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On the hydraulic conductance of three woody Devonian plants

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ABSTRACT

Most evolutionary innovations in plant vascular tissues, including secondary growth, occurred during the Devonian period (~420 to 360 million years ago). Such innovations had a major impact on land colonisation by plants and on their biodiversity. Here, we show the hydraulic conductance of the secondary xylem of three shrubby or arborescent plant fossils (a probably new genus of Cladoxylopsida, the archaeopteridalean genus *Callixylon* and the stenokolean genus *Brabantophyton*). Evidences come from the Ronquières fossil site (Belgium). This site is considered mid-late Givetian/earliest Frasnian in age. Results reveal that hydraulic conductivity of these early woody plants is more or less similar to that of modern gymnosperms, meaning that water transport was already as efficient in Devonian plants as it is in living plants. Our results further suggest that tracheids with features helping for optimised water transport were quickly selected in the evolutionary history of vascular plants.

Keywords: Conductance, Devonian, homogeneous pit, plant, secondary xylem, tracheid.

INTRODUCTION

The fossil record unambiguously indicates that plants acquired secondary growth early in their evolutionary history, much before seeds and laminated leaves (Gerrienne *et al.* 2011; Hoffman & Tomescu 2013). The earliest evidence of secondary xylem comes from several Early Devonian (~400 million years ago) euphyllophytes of small stature such as *Armoricaphyton* Strullu-Derrien *et al.* (2014) (Gerrienne & Gensel 2016). The early evolution of secondary xylem is obviously related to various factors such as the need of land plants for greater height, larger size and/or additional support and strength, but the consistent small size of the earliest woody plants and the large diameter (up to 100 µm in some cases) of the tracheids in their secondary xylem (Gerrienne *et al.* 2011; Hoffman & Tomescu 2013) suggest that the initial evolution of secondary xylem was, rather counterintuitively, mostly related with "optimizing hydraulics rather than immediately conferring mechanical advantages" (Rowe & Speck 2003, p. 310) such

ently in several lineages that all developed secondary growth (Meyer-Berthaud & Decombeix 2009): Lycopsida, Cladoxylopsida (plants with a dissected primary xylem, considered basal ferns sensu lato; only some genera are known to possess secondary growth) and lignophytes (plants with secondary xylem and phloem differentiated by a bifacial cambium).

In the course of their terrestrialisation, plants evolved more and more sophisticated water-conducting cells. This can be at least partly explained because plants with increasing height had to face the tricky problem of the trade-off between hydraulic efficiency and xylem reinforcement/safety. Tracheids with large lumen diameter and thin walls are indeed more efficient for conduction but also more vulnerable to implosion and to cavitation (formation of air bubbles that interrupt the water column) than tracheids with narrow lumen and comparatively thicker walls (Wilson 2013). Even though the Devonian period is highly critical with respect to the evolution of plant vascular tissues, especially of secondary xylem, and despite an initial attempt by Niklas as early as 1985, the hydraulic efficiency of only a few Devonian plants has to date been assessed. Three genera were evaluated, namely *Asteroxylon* (Lycopsida; Wilson &

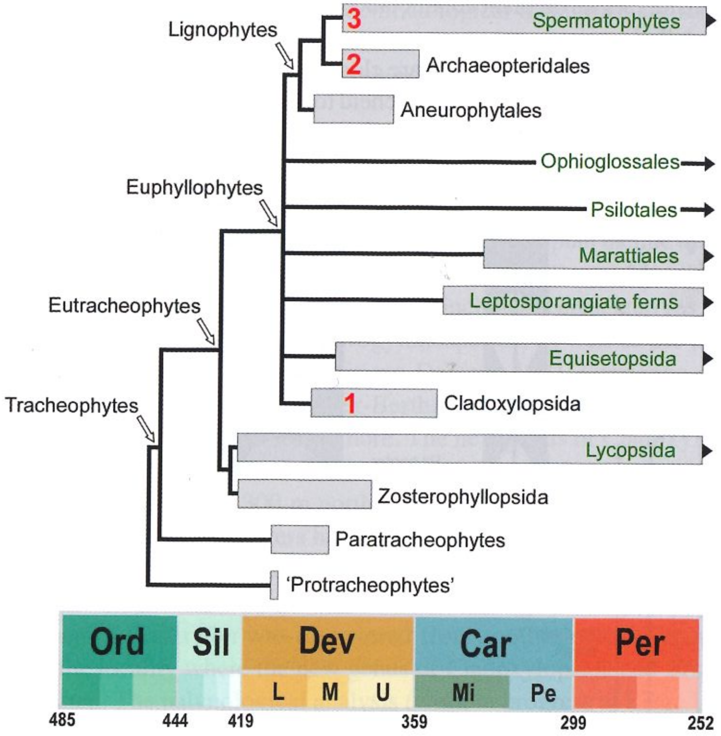

Figure 1. Time-calibrated consensus tree of polysporangiophytes, showing the phylogenetic and stratigraphic position of the three plants studied in this paper. 1 = cladoxylopsid (fern *sensu lato*) new genus; 2 = *Callixylon* sp. (Archaeopteridales); 3 = *Brabantophyton runcariense*

Fischer 2011), *Psilophyton* (basal euphyllophyte; Wilson 2016) and *Armoricaphyton* (basal euphyllophyte; Strullu-Derrien *et al.* 2014). The first two plants show primary growth only and, consequently, only the hydraulic parameters of the primary xylem could be evaluated. *Armoricaphyton* shows a limited amount of secondary xylem; the hydraulic efficiency of the latter was evaluated on the basis of lumen diameter and wall thickness, but the pitting between tracheids was not considered.

In this paper, we present the first study of the hydraulic properties of three different shrubby or arborescent Devonian plants with secondary xylem (Fig. 1). These taxa belong to three different euphyllophyte lineages and are the following: (i) a probably new genus of Cladoxylopsida (fern *sensu lato*); (ii) the archaeopteridalean genus *Callixylon* (an early lignophyte); (iii) the stenokolean genus *Brabantophyton* (an early lignophyte considered related to early spermatophytes).

Many of our calculations are based on pioneer works on Palaeozoic plant hydraulic conductance of, among others, Niklas (1985), Cichan (1986), Sperry (2003), Rowe & Speck (2003), Wilson *et al.* (2008), Wilson & Knoll (2010), Wilson & Fischer (2011), Wilson (2013, 2016), and Strullu-Derrien *et al.* (2013, 2014). We refer the reader to these papers as well as to Sperry & Hacke (2004) and Hacke *et al.* (2004) for more general considerations on plant conductance, and on how the flow of water in tracheids can be modelled.

Tracheids are elongated cells that are closed at both extremities (Fig. 2a). Water has therefore to move laterally from one tracheid to another through small holes in tracheid walls called pits (Fig. 2b,c). Two neighbouring tracheids are connected through pit pairs (Fig. 2b): the pit in the wall of a given tracheid always faces a pit in the wall of the adjacent tracheid. A pit includes a border and an aperture (Fig. 2b), and the water flows from one pit to the facing pit in the neighbouring tracheid through the pores of the pit membrane (that includes the middle lamella and the two primary walls of the adjacent tracheids). The flow of water through these pits has been described as comparable to the flow of electric current through resistors in parallel (in an analogy with Ohm's Law; van den Honert 1948). If more resistors are added in parallel to a circuit, the equivalent resistance of the circuit decreases and the total current of the circuit increases. Similarly, the more pits there are in the tracheid wall, the more water goes through the wall. Accordingly, individual pits are modelled as resistors in parallel (see Sperry & Hacke 2004; Hacke *et al.* 2004; Wilson *et al.* 2008 for more detail).

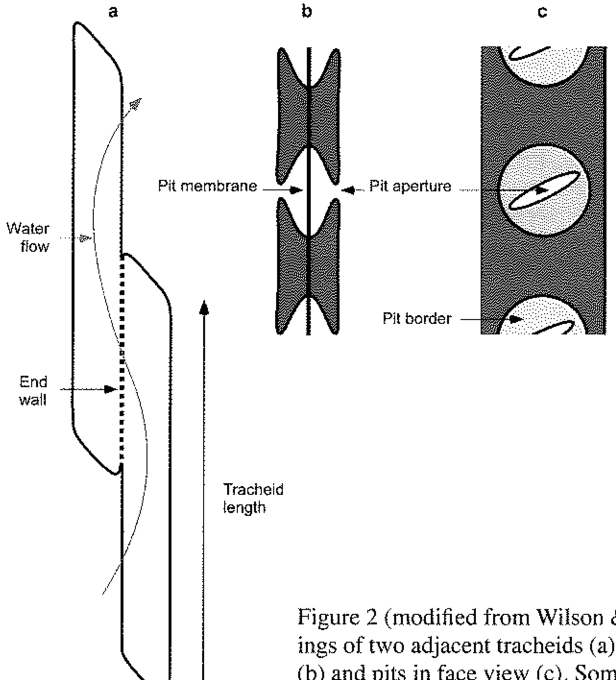

Figure 2 (modified from Wilson & Knoll 2010). Schematic drawings of two adjacent tracheids (a), pit pairs in longitudinal section (b) and pits in face view (c). Some characters used in the analysis

The three plants analysed herein come from two coeval, late Middle or earliest late Devonian localities next to the "plan incliné" at Ronquières (Belgium). We estimated various parameters linked to hydraulic conductance and resistance. Taking advantage of the co-occurrence of the three plants at the localities, and hence of their supposed common geographical and stratigraphical origins, we presume that the biases induced by the taphonomical and, possibly, palaeoclimatological conditions are reduced. The three plants are compared, first between them, and then with other plants. The mechanical support resulting from the secondary xylem is also discussed.

Locality and age

The specimens studied here were collected from the area of Ronquières (Belgium). The main Ronquières locality was described by Stockmans (1968) who reported an assemblage of 22 fossil plant taxa from the "Ronquières, tour du plan incliné (tête amont)" locality. The locality is considered having yielded the most diverse Middle Devonian flora from Belgium (Berry 2008). The assemblage includes representatives of zosterophyllopsids, lycopsids, cladoxylopsids, progymnosperms, as well as the proto-ovule *Runcaria* Stockmans (Stockmans 1968; Fairon-Demaret 1981; Gerrienne *et al.* 2004; Scheckler *et al.* 2006; Gerrienne & Meyer-Berthaud 2007; Berry 2008). Stockmans' Ronquières locality is not accessible anymore. The new genus of Cladoxylopsida and the specimen of *Brabantophyton* studied here were collected from an outcrop located along the "plan incliné", some 300 m northeast of Stockmans' locality (Cornet *et al.* 2012; Fig. 1). The fossiliferous layers have been attributed a mid-late Givetian or earliest Frasnian age (TA Oppel Zone or lowermost part of the TCo Oppel Zone; Streel *et al.* 1987; Momont *et al.* 2016a) on the basis of the miospore assemblage.

The specimen of *Callixylon* was discovered from another outcrop approximately 500 m east of the locality where the new genus of Cladoxylopsida and *Brabantophyton* were collected. The palynological analysis (work in progress at Liège University) indicates that the spore assemblage is identical to that discovered at the locality near the "plan incliné" (Momont *et al.* 2016a). This means that the outcrop has also a mid-late Givetian or earliest Frasnian age (TA Oppel Zone or lowermost part of the

Table 1. Raw measures of tracheid and pit parameters used in this study.

Target plant	Value	Tracheids					Pits					
		Minimum length (µm)	Cell diameter (µm)	Lumen area (µm)	Lumen diameter (µm)	Wall thickness (µm)	Pit number (pits)	Total pit area (mm^2)	Membrane area (µm)	Membrane diameter (µm)	Aperture area (µm)	Aperture diameter (µm)
Cladoxylopsid	Min.	1682	17.2	102	11.4	2.9	791	0.036	46	5.1	7	1.9
	Max.	2718	46.4	705	30.0	8.2	4437	0.454	102	7.6	23	3.6
	Mean	2102	27.9	246	17.3	5.3	1899	0.131	69	6.2	12	2.6
Callixylon	Min.	1733	22.2	303	19.6	1.3	242	0.010	43	7.4	11	3.7
	Max.	2833	45.1	1162	38.5	3.3	1244	0.112	90	10.7	35	6.7
	Mean	2259	29.7	518	25.2	2.3	506	0.034	67	9.2	20	5.0
Brabantophyton	Min.	1465	61.2	2592	57.4	1.9	3605	0.109	30	6.2	14	4.2
	Max.	2005	127.6	9880	119.2	4.2	11252	0.931	83	10.3	27	5.9
	Mean	1757	88.4	5515	82.4	3.0	6532	0.319	49	7.8	20	5.1

METHODS AND MATERIAL

Data acquisition

Photographs of sections were taken using a Nikon Df camera coupled to a Zeiss Stemi 2000-C stereomicroscope and Olympus BX51 microscope for detailed views. Plates were prepared with Canvas 9 (Deneba Systems, Inc.); measurements of lengths and areas were also performed with Canvas 9. Transformations made to the pictures include cropping, rotation, adjustment of brightness and contrast. At least 30 measurements were taken for each character (lumen diameter, wall thickness, number of pits/1000 µm^{2}···, pit membrane diameter, pit aperture diameter, pit aperture thickness), except for tracheid length because of technical limitations (see below).

Measurements for tracheids (Table 1; Fig. 2)

All measures were taken directly from photographs of sections. Because most areas measured (lumina, some pit membranes, pit apertures) are actually not circles, the area of the structure was determined and then the diameter was back-calculated using the formula for a circle.

– The *tracheid length* is only a minimum estimate, because most generally one or both end walls are not visible in the sections.

– The *tracheid diameter* (or *cell diameter*) was calculated by adding the lumen diameter and the double wall thickness (see below for an explanation of how it was calculated). The *cell radius* is half of the cell diameter.

– The *lumen area* was measured on photographs.

– The *lumen diameter* was calculated on the basis of the lumen area. The *lumen radius* is half of the lumen diameter.

– The *wall thickness* of a single tracheid was obtained by dividing by two the thickness of double walls between two tracheids.

– Considering that a whole tracheid and its lumen can be modelled as two concentric cylinders with similar length, *cell wall volume* (V) can be obtained in subtracting the lumen volume from the tracheid volume.

– The *cell wall area* (A_{wall}) was calculated by dividing the cell wall volume by the tracheid length. A_{wall} represents the amount of wall material per unit length; it gives a measurement of cell wall invested per tracheid.

– The *conduit area* ($A_{conduit}$) is the area of the tracheid (wall included) in transverse section. It was calculated using the cell radius.

Measurements for pits (Table 1; Fig. 2)

– The *pit membrane area* was measured directly on photographs.

– The *pit membrane diameter* was calculated from the pit membrane area.

– The *number of pits* (N_{pits}) has been calculated first in counting the number of pits in a given pitted area. It has then been extrapolated to the whole surface of the tracheid. For the cladoxylopsid, no correction factor was applied, because pits cover more or less are pitted, and (ii) the pits are placed in bands separated by unpitted areas, estimated at approximately half of the area of the tracheid radial wall. For *Brabantophyton*, a correction factor of 0.83 was applied to the number of pits because tangential walls include a smaller number of pits (approximately 66 % of the surface for tangential walls - 100 % for radial walls).

– The number of pores in pit membranes (N_{pores}) was calculated by dividing the pit membrane area by the surface area of a pore, assuming that all pores are of the same size (see below for further explanations). The size of the pores of the pit membrane is highly variable and depends on the type of pits. Torus-margo pits have much larger pores than homogeneous pits (Choat *et al.* 2008; Wilson *et al.* 2008; Wilson & Knoll 2010). In fossil plants, the pit membranes, and hence the pores, are very rarely preserved. In the case of homogeneous pits such as those of the three fossil plants studied here, the pore diameter has been estimated in previous studies at 40 nm (Wilson *et al.* 2008; Wilson & Knoll 2010), with the thickness of the surrounding microfibrils estimated at 15 nm; so, we applied this 40 nm + 15 nm dimension for the diameter of the pores of our three fossil plants.

– The *pit aperture area* was measured directly on photographs.

– The *pit aperture diameter* was calculated from the pit aperture area.

– The *pit aperture thickness* was measured directly on photographs.

Calculations (Tables 2 & 3)

In order to calculate the hydraulic conductance of a given tracheid, its resistance (which is the inverse of its conductance) has to be calculated. Most of our calculations are based on the works of, among others, Niklas (1985), Cichan (1986), Sperry (2003), Rowe & Speck (2003), Sperry & Hacke (2004), Hacke *et al.* (2004), Wilson *et al.* (2008), Wilson & Knoll (2010), Wilson & Fischer (2011), Wilson (2013, 2016), and Strullu-Derrien *et al.* (2013, 2014). The total resistance of a tracheid (R_{tot}) includes two components: the resistance to water flow through the cell lumen (R_{lumen}), equivalent to the resistance to flow in a tube, and the resistance to water flow through the wall (R_{wall}) of the two adjacent tracheids. R_{lumen} responds to the equation $R_{lumen} = 64 * vH_2O * L / n * D_{tracheid}^4$, where vH_2O is the dynamic viscosity of water at 25 °C expressed in $N * s/m^2$. R_{wall} depends on the number of pits that connect tracheids and on the resistance of the pits (R_{pit}) and responds to $R_{wall} = 2 * (R_{pit} / N_{pit})$. R_{pit} has to be doubled because when water moves from one cell to another, it goes through a pair of pits (Fig. 2b). R_{pit} comprises two components: the resistance of the pit membrane ($R_{membrane}$) and the resistance from the apertures of the two pits of the pit pair ($R_{aperture}$) (Fig. 2), and is calculated according to $R_{pit} = \alpha * R_{membrane} + 2 * R_{aperture}$. $R_{membrane}$ is equal to $24 * vH_2O * / N_{pore} * D_{pore}^4$, while $R_{aperture} = [128 * t_{ap} * vH_2O / n * D_{ap}^4] + [24 * vH_2O / D_{ap}^3]$, where t_{ap} is the thickness of pit aperture and D_{ap} its diameter. Finally, K_{sp} is the conductivity normalised to cross-sectional wall investment. It is calculated by dividing cell length (L) by the cell wall area (A_{wall}) multiplied by the total resistance (R_{tot}). K_{sc} is the conduit specific conductivity or conduit conductivity

Table 2. Calculations and modelling parameters of water conductance. See the "Methods and Material" section for details.

Target plant	Value	V_{wall} (m^3)	A_{wall} (m^2)	$A_{conduit}$ (m^2)	R_{lumen} (m^2)	$R_{aperture}$ (MPa*s/m3	N_{pores} (pores)	$R_{membrane}$ (MPa*s/m^3)	R_{pit} (MPa*s/m^3)	R_{wall} (MPa*s/m^3)	R_{tot} (MPa*s/m^3)	K_{sp} (m^2/MPa*s)	K_{sc} (m^2/MPa*s)
Cladoxylopsid	Min.	2.19E-13	1.30E-10	2.32E-10	6.57E+06	8.46E+08	13465	1.11E+10	1.12E+11	5.07E+07	5.72E+07	1.02E-02	5.72E-03
	Max.	2.67E-12	9.84E-10	1.69E-09	5.62E+08	1.53E+10	30112	2.48E+10	2.78E+11	7.04E+08	1.27E+09	4.83E-02	2.81E-02
	Mean	7.93E-13	3.77E-10	6.12E-10	6.26E+07	3.32E+09	20404	1.63E+10	1.70E+11	1.79E+08	2.42E+08	2.30E-02	1.42E-02
Callixylon	Min.	1.48E-13	8.54E-11	3.87E-10	7.59E+06	8.17E+07	12699	1.26E+10	1.28E+10	2.05E+07	2.81E+07	4.62E-02	1.02E-02
	Max.	1.23E-12	4.33E-10	1.60E-09	2.11E+08	6.53E+08	26458	2.63E+10	2.76E+10	2.28E+08	4.39E+08	2.33E-01	6.31E-02
	Mean	4.38E-13	1.94E-10	6.93E-10	5.26E+07	2.16E+08	19652	1.70E+10	1.74E+10	6.88E+07	1.21E+08	9.59E-02	2.69E-02
Brabantophyton	Min.	5.19E-13	3.54E-10	2.94E-09	1.00E+05	1.28E+08	8898	1.37E+10	1.62E+09	2.89E+05	3.89E+05	8.80E-01	9.59E-02
	Max.	3.26E-12	1.63E-09	1.28E-08	2.59E+06	4.74E+08	24366	3.75E+10	4.70E+09	2.60E+06	5.19E+06	3.17E+00	4.03E-01
	Mean	1.41E-12	8.05E-10	6.14E-09	5.21E+05	2.26E+08	14383	2.32E+10	2.77E+09	8.48E+05	1.37E+06	1.59E+00	2.09E-01

Table 3. Measures of thickness-to-span ratio of tracheids and the proportionality of lumen and wall resistances (R).

Target plant	Value	(2× wall thickness / lumen diameter)^2	R_{lumen} / R_{wall}
Cladoxylopsid	Min.	0.037	0.13
	Max.	2.070	0.80
	Mean	0.375	0.35
Callixylon	Min.	0.005	0.37
	Max.	0.113	0.93
	Mean	0.033	0.76
Brabantophyton	Min.	0.001	0.35
	Max.	0.021	0.99
	Mean	0.005	0.61

Note: value of α. In previous analyses (see Wilson *et al.* 2008 and references therein for further explanations), the constant α was introduced to modify the pit membrane resistance and allow it to vary. The values of α are chosen in order to preserve the proportionality of lumen to end-wall resistance (Sperry *et al.* 2005, 2006; Hacke *et al.* 2006; Wilson *et al.* 2008). Default values used by Wilson *et al.* (2008) were 1 and 16 for *Cordaites* and *Medullosa*, respectively. Following the procedure described by Wilson *et al.* (2008), we varied α, as a sensitivity analysis, between 0.1 and 100; the chosen default values for the new genus of Cladoxylopsida, *Callixylon* and *Brabantophyton* were 10, 1 and 0.1, respectively (Table 2). Those values were selected because they give a 35–76% mean proportionality of lumen to end-wall resistance (Table 3).

We also evaluated the “thickness-to-span” ratio of the three plants (Table 3). The “thickness-to-span” ratio is the square of the ratio of tracheid double wall thickness to lumen diameter (Woodruff *et al.* 2008); it gives an estimation of cell mechanical support against implosion. Wall thickness and lumen diameter are directly involved in the safety (of cell structure) – efficiency (of cell conductance) trade-off.

All the results of the measures and analyses performed on the three plants studied here are presented in Tables 1 to 3.

Plants studied (Fig. 3–17)

The three taxa studied here are preserved as pyrite permineralisations. The specimens have been cut transversely and longitudinally in order to observe all the characters of the secondary xylem. Peels and wafers of the specimens have been prepared following the standard techniques (Joy *et al.* 1956; Matten 1973; Galtier & Phillips 1999; Hass & Rowe 1999; Kenrick 1999).

New genus of Cladoxylopsida (Fig. 3–5, 10–12)

This new genus is represented by a single permineralised specimen (ULG-Ronq-17).

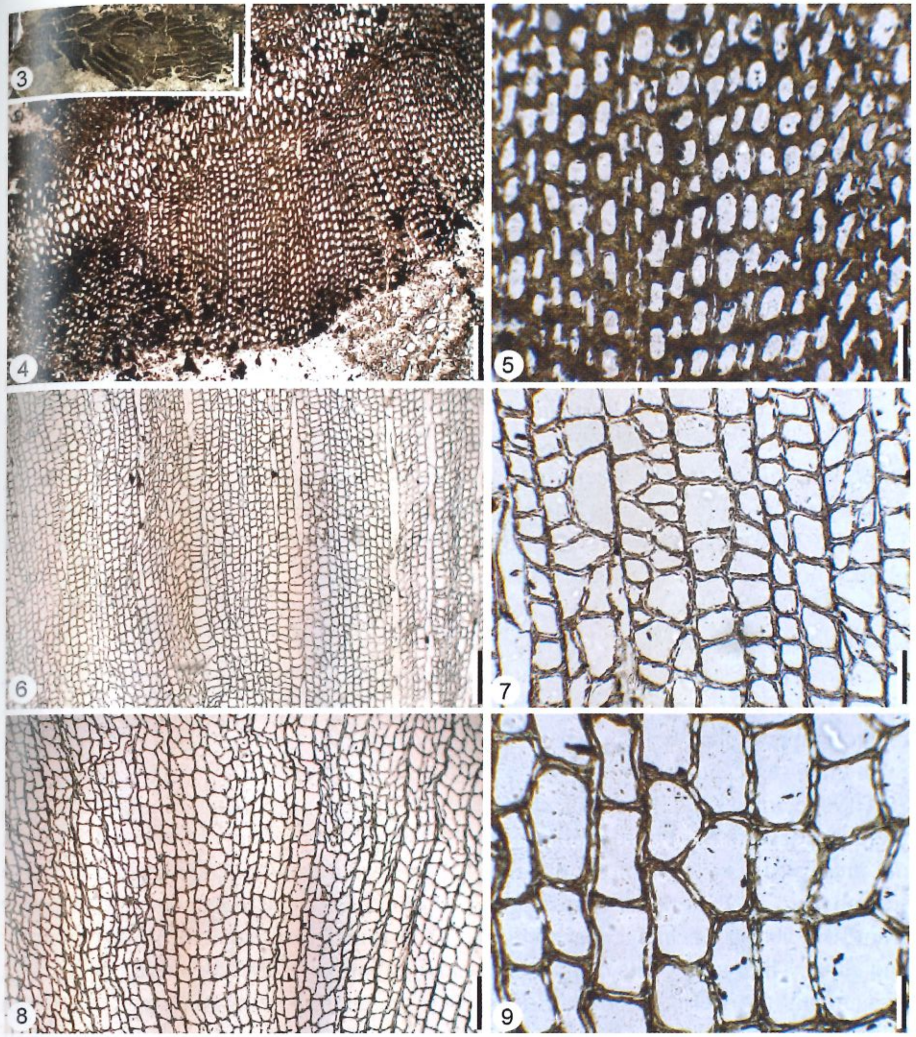

Figure 3–9. Transverse sections of the three plants studied in this paper. – Fig. 3–5: Cladoxylopsid new genus, TS. - 3. Gross view of specimen. - 4. Primary and secondary xylem of a vascular strand. - 5. Secondary xylem. – Fig. 6 & 7: *Callixylon* sp.; secondary xylem, TS. – Fig. 8 & 9: *Brabantophyton runcariense*; secondary xylem, TS. — Scale bars: 500 µm in 3; 100 µm in 4, 6, 8; 20 µm in 5, 7, 9.

have been prepared from two segments. The specimen is a decorticated fragment either of a juvenile plant or of a branch of an older plant, including approximately 20 vascular strands of variable shape and size (Fig. 3). All strands include a primary vascular strand surrounded by a ring of secondary xylem (Fig. 3, 4). The largest strands are elongated µm. The protoxylem strands are hardly distinguishable. The secondary xylem consists of radial rows of tracheids and narrow rays (Fig. 4, 5). In transverse section, tracheids are rectangular with thick walls and a circular to oval lumen; they range from 10 to 45 µm radially, and from 5 to 20 µm tangentially (Fig. 5). Both their tangential and radial walls include numerous circular to oval bordered pits closely arranged in one to two rows, with an elongated aperture (Fig. 10) (see Table 1 for detailed measurements). In longitudinal section (Fig. 10–12), tracheid walls show clearly the pit border and aperture. No torus is visible, and the pits are considered homogeneous. Between pits, the secondary wall material includes a core of unpreserved material that might correspond to the primary walls of the cells.

On the basis of its vascular system dissected into numerous vascular strands, this specimen is attributed to the Cladoxylopsida, a Middle Devonian to Mississippian class of basal ferns (Meyer-Berthaud & Decombeix 2007, 2009); most Cladoxylopsida were arborescent and could reach several metres high (Meyer-Berthaud & Decombeix 2009; Xu *et al.* 2017). The specimen from Ronquières probably represents a new genus, roughly comparable with some specimens of *Xenocladia* Arnold (1940) (see Mustafa 1980, Pl. 20: 7). It has however not been completely described yet and is currently left in open nomenclature.

Callixylon sp. (Fig. 6, 7, 13, 14)

Callixylon sp. is represented by a single permineralised specimen (ULg-RonqRui-01), approximately 6.0 cm long and 2.5 cm wide. It has been transversally cut into 9 segments of approximately 1 cm length. Radial and tangential sections have been prepared from three segments.

The specimen consists of secondary xylem only of a n-order axis (branch or trunk), including several (n+1)-order branch traces. The primary xylem of the n-order axis is not preserved. Tracheids of the secondary xylem are mostly rectangular in transverse section (Fig. 7). Their radial diameter ranges from 22 to 80 and their tangential diameter from 10 to 50 µm. The tangential section (not illustrated) shows that the rays are narrow and short. The radial walls of tracheids show radial bands of grouped pits (Fig. 13). Pits are uni- to triseriate, and in this case, they are alternate. Pits are bordered with an elliptical, tilted aperture. Their precise dimensions are given in Table 1. The bands of pits are separated by unpitted areas. At several places, the disposition of pits in groups is not regular; a few pits are evenly distributed within the unpitted areas. In longitudinal section (Fig. 14), tracheid walls show the thick pit border and aperture. No torus is visible, and the pits are considered homogeneous. Between pits, the secondary wall material includes a core of unpreserved material that might correspond to the primary walls of the cells.

Based on the presence of pits grouped into bands on the radial walls of the tracheids, the specimen is attributed to the archaeopteridalean genus *Callixylon* Zalesski (1911). A detailed description is currently under way at Liège University, and the definitive attribution of the specimen to a given *Callixylon* species is not possible yet. However,

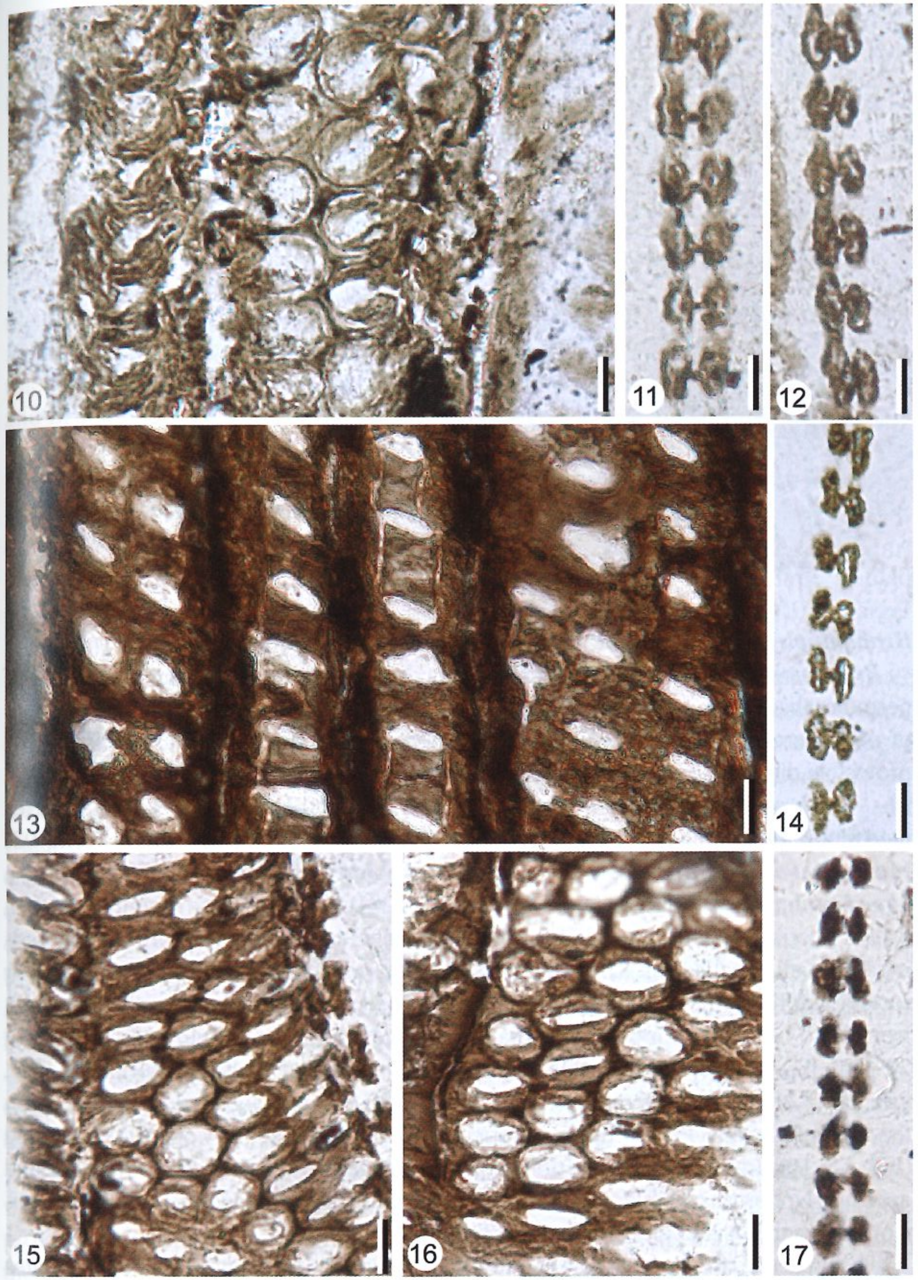

Figure 10–17. Longitudinal sections of the secondary xylem of three plants studied in this paper. Fig. 10–12. Cladoxylopsid new genus. - 10: Pits in face view, RLS. - 11 & 12: Pits in longitudinal section, TLS. – Fig. 13 & 14: *Callixylon* sp. - 13: Pits in face view, RLS. - 14: Pits

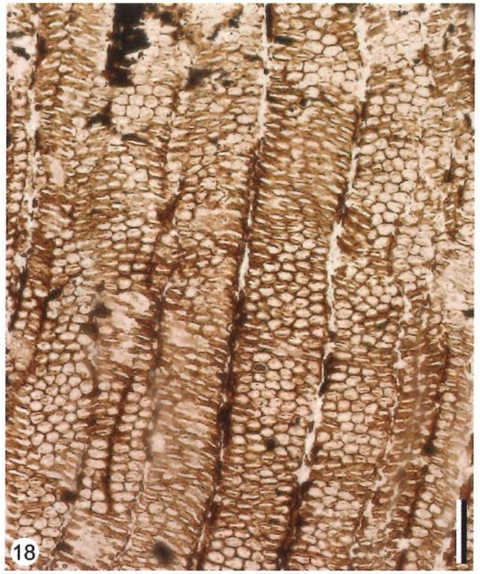

Figure 18. Radial section of the secondary xylem of *Brabantophyton runcariense*, showing the abundant pitting of the tracheids. – Scale bar: 50 μm.

Brabantophyton runcariense Momont *et al*. 2016a (Fig. 8, 9, 15–18)

Brabantophyton runcariense was described on the basis of a large collection of permineralised specimens (Momont *et al*. 2016a, b). Most of the specimens consist of permineralised stems showing primary growth only. They are characterised by a protostele dissected into three primary ribs, each of them dividing into two secondary ribs. The protostele includes a central protoxylem strand and several strands distributed along the midplanes of the ribs. The stem specimen studied here (ULg-Ronq-03; Momont *et al*. 2016b, plates I-III) is the only specimen of *Brabantophyton* to show a large amount of secondary xylem (Momont *et al*. 2016b, Pl. I, 3). The secondary xylem is characterised by radial rows of thin-walled tracheids interspersed with rays including thin-walled cells (Fig. 8). In transverse section, most of the tracheids are square or rectangular but some are polygonal (Fig. 8, 9). The radial diameter of tracheids ranges from 27 to 147 μm; their tangential diameter ranges from 39 to 87 μm (Momont *et al*. 2016 b). The tracheid radial walls are uniformly provided with multiseriate (2–10), circular to elliptical bordered pits (Fig. 15, 16, 18). Pits are slightly less abundant on the tangential walls. Pit aperture is elliptical, horizontally to slightly obliquely oriented (Fig. 15, 16). In longitudinal section (Fig. 17), tracheid walls show the pit border and aperture. No torus is visible, and the pits are considered homogeneous. Detailed dimensions of tracheids and pits are given in Table 1.

The presence of a central protoxylem strand in the stele of *Brabantophyton* indicates that the plant belongs to the radiatopses (Beck & Stein 1993; Stein 1993; Kenrick & Crane 1997). Despite the fact that a bifacial vascular cambium could not be demonstrated in the plant, it is considered an early representative of the lignophytes (Momont

Devonian to the Early Carboniferous (Beck & Stein 1993). The affinities of the order remain unclear, but its representatives are generally considered related to early seed plants (Matten 1992; Galtier & Meyer-Berthaud 1996).

RESULTS AND DISCUSSION

All the results of the measures and analyses performed on the three plants studied here are presented in Tables 1–3.

When only viewing the transverse sections (Fig. 5, 7, 9 – all are at the same magnification), it immediately appears that the three plants have different secondary xylem characteristics. The secondary xylem of the cladoxylopsid includes cells with a thick wall and a comparatively small lumen. The secondary xylem of both *Callixylon* and *Brabantophyton* consists of tracheids with a thin wall and a comparatively large lumen. The tracheids of *Brabantophyton* have a clearly larger lumen then those of the two other plants. These differences are expressed by the thickness-to-span (Table 3), indicating that *Callixylon* and *Brabantophyton* tracheids are presumably more devoted to conduction than those of the cladoxylopsid.

Major differences are also observed in the tracheid sizes of the three studied plants. Mean tracheid diameter in *Brabantophyton* is approximately three times larger (88.4 μm) than in the cladoxylopsid (27.9 μm) and in *Callixylon* (29.7 μm) (Table 1). Comparing with other measured Devonian plants, *i.e. Psilophyton* (tracheid diameter up to 70 μm; Niklas 1985) and *Asteroxylon* (tracheid diameter approximately 25 μm; Wilson & Fisher 2011; Table 1), we see that *Brabantophyton* tracheids have the larger diameter values. So, if we take into account the fact that flow rates are proportional to the diameter of the lumen raised to the fourth power, these measurements predict a good water transport capacity for *Brabantophyton*. In fact, *Brabantophyton* tracheid diameter ranges from 61 to 127 μm (Table 1). Such values are higher than those of modern conifers, whose tracheids very rarely reach more than 50 μm in diameter (Pitterman *et al*. 2006; Sperry *et al*. 2006). The *Brabantophyton* tracheid diameter range is within the range of measurements obtained from the Carboniferous seed plants *Callistophyton*, *Lyginopteris*, and partially *Medullosa*, a taxon for which high conductivity values have been modeled (see Wilson & Knoll 2010, fig. 6; Fig. 19).

For the cladoxylopsid tracheids, 17–46 μm in diameter and 1.6–2.7 mm in length, conductivity normalised to cross-sectional wall investment (K_{sp}) ranges from 0.0102 to 0.0483 $m^2/MPa*s$. Likewise, for *Callixylon* tracheids, 22–45 μm in diameter and 1.7–2.8 mm long, K_{sp} ranges from 0.0462 to 0.233 $m^2/MPa*s$. In *Brabantophyton*, we observe that the range of tracheid diameters, together with a 1.4–2.0 mm length, provides K_{sp} values ranging from 0.88 to 3.17 $m^2/MPa*s$. As expected because of the similarities and close size values of the tracheids of *Brabantophyton* and *Medullosa*, normalised conductivities are similar (Fig. 19). The two plants have higher conductivities than all the plants modeled by Wilson *et al*. (2008) (Fig. 19). This can be explained by the large diameter of their tracheids and the large number of

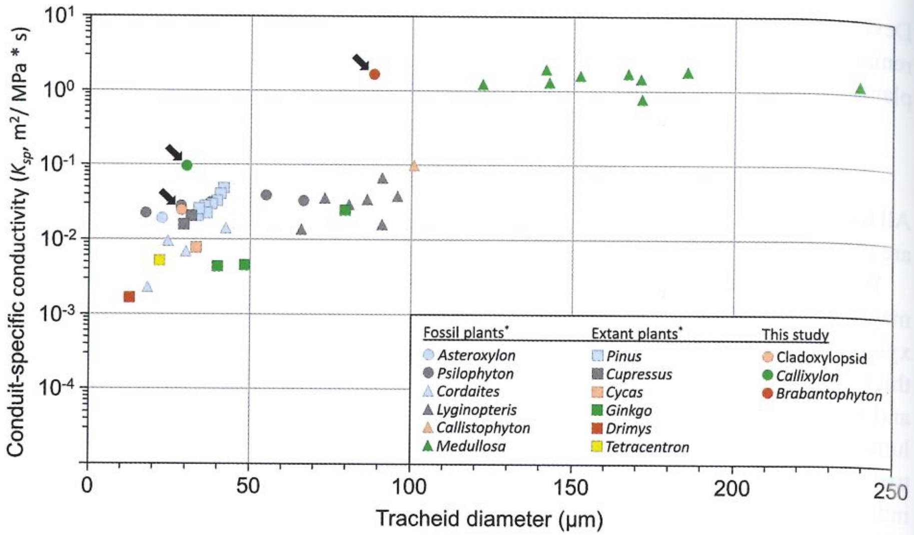

Figure 19. Morphospace of Wilson (2016)*, adapted. The three plants studied in this paper have been added (indicated by arrows).

membrane area. Although the three plants studied in this paper have homogeneous pits, their conductivities are of the same order of magnitude or higher than that of extant conifers with torus-margo pits (data for conifers from Hacke *et al.* (2004) and Wilson *et al.* (2008)). This suggests that soon after the origin of secondary xylem, tracheids were selected for optimised water transport rather than tracheids with high safety factors. This is also confirmed by the low thickness-to-span ratio shown by *Brabantophyton* (see below; Table 3).

The hydraulic conductivity of *Medullosa* and *Brabantophyton* is most probably underestimated because they are calculated on a minimum estimate for tracheid length. Nevertheless, apart from those calculated for *Sphenophyllum* (Cichan 1986), they are the highest values described for fossil plants, much higher than those of modern conifers, and only comparable with some living vessel-bearing angiosperms (Wilson & Knoll 2010; Wilson 2016; Fig. 19). In fact, in Wilson's (2016, fig. 8) morphospace, *Medullosa* appears into a range that no living seed plant occupies. Our analysis shows that conductivity values for the cladoxylopsid and *Callixylon*, all normalised to conduit cross-sectional or wall-specific area, are lower than in *Medullosa*. Both plants are placed roughly within the range of the extant gymnosperms. In contrast, the relationship between tracheid diameter and observed conductivity places *Brabantophyton* in a portion of Wilson's (2016) morphospace where no living or fossil plant has been registered yet (Fig. 19).

The "pit area resistance" is a measure of the water flow through the pit membrane: the higher the pit area resistance, the lower the water flow. The pit area resistance is

tracheid (N_{pits})" (Wilson *et al.* 2008). The pit area resistance of the tracheids of extant conifers has been estimated to be 59 times lower on average than the pit-area resistance of angiosperm vessels (Pittermann *et al.* 2005). This can be explained because extant conifers have torus-margo pits, in which the pit membrane has extremely large pores. Conifer tracheids and angiosperm vessels have, however, comparable total resistivities because the total pit area is much higher in angiosperm vessels (0.95 ± 0.51 mm²) than in conifer tracheids (0.016 ± 0.003 mm²) (Pittermann *et al.* 2005). Among the three plants studied here, the cladoxylopsid and *Callixylon* have low pit area resistance (Table 2), roughly comparable to that of extant conifers (data from Wilson *et al.* 2008), coupled with total pit areas (Table 2) that are also comparable with extant conifers. *Brabantophyton* has extremely low pit area resistance (Table 2), which is explained by the high total number of pits and the large total pit area (Table 2). This very low pit area resistance again highlights the fact that the *Brabantophyton* tracheids have exceptional conductance abilities.

The conduit "thickness-to-span" ratio is a measure of vulnerability to implosion (Woodruff *et al.* 2008). It is calculated as the square of the ratio between double tracheid wall thickness and tracheid lumen. This parameter positively correlates with wood density (Hacke *et al.* 2001, 2005). Conifer stem tracheids have generally higher thickness-to-span values than angiosperm vessels, presumably because conifer stem tracheids are also important for support, where fibres play this role in angiosperm woods (Hacke & Sperry 2001; Baas *et al.* 2004; Pittermann *et al.* 2006). The new cladoxylopsid has tracheids with a rather small lumen and thick walls: its thickness-to-span values are accordingly high (ranging from 0.037 to 2.070; Table 3), supporting the earlier suggestion (Xu *et al.* 2017) that cladoxylopsids favoured hydraulic safety/support instead of conductance, despite increased construction cost. This could be explained because the role in support of the tracheids, although generally present in smaller cross-sectional areas in cladoxylopsids than in lignophyte trees (Meyer-Berthaud *et al.* 2010), might have been essential for these early trees, as already suggested by Xu *et al.* (2017). The values for *Callixylon* are lower, and much lower than the lowest values obtained for *Cordaites* and *Pinus* by Wilson *et al.* (2008, fig. 8). This is surprising, because the *Callixylon*-type of wood is, at least in transverse section, anatomically close to the secondary xylem of conifers. These low values of thickness-to-span ratio in *Callixylon* could be tentatively explained because the plant favoured an efficient water transport in a stem/trunk that could reach several metres high, the support in this case being provided by the large proportion of wood rather than by wall thickness. In *Brabantophyton*, the lumen diameter is large and the tracheid walls are relatively thin: this explains why the thickness-to-span values of the plant are very low, ranging from 0.001 to 0.021. These values are within the same range as those of *Medullosa* (Wilson *et al.* 2008, fig. 8), a genus of which some species are vines (Dunn *et al.* 2003). We do not know if *Brabantophyton* had a vine-like habit, but again these very low values of thickness-to-span ratio, as well as the very large number of pits (Fig. 18), clearly

CONCLUSION

We present herein the first results on hydraulic efficiency of the secondary xylem of Devonian plants. Three taxa belonging to three different extinct lineages were evaluated: (i) a probably new genus of Cladoxylopsida (ferns *sensu lato*); (ii) the archaeopteridalean genus *Callixylon*, and (iii) the stenokolean genus *Brabantophyton*. All of them were collected from two coeval late Middle to earliest Upper Devonian localities at Ronquières (Belgium). The observed conductivity of these plants are roughly of comparable values, and of the same order of magnitude as extant gymnosperms with torus-margo pits, indicating that soon after the evolution of the earliest water-conducting cells, tracheids with features for optimised water transport were selected. It should however be noted that the detected high values of the thickness to span of the cladoxylopsid tracheids indicate that the latter are most probably also implicated in the hydraulic safety/support of the plant. Nevertheless, the hydraulic parameters of the secondary xylem of the three plants analysed here tend to confirm previous studies which indicated that water transport was presumably already as efficient in Devonian woody plants as it is in most extant plants (Fig. 19).

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