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Research paper

Drought avoidance and vulnerability in the Australian Araucariaceae

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The Araucariaceae is an iconic tree family. Once globally important, the Araucariaceae declined dramatically over the Cenozoic period. Increasing aridity is thought to be responsible for extinction and range contraction of Araucariaceae in Australia, yet little is known about how these trees respond to water stress. We examined the response to water stress of the recently discovered tree *Wollemia nobilis* Jones, W.G., Hill, K.D. & Allen, J.M. (Araucariaceae) and two closely related and widespread tree species, *Araucaria bidwillii* Hook. and *Araucaria cunninghamii* Mudie, and the island-endemic species, *Araucaria heterophylla* (Salisb.) Franco. Leaf water potential in all *Araucaria* spp. remained remarkably unchanged during both dehydration and rehydration, indicating strong isohydry. The xylem tensions at which shoot and stem hydraulic conductances were reduced to 50% (P50_{shoot} and P50_{stem}) were closely correlated in all species. Among the four species, *W. nobilis* exhibited greater resistance to xylem hydraulic dysfunction during water stress (as indicated by P50_{shoot} and P50_{stem}). Unexpectedly, *W. nobilis* also experienced the highest levels of crown mortality in response to dehydration, suggesting that this was the most drought-sensitive species in this study. Our results high-light that single traits (e.g., P50) should not be used in isolation to predict drought survival. Further, we found no clear correlation between species' P50 and rainfall across their distributional range. Diversity in drought response among these closely related Araucariaceae species was surprisingly high, considering their reputation as a functionally conservative family.

Keywords: Araucaria bidwillii, Araucaria cunninghamii, Araucaria heterophylla, conifer, hydraulic, water management, water stress, Wollemia nobilis.

Introduction

The geographic distributions of many plant species are defined by their water-stress tolerance (Brodribb and Hill 1999, Pockman and Sperry 2000, Engelbrecht et al. 2007). As plants transpire, tension increases in the xylem to draw water from the soil. As soil dries, there is an increased risk of hydraulic dysfunction, via cavitation (Tyree and Sperry 1989) and/or xylem wall collapse (Cochard 2004). Hydraulic dysfunction has a detrimental impact on photosynthesis and growth (Hsiao et al. 1976, Hubbard et al. 2001), and ultimately survival (McDowell et al. 2008, Brodribb and Cochard 2009). Vulnerability to hydraulic dysfunction varies among taxonomic groups and according to habitat. Hence, species' hydraulic traits can be related to their distributions. For example, P50 (the water potential resulting in a 50% loss of shoot hydraulic conductance (K_{shoot})) has been correlated with minimum dry season rainfall for a range of conifer species (Brodribb and Hill 1999). Similarly, P50 has been linked to survival under drought conditions (Blackman et al. 2009, Brodribb and Cochard 2009) and correlated with minimum rainfall availability (Blackman et al. 2012, Nardini and Luglio 2014). The difference between minimum stem water potential experienced in natural conditions and P50 is described as a safety margin (Choat et al. 2012). The size of this safety margin is potentially independent of rainfall and hence adds another axis of functional variation to the way plants manage hydration and use water (Choat et al. 2012).

Many studies point to variation in stomatal control of water loss during water stress as an important indicator of strategic variation in stress physiology among species. Different behaviours are often characterized as isohydric or anisohydric. Isohydric species maintain strict control of leaf water potential (ψ_{leaf}), closing stomata and restricting gas exchange to limit increases in xylem tension as water availability decreases. Anisohydric species, in contrast, do not maintain strict control of ψ_{leaft} continuing gas exchange as the environment dries and thus leading to a dynamic decline in ψ_{leaf} (Tardieu and Simonneau 1998). Anisohydric species are thus more exposed to dehydration under conditions of severe water shortage, but they are typically protected from injury by cavitation-resistant xylem (Brodribb et al. 2014). Anisohydry is generally more common in plants of drought-prone environments because their cavitation-resistant xylem allows maintenance levels of photosynthesis to continue under water-stressed conditions (McDowell et al. 2008, Kumagai and Porporato 2012).

In terms of stomatal control and xylem vulnerability, gymnosperms display considerable diversity in their responses to drought (Brodribb et al. 2014). Pinaceae are characterized by isohydry (Martínez-Vilalta et al. 2004, West et al. 2008), while many members of Cupressaceae behave anisohydrically in response to water stress (e.g., *Callitris*, Brodribb and McAdam 2013; *Juniperus*, McDowell et al. 2008). While drought responses of some conifers (e.g., Pinaceae and Cupressaceae) are well known, less is known about the drought responses and hydraulic vulnerability of other gymnosperm families, especially the dominant Southern Hemisphere conifer families Podocarpaceae and Araucariaceae (although see Pittermann et al. 2006).

Araucariaceae are an ancient and relictual gymnosperm family, first appearing in the fossil record of the Triassic (Kershaw and Wagstaff 2001). Once existing in both hemispheres, species of Araucariaceae are now restricted to the Southern Hemisphere (Stockey 1982), where they constitute one of three extant conifer families (Hill and Brodribb 1999). Gymnosperms, including the Araucariaceae, experienced a global decline in the Cretaceous associated with climatic drying and cooling, but Araucariaceae remained a major component of Australian vegetation until the late Cenozoic (Kershaw and Wagstaff 2001). There is a debate around the phylogenetic relationships within the Araucariaceae (Gilmore and Hill 1997, Setoquchi et al. 1998, Liu et al. 2009). The most recent study concluded that Agathis and Wollemia formed a clade separate to Araucaria that was distinct by the Cretaceous or Palaeogene (Escapa and Catalano 2013). Araucariaceae species are now restricted to moist and mainly mesothermal environments, as is typical in the southern conifers (Hill and Brodribb 1999, Kershaw and Wagstaff 2001). Despite their diversity, the majority of Araucariaceae species occur as low-density components of angiosperm-dominated rainforests (Enright 1995). Araucaria and Agathis spp. are typically large emergent trees in wet forests (Enright 1995, Hill and Brodribb

1999). Fragmented distributions and low levels of recruitment have resulted in a perception that the genus *Araucaria* is in decline (Enright 1995).

There are three genera in Araucariaceae: Araucaria, Agathis and Wollemia. Araucaria and Wollemia have natural distributions that extend into drier environments, along Australia's east coast from 11° to 32°S, which is why we focus on these two genera in this study. There are three species of Araucaria in Australia: (i) Araucaria heterophylla (Salisb.) Franco is restricted to Norfolk Island and has a natural distribution characterized by a subtropical climate moderated by the surrounding sea, (ii) Araucaria cunninghamii Mudie occurs in patches along the east coast of Australia from 30°S to New Guinea and (iii) Araucaria bidwillii Hook. occurs in disjunct populations in south-eastern and northeastern Queensland (Atlas of Living Australia). Wollemia nobilis Jones, W.G., Hill, K.D. & Allen, J.M. is a recently discovered monotypic genus within Araucariaceae. Its population is highly restricted, existing in a single catchment in the canyon landscape of eastern central New South Wales, where its habitat is riparian. Wollemia nobilis was discovered in 1994, and the mature population consists of fewer than 100 trees.

In this study, we set about to test the hypothesis that hydraulic vulnerability is related to distribution in Araucariaceae, and that *W. nobilis* is more hydraulically vulnerable than other Araucariaceae species, thus explaining, or strongly contributing to, the restricted distribution of *W. nobilis*.

Materials and methods

This study has two parts. The first experiments were undertaken to characterize species hydraulic conductance, using measurements from shoots and stems. The second experiment examines species responses to drought.

Part 1

Plants and growth conditions used for hydraulic conductance measurements The plants used for K_{shoot} measurements were grown in a glasshouse where temperature was maintained between 15 and 25 °C and shade was 40%. The plants used for stem hydraulic conductance (K_{stem}) measurements were grown outdoors in mean minimum temperatures of 14.8 and 10.6 °C and mean maximum temperatures of 22.9 and 23.8 °C (Royal Botanic Gardens in Sydney 33.868921 S, 151.215319 E, and the Australian Botanic Garden at Mount Annan 34.071442 S, 150.766241 E).

Shoot hydraulic vulnerability to dysfunction Due to the sessile leaf attachment in all species, it was impossible to measure hydraulic conductance in single leaves; hence, we measured short shoots with small numbers of leaves attached to a minimum length of stem. The proportion of stem in these samples was very small, and likely to be more cavitation resistant than the leaves (Hao et al. 2008, Blackman et al. 2010, Nolf et al. 2015), especially in

conifers (Brodribb and Cochard 2009); thus, we expect that hydraulic measurements reflect the leaf vulnerability. We measured K_{shoot} by cutting branches from *W. nobilis* (14 shoots from 3 trees), A. bidwillii (15 shoots from 3 trees), A. cunninghamii (17 shoots from 2 trees) and A. heterophylla (9 shoots from 2 trees). Trees were 4-5 years old and grown in pots. Individual branches were bench-dried to a range of water potentials and K_{shoot} was determined for leaves attached to short shoots (after Brodribb and Cochard 2009). Branches were dried under laboratory lighting and equilibrated in plastic bags before measurement. Water potentials of leaves attached to shoots were measured using a Scholander pressure chamber. The stems were then trimmed back under water and attached to a microflowmeter used to measure the flux of water rehydrating the target shoot (Melcher et al. 2012). Rehydration kinetics in flow were measured to calculate K_{shoot} (Brodribb and Cochard 2009). In a subsample of shoots, we measured initial and final $K_{\rm shoot}$ during the rehydration phase. Initial K_{shoot} was related to the initial rehydration flux and the initial ψ_{leaft} while the final K_{shoot} was calculated after the rehydration flux had declined to ~50% of the initial, and using the final ψ_{leaf} after the shoot was disconnected from the flowmeter. Rehydration time (up to 10 min) was chosen to allow relaxation of inward (hydrating) water flux to about half the initial value. Leaves remained illuminated under laboratory lighting. We tested for the possibility of exogenous embolism (Wheeler et al. 2013) by disconnecting and recutting shoots multiple times during rehydration to ensure that there was no flow increase (indicating the presence of an exogenous embolism). Shoot hydraulic conductance was measured at a range of water potentials beginning at -0.2 MPa and ending when K_{shoot} approached zero (-3 to -4 MPa). These data were used to determine the relationship between water potential and loss of K_{shoot} (normalization to leaf area was carried out by measuring leaf area on a flat-bed scanner). During all measurements, shoot temperature (equal to laboratory temperature, 21 °C) was measured with a copper-constantan thermocouple (30 American wire gauge, Omega Engineering, Stamford, Connecticut, USA) so that the effect of temperature on viscosity of water flux could be removed by normalizing all measurements to 20 °C (Sperry et al. 1988).

A linear function was fitted to K_{shoot} according to ψ_{leaf} in R (R Development Core Team 2014), and ψ_{leaf} at 50% conductance (P50_{shoot}) was calculated. Linear functions were used, rather than more complex functions, because they provided comparable fits while minimizing the potential for introducing error. P50_{shoot} was calculated as the water potential at which K_{shoot} was reduced to 50% of maximum. To assess whether the relationship between K_{shoot} and ψ_{leaf} varied according to species, we used an analysis of covariance (Crawley 2007), followed by a post hoc Scheffe test (Scheffe 1959). For each species, we compared mean annual rainfall (minimum and maximum; Atlas of Living Australia, Australian Government Bureau of Meteorology (BOM) 2014) with P50_{shoot} using Spearman's rank-order correlation.

Capacitance, an indication of stored water, was calculated as water loss over change in ψ_{leaft} using pre-turgor loss data (Blackman and Brodribb 2011) and using the samples from which we had measured K_{shoot} . Using the flowmeter output, it was possible to sum all water taken up during hydration between initial and final water potentials. Among-species differences in capacitance were assessed using analysis of variance (ANOVA) and Tukey's post hoc significance tests. All statistical analyses were undertaken in R (R Development Core Team 2014).

Stem hydraulic vulnerability to dysfunction Two branches were sampled in six mature individuals per species. Only 40-cm-long straight branches were selected in the upper part of the crown using a telescopic pole-pruner. Immediately after the sampling in the morning, leaves were removed and stems were wrapped up with humid paper and conditioned with plastic bags to avoid transpiration. Then, samples were sent to the high-throughput phenotyping platform (http://sylvain-delzon.com/caviplace, University of Bordeaux, Talence, France) and kept refrigerated at 4 °C until measurements.

Xylem cavitation was assessed with the CAVITRON, a centrifuge technique following the procedure described by Cochard (2002) and Cochard et al. (2005). Centrifugal force was used to establish negative pressure in the xylem and to provoke water-stressinduced cavitation, using a custom-built honeycomb aluminium rotor (Precis 2000, Bordeaux, France) mounted on a high-speed centrifuge (Sorvall RC5c+, Asheville, North Carolina, USA). This technique enables measurement of the conductance (K_i) of a stem under negative pressure. Xylem pressure (P_i) was first set to a reference pressure (-0.5 MPa), and K_i was determined by measuring the flux of a reference ionic solution (10 mmol dm⁻³ KCl and 1 mmol dm⁻³ CaCl₂ in deionized water) through the sample. The centrifugation speed was then set to a higher value for 3 min to expose the sample at a more negative pressure. For each pressure step, K_i was determined by measuring displacement speed of the air-water meniscus at the downstream extremity of the stem. This measurement was performed with a calibrated CCD camera (Scout sca640, Basler, Ahrensburg, Germany) coupled with a custom software (Cavisoft version 4.2, BIOGECO, University of Bordeaux, Bordeaux, France). Conductance was measured four times for each step, and the average was used to compute the per cent loss of K_{stem} (PLC in %). Per cent loss of K_{stem} was determined at each pressure step following the equation:

$$PLC = 100 \times \left(\frac{1 - K_i}{K_{max}}\right)$$
(1)

where K_{max} corresponds to the maximum hydraulic conductance measured at low speed (i.e., ψ_{stem} at -0.5 MPa). The procedure was repeated for at least eight pressure steps with a -0.5 or -1 MPa step increment until PLC reached at least 90%. Rotor velocity was monitored with a 10 r.p.m. resolution electronic tachymeter and xylem pressure was adjusted to about -0.02 MPa. The per cent loss of K_{stem} as a function of xylem pressure (MPa) represents the sample's vulnerability curve. A sigmoid function (Pammenter and Van der Willigen 1998) was fitted to the vulnerability curve from each sample using the following equation:

$$PLC = \frac{100}{[1 + \exp(S/25 \times (P_i - P50))]}$$
(2)

where P50 (MPa) is the xylem pressure inducing 50% loss of $K_{\rm stem}$ and S (% MPa⁻¹) is the slope of the vulnerability curve at the inflexion point. The stem hydraulic conductance ($K_{\rm stem}$) was estimated from $K_{\rm max}$ and the sapwood area of the branch, defining the transport efficiency.

Part 2

Plants and growth conditions used for dry-down experi*ment* The plants used in the dry-down experiment were grown from seed in 300-mm diameter pots (13.5 l) under optimal conditions, and they were ~2.5 years old. They had been grown outdoors in ~70% shade with annual mean minimum and maximum temperatures of 11.4 and 22.4 °C. All plants were well watered. Four individuals each of W. nobilis, A. bidwillii, A. cunninghamii and A. heterophylla were placed in a rainfall-exclusion (roofed) area, with ~50% shading (external photosynthetically active radiation was 45 mol m⁻² day⁻¹), where temperature and humidity were monitored. The mean height of A. bidwillii was 124 (±5 (1 SE)) cm and weight (plant, pot and soil) was 15,147 (±482) g, A. cunninghamii was 107 (±6) cm and 14,883 (±441) g, A. heterophylla was 137 (±5) cm and 16,612 (159) g and W. nobilis was 128 (±2) cm and 19,633 (±345) g. The aim of this part of the study was to compare responses of whole-plant gas exchange with water stress and recovery. Due to highly variable leaf morphology, it was neither practical nor meaningful to report gas exchange per unit leaf area; hence, we chose plants of similar size in terms of height and mass, and directly compared gas exchange dynamics with contrast differences in behaviour. Pots were covered in foil and bagged to prevent heating and evaporative water loss from soil.

Dry-down experiment Plants of each species were subjected to two different drought treatments; water was withheld for either a mild drought, with rewatering at 8 days and then again after 20 or 28 days (i.e., rewatering on Day 28 or 36 of the experiment), or a severe drought, with rewatering after 42 days. Water was then returned (plants were immersed in water for 1 h to ensure complete saturation) and recovery was monitored. Based on the species' wet forest distribution, we suggest that 42 days would constitute severe drought. To estimate transpiration, plants were weighed using scales accurate to 1 g (Sartorius Pty Ltd, Dandenong South, Victoria, Australia). Plants were weighed at 11 am and 12 pm every 1–3 days during Weeks 1 and 2, and every 3–4 days during Weeks 3–7. During initial measurements on Days 1–3, soil remained wet and these initial measurements were used

as unstressed references. Mean temperature and humidity during measurement period were 29.6 (\pm 12.6) °C and 33.1 (\pm 6.8) %. This resulted in a vapour pressure deficit of 3.98 kPa. Leaf water potential of the plants was measured using a Scholander pressure chamber. Recovery was calculated as the transpiration at 80% of mean transpiration on Days 1 and 2 (for each individual). If there was a large discrepancy between Days 1 and 2, then the mean was taken from Days 1 to 3. The per cent of brown branches on each tree was visually estimated at the end of the experiment. Crown mortality data (pooled for each species) were compared using a one-way ANOVA and Tukey's post hoc significance tests. Safety margin was calculated for each species as the difference between ψ_{leaf} responsible for 80% reduction in plant transpiration (indicative of stomatal closure) and P50_{shoot}.

Results

Part 1

Hydraulic vulnerability Wollemia nobilis showed the greatest resistance to hydraulic dysfunction with a P50_{shoot} of -2.22 MPa and P50_{stem} of -3.32 MPa (Figures 1 and 2, Table 1). While our study only included four species, there was a clear association between P50_{stem} and P50_{shoot} when they were compared across the species, with stems consistently more resistant to dysfunction than shoots (Figure 2). The slope of the relationship between K_{shoot} and ψ_{leaf} in *W. nobilis* was significantly different from that of A. bidwillii and A. heterophylla, but not of A. cunninghamii (Table 2 and Table S1 available as Supplementary Data at Tree Physiology Online). Alternatively, A. cunninghamii showed the least resistance to hydraulic dysfunction, with a P50_{shoot} of -1.63 MPa and P50_{stem} of -2.64 MPa. In addition, that there was no consistent increase in $K_{\rm shoot}$ after rehydration (data not shown) indicates that the decline in ${\it K}_{\rm shoot}$ was nonreversible in the short term. Differences in mean annual precipitation (MAP), particularly dry-end distribution limits, showed no clear correlation with P50_{shoot} or P50_{stem} among the species (minimum and maximum MAP; P > 0.05; Table 1). Wollemia nobilis had a low safety margin, 0.12 MPa, compared with the Araucaria spp. (0.53–0.69; Table 1).

Capacitance Capacitance before turgor loss was highest for *A. heterophylla* at 3075 (±568 (±1 SD), n = 5) mmol MPa⁻¹ m⁻². This was significantly different (P = 0.04) from *A. cunninghamii*, which had the lowest value at 1457 (±289, n = 7) mmol MPa⁻¹ m⁻², while the other species had intermediate values for capacitance (*A. bidwillii*: 1756 mmol MPa⁻¹ m⁻² ± 168, n = 5; *W. nobilis*: 1793 mmol MPa⁻¹ m⁻² ± 227, n = 3; not significantly different from either *A. heterophylla* or *A. cunninghamii*).

Part 2

Dry-down experiment During the dry-down experiment, water was withheld from *Araucaria* species and *W. nobilis* for up to



Figure 1. Hydraulic conductance (K_{shoot}) as a function of leaf water potential (LWP) for *A. bidwillii*, *A. cunninghamii*, *A. heterophylla* and *W. nobilis*. Regression parameters and R^2 values are reported in Table 2.



Figure 2. Relationship between P50_{shoot} and P50_{stem} for *A. bidwillii*, *A. cunninghamii*, *A. heterophylla* and *W. nobilis*. Solid line has slope of 1.

42 days. The rate of soil water decrease declined with time: plants that had water withheld for an initial mild drought of 8 days were reduced in total weight (plant plus soil) by 9.6% (±5), whereas after the severe drought of 42 days, weight was reduced by 20.8% (±3).

The trend for declining transpiration under increasing drought (mild and severe drought treatments) is clear for all species (Figure 3). It was not possible to present transpiration data in terms of normalized leaf area (the plants were of similar height and in pots of the same size, see Materials and methods). This is a limitation of the study. The ψ_{leaf} of the *Araucaria* species displayed little variation either during the water-stress period or following rewatering (Figure 3). In each of the *Araucaria* species, ψ_{leaf} was maintained just above P50_{shoot}. In contrast, ψ_{leaf} of *W. nobilis* fell markedly after 3 weeks drought (approaching P50_{stem}), but recovered to pre-drought ψ_{leaf} within 3 days of rewatering.

After rewatering, all species recovered transpiration to similar or higher levels than pre-water-stress conditions, although the mean rates of recovery differed (Figure 3). In terms of the trees exposed to mild drought, all *Araucaria* individuals recovered to at least 80% of their initial transpiration rates before Day 50 (2–3 weeks of rewatering). In contrast, the two *W. nobilis* individuals exposed to mild drought had not recovered by Day 50. The results for severe drought were similar. All but one of *Araucaria* individuals had recovered to at least 80% of initial

Table 1. P50 (95% CI), safety margin and mean annual precipitation (MAP) across distributional range for A. bidwillii, A. cunninghamii, A. heterophylla
and W. nobilis. Distributional rainfall data are World Clim Mean Annual Precipitation for Atlas of Living Australia records of non-cultivated specimens
within Australia. Unique values were used as indicators of different populations (widespread species; A. bidwillii and A. cunninghamii). Point data from
BOM weather stations were used to ascertain MAP for rarer species (A. heterophylla and W. nobilis). Note that rainfall means from BOM are calculated
over different time periods.

	P50 _{shoot} (MPa)	P50 _{stem} (MPa)	Safety margin (MPa)	MAP min. (mm)	MAP max. (mm)
A. bidwillii	-1.86 (-2.40, -1.40)	-3.01 (-3.05, -2.94)	0.53	746	2325
A. cunninghamii	–1.63 (–1.90, –1.37)	-2.64 (-2.68, -2.58)	0.69	702	2982
A. heterophylla	-1.72 (-2.14, -1.31)	-2.97 (-3.00, -2.91)	0.56	1135	1301
W. nobilis	-2.22 (-2.64, -1.84)	-3.32 (-3.39, -3.20)	0.12	953	953

Table 2. Hydraulic vulnerability curve fit (R^2) and linear regression parameters for *A. bidwillii*, *A. cunninghamii*, *A. heterophylla* and *W. nobilis*. Slope parameters sharing the same letter were not significantly different. K_{shoot} and ψ_{leaf} fits are presented in Figure 1. A post hoc Sheffe test was used to determine which slopes were significantly different.

	${\it K}_{\rm shoot}$ and $\psi_{\rm leaf}$ fit $({\it R}^2)$	Intercept (95% CI)	Slope (95% Cl)
A. bidwillii	0.37	4.91 (3.70, 6.34)	1.32 (2.04, 0.67) a
A. cunninghamii	0.55	3.53 (2.96, 4.10)	1.08 (1.41, 0.80) b
A. heterophylla	0.60	6.60 (5.02, 8.18)	1.91 (2.71, 1.12) a
W. nobilis	0.60	2.98 (2.47, 3.54)	0.67 (0.90, 0.49) b

transpiration by Day 50 (8 days of rewatering). Both of the *W. nobilis* exposed to severe drought had recovered to 80% of initial transpiration rates Day 50, and by Day 100, one individual had not recovered. Interestingly, transpiration had declined for some plants between Day 50 and Day 100. This was attributed to the high temperatures on that day (42.4 $^{\circ}$ C).

Wollemia nobilis plants experienced a large proportion of crown death, relative to the *Araucaria* spp. The droughted *Araucaria* spp. each had 0–10% leaf browning by Day 100 (i.e., watering resumed for 2 months). In contrast, the *W. nobilis* exposed to mild drought had 15 and 85% leaf browning, and those exposed to severe drought had 30 and 95% leaf browning. The plant with 95% leaf browning had re-sprouted (hence 5% green) at 100 days. *Wollemia nobilis* canopy health was significantly (P < 0.05) decreased compared with the other species, which showed minimal damage (mean = 3.5% leaf browning).

Discussion

Despite being closely related, *Wollemia* and *Araucaria* had distinct responses to water stress. The *Araucaria* species displayed remarkable isohydry, inferred from the constancy of their Ψ_{leaff} which was unchanged despite large variation in soil hydration and midday transpiration. In contrast, *W. nobilis* did not maintain constant Ψ_{leaff} Although *W. nobilis* had lower hydraulic vulnerability compared with the *Araucaria* species (i.e., more negative P50), it was less able to avoid damage by water stress than *Araucaria* species, as evidenced by crown mortality sustained during the dry-down experiment.

Extreme isohydry in Araucaria

The Araucaria species in this study exhibited extremely isohydric behaviour. Despite the imposition of water stress sufficient to completely close stomata in each of the Araucaria species, ψ_{leaf} remained relatively unchanged, and above P50_{shoot} (and P50_{stem}) during the 42-day dry-down treatment, or rewatering. That Araucaria species survived the drought without suffering major hydraulic dysfunction is evidenced by rapid recovery of transpiration and lack of leaf or branch mortality. Isohydry is commonly observed in the Northern Hemisphere conifer family Pinaceae (Martínez-Vilalta et al. 2004), but not in the other major Northern Hemisphere family Cupressacae (Brodribb et al. 2014). Across a range of environments, Pinaceae respond to drying similarly, closing stomata between -2 and -3 MPa (Richardson and Rundel 1998) to maintain static midday ψ_{leaft} This characteristic tends to correspond to relatively high vulnerability to cavitation (i.e., less negative P50, Delucia et al. 2000, Piñol and Sala 2000). The P50_{shoot} estimates in our study indicate that the vulnerability to shoot hydraulic dysfunction in these Araucaria spp. fell at the moderate-to-susceptible end of the spectrum when compared with angiosperms (Blackman et al. 2010, 2012, 2014; 130 species reviewed in Nardini and Luglio 2014) and was similar to conifers in the Pinaceae family (Charra-Vaskou et al. 2012, Brodribb et al. 2014). Furthermore, P50_{stem} suggested relatively high susceptibility in these Araucariaceae compared with other gymnosperms, such as the 96 species reviewed by Choat et al. (2012) that ranged in P50_{stem} from -1.74 MPa in Podocarpus latifolius to -14.10 MPa in Juniperus pinchotii. The Araucariaceae in our study were similar to the highly isohydric Pinaceae family (reviewed by Martínez-Vilalta et al. 2004), where the least negative P50_{stem} was -2.61 MPa in Pinus ponderosa (similar to A. cunninghamii with P50_{stem} at -2.64 MPa). These Australian Araucaria appear to have a drought response similar to Pinaceae, but with an even narrower range of ψ_{leaf} during which transpiration occurs.

Strong isohydry is mediated by high stomatal sensitivity to drought (Tyree and Ewers 1991, Martínez-Vilalta et al. 2004), with early stomatal closure likely to be driven by high levels of abscisic acid (ABA; Brodribb and McAdam 2013). Two other potential mechanisms for maintaining xylem function during water stress have been described. First, plants can invest in xylem



Figure 3. Araucaria bidwillii, A. cunninghamii, A. heterophylla and W. nobilis (a) mean leaf water potential (LWP \pm SE) with time (circles) and (b) mean midday transpiration ($T \pm$ SE) with time (squares) during drought recovery experiment. Arrows indicate re-watering. Plants are grouped by drought treatment: severe (42-day) drought in black and mild drought in white. For all species, mild drought incorporated rewatering at 8 days, and an additional rewatering at 28 or 36 days (i.e., 8 days drought followed by 20 or 28 days drought). Araucaria bidwillii and A. heterophylla were rewatered for the second time on Day 28 (20 days additional drought), and A. cunninghamii and W. nobilis were rewatered for the second time on Day 36 (28 days additional drought).

strengthening to increase cavitation resistance of xylem tissues (Tyree and Ewers 1991), but despite Southern Hemisphere conifers (Araucariaceae and Podocarpaceae) having on average 30% higher wood density than Northern Hemisphere conifers, their cavitation resistance is not proportionally higher (Pittermann et al. 2006). Second, plants can decrease their leaf-to-sapwood area ratio (Tyree and Ewers 1991). We saw no evidence of deciduousness in the *Araucaria* species. Rather than modifying xylem supply it appears that Araucarians use isohydry (i.e., highly drought-sensitive stomata), in concert with high capacitance (e.g., relative to 20 woody species, Blackman and Brodribb 2011), as their primary means of water-stress avoidance.

Drought avoidance may have contributed to the ability of the Araucaria species to maintain their distribution during Holocene drying in much of the temperate Southern Hemisphere, and particularly in Australia into the Tertiary. However, the relationship between physiological traits and distribution, according to MAP, was not clear in our study. Araucaria heterophylla had a dry-end distributional MAP of 1135 mm year⁻¹, whereas A. bidwillii and A. cunninghamii extend to areas of 746 and 702 mm year⁻¹. Contrary to A. heterophylla's high-rainfall distribution, A. heterophylla had similar resistance to hydraulic dysfunction (according to P50 shoot) to A. bidwillii and A. cunninghamii, although both A. bidwillii and A. cunninghamii extend into drier climes. Based on the theory that dry-adapted species should have high capacitance, the distributions of these species are again contrary to expectation, as A. heterophylla had the highest capacitance, and A. cunnighamii the lowest. Interestingly, both A. bidwillii and A. cunninghamii extend over a similar rainfall range yet A. cunninghamii has populations along the Queensland coast, while A. bidwillii's populations in south-eastern and north-eastern Queensland are disjunct. The influence of intraspecific variability in hydraulic function (e.g., Matzner et al. 2001) was not assessed in our study. Stomatal and xylem sensitivity to water stress during drought are clearly only two of many factors, including light, edaphic conditions, disturbance and competition, that drive plant species distributions (Raven et al. 2003).

The xylem vulnerabilities of these Araucarians were comparable to Pinaceae, but they contrast with those of Australia's most dominant conifer family: Cupressaceae. Pinaceae and Cupressaceae are frequently highlighted as examples from either end of the spectrum of plant responses to drought, especially in the case of piñon–juniper woodlands in the southwestern USA (Linton et al. 1998, McDowell et al. 2008, West et al. 2008, Breshears et al. 2009). In recent droughts, Cupressaceae (*Juniperus*) has recorded survival superior to that of *Pinus* (Breshears et al. 2009). Despite the remarkable isohydric control of ψ_{leaf} displayed by *Araucaria*, anisohydric *Callitris* (Cupressaceae) is the dominant conifer genus in Australia. *Callitris*' dominance highlights the ascendancy of anisohydric response to drought in the context of Australia—the world's driest vegetated continent. Stomatal control in *Callitris* is weaker, associated with lower levels of ABA (Brodribb and McAdam 2013), but allows *Callitris* to continue to photosynthesis for longer during water stress, and also to resume photosynthesis more quickly when water is returned. *Callitris* xylem is also fortified to withstand tensions of less than –8 MPa before P50_{stem} (Brodribb et al. 2010, Larter et al. 2015). This is in stark contrast to *Araucaria* with unvarying ψ_{leaf} and P50_{shoot} of –1.6 to –1.9 MPa and P50_{stem} of –2.6 to 3 MPa.

Disparity in Wollemia hydraulic vulnerability and drought response

Wollemia nobilis did not exhibit the extreme isohydry of the Araucaria species. Instead, ψ_{leaf} of *W. nobilis* fell in response to increasing duration of drought. Wollemia nobilis also exhibited more negative stem and shoot P50 than the other Araucariaceae, indicating reduced vulnerability to hydraulic dysfunction. These characters would typically imply enhanced drought tolerance (McDowell et al. 2008). However, a relatively anisohydric response of ψ_{leaf} to drought in *W. nobilis* appeared to expose leaves to damaging desiccation more quickly than the other Araucariaceae species (i.e., W. nobilis was the only species to experience ψ_{leaf} that posed a risk of hydraulic dysfunction). This eventually resulted in 85% crown mortality of one W. nobilis individual and 70% crown mortality of another, while the other Araucariaceae species sustained minimal damage. Wollemia nobilis does not exhibit isohydric behaviour compared with the Araucaria species, which maintained constant ψ_{leaf} throughout the drought period. Leaf water potential in W. nobilis declined consistently over a period of 2 weeks as plant transpiration decreased, exhibiting what would be strictly interpreted as anisohydric stomatal control.

However, W. nobilis xylem was not particularly resistant to dysfunction under water stress, compared with other conifers characterized by anisohydry in response to drought (Linton et al. 1998, West et al. 2008). Stomatal sensitivity (to water deficit) and plant hydraulic vulnerability to cavitation act together to determine plant drought tolerance (Martínez-Vilalta et al. 2004, Brodribb et al. 2014). Both Araucaria spp. and W. nobilis had low safety margins of <1 MPa. This is not uncommon (70% of 226 species reviewed by Choat et al. 2012 had safety margins <1 MPa), but the margin of only 0.12 MPa in W. nobilis is unusually small for conifers and may explain to some degree the sensitivity of this species to water stress. Our results highlight that single traits, such as P50, should not be used to predict drought survival; more negative P50 does not confer drought tolerance when transpiration is insensitive to drought conditions. Although W. nobilis was less vulnerable to hydraulic dysfunction compared with the Araucaria species, these among-species differences are minor when compared with the global range of cavitation resistance; for example, mean P50 in leaves is -1.54 MPa in tropical rainforest (Nardini and Luglio 2014) and -5.69 MPa in semiarid shrubland (Blackman et al. 2014).

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Wollemia nobilis's unique architecture may have contributed to its crown mortality: the xylem at W. nobilis branch bases is constricted, possibly facilitating the abscission of first-order branches (Burrows et al. 2007). According to Burrows et al. (2007), this may also restrict water transport and vascular supply to foliage-this may be one factor influencing W. nobilis' inability to avoid drought. Wollemia nobilis also was unusual in that damaged plants re-sprouted. Re-sprouting is unusual in gymnosperms (Del Tredici 2001) but is present in several members of the Araucariaceae (Burrows 1987, Burrows et al. 2003, Alabarce and Dillenburg 2012) as well as in a small number of species from other families, e.g., Pinus canariensis (Climent et al. 2004). Both Hill (1997) and Burrows et al. (2003) indicate that W. nobilis can re-sprout without injury; here, we show resprouting in response to crown mortality.

Does W. nobilis' response to drought explain its restricted distribution? Wollemia nobilis' distribution was characterized by a single value for MAP (953 mm year⁻¹) because it is limited to a single catchment. This value falls within the range of MAP for the more widespread species, A. bidwillii and A. cunninghamii, yet W. nobilis experienced major drought-induced crown mortality, whereas the other species did not. At the local scale, W. nobilis is limited to riparian habitat, indicating that it may be at the dry end of its potential distribution. Wollemia nobilis' drought sensitivity may have restricted it to areas that do not experience strong seasonal drought: within its current distribution, W. nobilis receives dry season monthly rainfall >50 mm (BOM 2014), as distinct from many adjacent regions (i.e., in New South Wales and Queensland) receiving <50 mm in the driest month, despite higher total annual rainfalls. Wollemia nobilis is also the least heat tolerant of the Araucariaceae (Offord 2012). Wollemia nobilis' drought response is unlikely to be the only driver of its restricted distribution, but it may provide important context for a broader suite of stressors that induced decline, particularly in the distant past (Kershaw and Wagstaff 2001). Wollemia nobilis is also affected by pathogenic Phytophthora spp. (Bullock et al. 2003, Puno et al. 2015), and the remnant population is characterized by low genetic variation (Peakall et al. 2003), which further limits adaptation to pathogens and changing environment (Hoffmann and Sgrò 2011).

Conclusions

Drought responses of the Araucaria species and W. nobilis were surprisingly divergent, given their phylogenetic proximity. The three Araucaria species displayed extreme isohydry, while *W. nobilis* did not maintain constant ψ_{leaf} and experienced crown mortality in the dry-down experiment. It appears Araucaria spp. use isohydry to avoid drought, while W. nobilis is less able to do so.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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