

# Lignin composition is related to xylem embolism resistance and leaf life span in trees in a tropical semiarid climate

Taysla R. A. Lima<sup>1</sup>, Ellen C. D. Carvalho<sup>2</sup>, Fernando R. Martins<sup>3</sup>, Rafael S. Oliveira<sup>3</sup> , Rafael S. Miranda<sup>4</sup> , Caroline S. Müller<sup>3</sup>, Luciano Pereira<sup>3</sup>, Paulo R. L. Bittencourt<sup>3</sup>, Jullyana C. M. S. M. Sobczak<sup>5</sup>, Enéas Gomes-Filho<sup>6</sup>, Rafael C. Costa<sup>2</sup> and Francisca S. Araújo<sup>2</sup>

<sup>1</sup>Ecology and Natural Resources Post-Graduate Program, Department of Biology, Federal University of Ceará, 60440-900 Fortaleza, CE, Brazil; <sup>2</sup>Department of Biology, Federal University of Ceará, 60440-900, Fortaleza, CE, Brazil; <sup>3</sup>Department of Plant Biology, Institute of Biology, University of Campinas – UNICAMP, PO Box 6109, 13083-970 Campinas, SP, Brazil; <sup>4</sup>Federal University of Piauí (UFPI/CPCE), Campus Professora Cinobelina Elvas, 64900-000 Bom Jesus, PI, Brazil; <sup>5</sup>Institute of Rural Development, University of International Integration of African-Brazilian Lusophony, 62790-000 Redenção, CE, Brazil; <sup>6</sup>Department of Biochemistry and Molecular Biology, Federal University of Ceará, 60440-554 Fortaleza, CE, Brazil

## Summary

Author for correspondence:

Taysla R. A. Lima

Tel: +55 85 997363285

Email: taysla-bt@hotmail.com

Received: 4 December 2017

Accepted: 9 April 2018

*New Phytologist* (2018) **219**: 1252–1262

doi: 10.1111/nph.15211

**Key words:** drought resistance, guaiacyl, phenology, plant hydraulics, seasonally dry tropical forest, syringyl, wood traits.

- Wood properties influence the leaf life span (LL) of tree crowns. As lignin is an important component of wood and the water transport system, we investigated its relationship with embolism resistance and the LL of several tree species in a seasonally dry tropical ecosystem.
- We determined total lignin and the monomer contents of guaiacyl (G) and syringyl (S) and related them to wood traits and xylem vulnerability to embolism ( $\Psi_{50}$ ) for the most common species of the Brazilian semiarid, locally known as Caatinga.
- Leaf life span was negatively related to  $\Psi_{50}$  and positively related to S : G, which was negatively related to  $\Psi_{50}$ . This means that greater S : G increases LL by reducing  $\Psi_{50}$ . Lignin content was not correlated with any variable.
- We found two apparently unrelated axes of drought resistance. One axis, associated with lignin monomeric composition, increases LL in the dry season as a result of lower xylem embolism vulnerability. The other, associated with wood density and stem water content, helps leafless trees to withstand drought and allows them to resprout at the end of the dry season. The monomeric composition of lignin (S : G) is therefore an important functional wood attribute affecting several key functional aspects of tropical tree species in a semiarid climate.

## Introduction

Wood attributes are recognized as key functional traits because they are involved in multiple vital functions of trees, such as water transport, carbohydrate storage, tree biomechanics, leaf phenology, and growth (Chave *et al.*, 2009; Poorter *et al.*, 2010; Markesteijn *et al.*, 2011). The most abundant compounds of wood are three structural polymers: cellulose, hemicellulose and lignin (Stephen *et al.*, 1987). Lignin is found in the secondary walls of particular types of cells, especially conduits and fibers (Fromm *et al.*, 2003; Awad *et al.*, 2012), but it also occurs in ray parenchyma cells (Fergus & Goring, 1970). Lignin is involved in many functions, including mechanical support and water conduction (Baucher *et al.*, 1998). In fact, both mechanical support and long-distance water transport in trees are only possible because of the evolution of lignin (Boyce *et al.*, 2004; Espíneira *et al.*, 2011). Lignin deposition in cell walls increases rigidity and impermeability to water and, consequently, it can alter water conduction, mechanical support and protection against pathogens (Pilate *et al.*, 2012). Lignin increases the rigidity of the

cell wall as a result of its hydrophobic features, keeping cellulose microfibrils dry and more resistant (Niklas, 1992). The rigidity and relative impermeability of cell walls create some resistance to high-tension water transport without the occurrence of conduit implosion (Hacke *et al.*, 2006).

Lignin is mainly composed of three monolignol monomers (Boerjan *et al.*, 2003; Morreel *et al.*, 2010): *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S). The monolignols are hydroxycinnamic alcohols (*p*-coumaryl, coniferyl and sinapyl), that is, phenylpropanoids with C3–C6 chains that differ from each other only by the degree of methoxylation (Baucher *et al.*, 1998). The lignin monomeric composition varies among species (Zhao *et al.*, 2010), tissue type, and response to environmental stress (Campbell & Sederoff, 1996). Syringyl is mostly deposited in fiber walls, whereas guaiacyl is largely deposited in the conduit walls (Saito *et al.*, 2012).

Low lignin content is related to higher vulnerability to embolism in both transgenic plants (Voelker *et al.*, 2011; Awad *et al.*, 2012) and native species (Pereira *et al.*, 2017). Because high tension occurs within the xylem conduits during water transport,

air from surrounding tissues may enter the conduits and induce embolism formation, interrupting the water column and the water supply to the leaves (Zimmerman, 1983). Therefore, embolism resistance is an important trait in understanding life-history strategies, such as drought resistance and leaf retention time in seasonally dry ecosystems (Choat *et al.*, 2005). A few studies have shown that plant fitness and water transport efficiency are related to lignin traits (Coleman *et al.*, 2008; Voelker *et al.*, 2011). Although lignin is related to embolism resistance, the mechanisms by which lignin directly or indirectly limits embolism are still unknown (Pereira *et al.*, 2017).

Plants in seasonally dry environments show a range of leaf life spans (LLs; Sobrado, 1997; Tyree *et al.*, 2003; Oliveira *et al.*, 2015), from early deciduous species that remain in leaf for 3–6 months a year to late deciduous species (mean LL 7–10 months) and evergreen species that remain in leaf throughout the year (Oliveira *et al.*, 2015). At one end of the spectrum, evergreen species are assumed to withstand water tension in the xylem, maintaining gas exchange during the dry season; at the other end, early deciduous species have traits that confer efficient water transport in the rainy season and avoid drought by reducing their metabolic activity during the dry season (Engelbrecht & Kursar, 2003; Tyree *et al.*, 2003). Therefore, hydraulic properties vary across phenological groups and correspond to a tradeoff between efficiency and safety (Hacke *et al.*, 2006). While much research has focused on species differences in wood characteristics determining hydraulic resistance, such as the anatomy of xylem conduits and pit membranes (Hacke *et al.*, 2001; Choat *et al.*, 2005; Jansen *et al.*, 2009), less is known about how chemical wood traits, such as lignin content and monomeric composition, are related to drought resistance strategies, such as hydraulic resistance to embolism and LL.

Here, we investigate the hypothesis that lignin content and composition determine drought resistance in tree species in a seasonally dry tropical forest. From this hypothesis, we make the following predictions: the total content and the monomeric composition of lignin are directly related to LL; the total content and the monomeric composition of lignin influence xylem embolism resistance; both the total lignin content and its monomeric composition are related to the wood traits that control LL (wood density, stem water saturation, and stem water potential in the dry season).

## Materials and Methods

### Study area

The semiarid tropical zone of northeastern Brazil extends over an area of 800 000 km<sup>2</sup> and is characterized by highly unpredictable rainfall concentrated in a single period of 3–5 months yr<sup>-1</sup>. The average annual rainfall is 775 mm, with a variation coefficient of 30% (MINTER, 1984). The prevailing vegetation is the largest South American stretch of the Seasonally Dry Tropical Forest, locally called Caatinga, which covers *c.* 70% of the Brazilian northeastern region, generally below 650 m of altitude (Moro *et al.*, 2014, 2016).

The regional climate of the studied area is BSh semiarid with rainfall concentrated in summer–autumn and drought around winter–spring, according to the Köppen–Geiger classification system (Peel *et al.*, 2007). Precipitation is often unpredictable, concentrated in time and scattered in space, and temperatures are high throughout the year, yielding twice as much evapotranspiration as precipitation (Nimer, 1989). The rainy season usually occurs between February and May (summer–autumn), the total average annual rainfall is 694 mm, and the average annual temperature is 28.7°C. Soils are shallow, with little water retention capacity. The region topography is flat with an average altitude of 75 m (Souza *et al.*, 2015).

### Species studied and variables measured

Samples were collected in May 2014, during the rainy season. Total precipitation in that year was 494 mm (below the normal average), and the average temperature was 29.8°C. We sampled 22 tree species (Table 1) in the Curu Valley Experimental Farm (3°47'S–39°16'W), Pentecoste municipality, Ceará state, northeastern Brazil. The sampling area and the species are exactly the same as those used by Oliveira *et al.* (2015). These authors measured wood density (WD, g cm<sup>-3</sup>), stem water saturation (QW<sub>sat</sub>, %), stem water potential in the dry season ( $\Psi_{\text{dry season}}$ , MPa) according to the method of Borchert (1994), and LL (LL, months) according to the method of Fournier (1974) in adult individuals with stem diameter at ground level  $\geq 3$  cm and height  $\geq 1$  m following Rodal *et al.* (1992). In 2012, the year in which Oliveira *et al.* (2015) collected their data, the annual precipitation was 416 mm (below the normal average).

We measured xylem embolism vulnerability ( $\Psi_{50}$ , MPa), total lignin content ( $\mu\text{g mg}^{-1}$  dry mass (DM)) and its monomeric composition (syringyl (S) and guaiacyl (G) in  $\mu\text{mol g}^{-1}$  DM) and calculated the S : G ratio (see later) for trees of the selected species. We assumed that these variables together could provide information on drought resistance (expressed by LL) resulting from both xylem traits (total lignin content, S, G, S : G, and  $\Psi_{50}$ ) and wood traits (WD, QW<sub>sat</sub> and  $\Psi_{\text{dry season}}$ ). The 22 species selected represent a wide range of LLs and leaf habit groups (from early deciduous to evergreen) and represent 73% of the species composition of the community. In addition, these species are among those with the highest occurrence rates in the Caatinga domain (Moro *et al.*, 2014). As our data were collected in exactly the same plots, from the same individuals and in a year with similar annual precipitation (below average) as those collected by Oliveira *et al.* (2015), we do not believe the difference in time of collection represents a significant bias in our results.

### Plant material preparation for lignin extraction

For each species, we selected three adult plants with stem diameter at ground level  $\geq 3$  cm (range of 3–20 cm) and height  $\geq 1$  m, following Rodal *et al.* (1992). From each individual tree studied, we collected a 10 cm segment from the apex of three mature branches and immediately froze them in liquid N<sub>2</sub>. Samples were then taken to laboratory and stored in a freezer at -70°C.

**Table 1** Tree species used in each leaf habit group and their mean values of leaf life span (LL, months), wood density (WD, g cm<sup>-3</sup>), stem water saturation (QW<sub>sat</sub>, %), stem water potential in the dry season ( $\Psi_{\text{dry season}}$ , MPa), lignin content (lignin,  $\mu\text{g mg}^{-1}$  dry mass (DM)), syringyl (S,  $\mu\text{mol g}^{-1}$  DM), guaiacyl (G,  $\mu\text{mol g}^{-1}$  DM), S : G ratio, and xylem vulnerability ( $\Psi_{50}$ , MPa)

Species	Family	Leaf habit groups <sup>1</sup>	LL <sup>1</sup>	WD <sup>1</sup>	QW <sub>sat</sub> <sup>1</sup>	$\Psi_{\text{dry season}}$ <sup>1</sup>	Lignin	S	G	S : G	$\Psi_{50}$
(1) <i>Ziziphus joazeiro</i> Mart.	Rhamnaceae	Evergreen	12	0.55	85	-2.7	132.94	36.92	34.58	1.1	-3.35
(2) <i>Cynophalla flexuosa</i> (L.) J. Presl	Capparaceae	Evergreen	12	0.56	79	-2.4	27.62	67.31	62.71	1.11	-2.68
(3) <i>Piptadenia viridiflora</i> (Kunth) Benth.	Fabaceae	Evergreen	12	0.59	69	-3.2	34.7	141.86	139.11	1.08	-3.45
(4) <i>Libidibia ferrea</i> (Mar. ex Tul.) L.P. Queiroz	Fabaceae	Late deciduous	10	0.65	55	-3.3	29.35	55.03	65.22	0.86	-3.17
(5) <i>Ximenia americana</i> L.	Olcaceae	Late deciduous	10	0.58	74	-7	135.83	27.41	48.54	0.93	—
(6) <i>Anadenanthera colubrina</i> (Griseb.) Altschul	Fabaceae	Late deciduous	9	0.61	64	-7.3	94.11	64.23	83.41	0.8	-4.22
(7) <i>Combretum leprosum</i> Mart.	Combretaceae	Late deciduous	7	0.55	83	-4.5	35.06	126.07	175.4	0.72	-4.18
(8) <i>Piptadenia stipulacea</i> (Benth.) Ducke	Fabaceae	Late deciduous	7	0.57	76	-5.5	98.65	60.28	94.97	0.63	-2.17
(9) <i>Aspidosperma pyrifolium</i> Mart.	Apocynaceae	Late deciduous	7	0.65	55	-6.5	112.55	50.68	55.39	0.95	-2.52
(10) <i>Poincianella bracteosa</i> (Tul.) L.P. Queiroz	Fabaceae	Early deciduous	6	0.65	54	-5.4	70.74	30.94	51.79	0.61	—
(11) <i>Lafoensia pacari</i> St. Hil.	Lythraceae	Early deciduous	5	0.62	62	-7.1	37.89	156.73	209.97	0.77	-3.63
(12) <i>Amburana cearensis</i> (Allemão) A.C. Sm.	Fabaceae	Early deciduous	5	0.46	118	-1.2	57.53	94.14	113.3	0.83	-2.42
(13) <i>Bauhinia cheilantha</i> (Bong.) D. Dietr.	Fabaceae	Early deciduous	5	0.67	50	-8	90.01	60.78	96.82	0.61	—
(14) <i>Cordia oncocalyx</i> Allemão	Boraginaceae	Early deciduous	5	0.55	83	-7.5	60.1	23.7	57.74	0.41	-1.65
(15) <i>Helicteres heptandra</i> L.B. Sm.	Malvaceae	Early deciduous	4	0.57	75	-8.3	5.88	48.66	86.71	0.56	—
(16) <i>Commiphora leptophloeos</i> (Mart.) J.B. Gillett	Burseraceae	Early deciduous	4	0.30	236	-1.5	26.81	37.03	69.16	0.53	—
(17) <i>Sebastiania macrocarpa</i> Müll. Arg.	Euphorbiaceae	Early deciduous	4	0.62	61	-6.8	17.48	79.45	99.30	0.83	—
(18) <i>Cordia trichotoma</i> (Vell.) Arráb. ex Steud.	Boraginaceae	Early deciduous	4	0.55	82	-8	35.88	120.99	189.04	0.65	—
(19) <i>Croton blanchetianus</i> Baill.	Euphorbiaceae	Early deciduous	4	0.62	61	-8.3	46.84	13.86	25.82	0.46	-0.13
(20) <i>Mimosa caesalpiniiifolia</i> Benth.	Fabaceae	Early deciduous	4	0.65	54	-8.2	123.05	21.18	36.67	0.55	-1.49
(21) <i>Cochlospermum vitifolium</i> (Willd.) Spreng.	Bixaceae	Early deciduous	3	0.20	416	-1.2	5.81	43.14	47.68	0.52	-1.19
(22) <i>Manihot carthaginensis</i> (Müll. Arg.) Allemão	Euphorbiaceae	Early deciduous	3	0.34	195	-1.1	33.35	27.55	201.88	0.38	-1.40

<sup>1</sup>Data from Oliveira *et al.* (2015).

Afterwards, each segment was macerated and lyophilized. Then, a 100 mg sample of the lyophilized material was washed three times with 1.5 ml of 80% ethanol to remove noncovalently bound compounds, followed by one wash with deionized water (see Day *et al.*, 2005). Finally, the washed sample was centrifuged at 12 000 g force for 15 min, and the precipitate was oven-dried overnight at 65°C. The dried precipitate was used for the lignin content analysis with thioglycolic acid and for lignin extraction by thioacidolysis.

## Lignin measurements

**Total lignin content** To determine the total lignin content, we followed the procedure described by Barber & Ride (1988), in which the application of thioglycolic acid leads to the formation of benzyl-alcohol thioesters, chemical groups typically found in lignin (Hatfield & Fukushima, 2005). We determined the lignin concentration using a spectrophotometer (AJX-1900; Micronal,

São Paulo, Brazil). The extracted lignin had its absorbance read at 280 nm, and its concentration was determined relative to the alkali lignin standard 2-hydroxypropyl ether (Sigma-Aldrich). Lignin values were expressed in  $\mu\text{g mg}^{-1}$  DM of stem tissue.

**Lignin monomeric composition** The monomeric composition of lignin was evaluated by extraction of thioacidolysis. This method extracts the lignin by degrading it through the selective degradation of the  $\beta$ -O-4 bonds and allows the monomers involved in these bonds to be specifically quantified. As the  $\beta$ -O-4 bond is the predominant type of bond under normal conditions for a number of species (Xiao *et al.*, 2011; Sun *et al.*, 2012) it is good for use in analysis. The thioacidolysis was performed according to Lapierre *et al.* (1995), and the final product (trimethylsilyl derivatives from the monomers guaiacyl and syringyl) was analyzed by GC-MS. Trimethylsilyl derivatives from the monomers guaiacyl and syringyl were analyzed according to Müsel *et al.* (1997). We used a GC coupled with a mass

detector (Shimadzu GCMS-QP 2010, Tokyo, Japan) equipped with a Split (Shimadzu auto-injector AOC-2i, Tokyo, Japan) a, with an electron ionization source operating at an ionization energy of 70 eV. G and S lignin monomers, analyzed with their trimethylsilyl derivatives, were identified by comparing their retention times and mass fragmentation spectra with those obtained from standard compounds of coniferyl and sinapyl alcohols (Sigma-Aldrich). They were then compared with reference spectra previously described in the literature (Rolando *et al.*, 1992). To construct the calibration curves, we referred to the external calibration method and used commercial (Sigma-Aldrich) standards of lignin monomers.

### Xylem vulnerability to embolism

We estimated the water potential inducing a loss of 50% ( $\Psi_{50}$ ) of xylem hydraulic conductivity using the pneumatic method (Pereira *et al.*, 2016). The pneumatic apparatus consists of a vacuum gauge connected to a system of tubes and valves that allow vacuum tension to be applied to the base of a cut branch with a syringe. The vacuum tension is applied for 2.5 min, and part of the air contained inside the branch is extracted (air discharged, AD), reducing the tension inside the tubes. The ideal gas equation allows the calculation of AD. The minimum AD content is obtained from hydrated branches, and the maximum AD is from dehydrated branches. The difference between maximum and minimum AD allows the percentage of discharged air (PAD) to be calculated, which acts as a proxy for percentage loss of conductivity. The relationship between PAD and xylem water potential ( $\Psi_x$ ) was fitted to a logistic function (Pereira *et al.*, 2016). We estimated  $\Psi_x$  by measuring the water potential of leaves of eight branch segments from each of the three individuals collected per species. Before estimating  $\Psi_x$ , each collected branch with leaves was kept in a dark plastic bag for 1 h to allow the water potential to equilibrate between the stem and leaf xylem. Then, we measured the leaf water potential with a pressure chamber (Model 3005F01; Soilmoisture, Santa Barbara, CA, USA). Leaf cuts were sealed with a PVC-based glue. We could only measure hydraulic vulnerability curves in 15 of the 22 species selected, because of latex exudation in some species. Despite this, we were able to perform xylem vulnerability measurements in evergreen, early and late deciduous species.

### Data analysis

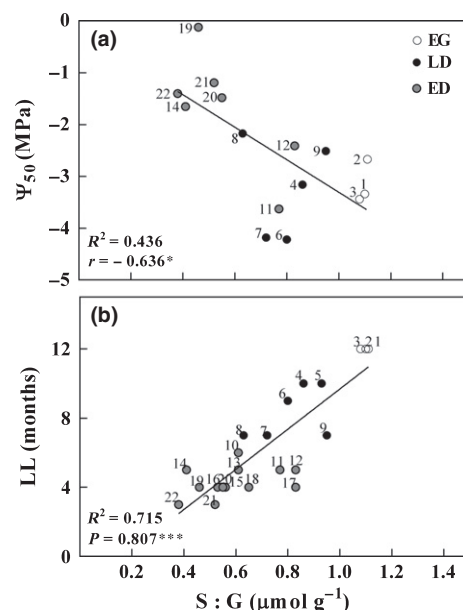
For all traits, we calculated mean species trait values. We used Student's *t*-test to test for differences between average values of G and S. To detect collinearity among variables, we performed pairwise linear correlation analyses of xylem vulnerability ( $\Psi_{50}$ ), lignin content (total lignin content), lignin composition (S, G, and S:G ratio), wood traits ( $QW_{\text{sat}}$ ,  $\Psi_{\text{dry season}}$ , and WD), and LL. We used Pearson correlation coefficients when variables were normally distributed (Shapiro–Wilk test), and Spearman's rank correlation coefficient when their distribution departed from normal. We performed a multiple regression analysis with noncollinear variables, which were considered the factor variables, to investigate the

influence of each variable on LL (the response variable). To eliminate collinear variables, we removed one variable of each pair with  $r \geq 0.7$  (Dormann *et al.*, 2013). Once the full model was selected, we applied a backward procedure to achieve the minimal model. We tested for normality of residuals with the Shapiro test and for homoscedasticity with the Fligner–Killeen test. We performed two sets of multiple regression analyses: one with all 22 species, and the other with the subset of 15 species for which data on xylem vulnerability ( $\Psi_{50}$ ) were available. All statistical analyses were performed in R (R Development Core Team, 2011) considering  $P < 0.05$  to indicate statistical significance.

Finally, we performed two principal components analyses (PCA) to investigate whether species with different LLs constituted distinct groups and to depict a whole picture of the relationships between species and all variables. One PCA was performed for all 22 species, and the other for the subset of 15 species with values of  $\Psi_{50}$ . In these analyses, we ordered the species as objects according to the noncollinear variables as descriptors, which were previously standardized through rank adjustment. All variables showed linear relationships among them (results not shown; see examples in Fig. 1). We transformed the cross-product matrix into a Pearson correlation matrix. The analyses were performed with PC-Ord 6.0 using the default settings and the biplot output.

## Results

Wood chemical traits varied significantly across the 22 co-occurring species (Table 1): lignin content of the branch apices varied 23-fold (5.81–135.83  $\mu\text{g mg}^{-1}$ ), G content varied eightfold (25.82–209.97  $\mu\text{g mg}^{-1}$ ), S content varied 11-fold (13.86–156.73  $\mu\text{g mg}^{-1}$ ), and S:G ratio varied threefold



**Fig. 1** Relationship between xylem vulnerability ( $\Psi_{50}$ ) (a) and leaf life span (LL) (b) and syringyl: guaiacyl ratio (S:G) of different tree species from a Brazilian seasonally dry forest. Leaf habit groups: EG, evergreen; LD, late deciduous; ED, early deciduous. Significance: \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ . Numbers refer to the species in Table 1.

(0.38–1.11  $\mu\text{g mg}^{-1}$ ). In general, the set of species had a higher amount of G than of S ( $t = -2.1542$   $P < 0.05$ ) (Table 1). Xylem vulnerability to embolism ( $\Psi_{50}$ ) varied from  $-1.05$  MPa in *Cochlospermum vitifolium*, an early deciduous species, to  $-4.94$  MPa in *Anadenanthera colubrina*, a late deciduous tree species (Table 1).

Total lignin content was not correlated with any variable (Table 2), indicating that total lignin content is not a good predictor of hydraulic functioning for semiarid tree species. G content was positively correlated solely with S content (Table 2), meaning that the content of one monomer tended to increase as the content of the other increased. S content was negatively correlated with  $\Psi_{50}$  (Table 2). The S : G ratio was positively correlated with LL and negatively with  $\Psi_{50}$  (Table 2; Fig. 1). This means that the higher the S content and the S : G ratio, the lower the  $\Psi_{50}$  and longer the LL (Figs 2, 3). All species with S : G  $> 0.83$  were late deciduous (7–10 months of LL) or evergreen (LL lasting 12 months), and all species with S : G  $< 0.63$  were early deciduous (LL from 3 to 6 months), whereas species with S : G between 0.63 and 0.83 could be early deciduous, late deciduous or evergreen. This suggests the existence of two thresholds, one (S : G  $> 0.83$ ) above which species retain their leaves for over 7 months  $\text{yr}^{-1}$ , the other (S : G  $< 0.63$ ) below which species remain leafy for up to 6 months. The relationships between S content, S : G,  $\Psi_{50}$  and LL were reinforced by the negative correlation of LL with  $\Psi_{50}$ , indicating that species with lower  $\Psi_{50}$  could retain their leaves for a longer time (Table 2; Fig. 3). Unexpectedly, WD was not related to lignin content or chemical composition, nor to  $\Psi_{50}$  (Table 2).

The minimum model yielded by the full multiple regression analysis (considering all 22 species) retained the S : G ratio as the only explanatory variable of LL:  $\text{LL} = -1.947 + 11.63 \text{ S : G}$ , with  $r^2 = 0.71$ ,  $P < 0.001$ ,  $F = 50.27$  (Table 3). S : G was also the only explanatory variable retained in the minimum model ( $\text{LL} = -1.574 + 11.52 \text{ S : G}$ , with  $r^2 = 0.76$ ,  $P < 0.001$ ,  $F = 42.57$ ; Table 3) when considering the subset of the 15 species with  $\Psi_{50}$  values. Both models showed LL to be directly related to S : G ratio.

In the PCA analysis of the 15-species subset, the first and second components, represented by axes 1 and 2, explained 44.0% and 30.2% of the total variance, respectively (Fig. 3). The first

axis eigenvalue (3.08) was significantly ( $P = 0.002$ ) greater than the mean randomized eigenvalue (2.14). Also, the second axis eigenvalue (2.12) was significantly ( $P = 0.002$ ) greater than the mean randomized eigenvalue (1.58). The overall disposition of the species over the ordination space described a large arch, characteristic of gradients (Wartenberg *et al.*, 1987), that is, the species did not constitute distinct groups. The first component (axis 1; Fig. 3) opposed S : G (score 0.89) to  $\Psi_{50}$  (score  $-0.89$ ), confirming the negative correlation between these two variables (Table 2). The species were ordinated from those with greater S : G (on the right) to those with more negative  $\Psi_{50}$  (at left), from the evergreen to the early deciduous species. The second PCA component (axis 2, Fig. 3) opposed WD (score 0.81) to  $\Psi_{\text{dry season}}$  (score  $-0.89$ ), confirming the negative correlation between these variables (Table 2). The species were ordinated from the greatest WD values (top) to the most negative  $\Psi_{\text{dry season}}$  (bottom), from the late deciduous species to the early deciduous ones. This confirmed the orthogonality between the two sets of correlated variables shown in Table 2: the S : G set and the WD set.

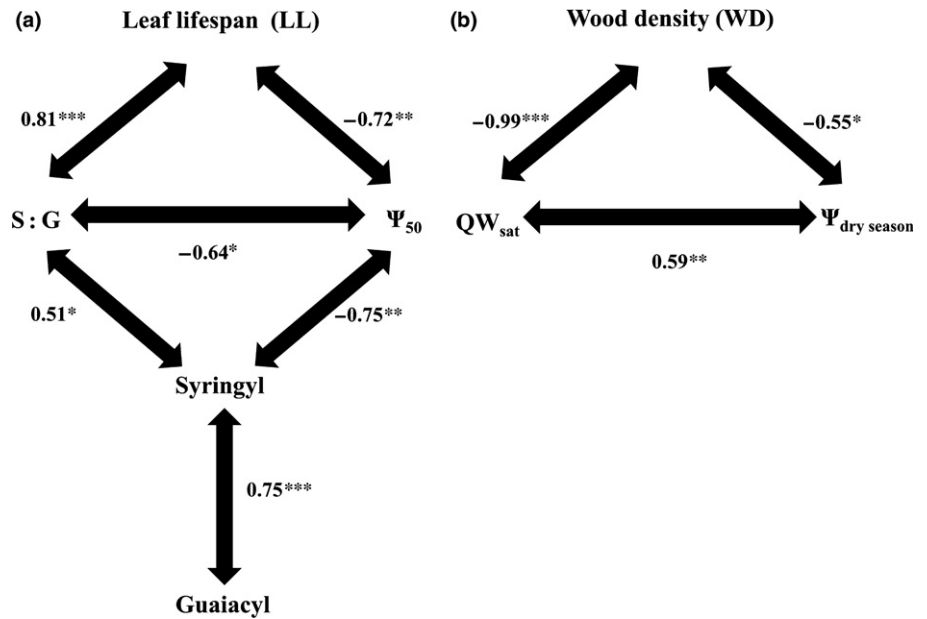
When considering all 22 species (Fig. 4), the first and second PCA components, represented by axes 1 and 2, explained 37.9% and 31.4% of the variance, respectively (Fig. 4). The first axis eigenvalue (2.28) was significantly higher ( $P = 0.009$ ) than the mean randomized eigenvalue (1.78). The second axis eigenvalue (1.89) was also significantly greater ( $P = 0.002$ ) than the mean randomized eigenvalue (1.37). As in Fig. 3, the overall disposition of the species described a great arch, indicating that early deciduous, late deciduous and evergreen species were arranged in a gradient-like continuum (Fig. 4). Species followed the same ordination set seen in Fig. 3: evergreen, late deciduous, and early deciduous species were arranged according to decreasing S : G values (score  $-0.91$ ). The first component (axis 1 in Fig. 4) ordinated the species from left to right mainly by the S : G diminution. As  $\Psi_{50}$  was not included in this analysis, and it was negatively correlated with S : G (Table 2; Fig. 3), the eigenvectors of the first PCA component did not show any opposition (Fig. 4). The second component (axis 2 in Fig. 4) opposed  $\Psi_{\text{dry season}}$  (score 0.81) to WD (score  $-0.68$ ), confirming both the negative correlation between these two variables and the orthogonality of WD with SG (Table 2).

**Table 2** Pairwise correlation coefficients among lignin content, syringyl (S), guaiacyl (G), S : G ratio, wood density (WD), stem water saturation ( $\text{QW}_{\text{sat}}$ ), stem water potential in the dry season ( $\Psi_{\text{dry season}}$ ), leaf life span (LL), and xylem vulnerability ( $\Psi_{50}$ )

	Lignin	G	S	S : G	WD <sup>1</sup>	$\text{QW}_{\text{sat}}^1$	$\Psi_{\text{dry season}}^1$	LL <sup>1</sup>
Lignin								
G	-0.342							
S	-0.289	<b>0.749***</b>						
S : G	0.203	0.04	<b>0.513*</b>					
WD <sup>1</sup>	0.367	-0.167	-0.011	0.219				
$\text{QW}_{\text{sat}}^1$	-0.344	0.16	0.024	-0.171	<b>-0.994***</b>			
$\Psi_{\text{dry season}}^1$	-0.299	0.131	0.158	0.22	<b>-0.553*</b>	<b>0.59**</b>		
LL <sup>1</sup>	0.408	-0.115	0.271	<b>0.807***</b>	0.247	-0.19	0.16	
$\Psi_{50}$	-0.071	-0.468	<b>-0.754**</b>	<b>-0.636*</b>	-0.205	0.159	0.013	<b>-0.723**</b>

<sup>1</sup>Data from Oliveira *et al.* (2015).

Significant correlations are highlighted in bold: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

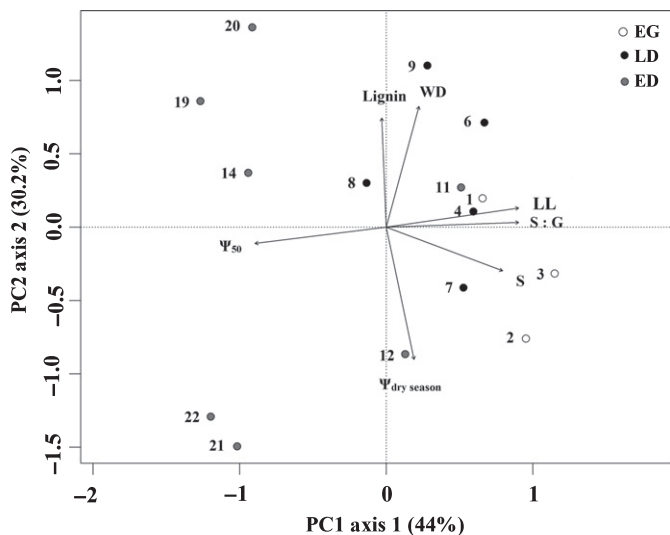


**Fig. 2** Lignin composition and wood density (WD) are the two axes of drought resistance in the correlation analyses. (a) Guaiacyl (G) and syringyl (S) contents are positively interrelated, the xylem vulnerability ( $\Psi_{50}$ ) diminishes as the S content increases, and a higher S : G ratio increases leaf life span (LL). (b) As WD decreases, the stem water saturation ( $QW_{sat}$ ) increases, which allows the stem to have greater water potential in the dry season ( $\Psi_{dry\ season}$ ). Significance: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

Both PCAs (Figs 3, 4) are consistent in their results and are congruent with the results obtained by the correlations (Table 2). Although the PCA results indicate the many variables that could be linked to drought resistance, the driving variable in leaf retention time was S : G.

### Discussion

Our results show the existence of two independent functional axes associated with drought resistance in woody species of semi-arid climate as indicated by two independent sets of correlations



**Fig. 3** Principal components analysis (PCA) biplot diagram ordering the subset of 15 species according to their xylem vulnerability ( $\Psi_{50}$ ), total lignin content (lignin), syringyl content (S), syringyl to guaiacyl ratio (S : G), wood density (WD), stem water potential in the dry season ( $\Psi_{dry\ season}$ ), and leaf life span (LL). Numbers correspond to the species in Table 1. Leaf habit groups: EG, evergreen; LD, late deciduous; ED, early deciduous.

among our variables. One axis is driven by WD, which is negatively related to both the capacity of the stem to store water ( $QW_{sat}$ ) and to maintain negative water potential during the dry season ( $\Psi_{dry\ season}$ ), as already demonstrated in Oliveira *et al.* (2015). The other axis is driven by the S : G ratio, an increase of which increases LL owing to lower xylem embolism vulnerability ( $\Psi_{50}$ ). Neither of these axes is associated with total lignin content. Although these two axes were revealed to be independent of one another, the species present a continuum of LL driven by S : G. Variation of the lignin monomeric composition (S : G) alone explained 65% (in the case of simple correlation) to 76% (in the case of multiple regression including  $\Psi_{50}$ ) of LL. Together, these results indicate that, although many variables can influence LL, the most important explanatory variable is S : G. Therefore, our first and second predictions, which proposed that the lignin total content and monomeric composition are related to LL and influence xylem embolism resistance, were partially confirmed; whereas our third prediction – stating that both the total lignin content and its monomeric composition are related to the wood traits that control LL – was completely rejected.

### Covariation among wood traits

Wood density was negatively correlated with dry season stem water potential and stem water saturation (Oliveira *et al.*, 2015) but not with  $\Psi_{50}$ , although many authors have associated WD with xylem hydraulic properties (Hacke *et al.*, 2001; Preston *et al.*, 2006; Jacobsen *et al.*, 2007; Chave *et al.*, 2009; Markesteijn *et al.*, 2011). In agreement with our findings, Awad *et al.* (2012) demonstrated, in transgenic lineages of poplars with modified composition of lignin, that there was no relationship between xylem's vulnerability to embolism and the mechanical properties of wood. Our results also agree with Preston *et al.* (2006), who concluded that WD and conduit traits describe two distinct plant functional axes.

Wood density alone is not a good indicator of lignin content, xylem vulnerability or LL for our studied species. WD expresses the total mass within a given volume of wood (Borchert, 1994). In fact, Gartner *et al.* (2004) found a linear positive correlation between WD and the proportion of cell wall per cross-sectional area. As lignin is deposited in cell walls and renders them thicker, a positive relationship between total lignin content and WD would thus be expected (Pereira *et al.*, 2017). However, our results did not show any relationship between WD and total lignin content, but rather a strong relationship between WD and stem water storage capacity in the dry season. Thus, it seems that, beyond lignin, a complex set of other characteristics, such as the relative proportions of cellulose, hemicellulose, extraneous components, gas and water content, are responsible for determining WD (Williamson & Wiemann, 2010), and its association with xylem vulnerability and LL may be indirect.

Increasing WD results in a decrease of  $QW_{\text{sat}}$  and  $\Psi_{\text{dry season}}$  (which became more negative) (Oliveira *et al.*, 2015). Low WD enables species in dry habitats to store water in their stem and to flush new leaves at the onset of rain (Borchert *et al.*, 2002; Wolfe & Kursar, 2015; Wolfe *et al.*, 2016). Indeed, Oliveira *et al.* (2015) observed that the species with the lowest WD were the first to shed leaves in the dry season, that is, they become dormant at the first signs of drought, generally 1 month before the end of the rains. However, Oliveira *et al.* (2015) also found that low-WD species can initiate bud-break before the end of the dry season probably as a result of high water storage. Bud-break requires turgor pressure for leaf expansion, which is not possible during the dry season unless water with a high hydric potential is stored in the stem. Storing water in the stem may be possible if plants decouple from the atmosphere by shedding their leaves before the soil dries too much, that is, early in the dry season. In fact, Oliveira *et al.* (2015) found a negative relationship between  $\Psi_{\text{dry season}}$  and leaf shedding. This combination of early bud-break with early leaf shedding for low-WD species would decouple WD from LL while coupling WD to timing of phenophases. This suggests that WD,  $QW_{\text{sat}}$  and  $\Psi_{\text{dry season}}$  constitute a water-driven leaf habit axis.

### Wood chemical traits driving leaf retention time

We found that LL and  $\Psi_{50}$  were not correlated with total lignin content. Indeed, we found that total lignin content was not correlated with any variable in this study. Apparently, our results contradict Fan *et al.* (2006), Coleman *et al.* (2008), Voelker *et al.* (2010, 2011), and Awad *et al.* (2012), who showed that smaller lignin content implies greater xylem vulnerability, a result recently confirmed by Pereira *et al.* (2017). However, Pereira *et al.* (2017) showed that, although smaller lignin content is related to greater xylem vulnerability, the opposite is not seen, that is, greater lignin content is not associated with higher embolism resistance. Moreover, Pereira *et al.* (2017) did not find any significant correlation between lignin content and xylem vulnerability for angiosperms, although low lignin content was always related to embolism vulnerability. It is not likely that a drought treatment could induce changes in the lignin content as an adaptive mechanism to tolerate

drought (Wildhagen *et al.*, 2017), albeit the lignin synthesis is determined by the expression of certain genes and can induce drought tolerance in transgenic plants (Xu *et al.*, 2017). Despite the low lignin content being related to embolism vulnerability, other functional effects of natural variation in lignin content are not known (Pereira *et al.*, 2017).

Our results showed that LL and  $\Psi_{50}$  were highly responsive to variation of the lignin monomeric composition. We showed that S : G determines the length of time trees can maintain leaves in their canopies during the dry season. Species with longer LL, such as the late deciduous and evergreen species that we studied, had more negative  $\Psi_{50}$ , corroborating the results of Choat *et al.* (2005) and Markesteijn & Poorter (2009), who demonstrated that embolism resistance is a key plant trait promoting drought resistance. The highly significant correlation between LL and  $\Psi_{50}$  that we found corroborates the results of Pereira *et al.* (2017), who hypothesized that lignin composition may explain the variation in embolism resistance across species.

### Possible mechanisms linking lignin and hydraulic traits

The total lignin content of Caatinga tree species did not show correlation with its monomeric composition, corroborating the result obtained by Wallis *et al.* (1996) for *Eucalyptus globulus*. Indeed, the relationship between the total lignin content and its monomeric composition is still not clear (e.g. Bose *et al.*, 2009). For instance, Lapiere *et al.* (1999) found that alterations in the expression of enzymes involved in the synthesis of the lignin monomers did not alter the total lignin content because the frequency of the  $\beta$ -O-4 bounds, the most frequent bound between the monolignols composing the lignin molecule (Baucher *et al.*, 1998), was not affected. Our results indicate that the proportions of monomers constituting lignin molecules are an important regulator of  $\Psi_{50}$  and LL in trees of semiarid climates and that there is no relationship between total lignin content and LL, wood traits (WD,  $QW_{\text{sat}}$ ,  $\Psi_{\text{dry season}}$ ) or xylem traits ( $\Psi_{50}$ , S, G, S : G).

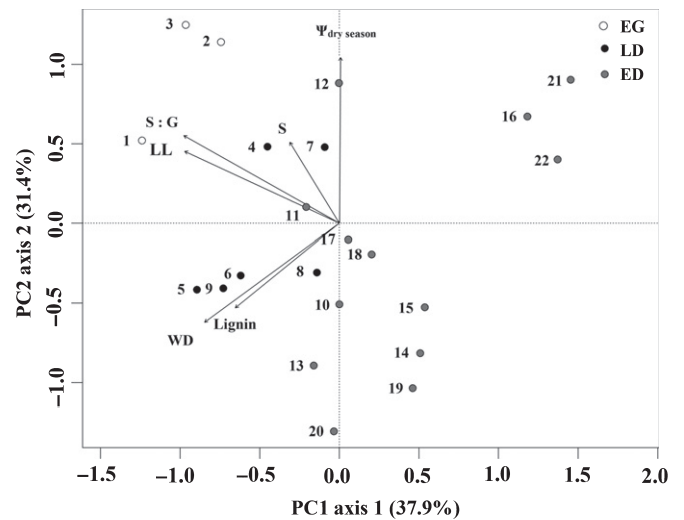
However, we found a strong correlation between LL,  $\Psi_{50}$  and S : G. This correlation implies that lignin composition, rather than content, explains the continuum of variation of plant embolism resistance. Although S and G contents were highly positively correlated, increasing S alone implied less xylem vulnerability. The fact that S content alone was correlated with less vulnerability, but not with LL, suggests that it is the combination of S and G that can confer longer LL.

How exactly the increase of S in relation to G can improve embolism resistance and extend LL is not yet fully understood. S is mostly deposited in fiber walls (Voelker *et al.*, 2011; Saito *et al.*, 2012), although it can also be found in secondary walls of ray parenchyma (Fergus & Goring, 1970). G is deposited in larger quantities in the conduit walls (Coleman *et al.*, 2008; Saito *et al.*, 2012). G-rich lignin is more rigid and hydrophobic than S-rich lignin (Koehler & Telewski, 2006; Bonawitz & Chapple, 2010), thus conferring rigidity to the conduit walls and allowing the conduit to withstand the tension of sap ascent (Higuchi, 1990). The conduit inner wall can be thought of as a scattering of hydrophobic and hydrophilic patches, which is hydrophilic

**Table 3** Multiple regression models considering leaf life span as the response variable, and the noncollinear factor variables – stem water saturation ( $QW_{sat}$ ), stem water potential in the dry season ( $\Psi_{dry\ season}$ ), total lignin content (lignin), syringyl to guaiacyl ratio (S : G) – for all 22 species and for a subset of 15 species for which values of xylem vulnerability ( $\Psi_{50}$ ) were available (the number of factor variables in each model is indicated by  $n$ )

	$n$	$r^2$	$F$	AICc	$P$ -value
<b>Variables – 22 species</b>					
S : G	1	0.715	50.267	89.95	<0.001
Lignin + S : G	2	0.733	26.059	91.576	<0.001
S : G + $QW_{sat}$	2	0.722	24.616	92.488	<0.001
S : G + $\Psi_{dry\ season}$	2	0.717	24.045	92.859	<0.001
S : G + $QW_{sat}$ + $\Psi_{dry\ season}$	3	0.742	17.239	94.221	<0.001
Lignin + S : G + $\Psi_{dry\ season}$	3	0.741	17.19	94.268	<0.001
Lignin + S : G + $QW_{sat}$	3	0.734	16.567	94.867	<0.001
Lignin + S : G + $QW_{sat}$ + $\Psi_{dry\ season}$	4	0.761	13.549	96.353	<0.001
$QW_{sat}$ + $\Psi_{dry\ season}$	2	0.431	7.19	108.217	0.005
Lignin + $QW_{sat}$ + $\Psi_{dry\ season}$	3	0.494	5.863	109.015	0.006
$QW_{sat}$	1	0.133	3.076	114.447	0.095
Lignin	1	0.115	2.608	114.899	0.122
Lignin + $\Psi_{dry\ season}$	2	0.22	2.678	115.151	0.095
Lignin + $QW_{sat}$	2	0.179	2.066	116.285	0.154
$\Psi_{dry\ season}$	1	0.044	0.926	116.599	0.347
<b>Variables – 15 species</b>					
S : G	1	0.766	42.57	63.498	<0.001
S : G + $QW_{sat}$	2	0.783	21.65	66.184	<0.001
S : G + $\Psi_{50}$	2	0.773	20.383	66.893	<0.001
S : G + $\Psi_{dry\ season}$	2	0.768	19.871	67.187	<0.001
Lignin + S : G	2	0.766	19.649	67.316	<0.001
S : G + $QW_{sat}$ + $\Psi_{dry\ season}$	3	0.788	13.667	70.473	<0.001
Lignin + S : G + $QW_{sat}$	3	0.787	13.511	70.609	<0.001
S : G + $QW_{sat}$ + $\Psi_{50}$	3	0.786	13.495	70.623	<0.001
S : G + $\Psi_{dry\ season}$ + $\Psi_{50}$	3	0.774	12.537	71.485	<0.001
Lignin + S : G + $\Psi_{50}$	3	0.773	12.458	71.558	<0.001
Lignin + S : G + $\Psi_{dry\ season}$	3	0.769	12.18	71.818	<0.001
S : G + $QW_{sat}$ + $\Psi_{dry\ season}$ + $\Psi_{50}$	4	0.792	9.51	76.066	0.002
Lignin + S : G + $QW_{sat}$ + $\Psi_{dry\ season}$	4	0.79	9.4	76.206	0.002
Lignin + S : G + $QW_{sat}$ + $\Psi_{50}$	4	0.789	9.352	76.264	0.002
Lignin + S : G + $\Psi_{dry\ season}$ + $\Psi_{50}$	4	0.774	8.558	77.350	0.003
$\Psi_{50}$	1	0.41	9.033	77.375	0.01
$QW_{sat}$ + $\Psi_{dry\ season}$	2	0.468	5.287	79.629	0.023
$QW_{sat}$ + $\Psi_{50}$	2	0.455	5.018	79.991	0.026
$QW_{sat}$ + $\Psi_{dry\ season}$ + $\Psi_{50}$	3	0.589	5.248	80.448	0.017
$\Psi_{dry\ season}$ + $\Psi_{50}$	2	0.432	4.561	80.626	0.034
Lignin + $\Psi_{50}$	2	0.419	4.323	80.967	0.039
$QW_{sat}$	1	0.188	3.012	82.163	0.106
Lignin + S : G + $QW_{sat}$ + $\Psi_{dry\ season}$ + $\Psi_{50}$	5	0.793	6.885	83.499	0.007
Lignin + $QW_{sat}$ + $\Psi_{dry\ season}$	3	0.474	3.309	84.126	0.061
Lignin + $\Psi_{dry\ season}$ + $\Psi_{50}$	3	0.463	3.166	84.436	0.068
Lignin + $QW_{sat}$ + $\Psi_{50}$	3	0.455	3.067	84.657	0.073
$\Psi_{dry\ season}$	1	0.039	0.53	84.689	0.479
Lignin	1	0.023	0.302	84.944	0.592
Lignin + $QW_{sat}$	2	0.191	1.415	85.93	0.281
Lignin + $QW_{sat}$ + $\Psi_{dry\ season}$ + $\Psi_{50}$	4	0.595	3.673	86.048	0.043
Lignin + $\Psi_{dry\ season}$	2	0.108	0.724	87.397	0.505

AICc, corrected Akaike information criterion.



**Fig. 4** Principal components analysis (PCA) biplot diagram ordering all 22 species according to their total lignin content (lignin), syringyl content (S), syringyl to guaiacyl ratio (S : G), wood density (WD), stem water potential in the dry season ( $\Psi_{dry\ season}$ ), and leaf life span (LL). Numbers correspond to the species in Table 1. Leaf habit groups: EG, evergreen; LD, late deciduous; ED, early deciduous.

when the conduit is full or being refilled and becoming hydrophobic when the conduit is embolized (McCully *et al.*, 2014). S presents a free phenolic hydroxyl group, as well as G. The difference is that the G has one methoxy group in the ring-3 position and the S unit has two methoxy groups in the ring-3,5 positions. In the polymerization, the position of ring 5 is reactive at G but inactive at S. These characteristics make the S-rich lignin more hydrophilic and with a highly flexible, less crosslinked structure, possibly resulting in a greater polymer flexibility (Pereira *et al.*, 2017). A more hydrophilic S-rich lignin, if present in the pit membrane, may increase the pressure needed for air-seeding to occur as it increases the contact angle in the pore meniscus (Pereira *et al.*, 2017). However, the conduit cell wall is not the only place of lignin deposition in the xylem.

Fergus & Goring (1970), Coleman *et al.* (2008) and Zheng *et al.* (2016) have detected the presence of S-rich lignin in the secondary wall of ray parenchyma cells. In seasonal climates, among other functions, the radial parenchyma stores resources and distributes them at the onset of the growing season (Larish *et al.*, 2012). Besides the radial parenchyma, axial parenchyma may also be present, and ‘the living parenchyma cells contribute lignin precursors to vessel elements after their death. However, the mechanism by which the monolignols are transported from living parenchyma cells to the secondary walls of dead vessel elements is unknown’ (Aloni, 2013). Lignification seems to be independent of cell type and cell wall sublayer, the *p*-hydroxyphenyl monolignol being deposited first, followed by G, and finally S, which is the last monolignol to be deposited in the cell wall (Terashima & Fukushima, 1989; Rencoret *et al.*, 2011). Lignin composition differs between the axial and radial systems, where the S : G ratio is higher in the radial parenchyma than in the axial fibers of the wood (Zheng *et al.*, 2016). The S : G ratio increases in sapwood towards heartwood in the radial parenchyma, while



in the wood axial fibers the lignin composition does not differ between the sapwood and the heartwood (Zheng *et al.*, 2016). Indeed, xylem ray and axial parenchyma and/or phloem have a vital role in the regulation and maintenance of xylem water conductance (Nardini *et al.*, 2011), but how exactly they work together deserves further research.

Therefore, xylem water transport not only depends on the conduit's characteristics, but it is also the outcome of synergistic, intricate processes performed by different structures at different organizational levels. For instance, a highly impermeable leaf cuticle, such as that of *Ziziphus joazeiro* (Oliveira *et al.*, 2003), or the ability to absorb water from dew, such that of *Combretum leprosum* (Pina *et al.*, 2016), can contribute to a longer LL. Also, tree height, mass and growth rate, conduit tapering, vessel packing and the ratio of sapwood to heartwood influence xylem transport, and variation in one or more traits is compensated by variation in other traits (Sperry *et al.*, 2012). Regarding our results, the fact that S content alone was correlated with less vulnerability, but not with LL, and the finding of a lower and an upper S : G threshold for LL and of two different axes related to drought resistance all advocate for the high complexity underpinning xylem transport. In spite of this complexity, S : G variation alone could explain most of the variation of LL through diminishing xylem embolism vulnerability, thus inferring that lignin chemical composition has a more important functional role in embolism resistance than previously thought.

## Conclusion

Studies of lignin chemistry variation and its relation to hydraulic traits and plant performance in ecological systems are virtually absent in the scientific literature. Our study provides a novel perspective on the interplay between wood chemical traits and plant hydraulic function. We found that lignin monomeric composition rather than total lignin content drives dry-season LL and resistance to xylem embolism among tree species in a semiarid environment. On the other hand, neither total lignin content nor its monomeric composition were related to wood density and dry-season stem water saturation and stem water potential.

Our results clearly show the existence of two axes of trait variation. One axis allows trees to keep their leaves for a longer time in the dry season by investing in the composition of lignin, which continuously varies among species. In this group, the increase in the S : G ratio results in lower xylem vulnerability and longer foliar retention in the dry season. The other axis allows the leafless tree to withstand drought by storing water in the stem and shedding leaves before the end of the rainy season, so as to keep higher stem water potential during the dry season and flush new leaves very early, still at end of the dry season. How these two axes are related to one another and how the mechanism relating S : G ratio to xylem embolism vulnerability works deserve further investigation.

## Acknowledgements



We thank the Cearense Foundation of Support for Scientific and Technological Development of the State of Ceará (FUNCAP)

for the grant to T.R.A.L. and for financial support (grant no. PPS-0088-00023.01.00/13); and the National Council for Scientific and Technological Development (CNPq) for the financial support granted to T.R.A.L. during internship at the University of Campinas and for the scholarship granted to F.R.M. We thank C. C. Oliveira for help in the field, and the staff of the Curu Valley Experimental Farm for local support. We thank Peter Stoltenberg Groenendyk for critical reading of the manuscript and suggestions on the final draft, and three anonymous reviewers for their insights and thoughtful comments which helped us improve our manuscript.

## Author contributions

F.R.M. led the study design with significant contributions from F.S.A., J.C.M.S.M.S., R.S.O. and T.R.A.L.; T.R.A.L., R.S.M., E.C.D.C., C.S.M., E.G.-F. and J.C.M.S.M.S. performed the experiments; T.R.A.L., E.C.D.C., R.S.O., L.P., P.R.L.B., J.C.M.S.M.S., R.C.C. and F.S.A. analyzed the data, under the supervision of F.R.M. All authors contributed to the writing of the manuscript.

## ORCID

Rafael S. Oliveira  <http://orcid.org/0000-0002-6392-2526>  
Rafael S. Miranda  <http://orcid.org/0000-0002-9087-6190>

## References

- Aloni R. 2013. Role of hormones in controlling vascular differentiation and the mechanism of lateral root initiation. *Planta* **238**: 819–830.
- Awad H, Herbette S, Brunel N, Tixier A, Pilate G, Cochar H, Badel E. 2012. No trade-off between hydraulic and mechanical properties in several transgenic poplars modified for lignins metabolism. *Environmental and Experimental Botany* **77**: 185–195.
- Barber MS, Ride JP. 1988. A quantitative assay for induced lignification in wounded wheat and its use to survey potential elicitors of the response. *Physiological and Molecular Plant Pathology* **32**: 185–197.
- Baucher M, Monties B, Montagu MV, Boerjan W. 1998. Biosynthesis and genetic engineering of lignin. *Critical Reviews in Plant Sciences* **17**: 125–197.
- Boerjan W, Ralph J, Baucher M. 2003. Lignin biosynthesis. *Annual Review Plant Biology* **54**: 519–546.
- Bonawitz ND, Chapple C. 2010. The genetics of lignin biosynthesis: connecting genotype to phenotype. *Annual Review of Genetics* **44**: 337–363.
- Borchert R. 1994. Soil and stem water storage determine phenology and distribution of tropical dry forest trees. *Ecology* **75**: 1437–1449.
- Borchert R, Rivera G, Hagnauer W. 2002. Modification of vegetative phenology in a tropical semi-deciduous forest by abnormal drought and rain. *Biotropica* **34**: 27–39.
- Bose SK, Francis RC, Govender M, Bush T, Spark A. 2009. Lignin content versus syringyl to guaiacyl ratio amongst poplars. *Bioresource Technology* **100**: 1628–1633.
- Boyce CK, Zwieniecki MA, Cody GD, Jacobsen C, Wirrick S, Knoll AH, Holbrook NM. 2004. Evolution of xylem lignification and hydrogel transport regulation. *Proceedings of the National Academy of Sciences, USA* **101**: 17555–17558.
- Campbell MM, Sederoff RR. 1996. Variation in lignin content and composition (mechanisms of control and implications for the genetic improvement of plants). *Plant Physiology* **110**: 3–13.
- Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* **12**: 351–366.

- Choat B, Ball MC, Luly JG, Holtum JAM. 2005. Hydraulic architecture of deciduous and evergreen dry rainforest tree species from north-eastern Australia. *Trees* 19: 305–311.
- Coleman HD, Samuels AL, Guy RD, Mansfield SD. 2008. Perturbed lignification impacts tree growth in hybrid poplar – a function of sink strength, vascular integrity and photosynthetic assimilation. *Plant Physiology* 148: 1229–1237.
- Day A, Ruel K, Neutelings G, Crônier D, David H, Hawkins S, Chabbert B. 2005. Lignification in the flax stem: evidence for an unusual lignin in bast fibers. *Planta* 222: 234–245.
- Dormann CF, Elith J, Bacher S, Buchmann C, Carl G, Carré G, Marquéz JRG, Gruber B, Lafourcade B, Leitão PJ *et al.* 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography* 36: 027–046.
- Engelbrecht BMJ, Kursar TA. 2003. Comparative drought-resistance of seedlings of 28 species of co-occurring tropical woody plants. *Oecologia* 136: 383–393.
- Espiñeira JM, Novo Uzal E, Gómez Ros LV, Carrión JS, Merino F, Ros Barceló A, Pomar F. 2011. Distribution of lignin monomers and the evolution of lignification among lower plants: evolution of lignification among lower plants. *Plant Biology* 13: 59–68.
- Fan L, Linker R, Gepstein S, Tanimoto E, Yamamoto R, Neumann PM. 2006. Progressive inhibition by water deficit of cell wall extensibility and growth along the elongation zone of maize roots is related to increased lignin metabolism and progressive stellar accumulation of wall phenolics. *Plant Physiology* 140: 603–612.
- Fergus BJ, Goring DAI. 1970. The location of guaiacyl and syringyl lignins in birch xylem tissue. *Holzforschung* 24: 113–117.
- Fournier LA. 1974. Um método quantitativo para lamedición de características fenológicas em árvores. *Turrialba* 24: 422–423.
- Fromm J, Rockel B, Lautner S, Windeisen E, Wanner G. 2003. Lignin distribution in wood cell walls determined by TEM and backscattered SEM techniques. *Journal of Structural Biology* 143: 77–84.
- Gartner BL, Moore JR, Gardiner BA. 2004. Gas in stems: abundance and potential consequences for tree biomechanics. *Tree Physiology* 24: 1239–1250.
- Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh K. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* 126: 457–461.
- Hacke UG, Sperry JS, Wheeler JK, Castro L. 2006. Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiology* 26: 689–701.
- Hatfield R, Fukushima RS. 2005. Can lignin be accurately measured? *Crop Science* 45: 832–839.
- Higuchi T. 1990. Lignin biochemistry: biosynthesis and biodegradation. *Wood Science and Technology* 24: 23–63.
- Jacobsen AM, Agenbag L, Esler KJ, Pratt RB, Ewers FW, Davis SD. 2007. Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. *Journal of Ecology* 95: 171–183.
- Jansen S, Choat B, Pletsers A. 2009. Morphological variation of intervessel pit membranes and implications to xylem function in angiosperms. *American Journal of Botany* 96: 409–419.
- Koehler L, Telewski FW. 2006. Biomechanics and transgenic wood. *American Journal of Botany* 93: 1433–1438.
- Lapierre C, Pollet B, Petit-Conil M, Toval G, Romero J, Pilate G, Leple JC, Boerjan W, Ferret V, Nadai V *et al.* 1999. Structural alterations of lignins in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acid O-methyltransferase activity have an opposite impact on the efficiency of industrial kraft pulping. *Plant Physiology* 119: 153–163.
- Lapierre C, Pollet B, Rolando C. 1995. New insights into the molecular architecture of hardwood lignins by chemical degradative methods. *Research on Chemical Intermediates* 21: 397–412.
- Larish C, Dittrich M, Wildhagen H, Lautner S, Fromm J, Polle A, Herich R, Rennenberg H, Müller T, Ache P. 2012. Poplar wood rays are involved in seasonal remodeling of tree physiology. *Plant Physiology* 160: 1515–1529.
- Markestijn L, Poorter L. 2009. Seedling root morphology and biomass allocation of 62 tropical tree species in relation to drought- and shade-tolerance. *Journal of Ecology* 97: 311–325.
- Markestijn L, Poorter L, Paz H, Sack L, Bongers F. 2011. Ecological differentiation in xylem cavitation resistance is associated with stem and leaf structural traits. *Plant, Cell & Environment* 34: 137–148.
- McCully M, Canny M, Baker A, Miller C. 2014. Some properties of the walls of metaxylem vessels of maize roots, including tests of the wettability of their luminal wall surfaces. *Annals of Botany* 113: 977–989.
- MINTER/SUDENE Ministério do Interior/Superintendência de Desenvolvimento do Nordeste. 1984. *Plano econômico-social de aproveitamento dos pequenos depósitos minerais do Nordeste Oriental*. Recife, PE, Brazil: SUDENE/CPRM.
- Moro MF, Lughadha EM, Araújo FS, Martins FR. 2016. A phytogeographical meta-analysis of the semiarid Caatinga domain in Brazil. *Botanical Review* 82: 91–148.
- Moro MF, Lughadha EM, Filer DL, Araújo FS, Martins FR. 2014. A catalogue of the vascular plants of the Caatinga phytogeographical domain: a synthesis of floristic and phytosociological surveys. *Phytotaxa* 160: 1–118.
- Morreel K, Kim H, Lu F, Dima O, Akiyama T, Vanholme R, Niculae C, Goeminne G, Inzé D, Messens E *et al.* 2010. Mass spectrometry-based fragmentation as an identification tool in lignomics. *Analytical Chemistry* 82: 8095–8105.
- Müsel G, Schindler T, Bergfeld R, Ruel K, Jacquet G, Lapierre C, Speth V, Schopfer P. 1997. Structure and distribution of lignin in primary and secondary cell walls of maize coleoptiles analyzed by chemical and immunological probes. *Planta* 201: 146–159.
- Nardini A, Salleo S, Jansen S. 2011. More than just a vulnerable pipeline: xylem physiology in the light of ion-mediated regulation of plant water transport. *Journal of Experimental Botany* 62: 4701–4718.
- Niklas K. 1992. *Plant biomechanics: an engineering approach to plant form and function*. Chicago, IL, USA: University of Chicago Press.
- Nimer E. 1989. *Climatologia do Brasil*. Rio de Janeiro, RJ, Brazil: Instituto Brasileiro de Geografia e Estatística, 419.
- Oliveira AFM, Meirelles ST, Salatino A. 2003. Epicuticular waxes from caatinga and cerrado species and their efficiency against water loss. *Anais da Academia Brasileira de Ciência* 75: 431–439.
- Oliveira CC, Zandavalli RB, Lima ALA, Rodal MJN. 2015. Functional groups of woody species in semi-arid regions at low latitudes. *Austral Ecology* 40: 40–49.
- Peel MC, Finlayson BL, McMahon TA. 2007. Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences* 11: 1633–1644.
- Pereira L, Bittencourt PRL, Oliveira RS, Junior MBM, Barros FV, Ribeiro RV, Mazzafera P. 2016. Plant pneumatics: stem air flow is related to embolism – new perspectives on methods in plant hydraulics. *New Phytologist* 211: 357–370.
- Pereira L, Domingues-Junior AP, Jansen S, Choat B, Mazzafera P. 2017. Is embolism resistance in plant xylem associated with quantity and characteristics of lignin? *Trees* 32: 349–358.
- Pilate G, Dejardin A, Leple JC. 2012. Field trials with lignin-modified transgenic trees. In: Jouanin L, Lapierre C, eds. *Lignins: biosynthesis, biodegradation and bioengineering*. Waltham, MA, USA: Academic Press, 1–36.
- Pina ALCB, Zandavalli RB, Oliveira RS, Martins FR, Soares AA. 2016. Dew absorption by the leaf trichomes of *Combretum leprosum* in the Brazilian semiarid region. *Functional Plant Biology* 43: 851–861.
- Poorter L, McDonald I, Alarcón A, Fichtler E, Licona JC, Peña-Claros M, Sterck F, Villegas Z, Sass-Klaassen U. 2010. The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytologist* 185: 481–492.
- Preston KA, Cornwell WK, DeNoyer JL. 2006. Wood density and vessel traits as distinct correlates of ecological strategy in 51 California coast range angiosperms. *New Phytologist* 170: 807–818.
- R Core Team. 2011. *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rencoret J, Gutiérrez A, Nieto L, Jiménez-Barbero J, Faulds CB, Kim H, Ralph J, Martínez AT, del Río JC. 2011. Lignin composition and structure in young versus adult *Eucalyptus globulus* plants. *Plant Physiology* 155: 667–682.
- Rodal MJN, Sampaio EVSB, Figueiredo MA. 1992. *Manual sobre métodos de estudo florístico e fitossociológico – ecossistema caatinga*, 1<sup>st</sup> edn. Brasília.

- Pernambuco, PE, Brazil: Sociedade Botânica do Brasil/Seção Regional de Pernambuco.
- Rolando C, Monties B, Lapierre C. 1992. Thioacidolysis. In: Lin SY, Dence CW, eds. *Methods in lignin chemistry*. Berlin, Germany: Springer-Verlag, 334–339.
- Saito K, Watanabe Y, Shirakawa M, Matsushita Y, Imai T, Koike T, Sano Y, Funada R, Fukazawa K, Fukushima K. 2012. Direct mapping of morphological distribution of syringyl and guaiacyl lignin in the xylem of maple by time-of-flight secondary ion mass spectrometry. *Plant Journal* 69: 542–552.
- Sobrado MA. 1997. Embolism vulnerability in drought-deciduous and evergreen species of a tropical dry forest. *Acta Oecologica* 18: 383–391.
- Souza BC, Oliveira RS, Araújo FS, Lima ALA, Rodal MJN. 2015. Divergências funcionais e estratégias de resistência à seca entre espécies decíduas e sempre verdes tropicais. *Rodriguésia* 66: 021–032.
- Sperry JS, Smith DD, Savage VM, Enquist BJ, McCulloh KA, Reich PB, Bentley LP, von Allmen EI. 2012. A species-level model for metabolic scaling in trees I. Exploring boundaries to scaling space within and across species. *Functional Ecology* 26: 1054–1065.
- Stephen SK, Timothy GR, Wolfgang GG. 1987. Relaxation behaviour of the amorphous components of wood. *Journal of Materials Science* 22: 617–624.
- Sun SN, Li MF, Yuan TQ, Xu F, Sun RC. 2012. Sequential extractions and structural characterization of lignin with ethanol and alkali from bamboo (*Neosinocalamus affinis*). *Industrial Crops and Products* 37: 51–60.
- Terashima N, Fukushima K. 1989. Biogenesis and structure of macromolecular lignin in the cell wall of tree xylem as studied by microautoradiography. In: Lewis NG, Paice MG, eds. *Plant cell wall polymers, biogenesis and biodegradation*. Washington DC, USA: American Chemical Society Symposium Series 399, 160–168.
- Tyree MT, Engelbrecht BMJ, Vargas G, Kursar TA. 2003. Desiccation tolerance of five tropical seedlings in Panama: relationship to a field assessment of drought performance. *Plant Physiology* 132: 1439–1447.
- Voelker SL, Lachenbruch B, Meinzer FC, Jourdes M, Ki C, Patten AM, Davin LB, Lewis NG, Tuskan GA, Gunter L *et al.* 2010. Antisense down-regulation of 4CL expression alters lignification, tree growth, and saccharification potential of field-grown poplar. *Plant Physiology* 154: 874–886.
- Voelker SL, Lachenbruch B, Meinzer FC, Kiti P, Strauss SH. 2011. Transgenic poplars with reduced lignin show impaired xylem conductivity, growth efficiency and survival. *Plant, Cell & Environment* 34: 655–668.
- Wallis AFA, Wearne RH, Wright PJ. 1996. Analytical characteristics of plantation eucalypt woods relating to kraft pulp yields. *Appita Journal* 49: 427–432.
- Wartenberg D, Ferson S, Rohlf FJ. 1987. Putting things in order: a critique of detrended correspondence analysis. *American Naturalist* 129: 434–448.
- Wildhagen H, Paul S, Allwright M, Smith HK, Malinowska M, Schnabel SK, Paulo MJ, Cattonaro F, Vendramin V, Scalabrin S *et al.* 2017. Genes and gene clusters related to genotype and drought-induced variation in saccharification potential, lignin content and wood anatomical traits in *Populus nigra*. *Tree Physiology* 38: 320–339.
- Williamson GB, Wiemann MC. 2010. Measuring wood specific gravity correctly. *American Journal of Botany* 97: 519–524.
- Wolfe BT, Kursar TA. 2015. Diverse patterns of stored water use among saplings in seasonally dry tropical forests. *Oecologia* 179: 925–936.
- Wolfe BT, Sperry JS, Kursar TA. 2016. Does leaf shedding protect stems from cavitation during seasonal droughts? A test of the hydraulic fuse hypothesis. *New Phytologist* 212: 1007–1018.
- Xiao L, Xu F, Sun RC. 2011. Chemical and structural characterization of lignins isolated from *Caragana sinica*. *Fibers and Polymers* 12: 316–323.
- Xu C, Fu X, Liu R, Guo L, Ran L, Li C, Tian Q, Jiao B, Wang B, Luo K. 2017. PtoMYB170 positively regulates lignin deposition during wood formation in poplar and confers drought tolerance in transgenic Arabidopsis. *Tree Physiology* 37: 1713–1726.
- Zhao Q, Wang H, Yin Y, Xu Y, Chen F, Dixon RA. 2010. Syringyl lignin biosynthesis is directly regulated by a secondary cell wall master switch. *Proceedings of the National Academy of Sciences, USA* 107: 14496–14501.
- Zheng P, Aoki D, Matsushita Y, Yagami S, Sano Y, Yoshida M, Fukushima K. 2016. Lignification of ray parenchyma cells (RPCs) in the xylem of *Phellodendron amurense* Rupr.: quantitative and structural investigation by TOF-SIMS and thioacidolysis of laser microdissection cuts of RPCs. *Holzforschung* 70: 641–652.
- Zimmerman MH. 1983. *Xylem structure and the ascend of sap*. New York, NY, USA: Springer.



## About New Phytologist

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Trust, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews and Tansley insights.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <26 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and all the latest information visit **www.newphytologist.com**