Maternal environment regulates morphological and physiological traits in *Eucalyptus grandis*

María Vivas[a,*], Víctor Rolo[b,c], Michael J. Wingfield[d], Bernard Slippers[a]

[a] *Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*
[b] *Conservation Ecology Research Unit, University of Pretoria, Pretoria, South Africa*
[c] *Forest Research Group, INDEHESA, University of Extremadura, Plasencia, Spain*
[d] *Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa*

ABSTRACT

The phenotype of a plant can be shaped by the biotic and abiotic conditions to which their parents are subjected. This phenotypic plasticity known as maternal environmental effect occurs regardless of the changes in the DNA sequences. Whereas the effect of the maternal environment on plant phenotype has been studied, its specific influence on plant physiology is less clear. This study considered the influence of the maternal environment on the regulation of plant morphology and physiology in progeny of *Eucalyptus grandis*. Plants were grown from seeds collected from two *E. grandis* clonal seed orchards that differed in environmental conditions (e.g. precipitation). Plant relative growth rate (RGR), leaf gas exchange and water use efficiency (WUE) were measured in the seedlings. RGR was 10% higher in the offspring from the maternal environment receiving higher precipitation levels. Leaf gas exchange, specifically leaves intercellular CO_2, and intrinsic WUE were also influenced by maternal environments. Intrinsic WUE was significantly lower in the orchard that received lower precipitation levels. The results demonstrate that the maternal environment can regulate the physiology of *E. grandis* in the subsequent generation. These analyses are useful to optimise tree improvement in a changing environment. Moreover, in a scenario of climate change, maternal environmental effects may be a crucial mechanism for certain species to get acclimated to sudden changes in environmental conditions.

1. Introduction

The plant phenotype is mainly determined by its genetic inheritance and environment. However, the phenotype of a plant is also shaped by the biotic and abiotic conditions to which its parents are subjected (Roach and Wulff, 1987; Jablonka and Raz, 2009; Herman and Sultan, 2011; Holeski et al., 2012). These influences on the phenotypic expression of the seedlings are known as maternal environmental effects, and they are not caused by DNA mutations (Roach and Wulff, 1987; Rossiter, 1996; Rix et al., 2012). Rather, the maternal effects are induced by seed provisioning and by epigenetic mechanisms (Herman and Sultan, 2011; Rix et al., 2012). Since the description of maternal environmental effects three decades ago, a number of studies have demonstrated its influence on morphological changes and resistance responses of the plants in the subsequent generation (e.g. Roach and Wulff, 1987; Violle et al., 2009; Elwell et al., 2011; Luna et al., 2012; Rasmann et al., 2012; Vivas et al., 2013, 2017). However, little is known regarding the physiological adaptation underlying these developmental and morphological changes.

Understanding maternal environmental effects is important from the perspective of predicting responses of plants to changing environments, as well as to harness their full productive potential through breeding programs. Failure to consider these effects could lead to misinterpretations of genetic studies in breeding programs. This is especially true in the light of climatic changes and the pressures these will place on natural distribution of plant communities and production areas alike (Allen et al., 2010; Koch et al., 1995). A relevant factor to consider in this regard, and that is expected to be influenced by maternal environments, is plant morphological and physiological responses to low water availability (Anderegg, 2015). Water availability is a key element shaping the distribution of current and future vegetation, as drought intensity and duration is expected to increase in many parts of the world (IPCC, 2014).

Water loss in plants is an inevitable consequence of photosynthesis, where CO_2 diffuses into the leaf and flux out water (i.e. leaf gas exchange). Minimizing water loss while maximizing CO_2 uptake (i.e.

---

* Corresponding author.
  E-mail address: maria.vivas@fabi.up.ac.za (M. Vivas).

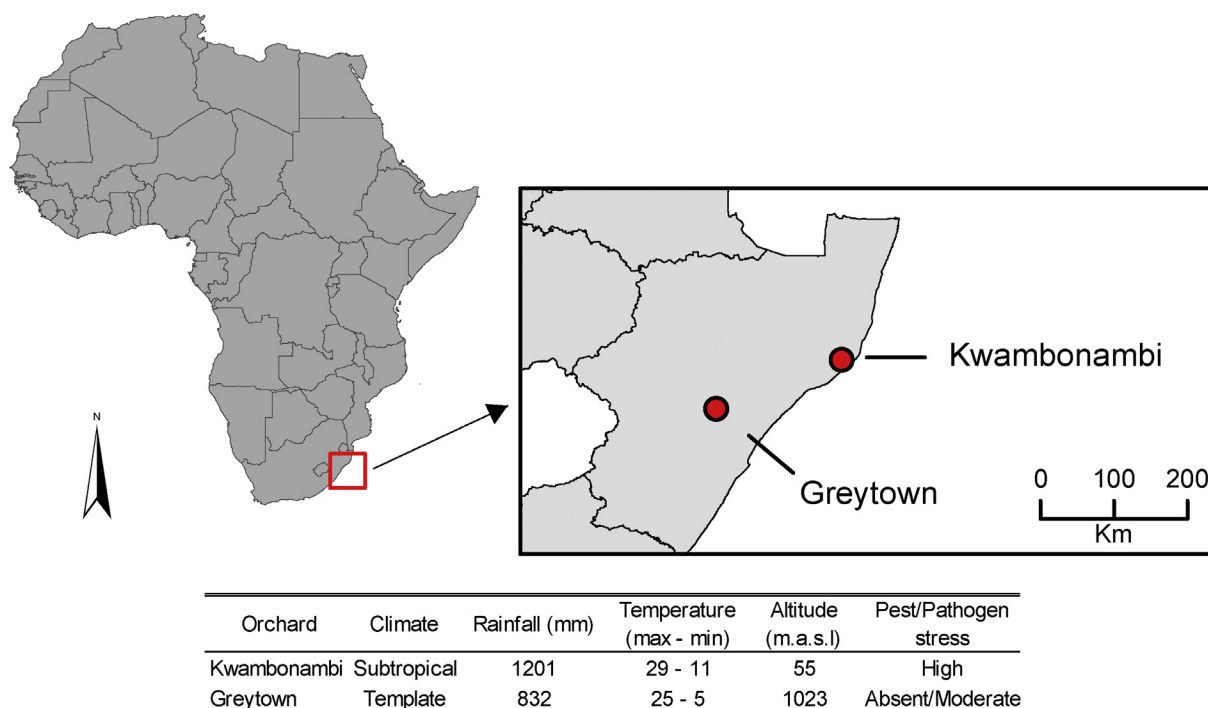| Orchard	Climate	Rainfall (mm)	Temperature (max - min)	Altitude (m.a.s.l)	Pest/Pathogen stress
Kwambonambi	Subtropical	1201	29 - 11	55	High
Greytown	Template	832	25 - 5	1023	Absent/Moderate

Fig. 1. Map showing the locations and the characteristics of the two *Eucalyptus grandis* orchards in South Africa. Data provided by the forest company.

increasing water use efficiency (WUE)) is a key physiological mechanism of plants adapted to low water availability (Gindaba et al., 2005; Choat et al., 2006). Plants achieve this by regulating the partial closure of the stomata (i.e. stomatal conductance (g_s)) to adjust leaf transpiration (Flexas and Medrano, 2002). The analysis of WUE evaluates plant biomass production as a function of water consumption (Monclus et al., 2006; Chamaillard et al., 2011; Battie-Laclau et al., 2016). Water use efficiency can be estimated at multiple spatial and temporal scales: from short (the ration between net photosynthesis and stomatal conductance; intrinsic WUE (Medrano et al., 2009)) to long (based on the composition in stable carbon isotopes ($\delta^{13}C$) (Farquhar and Richards, 1984)). This trait (WUE) typically has high within-species genetic variability and has therefore received substantial attention in tree breeding programs (e.g. Monclus et al., 2006; Aranda et al., 2012). However, the knowledge of how the maternal environment affects WUE in plant progeny, as well as related physiological traits such as stomatal control or net photosynthesis, remains limited (Latzel and Klimešová, 2009; Scotti et al., 2010).

Maternal effects on morphological traits of plants may result in changes in the relative growth rate (RGR) of the progeny, defined as the increase of dry mass production per unit of dry mass and time (Poorter and Werf, 1998). RGR can be divided into three components. Two of these are morphological components that describe how the plant allocates biomass to leaves (leaf mass ratio (LMR)), and how efficient the plant is in the construction of the leaf area (specific leaf area (SLA)). A third physiological component describes how plant biomass changes per unit leaf area and time (net assimilation rate) (Evans, 1972). Many studies have considered the relationship between RGR and its components in multiple plant species (e.g. Poorter and Remkes, 1990; Poorter and Werf, 1998; Loveys et al., 2002). However, the differences in RGR within a species are not well understood, and maternal environmental effects on RGR are less well-known. To understand the possible influence of maternal environments on plant morphology as well as its potential applications in plant breeding programs, it is necessary to consider the RGR within species.

Eucalyptus are widely used globally for the establishment of rapidly growing plantations and these are exposed to a wide variety of environmental conditions (Wingfield et al., 2015). Previous studies have reported maternal effects in *Eucalyptus* species. For example, maternal environment influenced seed mass and seed germination of *Eucalyptus globulus* in two different studies (Lopez et al., 2003; Rix et al., 2012). Moreover, there is evidence in *Eucalyptus grandis* that the maternal environment influences progeny performance, resistance to pest and pathogens as well as the structure of fungal communities in the foliage of the subsequent generation (Vivas et al., 2017). A relevant case study would thus be to explore the impact of maternal effects on morphological and physiological traits related to growth potential and leaf gas exchange of offspring plants.

In this study, we measured morphological and physiological traits of *E. grandis* seedlings originating from two different maternal environments when grown in a common garden. Given the cosmopolitan distribution of *Eucalyptus* species, their morphological and physiological responses to a wide variety of environmental conditions are well known (e.g. Correia et al., 2014, 2018; Navarrete-Campos et al., 2013). However, little is known about the underlying role of maternal effects on *Eucalyptus* morphology and physiology. We hypothesized that trees having the same genotype would acquire different morpho-physiological strategies according to the availability of resources (e.g. water) of their maternal environments. In particular, we considered whether (1) growth rates of *E. grandis* trees depend on the maternal environment. And, if there are differences due to morphological (SLA and LMR) attributes. Furthermore, we considered whether (2) the physiology of *E. grandis* trees, specifically leaf gas exchange and WUE, reflect the conditions of the maternal environment.

2. Materials and methods

2.1. Plant material

Seed material was collected from *E. grandis* trees open-pollinated and located in two clonal seed orchards in South Africa. The two orchards, Greytown (29°11′56.73″S, 30°39′34.46″E) and Kwambonambi (28°35′41.45″S, 32°11′38.98″E), included the same genetic material with identical spatial design, but different abiotic and biotic conditions (Fig. 1). The Kwambonambi orchard represents more favourable abiotic conditions (e.g. higher rainfall and temperatures) for

E. grandis development than the Greytown orchard. However, a higher infection in the Kwambonambi orchard by pests and pathogens decreased the height of the trees compared to the Greytown orchard (see Vivas et al., 2017, for more details about the seed orchards). Three *E. grandis* genotypes (G1, G2 and G3) represented by three ramets were selected in each orchard (2 maternal environments × 3 genotypes × 3 ramets).

Seeds were collected from each ramet in each orchard in March 2014. In July 2014, seeds were sown and germinated during two consecutive days as described in Vivas et al. (2017). Seedlings were grown in 5 L plastic bags containing a commercial substrate of compost pine bark in a common nursery. A total of 180 seedlings (2 maternal environments × 3 genotypes × 3 ramets × 10 seedlings) were randomly selected and distributed in a factorial design of two blocks in a greenhouse in July 2016. The average greenhouse conditions were: temperature 15 °C (max. 26 °C and min. 10 °C), 50% full sunlight and watered as needed. Ramet identity was confirmed and pollen contamination discarded, as described in a previous study (Vivas et al., 2017).

2.2. Morphological traits

For each plant, seedling height was measured monthly from month four after sowing until month 15, and thereafter at month 27. In September 2016 when the plants were 26-months-old, seedlings were cut above the root collar and divided into stem and leaf material. Just before seedlings were harvested, six leaves per seedling were collected and immediately scanned with a flatbed scanner (Canon Scan LiDE 220) to assess leaf area. Leaf area was calculated from the images using Image J software (Schneider et al., 2012). The collected stem and leaf material from the seedlings were oven-dried for 40 h at 70 °C and weighed to calculate the dry weight of the different components.

2.3. Leaf gas exchange measurements

Leaf gas exchange measurements were conducted on two young leaves fully developed per seedling on two consecutive days before harvesting. The results of the two young leaves were pooled per seedling. The measurements were carried out with a portable infrared gas analyzer (6400Li-Cor, Lincoln, NE, USA) equipped with a 6 cm² chamber, at 800 μmol m^{-2} s^{-1} photon flux density, 400 μmol CO_2 mol^{-1}. The gas exchange measurements recorded included: net photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (Ci, μmol CO_2 mol^{-1}) and transpiration rate (E, mmol H_2O m^{-2} s^{-1}). Leaf gas exchange measurements were calculated on a leaf area basis (6 cm²). When the leaf area was smaller than the 6 cm² chamber, the measurements were corrected by calculating the leaf area as above (see Morphological traits). Intrinsic water use efficiency (iWUE) was calculated as the ratio between A and gs (Medrano et al., 2009; Bartholome et al., 2015; Battie-Laclau et al., 2016).

2.4. Carbon isotopes ($\delta^{13}C$)

On a long time scale, water use efficiency (WUE) is related to the composition of stable carbon isotopes ($\delta^{13}C$) (Farquhar and Richards, 1984; Bartholome et al., 2015; Battie-Laclau et al., 2016). The foliar $\delta^{13}C$ signature was measured for each of the seedlings using the oven-dried leaves (see Morphological traits). The oven-dried leaves were ground to a fine powder with a ball mill and $\delta^{13}C$ composition was analyzed in a stable isotope ratio mass spectrometer (Flash EA 1112 Series) coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (Thermo Fischer, Bremen, Germany). Results were calculated using the standard equation:

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

where R represents $^{13}C/^{12}C$ and $R_{standard}$ is the molar ratio of the international standard Pee Dee Belemnite (Craig, 1957). Analytical precision was $< 0.08‰$ for $\delta^{13}C$.

2.5. Data analysis - relative growth rate

Total plant biomass was estimated at different time-steps based on the repeated measurements of seedling height, as it was not possible to harvest plants at different times. We first fitted a log-log model with final plant biomass as the response variable and final height as the predictor. We then fitted a growth curve for each seedling to calculate RGR at a common size (Paine et al., 2012) based on the estimated plant biomass. The power law model was the non-linear function that best fitted the data.

To estimate the influence of maternal environments and genotypes on the RGR and its morphological components, linear mixed models were used with RGR, SLA and LMR as response variables and, the maternal environments, the genotypes and their interaction as predictors. To account for different sources of variability present in the dataset the ramet and block were also included as random effects.

2.6. Data analysis - leaf gas exchange and water use efficiency

To assess whether overall morphological and physiological traits (i.e. leaf gas exchange, iWUE and $\delta^{13}C$) were influenced by the maternal environments and the genotypes, permutational multivariate ANOVA (PERMANOVA) was applied using Euclidean distance and 10,000 permutations. PERMANOVA mirrors the utility of classical ANOVA, such as assessing the effect of factors, but it allows the analysis of multivariate data (Anderson, 2017). Multivariate datasets were projected onto the first two principal component axes to assess visually differences between maternal environments and the genotypes.

Linear mixed models were used to assess the influenced of the maternal environments and the genotypes on each variable separately. The maternal environments, the genotypes and their interaction were included as explanatory variables. The dependent variables leaf gas exchange parameters (A, gs, C_i and E), iWUE and $\delta^{13}C$ fitted a normal distribution. Additionally, the ramet and block were considered as random variables in the model. Analyses were performed separately for each trait. In all cases, model validity was checked and 'lme4' package of the R software was used (R Core Team, 2014).

3. Results

3.1. Relative growth rate

Size standardized RGR showed significant differences between seedlings originating from the two maternal environments ($P < 0.01$, Fig. 2). The Greytown orchard showed lower RGR values than the Kwambonambi orchard (Table 1). Maternal genotypes or the interaction between orchards and genotypes didn't show significant differences in RGR (Table 1).

SLA and LMR were influenced by maternal genotypes ($P < 0.01$ and $P < 0.05$, respectively). Among maternal genotypes the G3 genotype had a significantly lower SLA and higher LMR than the rest of the genotypes (Table 1). SLA was also influenced by the interaction between maternal environments and genotypes $P < 0.05$. The G2 genotype showed a significantly higher SLA in the Kwambonambi orchard than in the Greytown orchard (Table 1).

3.2. Leaf gas exchange and water use efficiency

The maternal environment had a significant effect on the multivariate variation of physiological variables (A, gs, E, Ci, iWUE and $\delta^{13}C$) ($P = 0.008$). However, the maternal genotypes or the interaction between the maternal environment and genotypes did not influence the
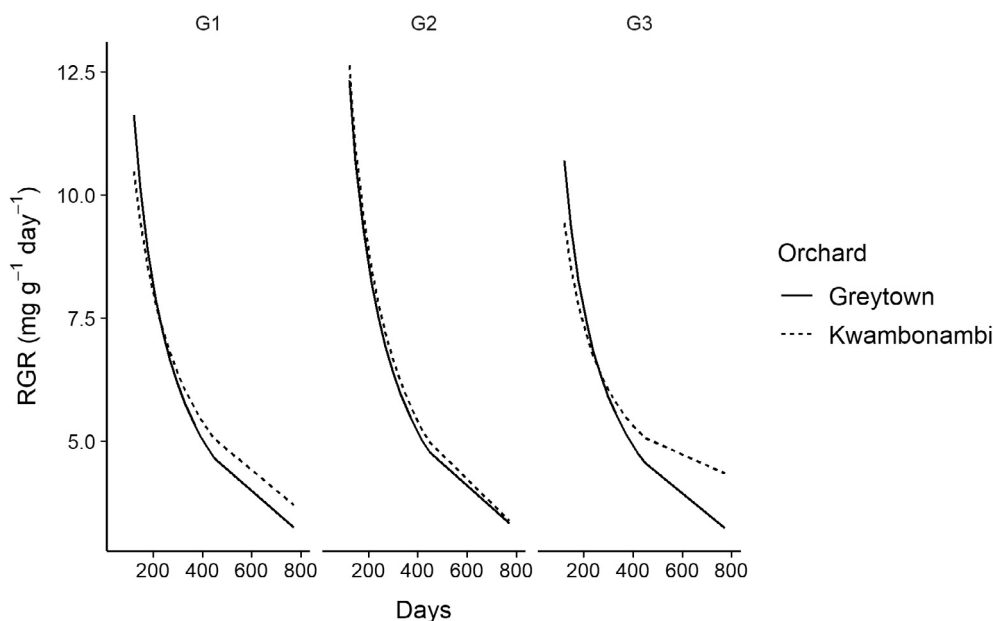
Fig. 2. Relative growth rate (RGR) of *Eucalyptus grandis* seedlings derived from three genotypes (G1, G2, G3), replicated in two maternal environments (Greytown and Kwambonambi).

physiological variables. The graphical exploration showed no overlapping confidence intervals between maternal environments for each genotype along the first axis which explained ~60% of the multivariate variation (Fig. 3).

Univariate linear mixed models showed that seedling Ci and iWUE were influenced by maternal environments ($P < 0.001$) and there were no significant differences between maternal genotypes ($P = 0.308$ and $P = 0.519$, Ci and iWUE, respectively). Ci was higher in seedlings from the Kwambonambi orchard than from Greytown. Plant iWUE showed the opposite pattern with higher values in seedlings from the Greytown orchard than from Kwambonambi (Table 2). Leaf δ^{13}C composition was not influenced by the maternal environments ($P = 0.603$), but there was a significant effect of the maternal genotypes ($P = 0.007$) and the interaction between maternal environments and genotypes ($P < 0.001$). Whereas seedlings from the maternal *Eucalyptus* genotype G1 showed higher values of leaf δ^{13}C composition in Kwambonambi than from Greytown orchard, genotype G3 showed the opposite pattern (Table 2). No significant differences were observed in plant *A*, gs, and *E* between maternal environments nor genotypes (Table 2).

4. Discussion

This is the first study to explore the maternal effects on the physiology of *E. grandis* progeny. The results demonstrated that the maternal environment regulates morphology and physiology in three genotypes of *E. grandis* from two different maternal environments. Specifically, RGR, Ci and iWUE revealed local adaptation to maternal
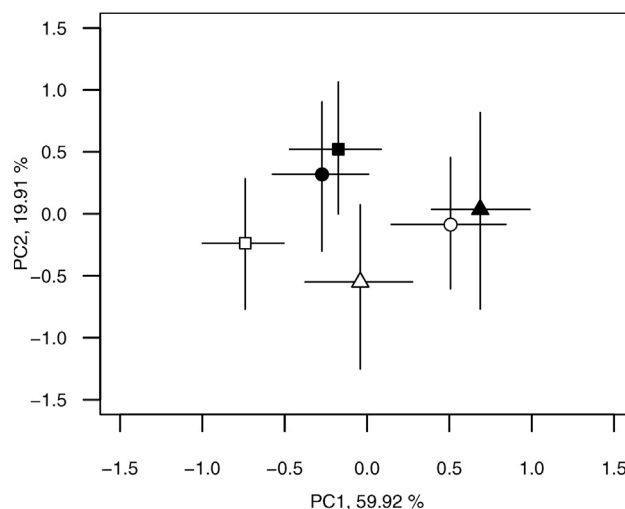

Fig. 3. Projected values of multivariate dataset of plant physiology (A, gs, E, Ci, iWUE and δ^{13}C) on the first two principal components of *Eucalyptus grandis* seedlings. Seedlings derived from three genotypes (● = G1, ■ = G2, ▲ = G3), replicated in two maternal environments (close symbols = Greytown and open symbols = Kwambonami). Bars depict 95% confidence intervals for each principal component.

Table 1

Morphological traits for the *Eucalyptus grandis* seedlings derived from three genotypes (G1, G2 and G3) clonally replicated in two maternal environments (Greytown and Kwambonambi). Mean values ± SE are shown. Different letters within a column indicate significant differences at $P < 0.05$.

Maternal environment	Genotype	RGR (mg g^{-1} day^{-1})	SLA (cm^2 g^{-1})	LMR (%)
Greytown	G1	4.03 ± 0.12[a]	1.26 ± 0.06[b]	0.188 ± 0.011[a]
	G2	4.22 ± 0.13[a]	1.10 ± 0.04[a]	0.210 ± 0.007[ac]
	G3	3.97 ± 0.13[a]	1.01 ± 0.07[a]	0.226 ± 0.010[bc]
Kwambonambi	G1	4.62 ± 0.11[a]	1.17 ± 0.04[ab]	0.221 ± 0.010[ac]
	G2	4.36 ± 0.14[a]	1.32 ± 0.06[b]	0.208 ± 0.009[ab]
	G3	4.79 ± 0.16[a]	1.01 ± 0.04[a]	0.246 ± 0.010[c]

RGR, relative growth rate; SLA, specific leaf area; LMR, leaf mass ratio.

Table 2

Physiological traits for the *Eucalyptus grandis* seedlings derived from three genotypes (G1, G2 and G3) clonally replicated in two maternal environments (Greytown and Kwambonambi). Mean values ± SE are shown. Different letters within a column indicate significant differences at $P < 0.05$.

Maternal environment	Genotype	A (μmol CO$_2$ m^{-2} s^{-1})	gs (mol H$_2$O m^{-2} s^{-1})	E (mmol H$_2$O m^{-2} s^{-1})	Ci (μmol CO$_2$ mol^{-1})	iWUE	δ^{13}C (‰)
Greytown	G1	8.78 ± 0.31[a]	0.165 ± 0.012[ab]	6.52 ± 0.26[a]	260.2 ± 4.1[a]	62.2 ± 2.5[b]	−30.3 ± 0.16[bc]
	G2	7.84 ± 0.41[a]	0.142 ± 0.011[a]	5.52 ± 0.26[a]	261.7 ± 4.1[a]	62.7 ± 2.4[b]	−30.3 ± 0.16[bc]
	G3	7.99 ± 0.37[a]	0.173 ± 0.014[ab]	5.85 ± 0.29[a]	264.2 ± 6.3[ab]	62.4 ± 3.8[b]	−31.3 ± 0.17[a]
Kwambonambi	G1	8.18 ± 0.36[a]	0.162 ± 0.011[ab]	5.94 ± 0.35[a]	270.8 ± 3.6[ac]	57.8 ± 2.2[ab]	−31.1 ± 0.18[a]
	G2	8.35 ± 0.26[a]	0.181 ± 0.011[ab]	6.66 ± 0.24[a]	276.0 ± 3.6[bc]	53.1 ± 2.1[a]	−29.9 ± 0.13[c]
	G3	8.40 ± 0.55[a]	0.202 ± 0.018[b]	6.04 ± 0.37[a]	280.2 ± 5.2[c]	51.6 ± 3.2[a]	−30.6 ± 0.18[b]

A, photosynthetic rate; gs, stomata conductance; Ci, intercellular CO$_2$ concentration; E, transpiration rate; iWUE, intrinsic water use efficiency; δ^{13}C, foliar stable carbon isotopes.

environments. To the best of our knowledge, this is the first report confirming that *E. grandis* trees with the same genotype may acquire different morpho-physiological strategies according to the conditions of their maternal environments. These results are important from the perspective of the global use as a plantation species across variable environments, many of which are changing due to climate change. Moreover, current dynamic vegetation models overlook maternal environmental effects, despite it may be a crucial mechanism to understand potential vegetation changes in a scenario of climate change.

RGR was higher in *E. grandis* trees from the Kwambonambi orchard than the Greytown orchard, reflecting an impact on the growth potential and biomass production of the offspring from maternal environments under varying conditions. A previous study (Vivas et al., 2017) showed that seedling height was significantly lower in seedlings grown from the Kwambonambi orchard than from the Greytown orchard. The present result suggests that seedling from the Kwambonambi orchard, although shorter, should have a greater biomass in comparison with Greytown. This significance should be taken into account in those programs focussed on tree selection that commonly seek to improve tree growth and thus maximize growth rates (Franklin et al., 2014).

Leaf gas exchange (Ci) and water use efficiency were influenced by the maternal environment. Specifically, Ci was lower and iWUE (i.e. A/gs) was higher in seedlings from Greytown compared to those from Kwambonambi. Similar patterns have been documented in previous single-generation studies, where plant species with a lower supply of water close the stomata inducing an initial decline of Ci inside the leaf and an increase of the iWUE (reviewed by Flexas and Medrano, 2002; Lambers and Poorter, 2004). These results suggest that the efficiency of the *E. grandis* seedlings to water availability can be determined by the conditions of the maternal environment. Maternal environments with less water availability (as in Greytown) can thus be expected to produce seedlings with more efficient water use than environments with more water availability. Imposing experimental drought levels to mother trees while controlling for other confounding effects could further confirm the results observed in our study under natural conditions.

Although *E. grandis* leaf δ^{13}C composition showed the same trend as iWUE (seedlings from the Greytown orchard showed negligible higher leaf δ^{13}C values than the Kwambonambi orchard), leaf δ^{13}C was not consistently influenced by the maternal environments across genotypes. Plant δ^{13}C composition has been used as a proxy for WUE (Farquhar and Richards, 1984). There are three non-excluding reasons for the lack of significance in the response of leaf δ^{13}C to the maternal effects in comparison with iWUE (Seibt et al., 2008). Firstly, plant iWUE and leaf δ^{13}C has two different temporal scales, where iWUE is measured at a very short time scale and leaf δ^{13}C is measured at longer timescale. Secondly, iWUE and leaf δ^{13}C are affected in a different way by changes in the environmental conditions and plant properties, so they can vary independently. Thirdly, leaf δ^{13}C cannot be used alone as a proxy of plant WUE, because the estimation of gas exchange and environmental condition should also be estimated. As a result, the relationship between leaf δ^{13}C variation and water availability may vary (Schulze

et al., 1998; Miller et al., 2001; Cernusak et al., 2011).

Our results indicate that the conditions of the maternal environment influence the morpho-physiological strategies in *E. grandis* progeny. These morpho-physiological strategies are important for plant performance and ecology (Poorter and Sack, 2012). The contrasting morpho-physiological strategies showed by *E. grandis* seedlings from the different maternal environment were expressed even though maternal environments were not controlled. Here, it is relevant to highlight the fact that mother trees in nature were exposed to numerous biotic and abiotic conditions (see Fig. 1). The likely multiplicity of stresses that a plant faces in a natural environment could have obscured a clearer signal of maternal effects in the progeny. However, this study provides a first approach to show the extent to which maternal environmental effects may regulate the physiology of their progeny in *E. grandis*. Further research considering the maternal environmental effects could make a significant contribution to the optimization of silvicultural practices and the improvement of growth of trees in a changing environment. Results of this study represent the first evidence that the environmental maternal effects could influence the physiology of *E. grandis* trees. And, the analyses used could be particularly useful in efforts to identify the responses of plants to different levels of water availability and to enhance our understanding of the within-species physiological variability.

References

Allen, C., Macalady, A., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D., Hogg, E., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J., Allard, G., Running, S., Semerci, A., Cobb, N., 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manage. 259, 660–684.

Anderegg, W., 2015. Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. New Phytol. 205, 1008–1014.

Anderson, M., 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). In: Wiley StatsRef: Statistics Reference Online. American Cancer Society, pp. 1–15.

Aranda, I., Gil-Pelegrín, E., Gascó, A., et al., 2012. Drought response in forest trees: from the species to the gene. In: Aroca, R. (Ed.), Plant Responses to Drought Stress. Springer-Verlag, Berlin, Heidelberg, pp. 293–334.

Bartholome, J., Mabiala, A., Savelli, B., Bert, D., Brendel, O., Plomion, C., Gion, J.M., 2015. Genetic architecture of carbon isotope composition and growth in *Eucalyptus* across multiple environments. New Phytol. 206, 1437–1449.

Battie-Laclau, P., Delgado-Rojas, J.S., Christina, M., Nouvellon, Y., Bouillet, J.-P., Piccolo, Md.C., Moreira, M.Z., Gonçalves, J.l.d.M., Roupsard, O., Laclau, J.-P., 2016. Potassium fertilization increases water-use efficiency for stem biomass production without affecting intrinsic water-use efficiency in *Eucalyptus grandis* plantations. For. Ecol. Manage. 364, 77–89.

Cernusak, L.A., Hutley, L.B., Beringer, J., Holtum, J.A.M., Turner, B.L., 2011. Photosynthetic physiology of eucalypts along a sub-continental rainfall gradient in northern Australia. Agric. For. Meteor. 151, 1462–1470.

Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12, 133–149.

Chamaillard, S., Fichot, R., Vincent-Barbaroux, C., Bastien, C., Depierreux, C., Dreyer, E., Villar, M., Brignolas, F., 2011. Variations in bulk leaf carbon isotope discrimination, growth and related leaf traits among three *Populus nigra* L. populations. Tree Physiol. 31, 1076–1087.

Choat, B., Ball, M.C., Luly, J.G., Donnelly, C.F., Holtum, J.A., 2006. Seasonal patterns of leaf gas exchange and water relations in dry rain forest trees of contrasting leaf phenology. Tree Physiol. 26, 657–664.

Correia, B., Pintó-Marijuan, M., Neves, L., Brossa, R., Dias, M., Costa, A., Castro, B., Araújo, C., Santos, C., Chaves, M., Pinto, G., 2014. Water stress and recovery in the performance of two *Eucalyptus globulus* clones: physiological and biochemical profiles. Physiol. Plant 150, 580–592.

Correia, B., Hancock, R., Amaral, J., Gomez-Cadenas, A., Valledor, L., Pinto, G., 2018. Combined drought and heat activates protective responses in *Eucalyptus globulus* that are not activated when subjected to drought or heat stress alone. Front. Plant. Sci. 9, 819.

Elwell, A.L., Gronwall, D.S., Miller, N.D., Spalding, E.P., Brooks, T.L.D., 2011. Separating parental environment from seed size effects on next generation growth and development in Arabidopsis. Plant Cell Environ. 34, 291–301.

Evans, G.C. (Ed.), 1972. The Quantitative Analysis of Plant Growth. University of California Press.

Farquhar, G., Richards, R., 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Funct. Plant. Biol. 11, 539–552.

Flexas, J., Medrano, H., 2002. Drought-inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitations revisited. Ann. Bot. 89, 183–189.

Franklin, O., Palmroth, S., Näsholm, T., 2014. How eco-evolutionary principles can guide tree breeding and tree biotechnology for enhanced productivity. Tree Physiol. 34, 1149–1166.

Gindaba, J., Rozanov, A., Negash, L., 2005. Photosynthetic gas exchange, growth and biomass allocation of two *Eucalyptus* and three indigenous tree species of Ethiopia under moisture deficit. For. Ecol. Manag. 205, 127–138.

Herman, J.J., Sultan, S.E., 2011. Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. Front. Plant. Sci. 2, 1–10.

Holeski, L.M., Jander, G., Agrawal, A.A., 2012. Transgenerational defense induction and epigenetic inheritance in plants. Trends Ecol. Evol. 27, 618–626.

IPCC, 2014. Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel of Climate Change. IPCC.

Jablonka, E., Raz, G., 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. Q. Rev. Biol. 84, 131–176.

Koch, G.W., Vitousek, P.M., Steffen, W.L., Walker, B.H., 1995. Terrestrial transects for global change research. Vegetatio 121, 53–65.

Lambers, H., Poorter, H., 2004. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. Adv. Ecol. Res. 34, 283–362.

Latzel, V., Klimešová, J., 2009. Fitness of resprouters versus seeders in relation to nutrient availability in two *Plantago* species. Acta Oecol. 35, 541–547.

Lopez, G.A., Potts, B.M., Vaillancourt, R.E., Apiolaza, L.A., 2003. Maternal and carryover effects on early growth of *Eucalyptus globulus*. Can. J. For. Res. 33, 2108–2115.

Loveys, B.R., Scheurwater, I., Pons, T.L., Fitter, A.H., Atkin, O.K., 2002. Growth temperature influences the underlying components of relative growth rate: an

investigation using inherently fast- and slow-growing plant species. Plant Cell Environ. 25, 975–988.

Luna, E., Bruce, T.J., Roberts, M.R., Flors, V., Ton, J., 2012. Next-generation systemic acquired resistance. Plant Physiol. 158, 844–853.

Medrano, H., Flexas, J., Galmés, J., 2009. Variability in water use efficiency at the leaf level among Mediterranean plants with different growth forms. Plant Soil 317, 17–29.

Miller, J.M., Williams, R.J., Farquhar, G.D., 2001. Carbon isotope discrimination by a sequence of *Eucalyptus* species along a subcontinental rainfall gradient in Australia. Funct. Ecol. 15, 222–232.

Monclus, R., Dreyer, E., Villar, M., Delmotte, F.M., 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 x Populus nigra*. New Phytol. 169, 765–777.

Navarrete-Campos, D., Bravo, L., Rubilar, R., et al., 2013. Drought effects on water use efficiency, freezing tolerance and survival of *Eucalyptus globulus* and *Eucalyptus globulus* × *nitens* cuttings. New For. 44, 119–134.

Paine, C.E.T., Marthews, T.R., Vogt, D.R., Purves, D., Rees, M., Hector, A., Turnbull, L.A., 2012. How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. Methods Ecol. Evol. 3, 245–256.

Poorter, H., Remkes, C., 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83, 553–559.

Poorter, H., Sack, L., 2012. Pitfalls and possibilities in the analysis of biomass allocation patterns in plants. Front. Plant Sci. 3.

Poorter, H., Avd, Werf, 1998. Is inherent variation in RGR determined by LAR at low irradiance and by NAR at high irradiance? A review of herbaceous species. In: Lambers, H., Poorter, H.P., VanVuuren, M. (Eds.), Inherent Variation in Plant Growth. Physiological Mechanisms and Ecological Consequences. Backhuys Publishers, Leiden, pp. 309–336.

Rasmann, S., De Vos, M., Casteel, C.L., Tian, D., Halitschke, R., Sun, J.Y., Agrawal, A.A., Felton, G.W., Jander, G., 2012. Herbivory in the previous generation primes plants for enhanced insect resistance. Plant Physiol. 158, 854–863.

R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rix, K., Gracie, A., Potts, B., Brown, P., Spurr, C., Gore, P., 2012. Paternal and maternal effects on the response of seed germination to high temperatures in *Eucalyptus globulus*. Ann. For. Sci. 69, 673–679.

Roach, D.A., Wulff, R.D., 1987. Maternal effects in plants. Annu. Rev. Ecol. Syst. 18, 209–235.

Rossiter, M., 1996. Incidence and consequences of inherited environmental effects. Annu. Rev. Ecol. Syst. 27, 451–476.

Scotti, I., Calvo-Vialettes, L., Scotti-Saintagne, C., Citterio, M., Degen, B., Bonal, D., 2010. Genetic variation for growth, morphological, and physiological traits in a wild population of the Neotropical shadetolerant rainforest tree *Sextonia rubra* (Mez) van der Werff (Lauraceae). Tree Genet. Genom. 6, 319–329.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Meth. 9, 671–675.

Schulze, E.-D., Williams, R.J., Farquhar, G.D., Schulze, W., Langridge, J., Miller, J.M., Walker, B.H., 1998. Carbon and nitrogen isotope discrimination and nitrogen nutrition of trees along a rainfall gradient in northern Australia. Aust. J. Plant Physiol. 25, 413–425.

Seibt, U., Rajabi, A., Griffiths, H., Berry, J.A., 2008. Carbon isotopes and water use efficiency: sense and sensitivity. Oecologia 155, 441.

Violle, C.H., Castro, H., Richarte, J., Navas, M.L., 2009. Intraspecific seed trait variations and competition: passive or adaptive response? Funct. Ecol. 23, 612–620.

Vivas, M., Kemler, M., Mphahlele, M.M., Wingfield, M.J., Slippers, B., 2017. Maternal effects on phenotype, resistance and the structuring of fungal communities in *Eucalyptus grandis*. Environ. Exp. Bot. 140, 120–127.

Vivas, M., Zas, R., Sampedro, L., Solla, A., 2013. Environmental maternal effects mediate the resistance of Maritime pine to biotic stress. PLoS One 8, e70148.

Wingfield, M.J., Brockerhoff, E.G., Wingfield, B.D., Slippers, B., 2015. Planted forest health: the need for a global strategy. Science 349, 832–836.