RESEARCH PAPER

Quantitative and qualitative changes in primary and secondary stem organization of *Aristolochia macrophylla* during ontogeny: functional growth analysis and experiments

Tom Masselter* and Thomas Speck

Plant Biomechanics Group, Botanic Garden, University of Freiburg, Faculty of Biology, Schänzlestr. 1, D-79104 Freiburg, Germany

Received 29 January 2008; Revised 18 April 2008; Accepted 6 May 2008

Abstract

The anatomy of young and old stems of *Aristolochia macrophylla* has been investigated for a better understanding of how secondary growth processes cause changes in the stem anatomy of a lianescent plant. In *A. macrophylla*, following an increase in volume of secondary vascular tissues, the cortical tissues are deformed and the outer sclerenchymatous cylinder ruptures. Morphometric measurements prove that the inner zone of the cortical parenchymatous tissue is compressed prior to the rupture of the outer sclerenchymatous cylinder. After the rupture has occurred, the radial width of the inner primary cortex slightly increases again. This could be caused by strain relaxation, suggesting that the inner primary cortex mechanically behaves similarly to cellular technical foam rubbers. Two different experiments were undertaken to test the outer cortical cylinders mechanically. The outer cortical cylinders comprise the outer sclerenchymatous cortical tissue and a collenchymatous sheath underneath the epidermis and the epidermis. In a first experiment, transverse compression loads were applied to the outside of the cortical cylinders causing ovalization of the cylinder until failure. This experiment allowed the Young’s Modulus of the outer cortical cylinders to be determined. In a second set of experiments, radial hydraulic pressure was applied to the inside of the cortical cylinders, mimicking the mechanical effects of internal growth processes. The increase of the internal pressure finally led to rupture of the cortical cylinders. The circumferential stresses acting on the inner surface of the cortical cylinders were calculated. These data allow quantitative estimates of the radial and circumferential pressures effected by vascular secondary growth processes during ontogeny in *A. macrophylla* stems. The experimental results further indicate that the outer sclerenchymatous cylinder is the main contributor to mechanical stability of young *A. macrophylla* stems.

Key words: *Aristolochia macrophylla*, biomechanics, ontogeny, primary organization, secondary growth, vine.

Introduction

Ontogeny and stem anatomy

The mechanical constraints acting on vines are very different from those acting on trees. In young ontogenetic stages, vines are stiff in bending and torsion, a characteristic that allows young ‘searcher twigs’ to span gaps between different host trees. In older ontogenetic stages, vines develop a high flexibility in bending and torsion that enables them to follow movement of the supporting host trees and to sustain branch and stem failures of their host trees without being damaged. Old vine stems are characterized by a high ability to resist tensile, bending and torsional stresses and strains (Speck and Vogellehner, 1992; Speck, 1994a; Speck and Rowe, 2001; Rowe and Speck, 2004), and show a significant wound healing ability (Wilson and Grange, 1984; Fisher and Ewers, 1989). In *Aristolochia macrophylla*, these properties are a consequence of the primary and secondary organization...
of the stems, and its variation during ontogeny (Figs 1, 2). There are three major adaptations to the lianescent growth strategy in *A. macrophylla* (Speck, 1994a, b; Masselter and Speck, 2006). (i) Embedding of the isolated vascular bundles in a soft parenchymatous matrix. (ii) Development of secondary wood with a high cell lumen to cell wall ratio containing many large-diameter vessels and huge parenchymatous wood rays (in older ontogenetic stages). (iii) Rupture of the outer sclerenchymatous and collenchymatous cylinders in older ontogenetic stages (Fig. 1B, D, F, G).

The embedding of stiff vascular bundles in soft parenchyma cells also influences the deformation of primary tissues during secondary growth in *A. macrophylla*. Unlike the growth processes observed in most self-supporting woody plants, in *A. macrophylla*, the abundant development of secondary xylem (Fig. 1) and secondary phloem deforms primary tissues not only peripheral to the vascular bundles but also toward the stem centre. The inner zone of the parenchymatous primary cortex is radially deformed, whereas the deformation of the pith takes place in a preferential direction. In the direction normal to this flattening the pith remains (nearly) undeformed (Fig. 1B, G).

As long as it is intact, the sclerenchymatous cortex prevents the deformation of the outermost cortex tissues, and contributes largely to the high bending and torsion stiffness observed in young stems (Fig. 1A, C, E; Speck, 1994a, b).

The development of secondary xylem comprising vessels with a high cell lumen to cell wall ratio and large parenchymatous wood rays, as well as the rupture of the sclerenchymatous and collenchymatous cylinders in older axes, leads to a significant decrease in Young’s Modulus and torsional modulus in older ontogenetic stages (Speck and Vogellehner, 1992; Speck, 1994a; Speck and Rowe, 1999).

Ongoing secondary growth, i.e. an increase in volume of the vascular tissues, causes increasing radial stresses and strains and finally significant compression of the inner parenchymatous cortex tissues, and circumferential (tangential) stresses and strains in the sclerenchymatous outer cortex cylinder. In older stems, the cells of the sclerenchymatous tissue debond and the sclerenchymatous cylinder finally ruptures into segments (Fig. 1B, D, F, G). The fissures are not distributed randomly in the stem cross-section, as they alternate with the remaining fragments of the ruptured sclerenchymatous cylinder which are situated ‘above’ the vascular bundles (Fig. 1G). The zones, where the fissures are formed, putatively represent regions of tangential stress concentrations. These fissures are quickly repaired by parenchymatous cells, which swell into the cracks and seal them. In later phases of the fissure repair, radial and tangential cell division of the repairing cells takes place, and the cell walls of the fissure-repairing cells may thicken and lignify (Haberlandt, 1924; Schöttgen, 1983; Speck et al., 2004a, b, 2006; Speck O. et al., 2006). By these processes the structural function of the sclerenchymatous cylinder can be fully or partly restored for a given time. Fissures occurring in older stem parts are still sealed by parenchymatous cells, but the cell walls of the repairing cells do not thicken and lignify any more. In this case, the decrease in the contribution of the sclerenchymatous cylinder to flexural and torsional stiffness of the plant becomes permanent (Speck, 1994a, b; Speck and Rowe, 1999, 2001; Speck et al., 2004c; Rowe and Speck, 2005).

In order to quantify stresses and strains occurring in the inner primary parenchymatous cortex and in the sclerenchymatous cylinder, experimental measurements of breaking strengths and stresses of the cortical cylinder were carried out by mechanically mimicking secondary vascular growth processes (Masselter and Speck, 2006).

### Materials and methods

#### Sampling and measuring of specimens

Material of *A. macrophylla* was collected from a specimen in the Botanical Garden, Freiburg. Particular attention was paid to use only plant axes without any visible damage. In cross-sections, tissue areas were measured with the digital image analysis software ‘Optimas’ (Bioscan Inc.) after scanning the outlines of these tissues previously drawn on acetate sheets. Values of the radial width of the inner parenchymatous primary cortex as well as of the radial width of the vascular cambial tissues (i.e. secondary wood and secondary phloem including the vascular cambium) are plotted against the distance from stem apex.

#### Normalizing of data

Stems of *A. macrophylla* show a large variability of stem girth, tissue geometry, and relative tissue areas from nodes to internodes as well as from one internode to the next (Fig. 2), i.e. a distinct ‘short-distance’ variability of tissue geometry and distribution. This variation tends to overlay the overall ‘long-distance’ ontogeny, in terms of increasing areas of secondary tissues and changes of relative size of the primary cortex. In order to deal with this problem, the measured values of the radial width of the inner primary cortex have been normalized, by dividing them by the width of a primary tissue that is not deformed by the secondary tissues. Although the pith in *A. macrophylla* is deformed during ontogeny (Fig. 1B, G), due to the non-uniformity of the deformation, there exists one direction in which the pith remains (relatively) undeformed by secondary growth and retains a (nearly) constant diameter throughout the entire ontogeny. This undeformed diameter (Fig. 1B, G; direction y) is arranged normal to the preferential direction of compression (Fig. 1B, G, direction x). The pith diameter in undeformed direction (diameter d; Fig. 1E) is used for normalizing the inner primary cortex by dividing the radial width of this tissue by the diameter d. This normalization allows a (semi-)quantitative analysis of the compression of the inner primary cortex due to stresses caused by cambial secondary growth.

#### Mechanical experiments

Plant segments were cut from the same specimen of *A. macrophylla*. Particular attention was paid to choosing undamaged and straight axes. These were cut into 3 cm long segments. The pith, the vascular bundles, and the inner parenchymatous primary cortex
Fig. 1. Cross-sections of stems of *Aristolochia macrophylla*. (A) One-year-old axis. (B) Two-year-old axis. 1, pith; 2, secondary xylem; 3, cambium; 4, secondary phloem; 5, inner parenchymatous zone of primary cortex; 6, sclerenchymatous cylinder; 7, outer parenchymatous zone of primary cortex; 8, collenchymatous cylinder; 9, epidermis. The deformed diameter of the pith in (B) ranges vertically from the uppermost vascular bundle to the lowermost vascular bundle, i.e. the pith is nearly uncompressed along direction y, whereas the highest compression occurs along direction x. (C) Detail of (A), showing a crushed vessel in the secondary xylem. (D) Detail of (B). (E) Schematic drawing of the cross-section of an *A. macrophylla* stem; d, undeformed diameter. (F) Detail of the sclerenchymatous cylinder in the cortex of *A. macrophylla*, showing a radial rupture. (G) Five-year-old axis. (A, B) scale bars = 3 mm; (C, D) scale bars = 200 μm; (E) scale bar = 1 mm; (F) scale bar = 100 μm; (G) scale bar = 5 mm.
were removed with standard drills, so that the sclerenchymatous cylinder and the more peripheral tissues (outer parenchymatous primary cortex, collenchymatous cylinder, epidermis) were still intact. It was not possible to remove the tissues outside the sclerenchymatous cylinder fully without damaging parts of the sclerenchyma. It is assumed that these parts do not significantly affect the data. (This is confirmed by experiments with set-up 2 in which the outer zone of the parenchymatous primary cortex (7 in Fig. 1) as well as the collenchymatous sheath (8 in Fig. 1) were lacerated with longitudinal cuts without damaging the sclerenchymatous cylinder. In each experiment, at least two of these cuts were made along the whole length of the plant segments.) Two different set-ups were used for determining the structural Young’s Modulus of the outer cortical cylinder in a circumferential direction (set-up 1), and the radial and circumferential (tangential) stresses (set-up 2) necessary to break it. With set-up 1 the structural Young’s Modulus of the cortical cylinder in a circumferential direction is measured by the transverse compression of the hollowed-out cortical cylinder. Significant transverse deformation occurs in plants with hollow stems as in the giant reed *Arundo donax* as a consequence of stem bending and will finally lead to local buckling (Spatz et al., 1995, 1997, 1998). It does not occur in plant with solid stems as in *A. macrophylla*. However, this test is well suited for determining the Young’s Modulus of the cortical cylinder in circumferential direction as: (i) the entire cortical cylinder is tested and not only a small part of it, thus reducing the odds of measuring an especially strong or an especially weak part accidentally; and (ii) measurement of the structural Young’s Modulus of the cortical cylinder in a circumferential direction would have otherwise required a tensile or bending test on curved sections of the cortical cylinder causing significant experimental problems.

Set-up 2 allows direct conclusions on the pressure exerted by secondary growth on the inner surface of the sclerenchymatous cylinder before rupturing.

**Set-up 1: Structural Young’s Modulus in a circumferential direction:** The hollowed out stem segments were transversally deformed (Fig. 3A, B) with an Instron testing machine which measured force and displacement during compression. The cortical cylinder of *A. macrophylla* is considered as thin walled, since its inner diameter/wall ratio is in the transition zone between thick (<25) and thin (>20) walled cylinders. For cylinders with this structure the error using thin-walled cylinder theories is still relatively small (<5%; Stephens, 1970), because shear strains can be neglected in a first approximation. It was possible to assess the structural Young’s Modulus in a circumferential direction with a numerical method for thin-walled cylinders developed by Spatz et al. (1995, 1997, 1998; see also Stephens, 1970).

The formulae below allow the structural Young’s Modulus to be calculated as a function of the force at a given elastic deformation of the cylinder. For determining the Young’s Modulus of the cortical cylinder, the Young’s Modulus was calculated using the force-displacement curves that were measured while transversally deforming the cortical cylinder. Values of the Young’s Modulus were calculated for all the data points for \( x > 0.1 \) mm and prior to failure of the cortical cylinder. We believe that thereby only data points that were in the elastic range were used.

\[
F = 2 \times S \left( \frac{1}{B} - \frac{A}{R^2} \right)
\]

where \( F \) is the force; \( S \) is the bending stiffness; \( R \) is the median radius of the original circle; \( 2A \) is the median short axis of the deformation ellipse; and \( 2B \) is the median long axis of the deformation ellipse.

The bending stiffness can be expressed as

\[
S = E_{\text{mean}} \times I \quad \text{and} \quad E_{\text{mean}} = \frac{S}{I}
\]

where \( E_{\text{mean}} \) is the structural Young’s Modulus in circumferential direction, and \( I \) is the axial second moment of area given by

\[
I = L \times \int_{r_1}^{r_2} (x - R)^2 \, dx \quad r_2 \leq x \leq r_1
\]

where \( L \) is the length of the segment under compression; \( r_2 \) is the inner radius of the original cylinder; and \( r_1 \) is the outer radius of the original cylinder.

\[
R = \frac{(r_1 + r_2)}{2}
\]
Set-up 2: Breaking stress of the cortical cylinder: The hollowed plant stems were glued to a steel cylinder which was connected to a syringe barrel (Fig. 4). An end cap was glued at the bottom of the plant stem to seal off the set-up. The plant-syringe system was filled with water and placed in an Instron testing machine, which exerted an increasing force onto the plunger of the syringe. The rising pressure $p$ in the water column of the set-up induced radial and circumferential stresses to the inner surface of the cortical cylinder, which failed at a certain load (Fig. 3C, D). This method is used for simulating stresses in the outer sterome induced by cambial secondary growth processes. It can be assumed that stresses due to actual secondary growth processes also act on the whole cortical cylinder.

For assessing the circumferential stress $\sigma_c$ at the point of failure, cylinder theories can be used, as described by Stephens (1970).

$$p = \frac{F}{\pi r_s^2}$$

$P$ is the pressure in the water column; $F$ is the force of the Instron testing machine; and $r_s$ is the inner radius of the syringe barrel.

With the inner radius $r_2$ and outer radius $r_1$ of the cortical cylinder, then:

$\sigma_r = p$ when $r=r_2$

$\sigma_c = 0$ when $r=r_1$

The maximum radial $\sigma_r$ and circumferential stresses $\sigma_c$ occur at $r=r_2$:

$$\sigma_c = -\frac{pr_1^2 - r_2^2}{r_1^2 - r_2^2}$$

the negative sign indicating tension.

In all, 144 axes were tested with the pressure device. 95 experiments failed for different experimental reasons, the most common problem was that the adhesive joints between the plant material and the steel or plastic failed before the pressure was high enough to rupture the cortical cylinder. Of the 49 successfully measured samples, 20 young axes with an intact cortex were tested. To analyse the influence that the sclerenchymatous and/or the collenchymatous tissues had on the pressure resistance of young specimens, the parenchymatous-collenchymatous (five successful tests) or the sclerenchymatous tissues (six successful tests) were artificially damaged. Laceration of the collenchymatous sheath and the outer parenchymatous primary cortex was done by using a modified scalpel allowing a well-defined depth of cutting. The sclerenchymatous component, on the other hand, was damaged by quick manual ovalization of the plant segment causing an audible and visible failure of the sclerenchymatous tissue. In addition, 13 axes with ontogenetically split sclerenchymatous cylinders were successfully tested. In five of the latter axes, the collenchymatous tissue was lacerated to test its mechanical importance.

Statistical significance was calculated with $t$-tests using the Scheffé-procedure.

**Results**

The values for the radial width of the vascular cambial tissues and the parenchymatous inner zone of the primary cortex differed considerably in the three axes tested (Fig. 5). This also held true to a lesser degree for the normalized radial width of the inner zone of the primary cortex. Nevertheless, it was possible to identify main trends for the changes in radial width of the vascular cambial tissues and of the inner parenchymatous zone of the primary cortex.

**Vascular cambial tissues**

The radial width of the vascular cambial tissues (VCT, including secondary xylem and secondary phloem) increased from young fully differentiated stem parts (arrows 1 in Fig. 5) to older stem parts in which rupturing of the sclerenchymatous cylinder was visible for the first time (arrows 2 in Fig. 5). In the young stem parts the values were 0.53 mm (axis 1), 0.26 mm (axis 2), and 0.21 mm (axis 3), respectively. At the point of rupture of the sclerenchymatous cylinder, the values had increased to 0.83 mm (axis 1), 0.53 mm (axis 2), and 0.62 mm (axis 3). In older ontogenetic stages beyond the region of the first rupturing of the sclerenchymatous cylinder, further increase in radial width of the VCT occurred in two of the stems analysed (to 4.6 mm in axis 1, and to 0.99 mm in axis 2). In stem 3, on the contrary, a slight decrease to 0.49 mm was found (final data points to the outmost right in Fig. 5). Some axes showed collapsed vessels, mostly
located at the onset of the second vegetation period (Fig. 1C).

**Inner parenchymatous zone of the primary cortex**

The normalized radial width of the unstrained parenchymatous inner primary cortex (arrows 1 in Fig. 5) amounted to 0.15 (axis 1), 0.12 (axis 2), and 0.08 (axis 3). At the point at which rupturing of the sclerenchymatous cylinder was visible for the first time (arrows 2 in Fig. 5), the normalized radial width of the parenchymatous inner cortex had decreased to 0.08 (axis 1), 0.07 (axis 2), and 0.03 (axis 3), respectively. After failure of the sclerenchymatous cylinder, relatively constant values for the normalized radial width of the parenchymatous inner primary cortex (data points to the outmost right in Fig. 5) were found in stem 1 (showing a further slight decrease to a value of 0.07). In the other two axes, on the contrary, an increase of the normalized radial width of the parenchymatous inner cortex occurred after rupturing of the sclerenchymatous cylinder. In stem 2 an increase of the normalized radial width to a value of 0.11, whereas in stem 3 an even more distinct increase to 0.09 occurred.

**Mechanical experiments**

*Set-up 1: structural Young’s Modulus in circumferential direction*: Of 120 tested samples, 12 could be used for assessing the circumferential structural Young’s Modulus of the cortical cylinder. The latter specimens showed abrupt decreases of force indicating brittle fracture after prior deformation (Figs 3A, B, 6A). The rest of the specimens did not show defined breaking events, but showed an entirely different stress–strain behaviour mode suggesting the presence of elastic deformation (e) followed by plastic deformation (p) and compaction (c) (Fig. 6B).

The hollowed plant stems typically failed in three regions. The first two cracks occurred at the outside at the two vertices in a transverse direction (direction normal to loading), followed by one lateral crack at the inside at one of the two remaining vertices (direction parallel to loading; Fig. 3B). This is reflected by typical force displacement curves with three failure events (Fig. 6A). In the circumferential direction, the mean value for the structural Young’s Modulus of the cortical cylinder was found to be $0.30 \pm 0.16$ GPa (Table 1).

*Set-up 2: breaking stress of the cortical cylinder*: Typically, the hollowed plant axes failed under increasing internal radial pressure in only one region (Fig. 3D). This event was marked by an abrupt decrease in internal water pressure which is mirrored by an abrupt decrease of the force applied to the plunger (Fig. 6C). The highest critical radial stresses (i.e. highest critical water pressure) were found in the hollowed axes of young stems with intact

---

**Fig. 5.** Bivariate plots of radial thickness of the vascular cambial tissues (VCT) and of the parenchymatous inner primary cortex versus the distance from the stem apex: absolute (i.e. measured) values (solid circles and triangles), normalized values (open triangles); (A) for axis 1; (B) for axis 2; (C) for axis 3. Normalized values are the measured values of the radial width of the parenchymatous inner primary cortex divided by the undeformed diameter of pith d. Arrow 1 indicates the position of the youngest anatomically entirely differentiated stem segment. Arrow 2 indicates the position of the first rupture in the sclerenchymatous cortex.
sclerenchymatous and collenchymatous cylinders, i.e. with all cortex tissues intact (V in Fig. 7A) and amounted to $r_{crit} = 1.54 \pm 0.26$ MPa. Cylinders of young stems with an artificially lacerated parenchymatous-collenchymatous components (W, $r_{crit} = 1.23 \pm 0.30$ MPa), and sclerenchymatous (X) components ($r_{crit} = 0.35 \pm 0.07$ MPa), respectively, showed lower values of critical radial stress. However, only in cylinders of young stems with an artificially split sclerenchyma cylinder (X) was the critical radial stress found to be significantly lower than in the other two types of young stems (V, W). Older axes with ontogenetically ruptured sclerenchymatous cylinders (Y, $r_{crit} = 1.31 \pm 0.32$ MPa) showed slightly lower values of critical radial stress compared with young axes with intact cylinders (V, Fig. 7B). However, this difference is not significant. The same holds for the difference between untreated old axes (Y) and old stems with additionally artificially lacerated parenchyma and collenchyma (Z, $r_{crit} = 0.90 \pm 0.44$ MPa), which showed a further but not statistically significant decrease in critical radial stress. By contrast, the difference found between values of critical radial stress for intact young axes (V) and old axes with artificially lacerated collenchyma (Z) is statistically significant.

Similar trends for the same groups (V–Z) were observed for the calculated circumferential critical (breaking) stress at the inner surface of the cortical cylinder (Fig. 7C, D). The difference between values for intact young axes (V, $r_{crit} = -9.42 \pm 1.93$ MPa) and young axes with artificially split sclerenchyma (X, $r_{crit} = -3.24 \pm 0.65$ MPa) is statistically significant. Cylinders of young stems with artificially lacerated parenchymatous-collenchymatous components (W, $r_{crit} = -5.54 \pm 1.03$ MPa) do not significantly differ from either of the former values. The values found for older axes with ontogenetically ruptured sclerenchymatous cylinders (Y, $r_{crit} = -9.51 \pm 2.42$ MPa) do not significantly differ from young axes with intact cylinders.

### Table 1. Results for the experimental set-up in which a transversal compression force was applied to the outside of the cortical cylinders of A. macrophylla (set-up 1)

|                  | Mean value | Standard deviation |
|------------------|------------|--------------------|
| $F$ [N]          | 5.06       | 2.11               |
| $r_1$ [mm]       | 2.12       | $3.2 \times 10^{-2}$ |
| $r_2$ [mm]       | 1.69       | $6.6 \times 10^{-2}$ |
| $L$ [mm]         | 11.73      | 1.71               |
| $I$ [$mm^4$]     | $7.99 \times 10^{-2}$ | $2.72 \times 10^{-2}$ |
| $E_{\text{mean}}$ [GPa] | 0.30       | 0.16               |

For determining the Young’s Modulus of the cortical cylinder, mean values were calculated for the data points of the force-displacement curves prior to failure of the cortical cylinder. $F$, force applied, $r_1$, outer radius of the cortical cylinders, $r_2$, inner radius of the cortical cylinders, $L$, length of the test segment, $I$, second moment of area of the cortical cylinders, $E_{\text{mean}}$, mean Young’s Modulus of the cortical cylinders. Number of samples ($n$) in which the cortical cylinder cracked = 12.
Old stems with additionally artificially lacerated parenchyma and collenchyma (Z, $\sigma_{c,cri}\approx-6.47\pm3.19$ MPa), showed a decrease in critical circumferential stress of about one-third compared to untreated old axes (Y) which, however, is not statistically significant.

Discussion

Variation of stem anatomy during ontogeny

For a discussion of stresses and strains measured in *A. macrophylla*, the cross-sections of axes can be approximated as a system of concentric cylinders (Fig. 8). Two different systems are necessary to describe the morphology of the axes prior to and after the rupture of the outer sclerenchymatous cortex.

- (i) A system built of three ‘closed’ cylindrical shells surrounding a central cylindrical core consisting of the vascular tissues (phloem, vascular cambium, and xylem) and the pith parenchyma (Fig. 8A, white central core). This system represents the arrangement of tissues in a young stem of *A. macrophylla*. The adjacent inner cylindrical shell represents the inner primary parenchymatous cortex tissue (Fig. 8A, hatched inner ring). The next cylinder represents the intact sclerenchymatous cortex (Fig. 8A, black ring), which is followed by the outer collenchymatous and parenchymatous cortex tissues and the epidermis (Fig. 8A, peripheral white ring). In the model, the vascular tissues, the inner parenchymatous cortex tissues, and the sclerenchyma...
are considered in a first order approximation to describe the structural changes due to secondary vascular growth. Stresses exerted by the vascular tissues due to their increasing girth caused by secondary vascular growth processes are transmitted through the inner zone of the primary parenchymatous cortex to the outer primary cortex. The ‘closed’ (i.e. intact) sclerenchymatous cortex forms a stiff cylinder and ‘encloses’ the other two tissues (Fig. 8A).

(ii) An ‘open’ system with a fragmented sclerenchymatous cylinder (Fig. 8B). This system represents the tissue distribution in an older stem of *A. macrophylla* with significant secondary vascular growth. In contrast to (i), the fragmented sclerenchymatous cylinder is of no major mechanical importance. Therefore, the system can be considered as ‘open’ (i.e. not enclosed) by the sclerenchymatous cylinder.

The results for the variation of the normalized radial thickness of the parenchymatous inner primary cortex can be interpreted accordingly. With rupture of the sclerenchymatous cylinder (arrows 2 in Fig. 5), the stem structure changes from (i) to (ii).

With increasing secondary vascular growth, i.e. increasing girth of the secondary vascular tissues, in all three stems a decrease of (normalized) radial width of the parenchymatous inner cortex occurs. This ‘squeezing’ of the parenchymatous inner cortex between the inner cylinder of the growing secondary vascular tissues and the closed sclerenchymatous cylinder is interpreted as a consequence of the increasing radial stresses caused by the secondary vascular growth. Similar findings have been reported for fossil plant stems by Stein and Hotton (1999) and Masselter *et al.* (2006, 2007). After rupturing of the sclerenchymatous cylinder in two of the tested axes (axes 2 and 3) the radial width of the parenchymatous inner cortex increases, whereas in axis 1 a slight further decrease is found (Fig. 5). This increase can be interpreted as a consequence of stress relaxation of the elastically strained (compressed) parts of the parenchymatous inner primary cortex which occurs after rupturing of the sclerenchymatous cylinder. This would indicate a relatively high amount of elastic or visco-elastic deformation in the inner primary cortex. The existence and kinetics of cell wall stress relaxations in plant tissues have been investigated by Yamamoto *et al.* (1970), Masuda *et al.* (1974), Cosgrove (1986, 1987), Hohl and Schopfer (1992), Yamamoto (1996), and Schopfer (2006).

**Deformation of cellular materials**

Deformations of cellular materials as seen in the inner zone of the primary parenchymatous cortex in *A. macrophylla* are complex. Therefore, a detailed account of the cellular deformations and of the growth stresses and strains in the inner primary cortex in *A. macrophylla* (Fig. 9B, E, H) is presented. For a better understanding of the processes occurring during secondary growth in the stems of *A. macrophylla*, the deformation patterns of cellular solids as predicted by theory (Fig. 9A, D, G), are compared to strains experimentally found in a closed-cell rubber foam (Fig. 9C, F, I). The deformations in

![Fig. 9. Cellular materials subjected to increasing compression.](image)
technical foams and living plant tissues can be quite similar, and the mechanical behaviour of cellular rubber foams (with closed cells) provides a conceptual framework for evaluating the mechanical behaviour of cellular parenchymatous plant tissues (Niklas, 1992). This comparative approach can be used to distinguish ‘elastic’ from ‘plastic’ deformations in these plant tissues.

In A. macrophylla differently deformed tissue zones (Fig. 9B, E, H) can be observed in the inner zone of the primary parenchymatous cortex. These deformations can be interpreted as ‘elastic’, i.e. reversible (Fig. 9A, B, D, E), or ‘plastic’, i.e. irreversible deformations (Fig. 9G, H). These types of deformation have been reported for plant tissues with a relatively soft cellular matrix (Gibson et al., 1981; Niklas, 1989, 1992; Gao et al., 1990; Gao and Pitt, 1991; Stein, 1993; Hutton and Stein, 1994; Stein and Hotton, 1999) for rubber foams (Gibson and Ashby, 1982; Gibson et al., 1982; Shim et al., 1992; Weaire and Fortes, 1994; Fig. 9C, F, I), other cellular materials (Weaire and Rivier, 1984; Shim and Stronge, 1986; Stronge and Shim, 1987, 1988; Shim et al., 1990; Poirier et al., 1992; Papka and Kyriakides, 1994; Lee et al., 2002), and has also been predicted by hypothetical models for cellular materials (Warren and Kraynik, 1987, 1988, 1991; Gibson and Ashby, 1988; Gibson, 1989; Kraynik and Warren, 1994). Common to these investigations is the occurrence of indented cells (Fig. 9E, F) which are first elastically compressed and finally become plastically deformed. These cells were in the originally unstrained state (Fig. 9B, C) more or less isodiametric and non-aligned. The initial so-called elastic buckling (Niklas, 1992), can, with increasing stress, develop into plastic cell buckling as well as (finally) into a total cell collapse (Fig. 9H, I). In this final stage the plant cells lose their turgor and are compacted cell wall against cell wall (Fig. 9H). Observations for measuring and modelling cellular material are given in Cunningham and Hilyard (1994) and Weaire and Fortes (1994). The similarity of tissue deformation patterns in biological and technical cellular materials indicates that the very different time factor (plant cells are strained much slower than the illustrated foam rubbers) is of no major significance for the geometry of stress–strain fields at equilibrium. Secondary growth-induced changes in geometry of parenchymatous tissues in A. macrophylla occur in a time span of weeks, months, and years. This indicates that effects of permeability and fluid exchange on the stress–strain behaviour of plant cells are negligible in a first order approximation.

Mechanical experiments

Set-up 1: structural Young’s Modulus in circumferential direction: The measured value of the structural Young’s Modulus in a circumferential direction of the cortical cylinder appears low for a mainly sclerenchymatous structure (0.30 GPa). Even if the experimental data are recalculated for the contribution of the sclerenchymatous cortex to the axial second moment of area of the cortical cylinder, which is about 65%, the structural Young’s Modulus would only increase by a factor of 1.5 to about 0.46 GPa. Our results are lower than the results of Spatz et al. (1995, 1997), where the structural Young’s Modulus of the hypodermal sterome of the giant reed Arundo donax which consists of sclerenchymatous fibres, was found to be about 10 times lower in a circumferential direction than in a longitudinal direction, and amounts to values of c. 1 GPa for the middle and lower stem segments. It is likely that the values of structural Young’s Modulus of the cortical cylinder in A. macrophylla are dominated by the contribution of the sclerenchymatous tissue. Twelve samples of cortical cylinders tested under increasing transversal compression failed consecutively first at the outside of the two vertices normal to the direction of loading, where the highest tension strains occur. The third failure was found on the inside at one of the two vertices parallel to the direction of loading where high tension stresses also occur. These regions of failure were also reported by Spatz et al. (1997) for tests with stem segments of A. donax. In stem segments of this plant, by contrast, the first cracks typically occurred at the inner surface of the vertices parallel to the direction of loading.

Set-up 2: breaking stress of the cortical cylinder: The sclerenchymatous cylinder is the main contributor to flexural and torsional stiffness of the young Aristolochia stems (Speck, 1994a, b; Speck et al., 1996).

The distinct and statistically significant decrease found for critical radial and tangential stresses in cortical cylinders with artificially damaged sclerenchyma cylinder and the finding that lacerating of the collenchyma only shows minor, non-significant effects on the radial breaking stress prove that the contribution of the collenchyma is of minor importance to mechanical properties in radial and tangential direction of young A. macrophylla stems.

The radial breaking stress found for the intact cortical cylinders (1.54±0.26 MPa) is in good accordance with radial growth stresses that plants are capable of exerting in a radial direction, which are approximately 1 MPa in Eucalyptus regnans (Boyd, 1950), and in timber in general by other authors (Jacobs, 1945; Kübler, 1959; Archer, 1986; Fournier et al., 1990), varying between 0.7 and 2.8 MPa. The radial breaking stress of the cylinder is also in line with the results for swelling stress in wood of the Norway spruce (Picea abies) by Virta et al. (2006), who measured a maximum swelling stress in the tangential direction of about 1.2 MPa. The circumferential or tangential breaking stress of the cortical cylinders is comparatively low: −9.42±1.93 MPa, if compared with values measured in a longitudinal direction (i.e. tensile breaking stress of the fibres, cf. Niklas (1993) who
reported a value for tensile breaking stress of sclerenchyma of –33.9 MPa in longitudinal direction. The measured critical circumferential stress is the stress at which the observed cell–cell debonding in a radial direction occurs. All fissures we have analysed showed this structure (Fig. 1F); i.e. no tangential (circumferential) rupturing of the sclerenchymatous cells themselves could be observed. The measured value of the critical stress in a circumferential direction is much higher than that reported for parenchyma (0.74 MPa according to Niklas, 1993). This corroborates the interpretation based on the laceration experiments, and further supports the idea that the sclerenchymatous cylinders represent by far the most important contributor to the critical stresses measured for the cortical cylinders (i.e. hollowed stems) of A. macrophylla (Fig. 7). This interpretation is further supported by qualitative observations during these tests. These prove that, in experiments with lacerated cortical cylinders, rupturing preferentially occurs in areas where the sclerenchymatous cylinder has been damaged, but the rupture does not preferentially occur in areas where the outer parenchymatous primary cortex (including epidermis) and the collenchymatous cylinder were artificially lacerated.

The observed collapse of large wood vessels (Fig. 1C) in A. macrophylla cannot be explained by the measured radial compressive stresses that occur prior to the rupture of the sclerenchymatous cylinder. These values are only c. 15% of the values measured by Ljungdahl et al. (2006) and Ljungdahl and Berglund (2007) for compression yield (stresses) in a radial direction of European oak, which holds early wood with large vessels of a comparable size than the large vessels of A. macrophylla (compare Müller et al., 2003). However, for physical reasons, it can be assumed that stresses in the vascular bundles caused by the secondary growth processes in A. macrophylla can be much higher than the stresses transferred to the inner surface of the sclerenchymatous cylinder. These stresses are likely to reach critical values for causing yield processes in the large early wood vessels. Recalculating the values of growth stresses at the periphery and within secondary vascular tissues will be the subject of a forthcoming paper.

Conclusions

The quantitative and qualitative data lead to a number of significant implications concerning ontogeny, structural anatomy, and biomechanics in A. macrophylla.

- (i) Secondary growth, i.e. the expansion of vascular cambial tissues causes stresses in the adjacent outer and inner primary tissues. Whereas in the inner primary tissues (mainly) radial compressive stresses occur, a combination of radial compressive and tangential tension stresses are found in the outer primary tissues. In combination with the existence of a peripheral cortical cylinder with a closed rigid sclerenchymatous cylinder, as found in A. macrophylla, these stresses cause compression of the pith, (sometimes) collapse of large early wood vessels, a decrease of the radial width of the parenchymatous inner zone of the primary cortex, and, finally, ruptures in the sclerenchymatous cylinder.
- (ii) The parenchymatous inner primary cortex is, in addition to a radial compression, at least partially elastically strained in a tangential direction. This zone is exposed to significant variations of mechanical stresses when the plant changes from a closed to an open cylinder organization caused by rupturing of the outer sclerenchymatous cylinder. After rupturing of the sclerenchymatous cylinder the inner primary cortex relaxes (visco-) elastically.
- (iii) The different ‘modes of deformation’ (elastic, plastic, compaction) in the inner parenchymatous cortex of A. macrophylla can also be found in compressed closed-cell rubber foams. Therefore, these foams are suitable material for testing deformation models of ‘soft’ plant tissues.
- (iv) The sclerenchymatous cylinder, while intact acts as a counter bearing to the secondary vascular tissues causing high stresses in the intermediate parenchymatous cortex tissues.

Acknowledgements

We gratefully acknowledge Professor Fink and his staff from the Forest-Botany Department, University of Freiburg for valuable help in preparing some of the thin sections, as well as our former scientific staff member E Danninger for carrying out some of the experiments. We would like to thank Professor Spatz from the Institute of Biology III, University of Freiburg, for valuable discussions about the experimental part of the study. We acknowledge Prof. Karl Niklas and the American Journal of Botany for the kind permission to reproduce parts of a figure.

References

Archer RR. 1986. Growth stresses and strains in trees. Berlin: Springer-Verlag.

Boyd JD. 1950. Tree growth stresses. I. Growth stress evaluation. *Australian Journal of Scientific Research* 3, 270–293.

Cosgrove DJ. 1986. Biophysical control of plant cell growth. *Annual Review of Plant Physiology* 37, 377–405.

Cosgrove DJ. 1987. Wall relaxation in growing stems: comparison of four species and assessment of measurement techniques. *Planta* 171, 266–278.

Cunningham A, Hillyard NC. 1994. Physical behaviour of polymeric foams: an overview. In: Hillyard NC, Cunningham A, eds. *Low density cellular plastics: physical basis of behaviour*. London: Chapman and Hall, 1–21.

Fisher JB, Ewers FW. 1989. Wound healing in stems of lianas after twisting and girdling injuries. *Botanical Gazette* 150, 251–265.

Fournier M, Bordonne PA, Guitard D. 1990. Growth stress patterns in tree stems. *Wood Science and Technology* 24, 131–142.
Gao Q, Pitt RE. 1991. Mechanics of parenchyma tissue based on cell orientation and microstructure. Transactions of the American Society of Agricultural Engineers 34, 232–238.

Gao Q, Pitt RE, Ruina R. 1990. A mechanics model of the compression of cells with finite initial contact area. Biorheology 27, 225–240.

Gibson LJ. 1989. Modelling the mechanical behaviour of cellular materials. Materials Science and Engineering A 110, 1–36.

Gibson LJ, Ashby MF. 1982. The mechanics of three-dimensional cellular materials. Proceedings of the Royal Society of London A 382, 43–59.

Gibson LJ, Ashby MF. 1988. Cellular solids: structures and properties. Oxford, New York, Beijing, Frankfurt, Sao Paulo, Sydney, Tokyo, Toronto: Pergamon Press.

Gibson LJ, Ashby MF, Schajer GS, Robertson CI. 1988. Low density cellular plastics: physical basis of behaviour and failure process during compressive and shear deformation of cellular solids. London: Chapman and Hall, 187–225.

Gibson LJ, Easterling KE, Ashby MF. 1981. The structure and mechanics of cork. Proceedings of the Royal Society of London A 377, 99–117.

Haberlandt GJF. 1924. Physiologische Pflanzenanatomie, 6th edn. Leipzig: Engelmann.

Hohl M, Schopfer P. 1994. The growth stresses of woody stems. Common-wealth Forestry Bureau Australia Bulletin 28.

Jacobs MR. 1945. The growth stresses of woody stems. Common-wealth Forestry Bureau Australia Bulletin 28.

Kraynik AM, Warren WE. 1994. The elastic behaviour of low density cellular plastics. In: Hilyard NC, Cunningham A, eds. Low density cellular plastics: physical basis of behaviour. London: Chapman and Hall, 187–225.

Kübler H. 1959. Studies on growth stresses in trees. Part I. The origin of growth stresses and the stresses in transverse direction. Holz als Roh-und Werkstoff 17, 1–9.

Lee HS, Hong SH, Lee JR, Kim YK. 2002. Mechanical behavior and failure process during compressive and shear deformation of honeycomb composite at elevated temperatures. Journal of Materials Science 37, 1265–1272.

Ljungdahl J, Berglund LA. 2007. Transverse mechanical behaviour and moisture absorption of waterlogged archaeological wood from the Vasa ship. Holzforschung 61, 279–284.

Ljungdahl J, Berglund LA, Burman M. 2006. Transverse anisotropy and moisture absorption of waterlogged archaeological wood from the Vasa ship. Holzforschung 60, 190–195.

Masselter T, Rowe NP, Speck T. 2007. Biomechanical reconstruction of the Carboniferous seed fern Lyginopteris oldhamia: implications for growth form reconstruction and habit. International Journal of Plant Sciences 168, 1177–1189.

Masselter T, Speck T. 2006. Evaluating secondary growth processes in Aristolochia macrophylla by experiments and modelling. In: Salmen L, ed. Proceedings of the 5th plant biomechanics conference. Stockholm: STFI-Packforsk AB, 49–54.

Masselter T, Speck T, Rowe NP. 2006. Ontogenetic reconstruction of the Carboniferous seed plant Lyginopteris oldhamia. International Journal of Plant Sciences 167, 147–166.

Masuda Y, Yamamoto R, Kawamura H, Yamagata Y. 1974. Stress relaxation properties of the cell wall of tissue segments under different growth conditions. Plant and Cell Physiology 15, 1083–1092.

Müller M, Gindl W, Teischinger A. 2003. Effects on cell anatomy on the plastic and elastic behaviour of different wood species loaded perpendicular to grain. IAWA Journal 24, 117–128.

Niklas KJ. 1989. Mechanical behavior of plant tissues as inferred from the theory of pressurized cellular solids. American Journal of Botany 76, 929–937.

Niklas KJ. 1992. Plant biomechanics; an engineering approach to plant form and function. Chicago: University of Chicago Press.

Niklas KJ. 1993. Influence of tissue density-specific mechanical properties on the scaling of plant height. Annals of Botany 72, 173–179.

Papka SD, Kyriakides S. 1994. In plane compressive response and crushing of honeycomb. Journal of the Mechanics and Physics of Solids 42, 1499–1532.

Poirier C, Ammi M, Bideau D, Trooade JP. 1992. Experimental study of the geometrical effects in the localization of deformation. Physical Review Letters 68, 216–219.

Rowe NP, Speck T. 2004. Hydraulics and mechanics of plants: novelty, innovation and evolution. In: Hemsley AR, Poole I, eds. The evolution of plant physiology. London: Elsevier, 301–330.

Rowe NP, Speck T. 2005. Plant growth forms: an ecological and evolutionary perspective. New Phytologist 166, 61–72.

Schopfer P. 2006. Biomechanics of plant growth. American Journal of Botany 93, 1415–1425.

Schöttgen J. 1983. Kompensative Histogenese beim Übergang vom primären zum sekundären Dickenwachstum am Spross von Aristolochia macrophylla. Master’s thesis, University of Freiburg, Germany.

Shim VPW, Stronge WJ. 1986. Lateral crushing in tightly packed arrays of thin-walled metal tubes. International Journal of Mechanical Sciences 28, 709–728.

Shim VPW, Tay BY, Stronge WJ. 1990. Dynamic crushing of strain-softening cellular structures: a one-dimensional analysis. Journal of Engineering Materials and Technology 112, 398–405.

Shim VPW, Yap KY, Stronge WJ. 1992. Effects of nonhomogeneity, cell damage and strain-rate on impact crushing of a strain-softening cellular chain. International Journal of Impact Engineering 12, 585–602.

Spatz H-Ch, Beismann H, Brüchert F, Emanns T, Speck T. 1997. Biomechanics of the giant reed Arundo donax. Philosophical Transactions of the Royal Society of London B 352, 1–10.

Spatz H-Ch, Beismann H, Emanns T, Speck T. 1999. Mechanical anisotropy and inhomogeneity in the tissues comprising the hollow stem of the giant reed Arundo donax. Biomimetics 3, 141–155.

Spatz H-Ch, Köhler L, Speck T. 1998. Biomechanics and functional anatomy of hollow-stemmed sphenopsids. 1. Equisetum giganteum (Equisetaceae). American Journal of Botany 85, 305–314.

Speck O, Luchsinger R, Busch S, Rüggeberg M, Speck T. 2006. Self-repairing membranes for pneumatic structures: transferring nature’s solutions into technical applications. In: Salmen L, ed. Proceedings of the 5th plant biomechanics conference. Stockholm: STFI-Packforsk AB, 115–120.

Speck T. 1994a. A biomechanical method to distinguish between self-supporting and non self-supporting fossil plants. Review of Palaeobotany and Palynology 81, 65–82.

Speck T. 1994b. Bending stability of plant stems; ontogenetical, ecological, and phylogenetical aspects. Biomimetics 2, 109–128.

Speck T, Luchsinger R, Busch S, Rüggeberg M, Speck O. 2006. Self-healing processes in nature and engineering: self-repairing biomimetic membranes for pneumatic structures. In: Brebbia CS, ed. Design and nature III. Southampton: WIT Press, 105–114.

Speck T, Masselter T, Prüm B, Speck O, Luchsinger R. 2004a. Smart materials: light-weight structures with variable stiffness and...
self-repair mechanisms. In: Jones TP, Rowe NP, eds. Fortschritt-Berichte VDI, Reihe15 Umwelttechnik, 249. Düsseldorf: VDI Verlag GmbH, 315–321.

Speck T, Masselter T, Prüm B, Speck O, Luchsinger R, Fink S. 2004b. Plants as concept generators for biomimetic light-weight structures with variable stiffness and self-repair mechanisms. Journal of Bionics Engineering 1, 199–205.

Speck T, Rowe NP. 1999. A quantitative approach for analytically defining size, growth form and habit in living and fossil plants. In: Kurmann MH, Hemsley AR, eds. The evolution of plant architecture. Kew: Royal Botanic Gardens, 447–449.

Speck T, Rowe NP. 2001. Die Wuchsform ‘Liane’: strukturelle Vorraussetzungen für eine erfolgreiche Einnischung als Kletterpflanze. Mitteilungen des Badischen Landesvereins für Naturkunde und Naturschutz 17, 875–893.

Speck T, Rowe NP, Brüchert F, Haberer W, Gallemüller F, Spatz H-C. 1996. How plants adjust the ‘material properties’ of their stems according to differing mechanical constraints during growth: an example of smart design in nature. In: Engin AE, ed. Bioengineering. PD-Volume 77. Proceedings of the 1996 Engineering Systems Design and Analysis Conference, Volume 5. ASME 1996, 233–241.

Speck T, Rowe NP, Civeyrel L, Claßen-Bockhoff R, Neinhuis C, H-Ch Spatz. 2004c. The potential of plant biomechanics in functional biology and systematics. In: Stuessy T, Hörandl F, Mayer V, eds. Deep morphology, towards a renaissance of morphology in plant systematics. Ruggell: Gantner Verlag, Königstein: Koeltz, 241–271.

Speck T, Vogellehner D. 1992. Fossile Bäume, Spreizklimmer und Lianen. Versuch einer biomechanischen Analyse der Stammstruktur. Courier Forschungsinstitut Senckenberg 147, 31–53.

Stein WE. 1993. Modelling the evolution of stelar architecture in vascular plants. International Journal of Plant Sciences 154, 229–263.

Stein WE, Hotton CL. 1999. Fabric analysis and plant anatomy. In: Jones TP, Rowe NP, eds. Fossil plants and spores: modern techniques. London: Geological Society, 97–104.

Stephens RC. 1970. Strength of materials. Theory and examples. London, New York, Melbourne, Auckland: Edward Arnold, Hodder.

Stronge WJ, Shim VPW. 1987. Dynamic crushing of a ductile cellular array. International Journal of Mechanical Sciences 29, 381–406.

Stronge WJ, Shim VPW. 1988. Microdynamics of crushing in cellular solids. Journal of Engineering Materials and Technology 110, 185–190.

Virta J, Koponen S, Absetz I. 2006. Measurement of swelling stresses in spruce (Picea abies) samples. Journal of Building and Environment 41, 1014–1018.

Warren WE, Kraynik AM. 1987. Foam mechanics: the linear elastic response of two-dimensional spatially periodic cellular materials. Mechanics of Materials 6, 27–37.

Warren WE, Kraynik AM. 1988. The linear elastic properties of open-cell foams. Journal of Applied Mechanics 55, 341–346.

Warren WE, Kraynik AM. 1991. The non-linear behaviour of open-cell foams. Journal of Applied Mechanics 58, 376–381.

Weaire D, Fortes MA. 1994. Stress and strain in liquid and solid foams. Advances in Physics 43, 685–738.

Weaire D, Rivier N. 1984. Soap, cells and statistics: random patterns in two dimensions. Contemporary Physics 25, 59–99.

Wilson JW, Grange RI. 1984. Regeneration of tissues in wounded stems: a quantitative study. Annals of Botany 53, 515–525.

Yamamoto R. 1996. Stress relaxation property of the cell wall and auxin-induced cell elongation. Journal of Plant Research 109, 75–84.

Yamamoto R, Shinozaki K, Masuda Y. 1970. Stress-relaxation properties of plant cell walls with special reference to auxin action. Plant and Cell Physiology 11, 947–956.