012) Differences in hydraulic architecture between Mesic and xeric *Pinus pinaster* populations at the seedling stage. *Tree Physiol* 32:1442–1457. <https://doi.org/10.1093/treephys/tps103>
- Cornic G (2000) Drought stress inhibits photosynthesis by decreasing stomatal aperture—not by affecting ATP synthesis. *Trends Plant Sci* 5:187–188. [https://doi.org/10.1016/S1360-1385\(00\)01625-3](https://doi.org/10.1016/S1360-1385(00)01625-3)
- Correia I, Almeida MH, Aguiar A, Alía R, David TS, Pereira JS (2008) Variations in growth, survival and carbon isotope composition (<sup>13</sup>C) among *Pinus pinaster* populations of different geographic origins. *Tree Physiol* 28:1545–1552. <https://doi.org/10.1093/treephys/28.10.1545>
- de la Mata R, Voltas J, Zas R (2012) Phenotypic plasticity and climatic adaptation in an Atlantic maritime pine breeding population. *Ann For Sci* 69:477–487. <https://doi.org/10.1007/s13595-011-0173-0>
- de la Mata R, Merlo E, Zas R (2014) Among-population variation and plasticity to drought of Atlantic, Mediterranean, and interprovenance hybrid populations of maritime pine. *Tree Genet Genomes* 10:1191–1203. <https://doi.org/10.1007/s11295-014-0753-x>
- de Miguel M, Sanchez-Gomez D, Cervera MT, Aranda I (2012) Functional and genetic characterization of gas exchange and intrinsic water use efficiency in a full-sib family of *Pinus pinaster* Ait. in response to drought. *Tree Physiol* 32:94–103. <https://doi.org/10.1093/treephys/tps122>
- de Miguel M, Cabezas J-A, de María N, Sánchez-Gómez D, Guevara M-Á, Vélez M-D, Sáez-Laguna E, Díaz L-M, Mancha J-A, Barbero M-C, Collada C, Díaz-Sala C, Aranda I, Cervera M-T (2014) Genetic control of functional traits related to photosynthesis and water use efficiency in *Pinus pinaster* Ait. drought response: integration of genome annotation, allele association and QTL detection for candidate gene identification. *BMC Genomics* 15:464. <https://doi.org/10.1186/1471-2164-15-464>
- DeWitt TJ, Scheiner SM (2004) Phenotypic plasticity: functional and conceptual approaches. Oxford University Press, Oxford
- Dietz K-J, Heber U (1983) Carbon dioxide gas exchange and the energy status of leaves of *Primula palinuri* under water stress. *Planta* 158:349–356. <https://doi.org/10.1007/BF00397337>

- Egea G, González-Lez-Real MM, Baille A, Nortes PA, Diaz-Espejo A (2011) Disentangling the contributions of ontogeny and water stress to photosynthetic limitations in almond trees: photosynthetic limitations in almond trees. *Plant Cell Environ* 34:962–979. <https://doi.org/10.1111/j.1365-3040.2011.02297.x>
- Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. *Annu Rev Plant Physiol* 33:317–345. <https://doi.org/10.1146/annurev.pp.33.060182.001533>
- Fernández M, Gil L, Pardos JA (1999) Response of *Pinus pinaster* Ait. provenances at early age to water supply. I. Water relation parameters. *Ann For Sci* 56:179–187. <https://doi.org/10.1051/forest:19990209>
- Fernández M, Gil L, Pardos JA (2000) Effects of water supply on gas exchange in *Pinus pinaster* Ait. provenances during their first growing season. *Ann For Sci* 57:9–16. <https://doi.org/10.1051/forest:2000107>
- Fernández M, Novillo C, Pardos JA (2006) Effects of water and nutrient availability in *Pinus pinaster* Ait. open pollinated families at an early age: growth, gas exchange and water relations. *New For* 31:321–342. <https://doi.org/10.1007/s11056-005-8196-8>
- Flexas J (2002) Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. *Ann Bot* 89:183–189. <https://doi.org/10.1093/aob/mcf027>
- Galmés J, Medrano H, Flexas J (2007) Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytol* 175:81–93. <https://doi.org/10.1111/j.1469-8137.2007.02087.x>
- Gaspar MJ, Velasco T, Feito I, Alía R, Majada J (2013) Genetic variation of drought tolerance in *Pinus pinaster* at three hierarchical levels: a comparison of induced osmotic stress and field testing. *PLoS One* 8: e79094. <https://doi.org/10.1371/journal.pone.0079094>
- Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct Ecol* 21:394–407. <https://doi.org/10.1111/j.1365-2435.2007.01283.x>
- Granier A, Loustau D (1994) Measuring and modelling the transpiration of a maritime pine canopy from sap-flow data. *Agric For Meteorol* 71:61–81. [https://doi.org/10.1016/0168-1923\(94\)90100-7](https://doi.org/10.1016/0168-1923(94)90100-7)
- Guehl JM, Picon C, Aussenac G, Gross P (1994) Interactive effects of elevated CO<sub>2</sub> and soil drought on growth and transpiration efficiency and its determinants in two European forest tree species. *Tree Physiol* 14:707–724
- Lamy J-B, Delzon S, Bouche PS, Alía R, Vendramin GG, Cochard H, Plomion C (2014) Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. *New Phytol* 201:874–886. <https://doi.org/10.1111/nph.12556>
- Lawlor DW, Cornic G (2002) Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ* 25:275–294. <https://doi.org/10.1046/j.0016-8025.2001.00814.x>
- Lebourgeois F, Lévy G, Aussenac G, Clerc B, Willm F (1998) Influence of soil drying on leaf water potential, photosynthesis, stomatal conductance and growth in two black pine varieties. *Ann Sci For* 55: 287–299. <https://doi.org/10.1051/forest:19980302>
- Limousin J-M, Misson L, Lavoire A-V, Martin NK, Rambal S (2010) Do photosynthetic limitations of evergreen *Quercus ilex* leaves change with long-term increased drought severity? *Plant Cell Environ*. <https://doi.org/10.1111/j.1365-3040.2009.02112.x>
- Marguerit E, Bouffier L, Chancerel E, Costa P, Lagane F, Guehl J-M, Plomion C, Brendel O (2014) The genetics of water-use efficiency and its relation to growth in maritime pine. *J Exp Bot* 65:4757–4768. <https://doi.org/10.1093/jxb/eru226>
- Martin-StPaul NK, Limousin J-M, Rodríguez-Calcerrada J, Ruffault J, Rambal S, Letts MG, Misson L (2013) Photosynthetic sensitivity to drought varies among populations of *Quercus ilex* along a rainfall gradient. *Funct Plant Biol* 39:25. <https://doi.org/10.1071/FP11090>
- Maseda PH, Fernández RJ (2006) Stay wet or else: three ways in which plants can adjust hydraulically to their environment. *J Exp Bot* 57: 3963–3977. <https://doi.org/10.1093/jxb/erl127>
- Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit J-M, Barbaroux C, Le Thiec D, Brechet C, Brignolas F (2006) Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. *New Phytol* 169:765–777. <https://doi.org/10.1111/j.1469-8137.2005.01630.x>
- Pachauri RK, Leo M, et Intergovernmental Panel on Climate Change (éds) (2015) Climate Change 2014: Synthesis Report. Intergovernmental Panel on Climate Change, Geneva
- Paiva JAP, Garnier-Géré PH, Rodrigues JC, Alves A, Santos S, Graça J, Le Provost G, Chaumeil P, Da Silva-Perez D, Bosc A, Fevereiro P, Plomion C (2008) Plasticity of maritime pine (*Pinus pinaster*) wood-forming tissues during a growing season. *New Phytol* 179:1180–1194. <https://doi.org/10.1111/j.1469-8137.2008.02536.x>
- Picon C, Guehl JM, Ferhi A (1996) Leaf gas exchange and carbon isotope composition responses to drought in a drought-avoiding (*Pinus pinaster*) and a drought-tolerant (*Quercus petraea*) species under present and elevated atmospheric CO<sub>2</sub> concentrations. *Plant Cell Environ* 19:182–190. <https://doi.org/10.1111/j.1365-3040.1996.tb00239.x>
- Picon-Cochard C, Guehl J-M (1999) Leaf gas exchange and carbohydrate concentrations in *Pinus pinaster* plants subjected to elevated CO<sub>2</sub> and a soil drying cycle. *Ann For Sci* 56:71–76. <https://doi.org/10.1051/forest:19990109>
- Plomion C, Bartholomé J, Bouffier L, Brendel O, Cochard H, De Miguel M, Delzon S, Gion J-M, Gonzalez-Martinez SC, Guehl J-M, Lagravelle H, Le Provost G, Marguerit E, Porté A (2016) Understanding the genetic bases of adaptation to soil water deficit in trees through the examination of water use efficiency and cavitation resistance: maritime pine as a case study. *J Plant Hydraul* 3(8): 008. <https://doi.org/10.20870/jph.2016.e008>
- R software (version 2.8.0, R 274Q8 Development Core Team 2008)
- Sánchez-Gómez D, Majada J, Alía R, Feito I, Aranda I (2010) Intraspecific variation in growth and allocation patterns in seedlings of *Pinus pinaster* Ait. submitted to contrasting watering regimes: can water availability explain regional variation? *Ann For Sci* 67: 505–504. <https://doi.org/10.1051/forest/2010007>
- Scheiner SM (2013) The genetics of phenotypic plasticity. XII. Temporal and spatial heterogeneity. *Ecol Evol* 3:4596–4609. <https://doi.org/10.1002/ece3.792>
- Schlichting CD (1986) The evolution of phenotypic plasticity in plants. *Annu Rev Ecol Syst* 17:667–693. <https://doi.org/10.1146/annurev.es.17.110186.003315>
- Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Science* 148:339–346
- Schulze ED, Turner NC, Gollan T, Shackel KA (1987) Stomatal responses to air humidity and to soil drought. *Stomatal Funct* 804713472:311–321
- Slaney M (2006) Impact of elevated temperature and [CO<sub>2</sub>] on spring phenology and photosynthetic recovery of boreal Norway spruce. Southern Swedish Forest research Centre. Swedish University of Agricultural Sciences, Alnarp
- Strand M, Lundmark T (1995) Recovery of photosynthesis in 1-year-old needles of unfertilized and fertilized Norway spruce (*Picea abies* (L.) karst.) during spring. *Tree Physiol* 15:151–158
- Strand M, Lundmark T, Söderbergh I, Mellander P-E (2002) Impacts of seasonal air and soil temperatures on photosynthesis in Scots pine trees. *Tree Physiol* 22:839–847
- Tardieu F (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. *J Exp Bot* 49:419–432. [https://doi.org/10.1093/jexbot/49.suppl\\_1.419](https://doi.org/10.1093/jexbot/49.suppl_1.419)

- Xu L, Baldocchi DD (2003) Seasonal trends in photosynthetic parameters and stomatal conductance of blue oak (*Quercus douglasii*) under prolonged summer drought and high temperature. *Tree Physiol* 23: 865–877. <https://doi.org/10.1093/treephys/23.13.865>
- Zhang J, Marshall JD, Jaquish BC (1993) Genetic differentiation in carbon isotope discrimination and gas exchange in *Pseudotsuga menziesii*: a common-garden experiment. *Oecologia* 93:80–87. <https://doi.org/10.1007/BF00321195>
- Zhang J, Fins L, Marshall JD (1994) Stable carbon isotope discrimination, photosynthetic gas exchange, and growth differences among western larch families. *Tree Physiol* 14:531–539. <https://doi.org/10.1093/treephys/14.5.531>

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