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Xylem Development and Xylem Conductivity of Furrowed Xylem in Stem Terminals of Five Species of *Aristolochia* (Aristolochiaceae)

Lance S. Evans[1] & Maya Carvalho-Evans[1]

[1] Pfizer Laboratory, New York Botanical Garden, The Bronx, NY 10458, USA

Correspondence: Lance S. Evans, Pfizer Laboratory, New York Botanical Garden, The Bronx, NY 10458, USA.

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Abstract

Xylem cells are responsible for water transport and mechanical support in most plants. Since vines depend upon other plants for mechanical support, the main responsibility of xylem cells in vines is primarily water transport and not mechanical support. The purpose of the current study was to study xylem characteristics in stems of five species of *Aristolochia*. Tissue samples from about 1.0 to 5.5 mm in diameter were processed with standard histological techniques. Anatomical characteristics were similar among all five species. Results show: (1) interfascicular cambia were not present, so stems had only furrowed xylem, (2) numbers of vascular bundles in stems were specific for each species and did not increase as stems enlarged, (3) radii of vessels were not dimorphic for any species, (4) numbers of vessels were linearly related with stem diameters, and (5) the largest half of all vessels supplied 95% of total xylem conductivity. To our knowledge, this is the first publication to document the development of furrowed xylem, describing both vessel characteristics and xylem conductivities in stems of *Aristolochia* species.

Keywords: *Aristolochia*, furrowed xylem, interfascicular cambium, xylem conductivity, vascular bundles.

1. Introduction

Most Lianas occur in tropical, moist, deciduous forests, but also occur in temperate rainforests and temperate deciduous forests. Lianas are long-stemmed, woody vines rooted in soils and use trees, as well as other means of vertical support, to climb the canopy for sunlight exposure. Their long slender stems must transport water from soils to the uppermost shoots and leaves to maintain growth (Rajput *et al*., 2017).

During normal development of woody plants with secondary growth, the vascular cambium forms and produces secondary xylem and secondary phloem to supplement the primary xylem and primary phloem, respectively. Initially, sections of the fascicular cambium originate between the primary xylem and primary phloem. At about the same time, some mature parenchyma cells between individual vascular bundles resume cell division to produce sections of interfascicular cambium (Mauseth, 2014). When the sections of interfascicular cambium link with sections of the fascicular cambium, the vascular cambium forms a complete circle. The complete vascular cambium then produces secondary xylem internally and secondary phloem externally (Mauseth, 2014).

Metcalf and Chalk (1950) describe many anomalous structures in stems and roots in many species. They describe ‘furrowed xylem’ in a stem of a species of *Aristolochia*, however, their description did not indicate how furrowed xylem develop. In furrowed xylem, it is presumed that the fascicular cambium develops normally but the absence of an interfascicular cambium does not allow for a complete vascular cambium. This research will focus on the expansion of vascular bundles, characteristics of cells proximal to bundles, and xylem conductivities during stem enlargement, with specific focus on the structure of the cells between vascular bundles that would ordinarily produce an interfascicular cambium. Overall, the purpose of this study was to determine the histological development of furrowed xylem, determine characteristics of vascular bundles, radii of xylem vessels and xylem conductivities in several species of *Aristolochia*. For the five species of *Aristolochia*, the hypotheses of this study are: (1) stem anatomies are similar among the five species, (2) interfascicular cambia are not present so the vascular cambium does not form a continuous ring, (3) numbers of vascular bundles in stems were specific for each species and were independent of stem diameters, (4) numbers of vessels were not similar among the species but were linearly related with stem diameters, (5) xylem conductivities were not similar among species but varied as a function of stem diameters, (6) the largest half of all vessel radii

provided 95% of the total xylem conductivity for all species.

2. Materials and Methods

2.1 Tissue Samples

Samples of *Aristolochia acutifolia* Duch. Accession number 1361/2001*A; *A. maxima* var. *augustofolia* Duch., Accession number 466/95*A; *A. pilosa* Kunth., Accession number 227/97; *A. spathulata* Duct. were obtained from the New York Botanical Garden on 07 July 2022. Three plants of *A. fimbriata* Cham. were obtained from Plant Delights Inc. in 09 July 2022. Tissues of this study were limited to stems between 0.5 and 7.0 mm in diameter. Tissue samples were selected along the stems in order to obtain tissues with only primary vascular tissues to tissues with secondary vascular tissues. Stem lengths were measured with a ruler. Stem diameters were measured with a digital caliper (Fisherbrand Traceable Digital Calipers cat. 06-664-16, Fisher Scientific, www.fishersci.com) to 0.1 mm.

2.2 Histological Processing

Tissue samples were taken for histology along the stems. Tissues were fixed in FAA (Formaldehyde Alcohol Acetic Acid) for 24 hours, dehydrated through a tertiary butanol series (Sass, 1958) and placed in wax (Histoplast, Thermo Scientific, Richard-Allen Scientific, Kalamazoo, MI). After tissues were embedded in Histoplast, sections were cut with a microtome at 20 μm and placed on microscope slides, stained with safranin/fast green and mounted with Canada balsam (Sass, 1958).

2.3 Vessel Measurements

All xylem vessels were counted for each sample. Photographs of samples were taken at 40× magnification. Photographic images of tissues were transferred to ImageJ (National Institutes of Health, http://rsb.info.nih.gov/ij; 1.53a, August 2022) to determine the radii of xylem vessels. One third of all bundles were sampled. For each microscopic tissue image, each bundle was numbered. Playing cards with numbers were randomly drawn to determine the bundles to be evaluated. All vessels in each selected bundles were evaluated. Since xylem vessels are not always circular (Tyree and Zimmermann, 2002), the derived conductivities are approximations. Two or three diameter measurements were obtained for each vessel, and mean diameters were converted to mean radii for each vessel.

2.4 Test for Vessel Dimorphism

A previous study (Bastos *et al*., 2016) postulated that vine species of the *Sapindaceae* have vessel dimorphism, large and small vessels only. Samples of the five species were studied to determine the distribution of vessel radii in all tissue samples. First, all vessel radii of each sample were sorted from smallest to largest. Second, differences between successive vessel radii were measured from smallest to largest. Third, the largest overall difference were determined for each sample. Lastly, radii of successive pairs of vessels were compared, the number of successive samples that were (1) more than 4 μm different, (2) between 2 to 3.99 μm different, (3) between 1 to 1.99 μm different, and (4) less than 1.0 μm different. Small differences in radii among successive vessels would indicate the lack vessel dimorphism while large differences would indicate the presence of dimorphism.

2.5 Xylem Conductivity Measurements

Xylem conductivity is a measure of the ability of plant tissues to transport water (McCulloh and Sperry, 2005a; Evans et al., 2016). Mean vessel diameters were converted to weighted mean vessel radii to calculate the xylem conductivity of each stem tissue section (McCulloh *et al*., 2009) using the Hagen-Poiseuille equation:

$$\frac{\pi * \text{number of vessels} * \text{mean radius of each vessel cm}^4}{8 * \text{viscosity of water}}$$

(K. McCulloh, personal communication). The units of xylem conductivity are g cm^{-1} MPa^{-1} s^{-1}.

Xylem conductivities of all stem sections for each species were compared. Statistical comparisons between two stem tissue sections were determined using t-test (socscistatistics.com). Regression analyses were performed with Microsoft Excel (Microsoft 8.0) to determine relationship between numbers of vessels versus stem diameters, xylem conductivities versus stem diameters, and versus stem diameters versus numbers of vessels.

3. Results

3.1 Stem Samples

Stem tapers were determined from diameter and distance measurements along stems since all data are expressed

as stem diameters. Mean tapers were 0.65 mm cm^{-1} for *A. acutifolia*, 0.07 mm cm^{-1} for *A. fimbriata* and *A. maxima*, 0.9 mm cm^{-1}, for *A. pilosa*, and 1.0 mm cm^{-1} for *A. spathulata*.

3.2 Comparative Anatomies among Species

3.2.1 Features in 1.7 to 2.1 mm Diameter Samples

Stem anatomies were similar among the five species. All species had a single-celled epidermis with seven to ten layers of cortical cells. All species had developing fused phloem fibers (Fig.1; Tamaio *et al*., 2010). Internally, additional individual phloem fibers were present. Parenchyma cells were present internal to the phloem fibers. The numbers of vascular bundles in stems were specific for each species and were independent of stem diameters but varied from 8 to 13 among the five species (Table 1).

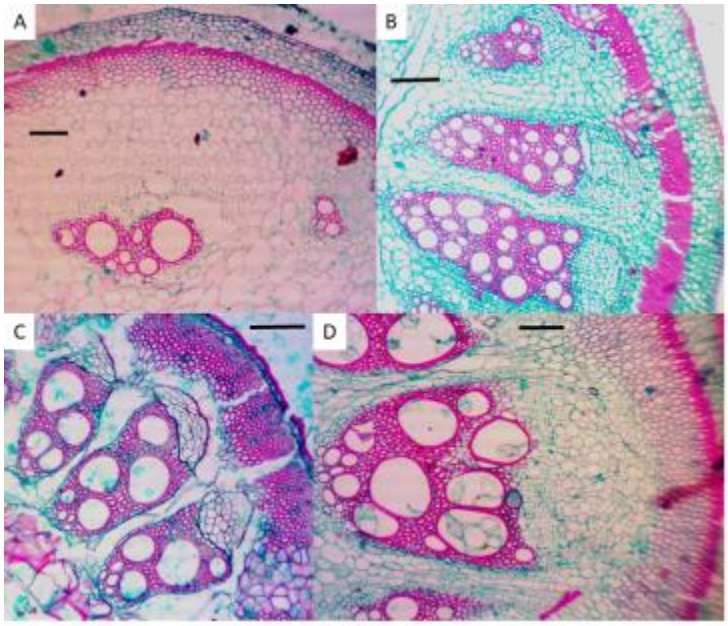

Fig. 1. Anatomical samples of *Aristolochia* between 1.7 and 2.1 mm in diameter

A: Sample of *A. pilosa* (1.7 mm in diameter) with developing phloem fibers with regularly spaced, small vascular bundles. B. Sample of *A. fimbriata* (1.8 mm) with fused fibers and varied vessel cell diameters. C: Sample of *A. maxima* (2.1 mm) with many phloem fused phloem fibers. D: Sample *A. acutifolia* (2.0 mm) showing several well developed vascular bundles. All bars = 100 μm.

Table 1. Number of vascular bundles in stem samples of *Aristolochia* species

	Species				
	A.	*A.*	*A.*	*A.*	*A.*
Stem sample	*acutifolia*	*fimbriata*	*maxima*	*pilosa*	*spathulata*
Smallest sample	11	9	13	7	9
Medium sample	11	9	13	6	10
Medium sample	---	7	13	7	12
Largest sample	11	9	16	7	10
Mean value	11	8.5	13.8	6.8	10.2
S.D.	--	1.0	1.5	0.5	1.2

3.2.2 Features in 2.2 to 4.5 mm Diameter Samples

As in smaller diameter samples, all species had a single-celled epidermis, seven to ten layers of cortical cells, and two or more cell layers of phloem fibers. All species, except *A. fimbriata*, had a two to nine-celled fused phloem fiber layer (Fig. 2A; 2B) internal to the phloem fibers. Parenchyma cells were present internal to the fused phloem fiber layer and collapsed phloem cells were present in all species except *A. fimbriata*. Some bundles were fused while there was a variety of bundle sizes (Fig. 2). For all species, stems were circular and

vascular bundles were distributed regularly. Diameters of vessels varied from small to large (Fig. 2C). Parenchyma cells of axial rays (Mauseth, 1988) were present between vascular bundles of all species. No traces of interfascicular cambia were present in any tissue samples. For most species, axial parenchyma cells were relatively uniform in size (Fig. 2A, 2C, 2D).

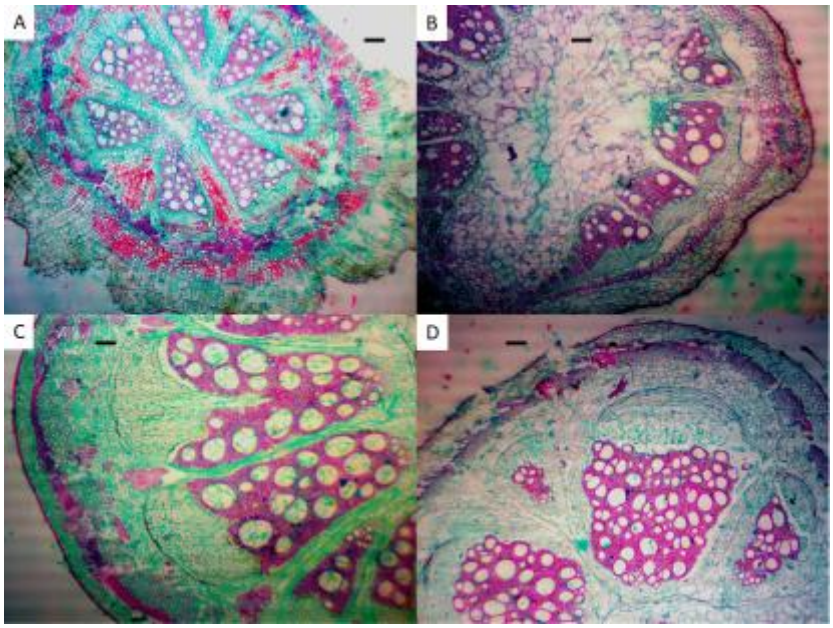

Fig. 2. Anatomical samples of *Aristolochia* between 2.2 and 4.5 mm in diameter

A: Sample of *A. fimbriata* (2.2 mm diameter) with few phloem fibers and few collapsed phloem cells near vascular bundles. Vascular bundles are regularly distributed, with peeled epidermis and uniform axial parenchyma. B: Sample of *A. spathulata* (3.0 mm) with developing phloem fibers and varied vessel diameters. C: Sample of *A. maxima* (4.5 mm) with many phloem fibers, one fused vascular bundle and axial parenchyma cells. D: Sample *A. pilosa* (3.8 mm) with large bundles and uniform shaped axial parenchyma cells. All bars = 100 μm.

3.2.3 Features in 4.0 to 5.5 mm Diameter Samples

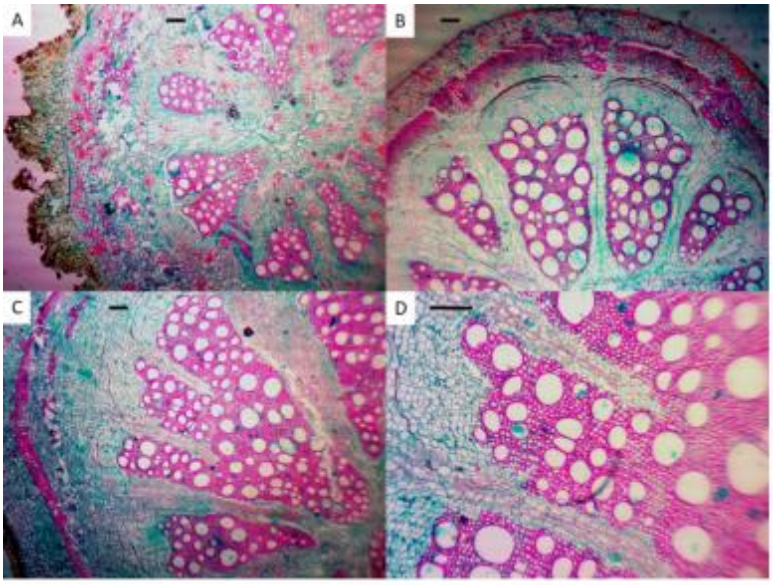

Fig. 3. Anatomical samples of *Aristolochia* between 3.9 and 5.5 mm in diameter

A: Sample of *A. fimbriata* (4.3 mm diameter) shows peeled epidermis and cortex, fused vascular bundles, few phloem fibers and no collapsed phloem cells near vascular bundles. Vascular bundles are regularly distributed. B: Sample of *A. spathulata* (3.9 mm) shows individual (not fused) vascular bundles. Note the uniform shapes of axial parenchyma cells. C: Sample of *A. pilosa* (5.5 mm) with fused vascular bundles and a variety of vessel

diameters. D: Enlargement of the sample in C of *A. pilosa* (5.5 mm) showing the uniform shapes of axial parenchyma cells. All bars = 100 μm.

All species had a single-celled epidermis. For all samples except *A. fimbriata* and *A. pilosa*, the epidermis and cortex were intact. For *A. fimbriata* and *A. pilosa* the outermost cortical cells peeled away (Fig. 3A). Two to three cell layers of phloem fibers were present in all samples. In all tissues except *A. fimbriata*, all species had a two to nine-celled fused phloem fibers layer (Fig 3B, Fahn 1967) internal to the phloem fibers. Parenchyma cells were present internal to the fused phloem cell layer. Collapsed phloem cells were present near vascular bundles in all species expect *A. fimbriata* (Fig. 3B, 3C). Some species (*A. acutifolia*, and *A. spathulata*) had separate individual bundles (Fig. 3B), while the other species had some individual bundles that were fused (Fig. 3C). Some bundles were large while others were smaller. All species had axial parenchyma with no trace of interfascicular cambia (Fig. 3D).

3.3 Vessel Radii and Xylem Conductivities

Stems of *A. acutifolia* had 2, 5 and 26 vessels above 60 μm in radius with 99, 128, 460 vessels with xylem conductivities of 38.7, 24.8 and 293 g cm^{-1} MPa^{-1} s^{-1} in stems of 2.0, 2.1 and 4.0 mm diameter, respectively (Table 2). In contrast with *A. acutifolia*, stems of *A. fimbriata* had no vessels above 60 μm in radius with 74, 187, 191, and 305 vessels with xylem conductivities of 0.03, 1.20, 3.63 and 4.05 g cm^{-1} MPa^{-1} s^{-1} in stems of 0.6, 1.8, 2.2 and 4.3 mm diameter, respectively (Table 3). Overall, xylem conducitivities for *A. acutifolia* were about 80 times higher than xylem conducitivities for *A. fimbriata.*

Table 2. Distribution of vessel radii in samples of *Aristolochia acutifolia*

Stem diameter (mm)	2.0	2.1	4.0
Number of vessels in stem	99	128	460
Number of vessels sampled	35	38	67
Xylem conductivities (g cm^{-1} MPa^{-1} s^{-1})	38.7	24.8	293
Vessel radii - Mean (number of cells)			
Less than 20 μm	15.1 (7)	15.4 (13)	18.5 (2)
Between 20 and 39.9 μm	26.7 (10)	28.8 (15)	30.3 (18)
Between 40 and 59.9 μm	46.8 (9)	46.7 (5)	51.5 (21)
Above 60 μm	85.6 (2)	70.6 (5)	73.5 (26)
Mean of all samples (μm)	41.8	32.5	53.4
T-test results	t-value	probability	
2.0 vs 2.1 samples	1.88	0.032	
2.0 vs 4.0 samples	-2.57	0.0058	
2.1 vs 4.0 samples	-5.09	0.00001	

Table 3. Distribution of vessel radii in samples of *Aristolochia fimbriata*

Stem diameter (mm)	0.6	1.8	2.2	4.3
Number of vessels in stem	74	187	191	305
Number of vessels sampled	20	39	74	59
Xylem conductivities (g cm^{-1} MPa^{-1} s^{-1})	0.04	1.20	3.63	4.05
Vessel radii Mean (number)				
Less than 20 μm	9.75 (20)	15.3 (16)	15.7 (13)	15.4 (16)
Between 20 and 39.9 μm	(0)	25.3 (11)	26.4 (30)	26.6 (24)
Between 40 and 59.9 μm	(0)	(0)	51.0 (2)	44.6 (1)
Mean of all samples (μm)	9.75	16.9	20.2	19.6
T-test results	t-value		probability	
0.6 vs 1.8 samples	-4.97		<0.00001	
0.6 vs 2.2 samples	-5.10		<0.00001	
0.6 vs 4.3 samples	-5.31		<0.00001	
1.8 vs 2.2 samples	-2.07		0.040	
1.8 vs 4.3 samples	-1.83		0.069	
2.2 vs 4.3 samples	-0.38		0.70	

Stems of *A. maxima* had 0, 0, 2, and 13 vessels above 60 μm in radius with 57, 116, 97, 220 vessels with xylem conductivities of 0.40, 4.57, 8.0, and 103 g cm^{-1} MPa^{-1} s^{-1} in stems of 0.6, 1.5, 2.0, and 4.5 mm in diameter, respectively (Table 4). Stems of *A. pilosa* had 36, 62, 240, and 562 vessels with xylem conductivities of 23, 5.06, 50.3, and 84.8 g cm^{-1} MPa^{-1} s^{-1} in stems of 1.7, 2.0, 3.8, and 5.5 mm diameter, respectively (Table 5). Xylem conductivities for *A. spathulata* were 0.19, 1.55, 4.75, and 39.9 g cm^{-1} MPa^{-1} s^{-1} for 56, 133, 149, and 279 vessels in stems of 1.0, 2.0, 3.0, and 3.9 mm diameter, respectively (Table 6). Within each of the five species, stems with the smallest diameter had significantly smaller vessel radii compared with those of the largest stems.

Table 4. Distribution of vessel radii in samples of *Aristolochia maxima*

Stem diameter (mm)	0.6	1.5	2.0	4.5
Number of vessels in stem	57	116	97	220
Number of vessels sampled	19	29	36	4
Xylem conductivities (g cm^{-1} MPa^{-1} s^{-1})	0.04	4.57	8.00	103
Vessel radii Mean (s.d.)				
Less than 20 μm	9.7 (19)	18.4 (14)	15.4 (12)	16.8 (9)
Between 20 and 39.9 μm	(0)	24.8 (13)	26.6 (13)	25.7 (15)
Between 40 and 59.9 μm	(0)	49.9 (3)	49.0 (6)	55.9 (7)
Above 60 μm	(0)	(0)	64.7 (2)	74.8 (13)
Mean of all samples (μm)	9.69	23.2	28.7	43.2
T-test results	t-value		probability	
0.5 vs 1.5 samples	-4.89		<0.0001	
0.5 vs 2.0 samples	-5.44		<0.0001	
0.5 vs 4.5 samples	-5.81		<0.0001	
1.5 vs 2.0 samples	-1.61		0.11	
1.5 vs 4.5 samples	-4.02		0.00014	

Table 5. Distribution of vessel radii in samples of *Aristolochia pilosa*

Stem diameter (mm)	1.7	2.0	3.8	5.5
Number of vessels in stem	36	62	240	562
Number of vessels sampled	24	33	55	56
Xylem conductivities (g cm^{-1} MPa^{-1} s^{-1})	1.23	5.06	50.3	84.8
Vessel radii Mean (s.d.)				
Less than 20 μm	13.6 (12)	15.4 (6)	14.4 (4)	18.6 (3)
Between 20 and 39.9 μm	24.3 (10)	28.6 (19)	30.7 (25)	29.3 (28)
Between 40 and 59.9 μm	52.1 (2)	48.3 (9)	51.5 (15)	48.2 (14)
Above 60 μm	(0)	(0)	69.0 (5)	66.6 (4)
Mean of all samples (μm)	21.3	31.5	39.8	37.1
T-test results	t-value		probability	
1.7 vs 2.0 samples	-3.12		0.0028	
1.7 vs 3.8 samples	-5.02		<0.00001	
1.7 vs 5.5 samples	-4.85		<0.00001	
2.0 vs 3.8 samples	-2.52		0.0134	
2.0 vs 5.5 samples	-1.90		0.063	
3.8 vs 5.5 samples	0.89		0.376	

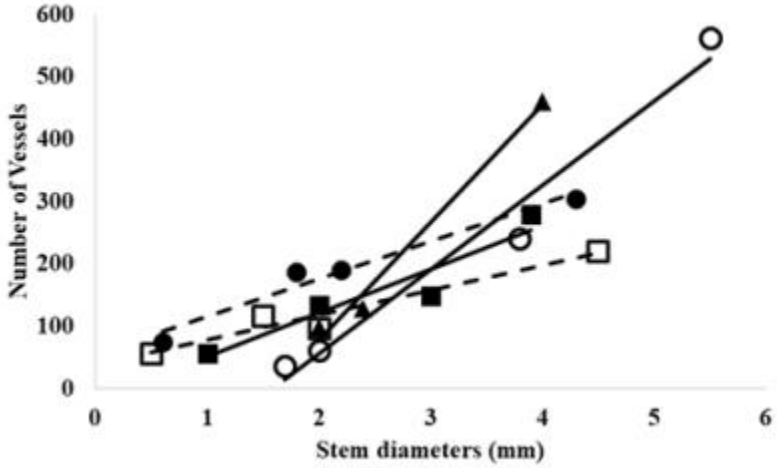

Fig. 4. Relationship between stem diameters and number of xylem vessels for five species of *Aristolochia*

Closed triangles, solid line: *A. acutifolia, slope* 47.9; Closed circles, dashed line: *A. fimbriata, slope* 6.010; Open squares, dashed line: *A. maxima, slope* 39.8; Open circles, solid line: *A. pilosa, slope* 135. Solid squares, solid line: *A. spathulata, slope* 70.1. All r^2 values were above 0.92.

Numbers of vessels were linearly related with stem diameters with slopes 47.9, 6.01, 39.8, 135, and 70.1 for *A. acutifolia, A. fimbriata, A. maxima, A. pilosa,* and *A. spathulata,* respectively (Fig. 4). All r^2 values were above 0.92. For *A. fimbriata,* there were 74 vessels for the 0.6 mm diameter sample and 305 vessels for the 4.3 mm diameter sample to produce a slope of 6.01. In contrast, for *A. pilosa* there were 36 vessels for the 1.7 mm diameter sample and 562 vessels for the 5.5 mm diameter sample to give a slope of 135.

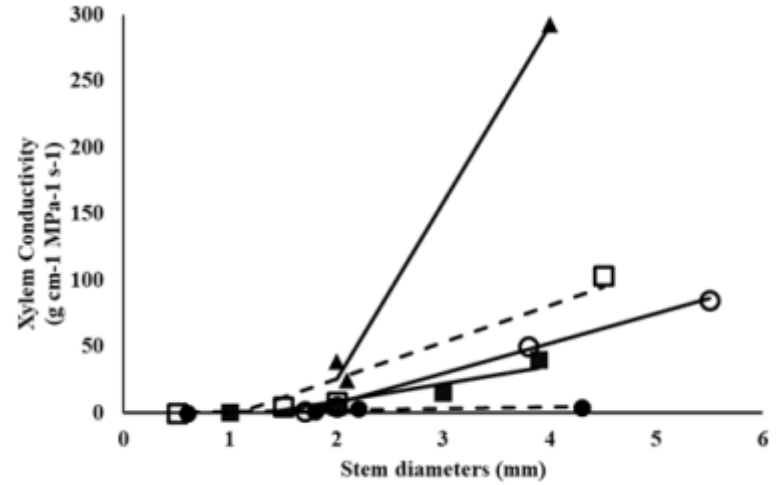

Fig. 5. Relationship between stem diameters and xylem conductivities for five species of *Aristolochia*

Closed triangles, solid line: *A. acutifolia, slope* 138; Closed circles, dashed line: *A. fimbriata, slope* 1.06; Open squares, dashed line: *A. maxima, slope* 27.7; Open circles, solid line: *A. pilosa, slope* 22.5. Solid squares, solid line: *A. spathulata, slope* 13.3. All r^2 values were above 0.89

Xylem conductivities were not similar among species but they varied as a function of stem diameters. Slopes of relationships of xylem conductivities and stem diameters were 138, 1.06, 27.7, 22.5, and 13.3 for *A. acutifolia, A. fimbriata, A. maxima, A. pilosa,* and *A. spathulata,* respectively. All r^2 values were above 0.78 (Fig. 5). For *A. fimbriata* the xylem conductivity was 0.04 g cm^{-1} MPa^{-1} s^{-1} for the 0.6 mm diameter sample and 4.05 g cm^{-1} MPa^{-1} s^{-1} for the 4.3 mm diameter sample to produce a slope of 1.06. In contrast, for *A. acutifolia,* the xylem conductivity was 38.7 g cm^{-1} MPa^{-1} s^{-1} for the 2.0 mm diameter sample and 293 g cm^{-1} MPa^{-1} s^{-1} for the 4.0 mm diameter sample to produce the slope of 138.

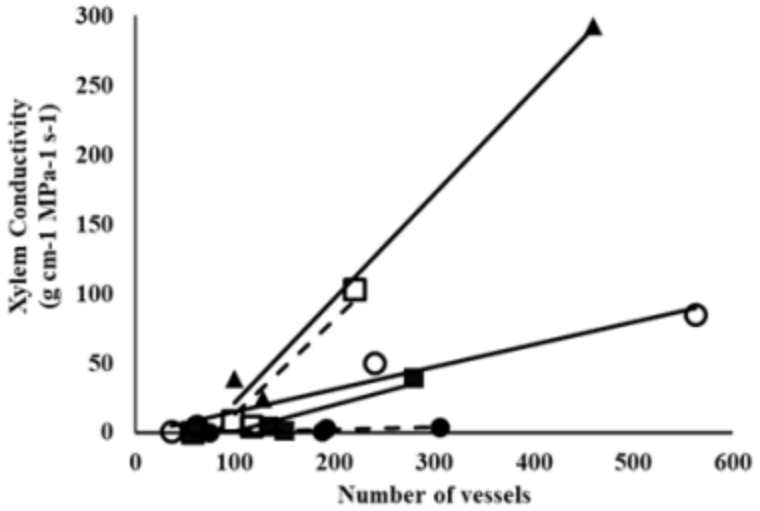

Fig. 6. Relationship between number of vessels and xylem conductivities for five species of *Aristolochia*

Closed triangles, solid line: *A. acutifolia, slope* 0.78; Closed circles, dashed line: *A. fimbriata, slope* 0.17; Open squares, dashed line: *A. maxima, slope* 0.68; Open circles, solid line: *A. pilosa, slope* 0.16. Solid squares, solid line: *A. spathulata, slope* 0.19. All r^2 values were above 0.82

Slopes of relationships of xylem conductivities and numbers of vessels were 0.78, 0.17, 0.68, 0.16, and 0.19 for *A. acutifolia, A. fimbriata, A. maxima, A. pilosa,* and *A. spathulata,* respectively. All r^2 values were above 0.78 (Fig. 6). The lower slope values for *A. pilosa* and *A. fimbriata* may be attributed to the low mean vessel radii of 19.6 and 37.1 mm, respectively for their largest diameter samples. In contrast, the high slope value may be attributed to the large mean vessel radius of 73.5 mm for the largest diameter sample of *A. acutifolia*.

Analyses were performed to determine the contributions of vessel radii to xylem conductivities. Since values of μm^4 radius are the basis to calculate xylem conductivities, values of μm^4 radius were determined for the largest diameter samples of each species (Table 7). For each species values of μm^4 radius with (1) all vessels, (2) without the smallest vessels, (3) without the smaller half of vessels and (4) without the smaller three quarters of vessels were determined. For the above four categories, mean percentages of μm^4 radius values were 98.9, 94.7 and 80.1% of all the vessels, respectively. The data indicate that the smallest vessels made little to no contribution while the smallest half of vessels added only 5% to all μm^4 radius values. Therefore, the largest half of all vessels supplied 95% of total xylem conductivities.

Table 6. Distribution of vessel radii in samples of *Aristolochia spathulata*.

Stem diameter (mm)	1.0	2.0	3.0	3.9
Number of vessels in stem	56	133	149	279
Number of vessels sampled	17	37	33	44
Xylem conductivities (g cm^{-1} MPa^{-1} s^{-1})	0.19	1.55	4.75	39.9
Vessel radii Mean (s. d.)				
Less than 20 μm	11.3 (14)	15.3 (16)	15.7 (13)	15.4 (16)
Between 20 and 39.9 μm	24.1 (3)	25.7 (16)	28.5 (10)	27.4 (17)
Between 40 and 59.9 μm	(0)	45.7 (5)	50.3 (9)	51.4 (8)
Above 60 μm	(0)	(0)	66.3 (1)	73.6 (3)
Mean of all samples (μm)	13.5	30.5	23.9	30.6
T-test results	t-value		probability	
1.0 vs 2.0 samples	-3.59		0.0001	
1.0 vs 3.0 samples	-4.23		0.0001	
1.0 vs 3.9 samples	-3.86		0.0003	
2.0 vs 3.0 samples	2.03		0.046	
2.0 vs 3.9 samples	-1.98		0.0514	
3.0 vs 3.9 samples	-0.008		0.99	

Table 7. Influence of vessel radii on the calculations of the Hagen-Poiseuille equation for the stem samples of *Aristolochia*

	Sample					
	A. Acutifolia	*A. fimbriata*	A. *maxima*	A. *pilosa*	*A. spathulata*	Mean (s.d.)
Stem diameter (mm)	4.0	4.3	4.5	5.5	3.9	
Number of vessels in sample	460	305	220	562	279	
Number of vessels sampled	67	59	44	56	44	
Radius of largest vessel (μm)	109	44.5	101	72.2	76.1	
Radius smallest vessel (μm)	17.3	8.6	11.0	16.3	10.8	
Vessel radii (μm)						
Smallest quarter	35.6>	13.4>	20.5>	26.8>	16.5>	
Smallest half	53.3>	17.8>	31.0>	32.5>	24.5>	
Smallest three-quarters	63.8>	24.8>	66.7>	45.5>	34.8>	
Sum of all μm^4 radius values (percent of all)						
Without smallest quarter	1.07×10^9 (98.1)	1.97×10^7 (98.5)	5.27×10^7 (99.8)	1.84×10^8 (97.9)	1.60×10^8 (100)	98.9 (0.98)
Without smallest half	9.85×10^8 (90.3)	1.88×10^7 (94.0)	5.21×10^7 (98.6)	1.74×10^8 (92.6)	1.57×10^8 (98.2)	94.7 (3.6)
Without smallest three quarters	7.92×10^8 (72.7)	1.55×10^7 (78.0)	4.30×10^7 (81.4)	1.43×10^8 (76.1)	1.47×10^8 (92.1)	80.1 (7.6)
All	1.09×10^9 (100)	2.00×10^7 (100)	5.00×10^7 (100)	1.88×10^8 (100)	1.60×10^8 (100)	

4. Discussion

Vines need support to grow vertically and wrap around supports. Some of the mechanical aspects of vines and vine growth has been described by Niklas (1992). Values of flexural stiffness, the product of the second moment of area and elastic modulus, are low for vines (Niklas, 1992). The low values of flexural stiffness of vine stems may result from the furrowed xylem structure that includes the lack of a continuous, circular cambium with resultant compact secondary xylem and fused or continuous phloem fiber layers. Moreover, the thin walled axil parenchyma tissues between individual vascular bundles and the lack of fused phloem fibers in species of *Aristolochia* may aid in the flexibility for stems to wrap around supports.

Tamaio *et al*. (2010) described the development of new vascular bundles in stems of *Cissampelos andromorpha* related to xylem furrowing. In this study, stems of *A. fimbriata*, *A. maxima*, and *A. pilosa* produced new vascular bundles adjacent to existing vascular bundles. In stems of *A. pilosa*, two new vascular bundles were produced near each existing vascular bundle.

Tamaio *et al*. (2010) stated that new vascular bundles are produced from cambium cells that separate from exiting cambium (fragmented cambium) to produce independent vascular bundles. A fragmented cambium may produce new of vascular bundles in the species of this study.

Xylem furrowing occurred in roots of *Urvillea rufescens*, *Serjania lethalis* and *S. caracasana* that was attributed to phloem wedges, additional phloem cells that occur in vascular strands and cylinders formed with secondary phloem that connects with the central cylinder during root enlargement (Bastos *et al*., 2016). Another interpretation of their images is that the vascular cambium ceased to produce xylem cells but continued to produce phloem cells. This explanation fits the images shown by Bastos *et al*. (2016). For species of *Aristolochia* phloem wedges were not present. The only axial parenchyma were present between vascular bundles for the *Aristolochia.* For *Aristolochia*, a complete vascular cambium never developed, thus the interfascicular cambium never formed.

Bastos *et al*. (2016) suggested that several species of the Sapindaceae have vessel dimorphism, large and small vessels only. Based upon the images of Bastos *et al*. (2016) *S. lethalis* and *P. carpopoda* have primarily large and small vessel diameters. However, images of *S. caracasana*, *T. scadens* and *U. rufescens* exhibit a variety of vessel diameters. To determine if vessel radii were dimorphic for species of *Aristolochia*, analyses were performed to determine the differences in radii among vessels. Large differences occurring among vessel radii may indicate vessel dimorphism. However, if only small differences occurred among vessel radii, then the idea of dimorphisms is not supported. The largest difference between samples was 9.6, 6.25, 23.6, 4.53 and 12.0 μm

in which the entire range of values was 88.2, 35.9, 90.4, 55.9 and 63.3 μm for the largest diameter stem samples of *A. acutifolia, A. fimbriata, A. maxima, A. pilosa,* and *A. spathulata,* respectively (Table 8). For these same largest stem samples, the number of vessel differences of more than 4.0 μm were 5, 1, 3, 2 and 6 for *A. acutifolia, A. fimbriata, A. maxima, A. pilosa,* and *A. spathulata,* respectively. These data do not support the idea of vessel radii dimorphism for the *Aristolochia* species.

Table 8. Vessel characteristics of stem samples of *Aristolochia* species

	Species				
	A.	*A.*	*A.*	*A.*	*A.*
Stem sample	a*cutifolia*	*fimbriata*	*maxima*	*pilosa*	*spathulata*
Smallest sample					
Number of vessels sampled	35	20	29	33	17
Largest radius (μm)	85.6	13.8	58.5	59.8	27.8
Smallest radius (μm)	12.8	6.3	8.9	11.1	7.5
Range of radii (μm)	72.8	7.5	49.6	48.7	20.3
Differences in radii among successive vessel.					
Largest difference (μm) [1]	7.2	1.5	10.3	5.38	4.2
Number of samples (differences) [2]					
More than 4.0 μm	6	0	3	3	1
Between 2 to 3.99 μm	8	0	3	3	2
Between 1 to 1.99 μm	7	1	6	12	3
Less than 1.0 μm	13	19	16	14	10
Medium sample					
Number of vessels sampled	38	74	37	55	37
Largest radius (μm)	85.1	57.5	65.2	76.5	51.4
Smallest radius (μm)	12.0	8.1	8.2	9.6	8.2
Range of radii (μm)	72.1	49.4	57.0	66.9	43.2
Differences in radii among successive vessel.					
Largest difference (μm) [1]	15.8	11.8	12.5	6.75	8.1
Number of samples (differences) [2]					
More than 4.0 μm	3	2	2	3	1
Between 2 to 3.99 μm	9	3	5	9	7
Between 1 to 1.99 μm	7	6	10	12	5
Less than 1.0 μm	18	62	19	30	23
Largest sample					
Number of vessels sampled	67	59	44	56	44
Largest radius (μm)	105.5	44.5	101	72.2	76.1
Smallest radius (μm)	17.3	8.6	11.0	16.3	10.8
Range of radii (μm)	88.2	35.9	90.4	55.9	63.3
Differences in radii among successive vessel.					
Largest difference (μm) [1]	9.6	6.25	23.6	4.53	12.0
Number of samples (differences) [2]					
More than 4.0 μm	5	1	3	2	6
Between 2 to 3.99 μm	8	1	6	6	3
Between 1 to 1.99 μm	16	8	14	11	7
Less than 1.0 μm	37	48	20	36	27

A variety of vessel diameters is advantageous for plants exposed to environmental extremes. Conductivity is proportional to vessel diameter to the fourth power according to the Poiseuille's law (Zimmermann, 1983; Sperry *et al*., 2006), so the contribution of large vessels may make the largest contribution to xylem conductivity. However, if embolisms occur to larger vessels (Ewers *et al*., 1997), smaller diameter vessels may ensure the water column is maintained (Brodersen *et al*., 2013). Lianas are well known for having wider vessels in their wood than the vessels of self-supporting relatives (Ewers and Fisher, 1991; Angyalossy *et al*., 2012; Wyka *et al*., 2013). Ewers and Fisher (1991) demonstrate that lianas and their free-standing relatives had mean maximum vessel diameters of 234 and 116 μm, respectively. According to Poiseuille's law, the larger vessels for Lianas should provide 16 times more efficient conductivity than free-standing relatives (Ewers and Fisher, 1991).

Values of xylem conductivities involving the Hagen-Poiseuille equation are most relevant in non-woody tissues in which xylem cells are mostly involved with water transport and are less involved with mechanical support. Examples of plant tissues less involved with mechanical support are compound leaves (McCulloh *et al*., 2009), young plants of *Psilotum nudum* (McCulloh and Sperry, 2005b), terminal cactus stems (Evans and Skonieczny, 2015), terminal shoot meristems (Evans *et al*., 2016), grass leaves (Evans and Ortega, 2019) and the stems of *Aristolochia* of this study. Xylem conductivities of many stems of the present study were small between 3 and 5 g cm^{-1} MPa^{-1} s^{-1}. In contrast primary leaf veins of several plants such as *Catalpa bignoniodies*, *C. speciosa*, *Oxydendrum arboretum*, and *Phytolacca americana* had large xylem conductivities of 20.4, 12.9, 4.47, and 5.08 g cm^{-1} MPa^{-1} s^{-1}, respectively (Evans *et al*., 2020). The xylem conductivities of stems of *Aristolochia* were smaller than primary veins of some leaves. In summary, the xylem conductivities of the stems of *Aristolochia* in this study are small.

To our knowledge, this is the first publication to document the development of furrowed xylem in *Aristolochia* stems. Results exhibit the following: (1) interfascicular cambia were not present, since only axial cells were present, (2) numbers of vascular bundles in stems were specific for each species and did not increase as stems enlarged, (3) radii of vessels were not dimorphic for any species, (4) numbers of vessels were linearly related with stem diameters, and (5) the largest half of all vessels supplied 95% of total xylem conductivity.

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Declaration of competing interest

The authors have no competing interests.

Author contributions

Experimental design, sample procurement, microscopic preparation, data analysis, line figure and figure preparation, manuscript preparation and editing was performed by LSE. All photographs of tissue samples, all vessel diameters, analyses of vessels and xylem conductivity calculations and figure preparations were done by MCE.

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