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Seedling Growth and Physiological Responses of Sixteen Eucalypt Taxa under Controlled Water Regime

Paulo H. M. Silva [1,2,*], Otavio C. Campoe [1,3], Rinaldo C. de Paula [2] and David J. Lee [4]

[1] Instituto de Pesquisas e Estudos Florestais, Av. Pádua Dias 11, C. P. 530, CEP 13400-970 Piracicaba, SP, Brazil; otavio.campoe@ufsc.br
[2] Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Via de Acesso Prof. Paulo Donato Castellane, km 5, CEP 14.884-900 Jaboticabal, SP, Bolsista PQ 2-CNPq, Brazil; rcpaula@fcav.unesp.br
[3] Federal University of Santa Catarina, Ulysses Gaboardi hwy, km 3, C.P. 101, CEP 89520-000 Curitibanos, SC, Brazil
[4] Forest Industries Research Centre, University of the Sunshine Coast, Maroochydore DC 4558, Queensland, Australia; DLee@usc.edu.au
* Correspondence: paulohenrique@ipef.br; Tel.: +55-192-105-8600

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Abstract: We assessed growth and physiological responses of *Eucalyptus* and *Corymbia* species to water limitation aiming to widen possibilities for plantations in dry climatic conditions. We selected 16 taxa: 4 *Corymbia* and 12 *Eucalyptus* species from the Subgenera *Symphyomyrtus*. Seedlings were evaluated from 100 to 170 days after sowing. Growth and physiological traits showed significant differences among taxa and between two levels of water availability. Water limitation significantly impacted biomass production and physiological characteristics, however in different levels. Leaf area and biomass production decreased 15%–48% under water limitation among taxa. *Eucalyptus moluccana*, CCV 2, and VM1 (drought tolerant clone) showed the largest decrease in leaf area. Transpiration across taxa decreased 30%–57% and photosynthesis 14%–48% under water limited condition. Taxa from cold environments were less responsive in leaf area reduction under water limitation, and taxa from *Exsertaria* section showed lower reduction in photosynthesis (*E. camaldulensis* showed the lowest reduction). Responses to water limitation are related to the environment of origin. *E. molucana*, the only *Adnataria* species from a high precipitation region (>1500 mm year^{-1}), was one of the most sensitive in reduction of biomass production, different behavior from the other *Adnataria* species, originated in regions with rainfall <750 mm year^{-1}. Water limitation increased leaf-level water use efficiency by 18% on average, 8% in *E. longirostrata*, and 28% in *E. camaldulensis*, *E. brassiana*, and *E. crebra*. Growth and physiological responses observed show the potential of different eucalypts taxa to tolerate water limited environments.

Keywords: *Eucalyptus*; *Corymbia*; water stress; productivity; drought tolerance

1. Introduction

The majority of commercial forest plantations in Brazil are generally planted with eucalypts (*Eucalyptus* and *Corymbia* species and hybrids), due to high wood productivity, ease of vegetative propagation, and desirable wood traits [1,2]. The expansion of these plantations is underpinned by substantial investment in research and operational improvements in breeding and silvicultural management over the last 30 years [3]. Part of the expansion of forest plantations is occurring in regions with different climatic conditions to the traditional silviculture areas, in some cases with strong environmental stresses (e.g., extended dry periods and high temperatures), negatively impacting tree survival and growth. Additionally, climate change is threatening traditional regions of *Eucalyptus* and *Corymbia* plantations due to the potential of increase in intensity and longer periods of drought, resulting in significant decrease of forest plantation productivity [4].

Eucalypts show several adaptations to avoid or tolerate environmental stresses [5] such as reduction in canopy leaf area; increased partitioning of carbohydrates to root growth; change in size, thickness, and distribution of leaves; stomatal closure; and production of osmoregulators [6–9]. However, depending on the frequency, duration, and severity of droughts, plant age and plasticity (within and among species), and changes in structural, physiological, biochemical characteristics may not be enough to avoid significant reduction in growth and mortality [4,10,11].

Studies focusing on effects of water stress on eucalypts show a wide range of behaviors among and within species. Ngugi *et al.* [12] studied the effects of duration and intensity of water stress on gas exchange and leaf water potential in *E. cloeziana* F. Muel from humid (Gympie) and dry regions (Hungry Hills), and *E. argophloia* Blakely from dry region (Chinchilla). They found that *E. argophloia* showed a higher net photosynthetic rate and stomatal conductance than both provenances of *E. cloeziana*. Ladiges [13] found that seedlings of *E. viminalis* Labil from four provenances showed differences in drought tolerance that are related to the severity of the drought in the occurrence area. The provenance with increased drought tolerance was able to maintain relatively high transpiration rates under moderate stress. Costa and Silva *et al.* [14] studied the mechanisms of drought tolerance in a susceptible and tolerant *E. globulus* Labil clone. They observed that the drought tolerant clone maintained higher leaf water potential throughout the day and sustained greater biomass production than the drought susceptible clone.

The behavior of plants under environmental stress varies among and within species and provenances due to divergent evolutionary processes under environmental variation in the original region, thus the knowledge of different strategies to cope with drought is useful in breeding programs. Usually, productivity and drought tolerance are inversely proportional and adaptations result in wide range of growth rates to different taxa under water limited conditions [15].

A limited number of eucalypts species are significantly used in plantations worldwide [16]; however, there are several species or hybrids with potential to be developed for stressful climatic conditions. There is also great interest in hybrids to improve desirable combinations [17]. Clonal propagation has allowed the adoption of hybridization in breeding programs for commercial exploitation of the heterosis in *Eucalyptus* genus. The technique is commonly applied to identify eucalypts hybrids with increased stress tolerance [18,19].

Our objective was to evaluate responses of currently non-commercial species to changes in water availability aiming to identify desirable physiological and morphological characteristics related to drought tolerance.

2. Material and Methods

2.1. Seedling Production and Experimental Site

The experiment was conducted in a greenhouse at the São Paulo State University, in Jaboticabal-SP, Brazil (21°15′ S, 48°19′ W, 605 m a.s.l.). The Köppen climatic classification for the region is Cwa [20]. The seedlings and cuttings (two commercial clones) were grown in polypropylene containers filled with 55 cm^3 of organic substrate (20% rice husk, 35% pine bark, 30% coconut fiber; and 15% vermiculite). Fertilization before 100 days was performed four times a week, with 5 mm of solution (Calcium nitrate 450 g; Ammonium nitrate 300 g; Monoammonium Phosphate 250 g; Potassium nitrate 300 g; Magnesium sulfate 250 g; Ammonium sulfate 250 g ; Tenso iron 2.5 g; Manganese sulfate 0.85 g; Boric Acid 0.75 g; Zinc sulfate 0.325 g; Copper sulfate 0.1 g; and Sodium molybdate 0.005 g in 1000 L of water). After 100 days, seedlings were transplanted into plastic containers filled with 7 dm^3 of sand and acclimated (with irrigation and fertigation) for 30 days prior to the imposition of water availability treatment. During the trial, plants were fertigated weekly (210 g of nitrogen, 31 of P, 234 g of K, 200 g of Ca, 48 g of Mg and 64 g of S in 1000 L of water) aiming to eliminate potential nutritional limitation [21].

2.2. Experimental Design

The experiment was established as a complete randomized block design with eight replicates of the 32 treatments, with 256 seedlings, comprising 16 taxa (Table 1) and two water regimes.

- Irrigated (IRR): plants received daily irrigation of 400 mL to maintain at least 60% of the maximum water retention in the soil (soil moisture > 20%; $\Psi_s < 0.02$ bar), eliminating water limitation; and
- Water limited (WL): irrigation was suspended until the occurrence of visual symptoms of stress (leaf wilting), when the plants were irrigated with 500 mL, corresponding to approximately 20% of soil capacity retention, repeating the cycle (soil moisture 3% to 10% v/v; $0.1 < \Psi_s < 1$ bar).

2.3. Physiological and Biometric Measurements

Plants were submitted to 16 cycles of water stress over the study period, considering day zero as the beginning of the treatment imposition. At days 15, 25, 55, and 65, we measured photosynthesis (A), stomatal conductance (g_s) and transpiration (E) using a portable infrared gas analyzer (LCPro, ADC, England). Measurements were carried out between 7:30 and 11:00 h, using photosynthetically active photon flux of 1000 μmol $m^{-2}\cdot s^{-1}$ and CO_2 concentration of 380 ± 10 ppm. Measurements were performed on one fully expanded leaf from the upper third of the crown of four plants per treatment. Leaf-level instantaneous water use efficiency (WUE_i) was calculated by the ratio between A and E. Due to the large number of samples, analyses were performed on two consecutive days (two blocks a day) in each evaluation.

Total height, ground-level stem diameter, total leaf area, number of leaves, shoot and root biomass, were evaluated in the end of the experiment (at day 70). Total plant leaf area was determined using the LI-3100 Area Meter (LI-Cor Biosciences, Lincoln, NE, USA). Shoot and root dry biomass was determined on a precision scale (0.001 g) after drying for 96 h at 65 °C.

2.4. Statistical Analysis

Analyses of variance were performed to evaluate significant statistical differences between levels of water availability (WL and IRR), among species and interaction between both for biometric (total height, ground-level stem diameter, total plant leaf area, aboveground and root biomass and specific leaf area) and physiological (photosynthesis, stomatal conductance, transpiration and instantaneous water use efficiency) traits. Physiological data was averaged and analyzed across the four measurements that showed little decline in physiological data along the time for all treatments (days 15, 25, 55, and 65 after water manipulation imposition). Significant differences were analyzed with a *post hoc* Scott-Knott Algorithm to separate treatment means into discrete groups. Linear regressions between stomatal conductance and transpiration, stomatal conductance, and photosynthetic rate, and photosynthetic rate and transpiration were fitted and compared using analysis of variance. All datasets passed on normality and variance homoscedasticity tests, and no transformations were needed. The probability level used to determine significance on all the analysis was $p < 0.05$. The analyses were performed using R 3.1.0 and the package ExpDes (R Core Team).

Table 1. Studied taxa of *Corymbia* and *Eucalyptus* with their respective abbreviation, section, origin, seed lot, and provenance-location with respective climate.

Species	Abbreviation	Section	Origin	Seed lot	Provenance-Source	AAT (°C)	Annual Rainfall (mm)	Koppen
Corymbia. Citriodora (Hook) subsp. *variegata* F. Muell	CCV 1		CSIRO	19,664	Barakula, AU (WS)	21.6	656	BSh
Corymbia citriodora subsp. *variegata*	CCV 2		CSIRO	20,787	Barclays Deniliquin, AU (SO)	18.7	394	BSk
Corymbia torelliana F. Muell	CT		IPEF	AN0255N01	Anhembi, BR (SO)	22.2	1305	Aw/Cwa
Corymbia henryi S.T. Blake	CH		DAFF	10,250	Lockyer, AU (WS)	21.2	820	CFa
Eucalyptus argophloia Blakely	E. argo	Symphyomyrtus Adnataria	DAFF	12,716	Dalby, AU (SO)	20.8	676	BSh
Eucalyptus amplifolia Brooker & A. Slee	E.ampl 1	Symphyomyrtus Exsertaria	CSIRO	15,281	Nerong S.F (WS)	19.2	1321	Cfb
Eucalyptus amplifolia	E. ampl 2	Symphyomyrtus Exsertaria	CSIRO	18,731	Clouds CK SF & TSR (WS)	16.5	1434	Cfb
Eucalyptus crebra F. Muell	E. crebra	Symphyomyrtus Adnataria	CSIRO	15,146	NW Baradine, AU (WS)	18.9	747	BSh
Eucalyptus moluccana Roxb.	E. molu	Symphyomyrtus Adnataria	CSIRO	20,010	Crediton, AU (WS)	22.0	1539	BSh
Eucalyptus brassiana S.T. Blake	E. bras	Symphyomyrtus Exsertaria	IPEF	US002N01	Urbano Santos, BR (SO)	27.0	1700	Aw
Eucalyptus camaldulensis Dehnh	E. camal	Symphyomyrtus Exsertaria	IPEF	SE007N01	Selviria, BR (SO)	23.6	1300	Aw/Cwa
Eucalyptus brookeriana A. M. Gray	E. brook	Symphyomyrtus Maidenaria	CSIRO	18,317	Otways, AU (WS)	12.9	1539	Cs
Eucalyptus macarthurii Deane & Maiden	E. maca	Symphyomyrtus Maidenaria	CSIRO	20,897	Paddys River, AU (WS)	12.3	1248	Cfb
Eucalyptus longirostrata (Blakely) L. Johnson & K. Hill	E. long	Symphyomyrtus Latoangulate	DAFF	20,464	Coominglah, AU (WS)	22.0	690	Cfa
Eucalyptus urophylla S.T. Blake	GG100 *	Symphyomyrtus Latoangulate	Gerdau/Vallourec	Clone GG 100	NA	NA	NA	NA
Eucalyptus urophylla x E. camaldulensis	VM1 *	Symp. Lateangulate x Exsertaria	Vallourec	Clone VM 1	NA	NA	NA	NA

CSIRO: Commonwealth Scientific and Industrial Research Organization, Australia; DAFF: Queensland Department of Agriculture, Fisheries and Forestry, Australia, IPEF: Instituto de Pesquisas e Estudos Florestais, Brazil; Vallourec: Vallourec Tubes, Brazil. AAT: Annual Average Temperature; AU: Australia; BR: Brazil; SO: Seed Orchard; WS: Wild seed; NA: Not Applicable; * Commercial clones obtained in dry Brazilian region.

3. Results

Growth and physiological variables showed significant differences among taxa and between water availability (except specific leaf area to water availability). Total height, root, stem, total biomass, and transpiration showed significant interaction between taxa and water availability (Table 2).

The negative effect of water limitation was higher on leaf area (decreasing from 1098 to 722 cm^2 $tree^{-1}$) and biomass production. Above-ground biomass decreased from 21.5 to 14.4 g $tree^{-1}$ and root biomass from 7.2 to 4.2 g· $tree^{-1}$, leading to an average decrease of 33% compared to the irrigated seedlings for all taxa (Table 3). *Eucalyptus moluccana*, CCV 2 and VM1 (a current commercial clone) showed the largest decrease in leaf area (~48%) whereas the least affected taxa were CT and *E. brookeriana* (less than a 20% decrease in leaf area). The total number of leaves per plant decreased on average from 158 to 115 (−27%); this reduction was less pronounced in species belonging to the *Corymbia* genus. Total height and diameter decreased on average 15%, from 70.1 cm to 58.8 cm, and from 7.8 cm to 6.6 cm, respectively, under water limited conditions. Specific leaf area (SLA) did not show a regular overall pattern, with increases in five taxa and decreases in 11 taxa, however all *Corymbia* species showed decrease in SLA under water limited condition.

Water limitation reduced all physiological variables. Average transpiration in irrigated plants was 4.15 mmol $m^{-2}\cdot s^{-1}$, decreasing to 2.38 mmol $m^{-2}\cdot s^{-1}$ under water limited condition (Table 4). The most conservative taxa were *CT* and *E. moluccana*, showing the largest decrease under water limited conditions, from 3.48 mmol $m^{-2}\cdot s^{-1}$ to 1.47 mmol $m^{-2}\cdot s^{-1}$ (reduction of 58%) and from 3.97 mmol $m^{-2}\cdot s^{-1}$ to 1.69 mmol $m^{-2}\cdot s^{-1}$ (reduction of 57%), respectively. Stomatal conductance decreased on average 39% due to water limitation, from 0.33 mol $m^{-2}\cdot s^{-1}$ to 0.2 mol $m^{-2}\cdot s^{-1}$ among plants. The most responsive was CT (reduction of 64%) and the least was *E. amplifolia* (reduction of 10%). The average photosynthetic rate decreased from 12.3 μmol $m^{-2}\cdot s^{-1}$ to 8.3 μmol $m^{-2}\cdot s^{-1}$ under the water limited conditions. Similarly, the most conservative taxa was CT, which decreased 53% (from 10.41 μmol $m^{-2}\cdot s^{-1}$ to 4.90 μmol $m^{-2}\cdot s^{-1}$); the least conservative was *E. camaldulensis*, with a reduction of only 14% (from 10.34 μmol $m^{-2}\cdot s^{-1}$ to 8.85 μmol $m^{-2}\cdot s^{-1}$). *E. argophloia*, *E. amplifolia*, and *E. crebra* showed the highest photosynthetic rate among taxa, higher than 11 μmol $m^{-2}\cdot s^{-1}$ on water limited plants and higher than 15.5 μmol $m^{-2}\cdot s^{-1}$ on water limited plants. The range in leaf-level instantaneous water use efficiency due to water limitation increased from 8% in *E. longirostrata* to ~28% in *E. camaldulensis*, *E. brassiana*, and *E. crebra*.

Table 2. Levels of significance (*p*-values) from the analysis of variance performed to evaluate the effects of taxa, water availability, and their interaction on the studied variables.

Source of Variation	Total Height	Basal Stem Diameter	Leaf Area	Specific Leaf Area	Biomass				E	A	g_s	WUE_i
					Root	Stem	Leaves	Total				
Taxa	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Water availability	<0.0001	<0.0001	<0.0001	0.613	<0.0001	<0.0001	0.0361	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Taxa x Water availability	<0.0001	0.068	0.0876	0.125	0.0161	0.0006	0.2528	0.0216	0.0006	0.4914	0.9114	0.968

Table 3. Growth and biomass production of the 16 studied taxa at the end of the 70 days of the experiment for soil water limited and irrigated plants. Numbers followed by different letters within each column are statistically different. IRR: irrigated; WL: soil water limited.

Taxa	Total Height cm				Basal Stem Diameter cm				Number of Leaves Leaf $Tree^{-1}$				Leaf Area $cm^2 \cdot Tree^{-1}$				Specific Leaf Area $m^2 \cdot kg^{-1}$				Shoot Biomass g $Tree^{-1}$				Root Biomass g $Tree^{-1}$				Total Biomass g $Tree^{-1}$			
	IRR		**WL**		**IRR**		**WL**		**IRR**		**WL**		**IRR**		**WL**		**IRR**		**WL**		**IRR**		**WL**		**IRR**		**WL**		**IRR**		**WL**	
CCV 1	76.7	A	59.7	A	7.9	B	6.2	B	48	F	35	C	946	B	490	B	11.9	A	8.1	B	18.0	B	10.6	B	7.0	B	3.4	C	25.0	B	14.0	B
CCV 2	73.3	A	49.9	B	8.5	B	6.1	B	30	F	32	C	1158	A	836	A	7.3	B	7.2	B	24.6	A	15.6	A	8.0	A	5.3	B	32.5	A	20.9	A
CT	73.0	A	64.3	A	9.2	A	7.8	A	39	F	24	C	1081	B	880	A	9.9	A	8.9	A	21.9	B	17.4	A	7.8	A	5.4	B	29.7	A	22.9	A
CH	64.3	B	53.3	B	7.4	C	6.6	B	23	F	18	C	1043	B	707	B	7.5	B	6.5	B	21.3	B	15.8	A	6.9	B	5.1	B	28.1	B	20.8	A
E. argo	73.9	A	61.1	A	6.4	C	5.9	B	662	A	344	A	925	B	588	B	6.3	B	7.5	B	24.6	A	14.5	B	6.0	B	3.8	C	30.7	A	18.3	B
E.ampl 1	66.7	B	49.0	B	6.6	C	5.8	B	36	F	20	C	1189	A	684	B	5.8	B	5.4	B	28.0	A	16.8	A	5.1	B	3.7	C	33.1	A	20.4	A
E. ampl 2	69.4	B	63.8	A	9.2	A	8.5	A	47	F	40	C	1132	A	872	A	9.9	A	8.6	B	21.1	B	16.2	A	9.3	A	8.2	A	30.4	A	24.4	A
E. crebra	67.3	B	51.1	B	6.2	C	5.2	B	437	B	320	A	817	B	458	B	6.0	B	4.9	B	21.3	B	14.2	B	5.9	B	3.9	C	27.2	B	18.1	B
E. molu	59.5	B	57.9	A	8.7	A	6.5	B	110	E	99	C	1388	A	721	B	8.2	B	12.6	A	24.7	A	13.0	B	8.9	A	5.0	B	33.7	A	18.0	B
E. bras	84.6	A	65.4	A	7.9	B	6.8	B	88	E	73	C	1081	B	682	B	10.3	A	9.8	A	19.7	B	12.6	B	7.6	A	4.6	B	27.3	B	17.1	B
E. camal	77.8	A	64.0	A	9.1	A	7.5	A	63	F	46	C	997	B	606	B	8.2	B	7.6	B	21.3	B	13.8	B	9.4	A	7.4	A	30.7	A	21.3	A
E. brook	62.6	B	62.0	A	6.3	C	6.3	B	238	D	220	B	923	B	783	A	9.4	A	9.5	A	17.4	B	15.3	A	4.3	B	2.6	C	21.8	B	18.0	B
E. maca	63.4	B	57.6	A	6.7	C	5.8	B	317	C	255	B	1320	A	1003	A	10.0	A	12.9	A	21.3	B	13.9	B	7.4	A	4.7	B	28.6	A	18.6	B
E. long	77.9	A	62.4	A	9.2	A	8.4	A	214	D	182	B	827	B	586	B	10.0	A	11.2	A	17.7	B	11.8	B	6.6	B	4.9	B	24.3	B	16.6	B
GG100	63.5	B	58.3	A	7.6	C	6.3	B	83	E	69	C	1461	A	976	A	11.2	A	10.1	A	21.1	B	16.1	A	7.7	A	5.5	B	28.8	A	21.6	A
VM1	68.4	B	60.5	A	7.1	C	5.8	B	88	E	64	C	1273	A	678	B	11.1	A	9.1	A	19.9	B	13.5	B	7.0	B	4.0	C	26.9	B	17.4	B
Average	**70.1**		**58.8**		**7.8**		**6.6**		**158**		**115**		**1098**		**722**		**8.9**		**8.7**		**21.5**		**14.4**		**7.2**		**4.8**		**28.7**		**19.3**	
Variation (%)			**−16**				**−15**				**−27**				**−34**				**−2**				**−33**				**−33**				**−33**	

Table 4. Average of four ages of evaluations of physiological variables measured on water limited and irrigated plants. Numbers followed by different letters within each column are statistically different. WUE_i: instantaneous water use efficiency in leaf scale; IRR: irrigated; WL: soil water limited.

Taxa	**Transpiration mmol $m^{-2}\cdot s^{-1}$**				**Stomatal Conductance mol $m^{-2}\cdot s^{-1}$**				**Photosynthesis μmol $m^{-2}\cdot s^{-1}$**				**WUE_i μmol $mmol^{-1}$**			
	IRR		**WL**		**IRR**		**WL**		**IRR**		**WL**		**IRR**		**WL**	
CCV 1	3.82	B	2.67	B	0.31	B	0.23	B	12.31	B	9.53	B	3.27	A	3.62	A
CCV 2	4.30	B	2.38	B	0.39	A	0.22	B	13.89	A	8.77	B	3.25	A	3.73	A
CT	3.48	B	1.47	C	0.25	B	0.09	B	10.41	B	4.90	C	3.00	A	3.37	B
CH	3.96	B	2.32	B	0.32	B	0.21	B	13.09	B	9.21	B	3.32	A	4.00	A
E. argo	6.82	A	3.91	A	0.38	A	0.32	A	16.80	A	11.17	A	2.44	B	2.82	B
E.ampl 1	4.46	B	2.98	A	0.48	A	0.43	A	15.55	A	11.37	A	3.48	A	3.92	A
E. ampl 2	3.85	B	2.23	B	0.39	A	0.19	B	12.51	B	9.02	B	3.26	A	4.06	A
E. crebra	6.66	A	3.56	A	0.35	A	0.25	B	15.93	A	11.03	A	2.42	B	3.12	B
E. molu	3.97	B	1.69	C	0.33	B	0.19	B	12.50	B	6.44	C	3.16	A	3.77	A
E. bras	3.92	B	2.39	B	0.35	A	0.20	B	11.09	B	8.39	B	2.82	B	3.57	A
E. camal	3.32	B	2.25	B	0.28	B	0.16	B	10.34	B	8.85	B	3.12	A	3.99	A
E. brook	3.66	B	2.14	B	0.31	B	0.22	B	11.31	B	8.07	B	3.11	A	3.83	A
E. maca	3.47	B	1.65	C	0.26	B	0.12	B	9.13	B	5.03	C	2.63	B	3.00	B
E. long	3.78	B	2.53	B	0.31	B	0.15	B	11.21	B	7.94	B	2.96	A	3.20	B
GG100	3.50	B	1.84	C	0.26	B	0.11	B	10.20	B	6.11	C	2.95	A	3.27	B
VM1	3.35	B	2.04	B	0.24	B	0.10	B	10.50	B	7.50	C	3.14	A	3.70	A
Average	**4.15**		**2.38**		**0.33**		**0.20**		**12.30**		**8.33**		**3.02**		**3.56**	
Variation (%)			**−43**				**−39**				**−32**				**+18**	

E. amplifolia provenance Clouds CK showed the highest biomass production and reduced susceptibility to water limitation. *C. torelliana* had a relative high leaf area production and a small decrease under water limited condition. *E. moluccana* showed the largest reduction of leaf area under water limitation. *E. amplifolia, E. argophloia*, and *E. crebra* showed the highest stomatal conductance and photosynthetic rates under water limited condition and the smallest decrease in these variables due to water limitation. *C. torelliana*, *E. macarthurii*, GG100, and *E. moluccana* showed the lowest stomatal conductance and the largest reduction in photosynthetic rate under water limitation (Figure 1).

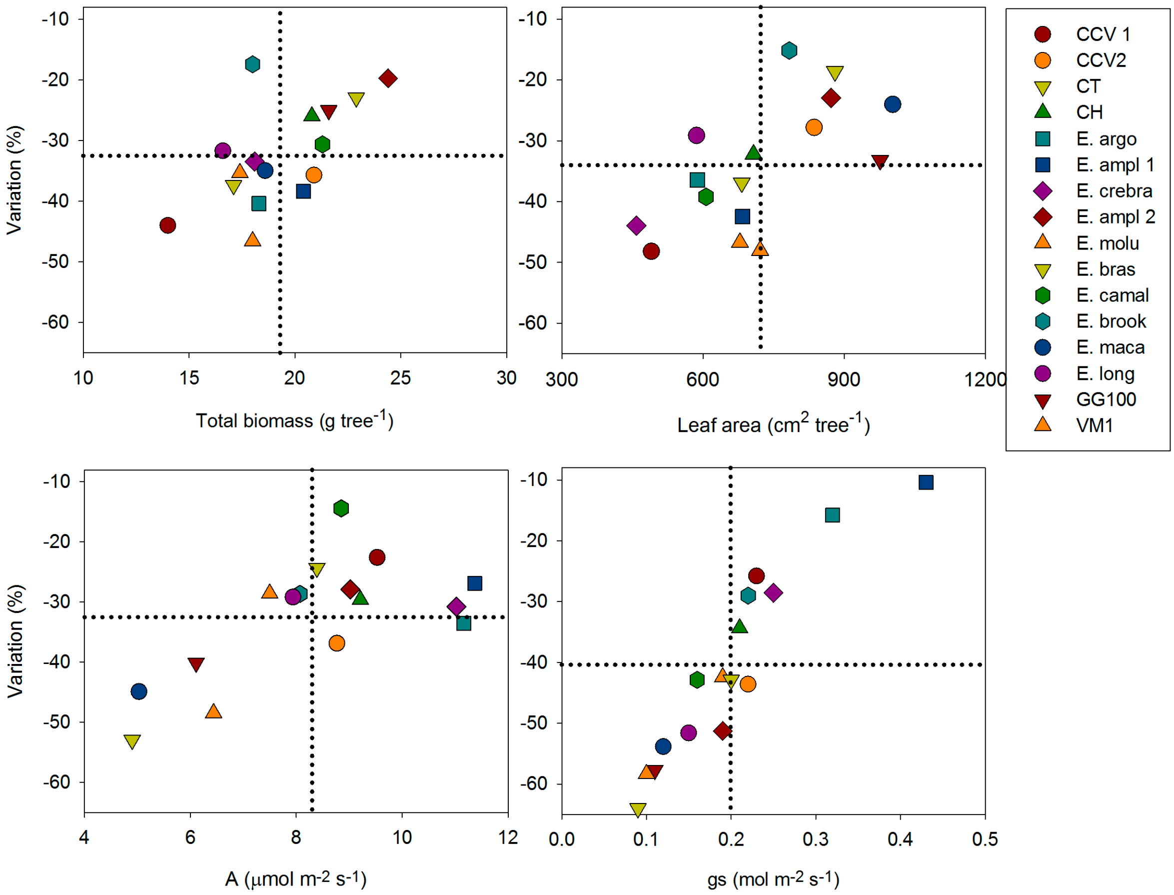

Figure 1. Absolute total biomass, stomatal conductance (gs), photosynthetic rate (A), and leaf area under water limited conditions for the 16 taxa and their relative decrease under water limited regime. Dotted lines represent the average values of all taxa at both axes.

The relation between physiological variables were statistically similar (p-value > 0.05), showing a gradient of behavior among taxa and water availability (Figure 2).

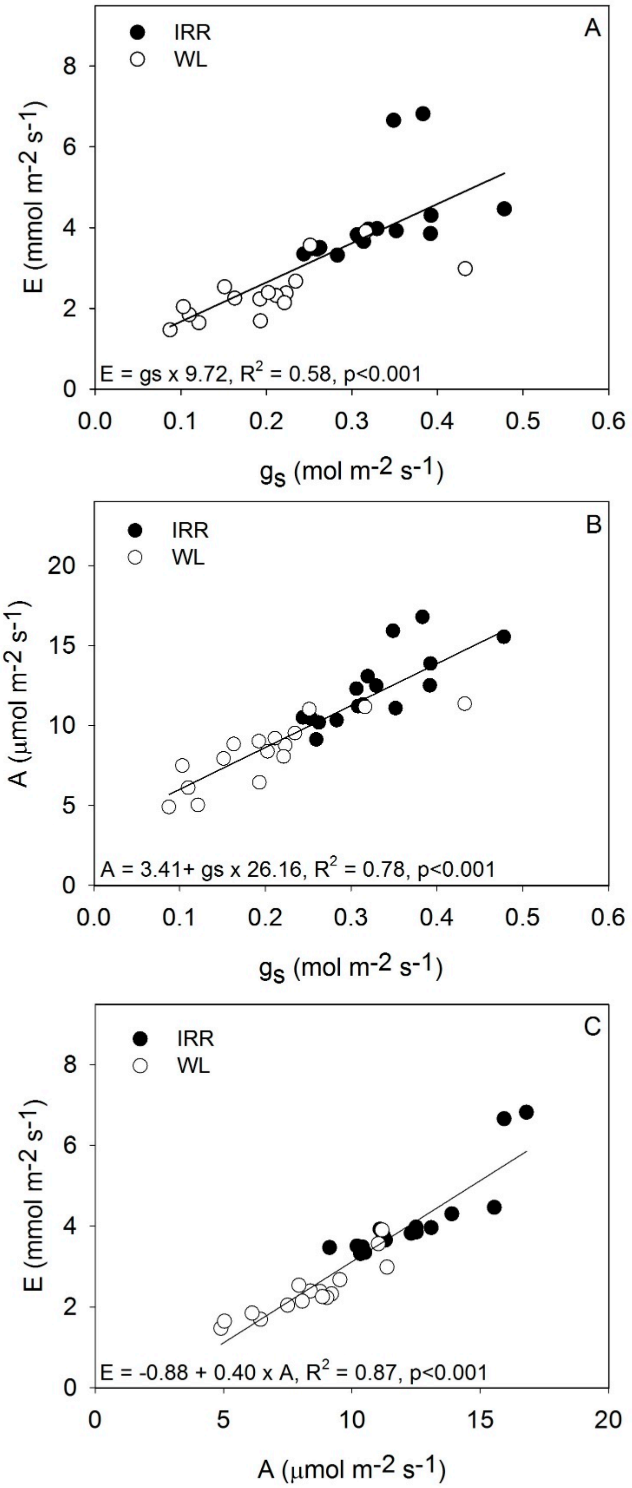

Figure 2. Relationship between stomatal conductance and transpiration (**A**); stomatal conductance and photosynthetic rate (**B**); and photosynthetic rate and transpiration (**C**) for the 16 taxa under water limited (WL) and irrigated (IRR) conditions.

4. Discussion

The productivity of the eucalypts is strongly related to water supply [22,23], and drought conditions affects growth, biomass production, and harvestable yield [24]. Water limitation applied to the 16 eucalypt taxa in our experiment was moderate and relatively long for seedlings, over two months, resulting in decreased total biomass production ranging from 18% to 47% (Table 3). This type of water stress (moderate and prolonged) is known to result in greater impact on biomass production than severe drought stress for a short period [25,26].

Our results, under controlled greenhouse conditions with seedlings in pots, are in accordance with experimental field results. Christina *et al.* [27] studied, under field conditions, the response of 37% of rainfall exclusion in three year old *E. grandis* Hill ex Maiden clonal stands. They found significant decrease in growth (53% in leaf area and 30% in total height) and in physiological characteristics. Similarly, we also observed a significant reduction in leaf area, a structural adaptation to reduce the transpiration surface of the canopy.

The *E. camaldulensis* showed similar changes in the shoot/root ratio under soil water limited conditions to those presented by Chaves *et al.* [28] with a greater decrease of shoot than root system. On the other hand, the *E. brookeriana* expressed the opposite behavior under soil water limited conditions. Contrasting responses in shoot and root biomass production reflect the different strategies that the 16 studied taxa show in relation to carbon allocation. In general, the decrease in soil water availability increase carbon partitioning to root production, aiming to increase fine root surface, and water absorption capacity [29,30]. Despite the general behavior, our results show that a range of responses to soil water deficit has significant impacts on growth, leading to variable wood biomass production under stressful conditions.

Physiological and growth responses of related species like *Corymbia citriodora* subsp. *variegata* and *Corymbia henryi*, that are molecularly homogeneous [31], or the two sources of both *C. citriodora* subsp. *variegata* and *E. amplifolia*, show that the variation within the species (different provenances) was similar or higher than the variation among species. Significant intra-specific variation in these traits is commonly observed in eucalypts [12,32]. This variation in widespread species is more often related to the provenance's environmental conditions [12,13] than traits specific to the species. Hence, plants sourced from areas with high water stress are generally more drought-tolerant, however they are likely to be less responsive under well-watered conditions showing lower production [5]. Therefore inter/intra species hybridization is an effective method to obtain satisfactory productivity rates while incorporating drought-tolerance characteristics from more drought tolerant parents.

Both *Eucalyptus* species belonging to the Maidenaria section (known as the cold tolerant section), *C. torelliana*, one *E. amplifolia* provenance, and the *C. citriodora* subsp. *variegata* (CCV2) from the Deniliquin seed orchard were less responsive in leaf area reduction under water limitation conditions, as observed in other genera [33]. These taxa were from cold environments, either from cold located provenances or colder *ex situ* growing conditions, than the other sources of the same taxa. Probably, the morphological and physiological characteristics that provide cold tolerance are related to drought tolerance, affecting plant hydraulics to maintain the photosynthetic rate in stressful conditions [34,35]. Adaptation to colder environments may be due to cold stress reducing hydro activity, causing osmotic stress within the cell [36].

We found significant reductions in leaf area of *Eucalyptus* and *Corymbia* genus (>30%), however, *C. torelliana* was the least affected by water limitation. Similar results were found by Myers and Landsberg [25] , who reported reduction of 20% in leaf area under water stressed condition in what was then called *E. maculata* Hook (no provenance details were provided, therefore, it could be either *C. maculata*, *C. citriodora* subsp. *variegata* or even *C. henryi*, following recent taxonomic revisions: e.g., [37]). In accordance with our results, Cernusak *et al.* [38] described that the variation during the dry periods in ecosystem gas exchange was more related to changes in leaf area than changes in the photosynthetic rate.

Eucalypt species show a wide range of responses to water stress that are related to the environment of origin, resulting in different combinations of morphological and physiological attributes to provide drought tolerance [39]. For example, in our study *E. molucana* is the only *Adnataria* specie from high precipitation region (>1500 mm year^{-1}), and one of the most sensitive in biomass production and leaf area under water limited conditions. *E. argophloia* and *E. crebra*, from the *Adnataria* section, showed high transpiration, with high reduction of leaf area under water limitation, from provenances from regions with average annual rainfall lower than 750 mm year^{-1}. Taxa from stressful environmental conditions have the ability to respond strongly to avoid desiccation under water stress conditions by a range of mechanisms including shedding leaves, the arrangement of their leaves, and cellular processes that allow them to withstand low water potentials [5].

The highest increase in leaf-level water use efficiency under water stress conditions was shown by *E. camaldulensis*, *E. brassiana* (both *Exsertaria* section), and *E. crebra*. White *et al.* [40] described that water use efficiency at leaf level is not correlated to plantation water productivity efficiency in eucalypt stands. Silva *et al.* [41] discuss that increase in water efficiency in leaf scale is result of mechanisms aimed to allow plant survival under stressful conditions. *E. camaldulensis* is one of most suitable species for regions with drought stress [42]. *Eucalyptus camaldulensis* and *E. brassiana,* from the *Exsertaria* section, are widely used in commercial plantations to improve drought tolerance. However, both species are susceptible to gall wasp—*Leptocybe invasa* Fisher & La Salle [43]. This ubiquitous pest of eucalypts stands, was first observed in Brazil in 2007, and is currently spread across several states, causing widespread damage in nurseries and stands. Mendel *et al.* [44] tested several eucalypt species and observed hosts from *Exsertaria*, *Latoangulata*, and *Maidenaria* sections, three important sections of *Eucalyptus* plantation worldwide [16].

Our study was developed under controlled conditions of water availability to the seedlings, however under field conditions the physiological behavior is influenced by other abiotic stresses (e.g., temperature and nutritional status) interacting with drought [45] . Therefore, future research will focus on understanding how the relations between the productivity and physiological behavior under controlled and field conditions are important to improve the ability to select adapted taxa to stressful environments.

5. Conclusion

Our results indicate that non-commercial taxa that evolved in regions with different environmental conditions/stresses can be a source of different mechanisms of stress tolerance through pure specie stands or hybridization aiming introgression of these characteristics into commercial plantations.

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References

1. Gonçalves, J.L.M.; Stape, J.L.; Laclau, J.-P.; Smethurst, P.; Gava, J.L. Silvicultural effects on the productivity and wood quality of eucalypt plantations. *For. Ecol. Manag.* **2004**, *193*, 45–61. [CrossRef]
2. Gonçalves, J.L.M.; Stape, J.L.; Laclau, J.P.; Bouillet, J.P.; Ranger, J. Assessing the effects of early silvicultural management on long-term site productivity of fast-growing eucalypt plantations: The Brazilian experience. *South For. J. For. Sci.* **2008**, *70*, 105–118. [CrossRef]
3. Gonçalves, J.L.M.; Alvares, C.A.; Higa, A.R.; Silva, L.D.; Alfenas, A.C.; Stahl, J.; Ferraz, S.F.; de Paula, W.L.; Brancalion, P.H.S.; Hubner, A.; *et al.* Integrating genetic and silvicultural strategies to minimize abiotic and biotic constraints in Brazilian eucalypt plantations. *For. Ecol. Manag.* **2013**, *301*, 6–27. [CrossRef]
4. Allen, C.D.; Macalady, A.K.; Chenchouni, H.; Bachelet, D.; McDowell, N.; Vennetier, M.; Kitzberger, T.; Rigling, A.; Breshears, D.D.; Hogg, E.H.; *et al.* A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manag.* **2010**, *259*, 660–684. [CrossRef]
5. Florence, R.G. *Ecology and Silviculture of Eucalypt Forest*; Collingwood: CSIRO, Australia, 2004; p. 413.
6. Ryan, M.G. Tree responses to drought. *Tree Physiol.* **2011**, *31*, 237–239. [CrossRef] [PubMed]

7. Way, D.A.; Domec, J.-C.; Jackson, R. Elevated growth temperatures alter hydraulic characteristics in trembling aspen (*Populus tremuloides*) seedlings: implications for tree drought tolerance. *Plant Cell Environ.* **2012**, *36*, 103–115. [CrossRef] [PubMed]
8. Battie-Laclau, P.; Laclau, J.-P.; Domec, J.-C.; Christina, M.; Bouillet, J.-P.; Piccolo, M.C.; Gonçalves, J.L.M.; Moreira, R.M.; Krusche, A.V.; Bouvet, J.-M.; *et al.* Effects of potassium and sodium supply on drought-adaptive mechanisms in Eucalyptus grandis plantations. *New Phytol.* **2014**, *203*, 401–413. [CrossRef] [PubMed]
9. Granda, V.; Delatorre, C.; Cuesta, C.; Centeno, M.L.; Fernández, B.; Rodríguez, A.; Feito, I. Physiological and biochemical responses to severe drought stress of nine *Eucalyptus globulus* clones: A multivariate approach. *Tree Physiol.* **2014**, *34*, 778–786. [CrossRef] [PubMed]
10. Breda, N.; Huc, R.; Granier, A.; Dreyer, E. Temperate forest trees and stands under severe drought: A review of ecophysiological responses, adaptation processes and long-term consequences. *Ann. For. Sci.* **2006**, *63*, 625–644. [CrossRef]
11. McDowell, N.; Pockman, W.T.; Allen, C.D.; Breshears, D.D.; Cobb, N.; Kolb, T.; Plaut, J.; Sperry, J.; West, A.; Williams, D.G.; *et al.* Mechanisms of plant survival and mortality during drought: Why do some plants survive while others succumb to drought? *New Phytol.* **2008**, *178*, 719–739. [CrossRef] [PubMed]
12. Ngugi, M.; Doley, D.; Hunt, M.; Ryan, P.; Dart, P. Physiological responses to water stress in *Eucalyptus cloeziana* and *E. argophloia* seedlings. *Trees* **2004**, *18*, 381–389. [CrossRef]
13. Ladiges, P.Y. Variation in drought tolerance in *Eucalyptus viminalis* Labill. *Aust. J. Bot.* **1974**, *22*, 489–500. [CrossRef]
14. Costa e Silva, F.; Shvaleva, A.; Maroco, J.P.; Almeida, M.H.; Chaves, M.M.; Pereira, J.S. Responses to water stress in two *Eucalyptus globulus* clones differing in drought tolerance. *Tree Physiol.* **2004**, *24*, 1165–1172. [CrossRef] [PubMed]
15. Claeys, H.; Inzé, D. The agony of choice: How plants balance growth and survival under water-limiting conditions. *Plant Physiol.* **2013**, *162*, 1768–1779. [CrossRef] [PubMed]
16. Harwood, C. *New Introductions—Doing It Right In Developing a Eucalypt Resource: Learning from Australia and Elsewhere*; Walker, J., Ed.; Wood Technology Research Centre, University of Canterbury: Christchurch, New Zealand, 2011; pp. 125–136.
17. Potts, B.M.; Dungey, H.S. Hybridisation of Eucalyptus: Key issues for breeders and geneticists. *New For.* **2004**, *27*, 115–138. [CrossRef]
18. Dungey, H.S.; Potts, B.M.; Carnegie, A.J.; Ades, P.K. Mycosphaerella leaf disease: Genetic variation in damage to Eucalyptus nitens, Eucalyptus globulus, and their F1 hybrid. *Can J. For. Res.* **1997**, *27*, 750–759. [CrossRef]
19. Reis, G.G.; Reis, M.D.G.F.; Fontan, I.D.C.I.; Monte, M.A.; Gomes, A.N.; de Oliveira, C.H.R. Crescimento de raízes e da parte aérea de clones de híbridos de *Eucalyptus grandis x Eucalyptus urophylla* e de *Eucalyptus camaldulensis x Eucalyptus spp* submetidos a dois regimes de irrigação no campo. *R Árvore* **2006**, *30*, 921–931. [CrossRef]
20. Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; Gonçalves, J.L.M.; Sparovek, G. Köppen's climate classification map for Brazil. *Meteorol Z* **2013**, *22*, 711–728. [CrossRef]
21. Sarruge, J.R. Soluções nutritivas. *Summa Phytop* **1975**, *1*, 231–233.
22. Stape, J.; Binkley, D.; Ryan, M. Eucalyptus production and the supply, use and efficiency of use of water, light and nitrogen across a geographic gradient in Brazil. *For. Ecol. Manag.* **2004**, *193*, 17–31. [CrossRef]
23. Stape, J.L.; Binkley, D.; Ryan, M.G.; Fonseca, S.; Loos, R.A.; Takahashi, E.N.; Silva, C.R.; Silva, S.R.; Hakamada, R.E.; Ferreira, J.M.; *et al.* The Brazil Eucalyptus Potential Productivity Project: Influence of water, nutrients and stand uniformity on wood production. *For. Ecol. Manag.* **2010**, *259*, 1684–1694. [CrossRef]
24. Jaleel, C.A.; Manivannan, P.; Wahid, A.; Farooq, M.; Somasundaram, R.; Panneerselvam, R. Drought stress in plants: A review on morphological characteristics and pigments composition. *Int. J. Agric. Biol.* **2009**, *11*, 100–105.
25. Myers, B.J.; Landsberg, J.J. Water stress and seedling growth of two eucalypt species from contrasting habitats. *Tree Physiol.* **1989**, *5*, 207–218. [CrossRef] [PubMed]
26. Bauerle, W.L.; Wang, G.G.; Bowden, J.D.; Hong, C.M. An analysis of ecophysiological responses to drought in American Chestnut. *Ann. For. Sci.* **2006**, *63*, 833–842. [CrossRef]

27. Christina, M.; Le Maire, G.; Battie-Laclau, P.; Nouvellon, Y.; Bouillet, J.-P.; Jourdan, C.; de Moraes Gonçalves, J.L.; Laclau, J.-P. Measured and modeled interactive effects of potassium deficiency and water deficit on gross primary productivity and light-use efficiency in *Eucalyptus grandis* plantations. *Glob. Chang. Biol.* **2015**, *21*, 2022–2039. [CrossRef] [PubMed]
28. Chaves, M.M.; Pereira, J.S.; Maroco, J.; Rodrigues, M.L.; Ricardo C, P.P.; Osório, M.L.; Pinheiro, C. How plants cope with water stress in the field? Photosynthesis and growth. *Ann. Bot.* **2002**, *89*, 907–916. [CrossRef] [PubMed]
29. Litton, C.M.; Raich, J.W.; Ryan, M.G. Carbon allocation in forest ecosystems. *Glob. Change Biol.* **2007**, *13*, 2089–2109. [CrossRef]
30. Ryan, M.G.; Stape, J.L.; Binkley, D.; Fonseca, S.; Loos, R.A.; Takahashi, E.N.; Silva, C.R.; Silva, S.R.; Hakamada, R.E.; Ferreira, J.M.; *et al.* Factors controlling Eucalyptus productivity: How resource availability and stand structure alter production and carbon allocation. *For. Ecol. Manag.* **2010**, *259*, 1695–1703. [CrossRef]
31. Ochieng, J.W.; Shepherd, M.; Baverstock, P.R.; Nikles, G.; Lee, D.J.; Henry, R.J. Two sympatric spotted gum species are molecularly homogeneous. *Conserv. Genet* **2010**, *11*, 45–56. [CrossRef]
32. Wang, D.; Bachelard, E.P.; Banks, J.C.G. Growth and water relations of seedlings of two subspecies of Eucalyptus globulus. *Tree Physiol.* **1988**, *4*, 129–138. [CrossRef] [PubMed]
33. Teskey, R.O.; Bongarten, B.C.; Cregg, B.M.; Dougherty, P.M.; Hennessey, T.C. Physiology and genetics of tree growth response to moisture and temperature stress: an examination of the characteristics of loblolly pine (*Pinus taeda* L.). *Tree Physiol.* **1987**, *3*, 41–61. [CrossRef] [PubMed]
34. Valentini, R.; Mugnozza, G.S.; Giordano, E.; Kuzminsky, E. Influence of cold hardening on water relations of three Eucalyptus species. *Tree Physiol.* **1990**, *6*, 1–10. [CrossRef] [PubMed]
35. Choat, B.; Medek, D.E.; Stuart, S.A.; Pasquet-Kok, J.; Egerton, J.J.G.; Salari, H.; Sack, L.; Ball, M. Xylem traits mediate a trade-off between resistance to freeze–thaw-induced embolism and photosynthetic capacity in overwintering evergreens. *New Phytol.* **2011**, *191*, 996–1005. [CrossRef] [PubMed]
36. Taiz, L.; Zeiger, E. *Plant Physiology*, 5th ed.; Sinauer Associates Inc: Sunderland, UK, 2010.
37. Parra-O, C.; Bayly, M.J.; Drinnan, A.; Udovicic, F.; Ladiges, P. Phylogeny, major clades and infrageneric classification of *Corymbia* (Myrtaceae), based on nuclear ribosomal DNA and morphology. *Aust. Syst. Bot.* **2010**, *22*, 384–399. [CrossRef]
38. Cernusak, L.A.; Hutley, L.B.; Beringer, J.; Holtum, J.A.; Turner, B.L. Photosynthetic physiology of eucalypts along a sub-continental rainfall gradient in northern Australia. *Agric. For. Meteorol.* **2011**, *151*, 1462–1470. [CrossRef]
39. Merchant, A.; Callister, A.; Arndt, S.; Tausz, M.; Adams, M.A. Contrasting physiological responses of six *Eucalyptus* species to water deficit. *Ann. Bot.* **2007**, *100*, 1507–1515. [CrossRef] [PubMed]
40. White, D.A.; Beadle, C.L.; Worledge, D.; Honeysett, J.L. Wood production per evapotranspiration was increased by irrigation in plantations of *Eucalyptus globulus* and *E. nitens*. *New For.* **2016**, *47*, 303–317. [CrossRef]
41. Silva, P.H.M.; Campoe, O.C.; Vieira, I.G.; Paula, R.C.D. Aplicação foliar de boro em eucalipto sob estresse hídrico. *Scientia Forestalis* **2015**, *43*, 395–405.
42. Lemcoff, J.H.; Guarnaschelli, A.B.; Garau, A.M.; Prystupa, P. Elastic and osmotic adjustments in rooted cuttings of several clones of *Eucalyptus camaldulensis* Dehnh. from southeastern Australia after a drought. *Flora* **2002**, *197*, 134–142. [CrossRef]
43. Silva, P.H.M.; Paula, R.C.; Miranda, A.C.; Moraes, M.L.T.; Furtado, E.L.; de Pieri, C.; Brawner, J.; Lee, D. Screening of rust and gall wasp in eucalypts species and provenances. *Pesq Flor Brasil* **2015**, *35*, 323–328. [CrossRef]
44. Mendel, Z.; Protasov, A.; Fisher, N.; La Salle, J. Taxonomy and Biology of *Leptocybe invasa* (Eulophidae: Hymenoptera) an invasive gall inducer on eucalyptus. *Aust. J. Entomol.* **2004**, *43*, 101–113. [CrossRef]
45. Waraich, E.A.; Ahmad, R.; Ashraf, M.Y. Role of mineral nutrition in alleviation of drought stress in plants. *Aust. J. Crop. Sci.* **2011**, *5*, 764.