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The toughness of plant cell walls

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SUMMARY

All plant tissues and plant-based materials are composites and therefore, during standard fracture mechanics tests, cracks within them tend to arrest and deflect because of crack-stopping mechanisms at cell boundaries or in air-spaces. Due to this change of direction cracks do not cross the toughest structures, frustrating both their measurement and the understanding of the cracking process. Accordingly, there are no accurate values for the toughness of plant cell walls. We have attempted to solve this problem here by driving the crack with blades. We show from cutting experiments on twenty individual plant tissues and plant-based materials that the intrinsic toughness of plant cell wall, independent of cell form, is between 3.4–4.2 kJ m^{-2}; for any tissue it is directly proportional to the volume fraction that the cell wall occupies. Plastic work, which is dependent on cellular geometry, can increase toughness to a value of at least 30 kJ m^{-2} in woody tissues, but this capacity is probably not linearly related to cell wall volume fraction.

1. INTRODUCTION

Plants combine the crystalline polymer cellulose, in the form of long strands, together with variable quantities of other polysaccharides, proteins and lignins, to produce a stiff, strong and tough cell wall (Preston 1974; McCann & Roberts 1991; Carpita & Gibeaut 1993). However, cell wall toughness is not reliably known, even though this is a most important property for its commercial exploitation in the wood (Dinwoodie 1981; Bodig & Jayne 1982), paper (Page *et al.* 1971; Westerlind *et al.* 1991), pharmaceutical (Podczeck & Newton 1992), food (Purslow 1991; Okiyama *et al.* 1992) and textile industries (Chapman 1974). Cell wall 'fibre' is also the major textural constituent of plant foods in human diets and an important influence on the feeding ecology of vertebrate and invertebrate herbivores (Coley 1983; Waterman *et al.* 1988) where 'fibre' toughness is probably the major impediment (Coley 1983; Choong *et al.* 1992; Shipley & Spalinger 1992).

The toughness of plant materials appears to vary with their relative density: the volume fraction of cell wall, V_c (Gibson & Ashby 1988). However, cellular geometry is also an important influence as probably is turgidity in non-lignified tissues (Atkins & Vincent 1984). Tissues containing isodiametric cells have a low toughness, typically only 200–300 J m^{-2} for parenchyma (Vincent 1990). Elongated lignified cells found in wood have high toughness (> 20 kJ m^{-2}) when the crack direction is directed at right-angles to cell length (Jeronimidis 1980). This woody toughness is not in proportion to V_c and depends on the helical winding of cellulose microfibrils that develop in the wall following cell elongation (Preston 1974).

The toughness of woods across the grain cannot be used to calculate cell wall toughness because cracks in all except light woods follow a zigzag path, crossing fibres only at their weak points (Ashby *et al.* 1985). If cell wall toughness is not known, then the understanding of the conditions under which cracks turn from a straight path cannot be derived. This is because crack deflection is not itself a toughness mechanism but a result of the anisotropy of toughness which leads to crack-stopping. Toughness values for woods would be even higher and reflect actual cell wall values only if the crack remained straight. Because cracks deviate from a direct path across dense woody fibres since the middle lamella between fibres is more brittle (e.g. Jeronimidis 1980 for wood; Lucas *et al.* 1991 for seed shell), the toughness of cell wall *per se* is known only from extrapolation from low-density woods (Ashby *et al.* 1985).

Fractures can also deflect in low-density tissues for other reasons. Air spaces in apple flesh, for example, blunt cracks (Vincent & Khan 1993). Though free-running fracture tests may be very useful, for example, in mimicking how cracks run during a bite by the incisor teeth, understanding the conditions for crack deflection still demands an accurate value for cell wall toughness.

We have attempted to solve this problem by cutting tests in which a straight crack is forced through sheets of material. These must be sufficiently thin to control crack velocity because elastic strain energy increases with specimen thickness. It is necessary to prevent energy storage in excess of that required to push the crack slowly because it might then deflect. Some frictional work is involved in cutting but, in biological materials, this is liable to be small. Even so, a simple

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practical way of accounting for it is to make a second pass of the cutting blade with or without the cut material still in place (Atkins & Mai 1979). There are two other problems: fracture may not be in mode I in cutting tests and also the plastic response of the solid is possibly more localized due to low strain levels away from the crack, as compared with 'natural' fractures. These factors can be checked by comparing results of cutting with other tests: at least, for those low V_c solids where crack direction can be maintained (Gibson & Ashby 1988).

Cutting tests are usually only employed on low V_c cellular plant tissues but, by making thin sections of high V_c tissues with a diamond wheel, we have succeeded in obtaining the toughness of almost completely solid plant tissue. The aim of this study was to produce a reliable value for the toughness of cell walls.

2. MATERIALS AND METHODS

Twenty materials having a broad range of V_c and cellular geometries were selected (see table 1). All were either plant tissues themselves or derivatives. All were substantially free from silica which might wear the cutting surface. This was checked in seed shells and wood by qualitative energy-dispersive X-ray micro-analysis. The turgidity of all low V_c tissues was maintained until testing. Wood and shell were tested in saturated condition to minimize friction. Papers were allowed to equilibriate to ambient temperature (23 °C) and relative humidity (50–60 %) before tests, whereas seed coats and pods were cut at the moisture contents at which they were collected. Sections for testing were produced by two methods. Freehand sections were cut from blocks of low V_c tissues using surgical scalpel blades. These blocks often tapered. The direction of taper was established by a micrometer screw gauge and the cut was then directed across an even thickness. After testing, the region immediately around the crack was cut out and thickness measurements made on this isolated strip. Harder materials such as wood and seed shell were cut using a diamond wheel. The thickness of each section was also measured with a micrometer screw gauge. The seed coats, pods and papers were already in sheet form so the size of these materials in tests was varied by layering, gripped to prevent slipping.

Toughness was obtained from cutting tests with hairdressing scissors (Dovo, Germany) with polished cobalt steel blades (Lucas *et al.* 1991; Vincent, 1992). The included angle of the blades was 45 ° and the radius of curvature of the cutting edges, obtained by casting with an elastomeric dental impression material, was 1.6 μm. The crosshead speed was 14 mm min^{-1} which produced controlled cracking. Metal-to-metal friction was estimated by making a second empty pass of the scissors for each test (Atkins & Mai 1979). The toughness of green turnip, an isotropic low V_c tissue without apparent air-spaces, was also tested by a 15° wedge test (Vincent *et al.* 1991) which served for a comparison with the scissors tests.

In tissues where fibres were parallel, the crack was directed perpendicular to fibre direction. The maximum section thickness that could be tested (0.5–4.0 mm) depended on gross distortion of thick low V_c specimens and on the point at which control of crack growth was lost in tissues with high V_c. The effectiveness of gripping layered sheets of paper was checked on one material (filter paper No. 542) by glueing between 2–4 layers together about 5 mm away from each side of the cut before testing. No differences in toughness were found.

The structure of all materials was investigated by preparing samples for light microscopy (LM), laser-scanning confocal microscopy (LSCM: Biorad MRC-600) and scanning electron microscopy (SSEM: Cambridge S360 and S440). Sections for LM and LSCM were usually cut freehand. Semi-thin (1 μm thick) sections of *Milletia atropurpurea* cotyledon were cut, stained with toluidine blue, and observed under LM.

All test specimens were viewed under LSCM. Lignified tissues fluoresced strongly and did not require staining. However, lignification was also checked by viewing slides stained with 1 % phloroglucinol under LM. Non-lignified tissues were stained with safranin or fast green to provide contrast under LSCM. SSEM was used, particularly for higher V_c tissues, to assess damage that was associated with the tests and also to view normal structure. The materials were categorized as either isodiametric or fibrous. One material, the shell (middle integument) of the seed of *Mezzettia parviflora* (Annonaceae) contained both isodiametric cells and fibres of very similar high density (Lucas *et al.* 1991). The fibres were arranged in parallel in the outer portion of the shell, zone I (Lucas *et al.* 1991) and as haphazardly directed bundles in much of the rest of the shell (zone II). However, there was a thin band of isodiametric cells surrounding the shell at its equator (zone III). By testing each region of the shell separately, the influence of cellular geometry on toughness values could be evaluated without variation in V_c. Zone I was always < 1 mm wide and it was difficult to verify the direction of cutting in relation to fibre orientation. Therefore, the angle between the cut and fibre direction was measured on LSCM images. All tests in which this angle was $90° \pm 20°$ were accepted.

Histological measurements to estimate V_c were made on representative sections of all homogeneous tissues. For low V_c tissues with isodiametric cells, cell wall thickness was divided by cell wall length to give V_c (Gibson & Ashby 1988). For tissues with parallel fibres, V_c was assessed as the area fraction of cell wall, measured by image analysis, when the fibre was viewed end-on. In materials where cellular contents were either minimal or missing, such as papers, seed coats, pods, wood and most seed shells, V_c was determined by cutting out a convenient shape, measuring the volume of the tissue, drying where necessary and then weighing to calculate the density. The result was then normalized to a cell wall density of 1.5 Mg m^{-3} (Gibson & Ashby 1988).

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3. RESULTS

For all materials, the dependence of toughness on section thickness was linear when thickness was small. However, as thickness was increased, measured toughness levelled off. In most tissues, this plateau appeared to be at about 1 mm in section thickness (e.g. green turnip in figure 1). For green turnip, plateau toughness values were very similar to the mean for the crack-opening test (see figure 1*a*). The toughness of watermelon tissue appeared to plateau at a much higher thickness, beyond the limit of testing. Cell sizes in

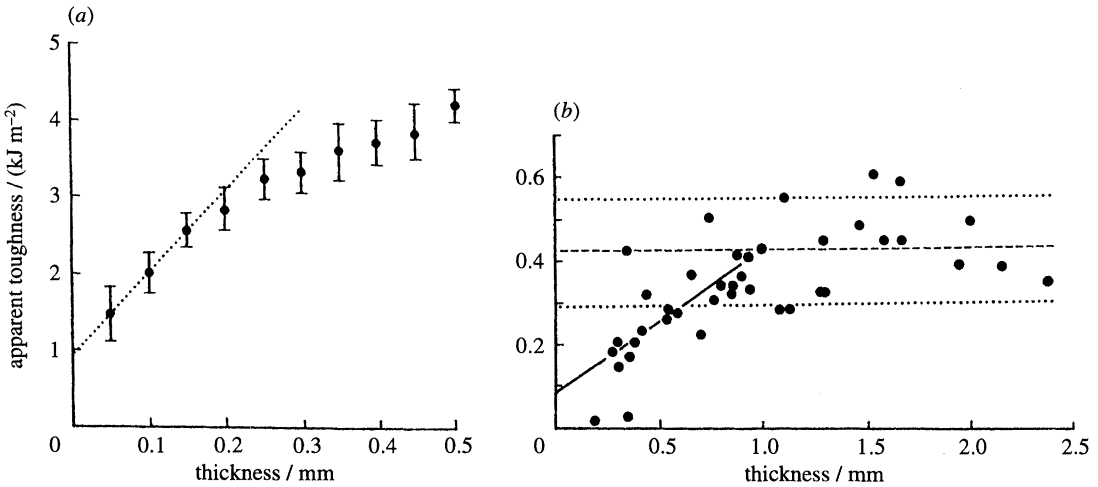

Figure 1. (*a*) The apparent toughness of bank paper increases linearly (dotted line) with specimen thickness (varied by layering) when thickness < 0.2 mm. (*b*) The solid line shows the regression for toughness values on section thickness for green turnip; these level off at about 1 mm. The dashed line shows the mean toughness from a wedge test; the dotted lines lie two standard deviations away.

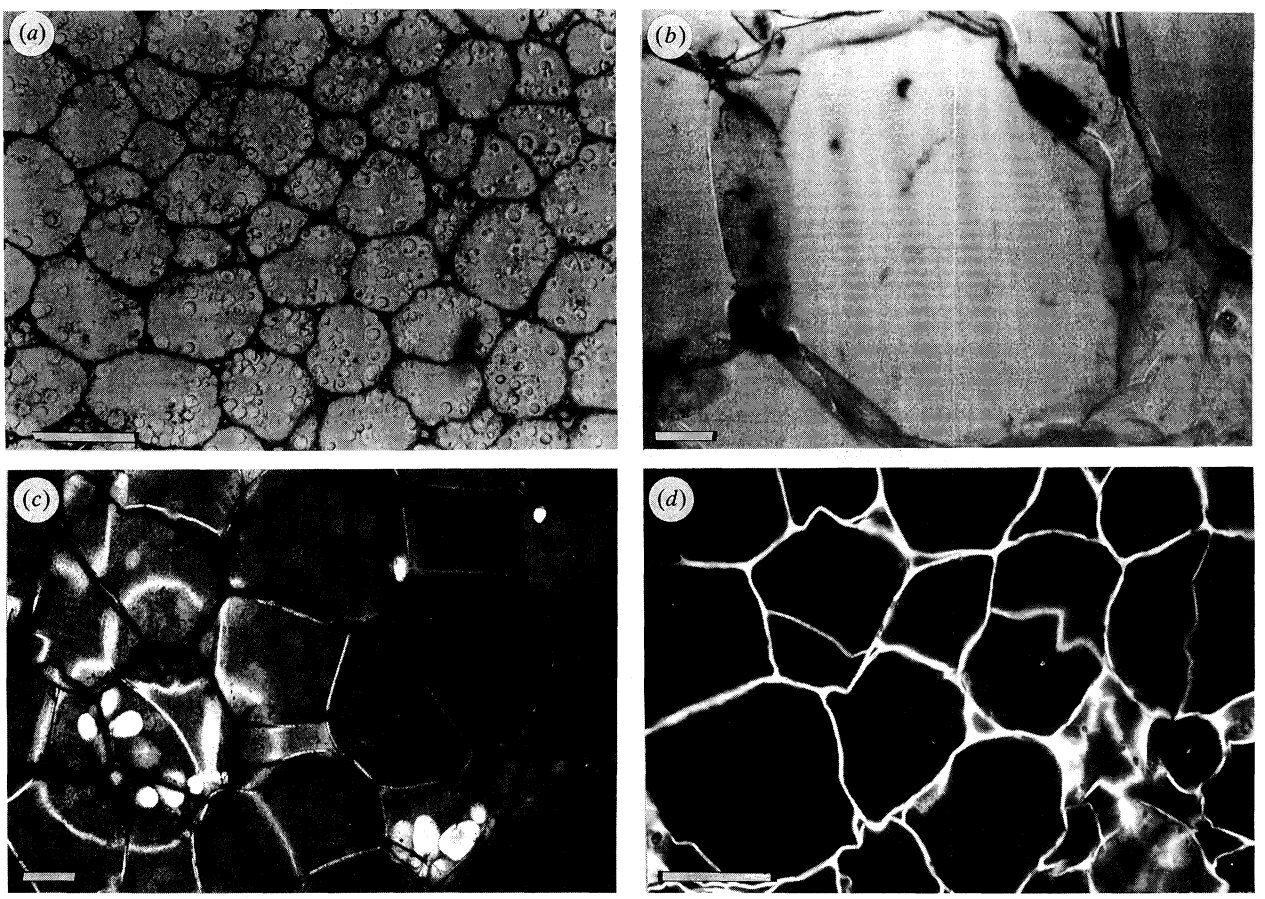

Figure 2. The structure of tissues with isodiametric cell geometry and low cell wall volume fraction. (*a*) *M. atropurpurea* cotyledon (LM). Cells contain a large number of starch granules, scale bar, 50 μm. The other photographs are from LSCM, all with scale bars of 100 μm. (*b*) Watermelon flesh has cells of about 500 μm in diameter, (*c*) potato flesh and (*d*) green turnip.

Table 1. *The tissues or materials tested in this study*

(The number of data points, correlation coefficients, intercepts and slopes refer to the results of regression of toughness on section thickness described in the text. I indicates isodiametric component cells (termed a 'foam') and F means a largely or wholly fibrous structure. *n* is the number of data points for the regression calculation of *r*, the correlation coefficient; the bracketed figures under slope and intercept are the standard errors.)

material	description	cell geometry	relative density	*n*	*r*	slope (kJ m^{-2})	intercept (MJ m^{-3})
Citrullus vulgaris red watermelon (Cucurbitaceae)	foam	I	0.0031	22	0.815	21.9 (3.5)	9.5 (6.8)
Brassica rapa green turnip (Brassicaceae)	foam	I	0.0192	25	0.731	341.0 (66.4)	84.5 (43.2)
Solanum tuberosum Russet Burbank potato (Solanaceae)	foam	I	0.0253	35	0.467	343.5 (113.4)	89.6 (50.7)
Gossypium sp.[a] cotton pads (Malvaceae)	three-dimensional disconnected mat of hairs	F	0.089	20	0.909	455.7 (49.3)	300.8 (101.2)
Milletia atropurpurea cotyledon (Leguminosae)	foam	I	0.127	31	0.720	1039.5 (186.2)	267.6 (123.5)
Leucaena leucocephala pod – inner brown layer only (Leguminosae)	variable two-dimensional xylem mat	F	0.242	27	0.822	5775.3 (799.4)	761.3 (194.1)
filter paper[b] no. 1	pseudo-random three-dimensional mat	F	0.343	15	0.963	7650.0 (597.6)	1648.9 (217.3)
filter paper[b] no. 42	pseudo-random three-dimensional mat	F	0.347	17	0.931	6793.3 (689.5)	1980.2 (313.8)
M. atropurpurea seed coat	foam	I	0.349	25	0.633	894.6 (217.4)	1681.3 (210.7)
Albizia splendens pod (Leguminosae)	irregular fibrous mat and epidermis and brachysclereids	F	0.349	41	0.821	3143.2 (349.8)	970.0 (259.2)
filter paper[b] no. 542	pseudo-random three-dimensional mat	F	0.411	30	0.924	11840 (923)	1368.3 (218.9)
bank paper	pseudo-random three-dimensional mat	F	0.413	45	0.855	10853 (1005)	913.8 (108.6)
newsprint	pseudo-random three-dimensional mat	F	0.449	39	0.921	9225 (640)	1232 (147)
Eusideroxlon zwageri Bornean ironwood (Lauraceae)	parallel fibres	F	0.617	26	0.905	31333 (3005)	1216 (994)
Aleurites moluccana candlenut shell (Euphorbiaceae)	parallel fibres	F	0.90	40	0.801	14630 (1778)	2638 (515)
Schinziophyton rautenenii shell of mongongo seed (Anacardiaceae)	three-dimensional fibrous mat	F	0.94	21	0.491	11349 (4617)	3767 (1005)
Mezzettia (I):	parallel fibres	F	0.95	16	0.576	24840	4558

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Table 1 (*cont.*)

material	description	cell geometry	relative density	*n*	*r*	slope (kJ m^{-2})	intercept (MJ m^{-3})
parviflora seed shell (Annonaceae)						(9421)	(1935)
(II):	three-dimensional fibrous mat	F	0.95	29	0.577	12011 (2822)	3739 (680)
(III):	foam	I	0.95	12	0.184	2310 (3905)	4192 (1136)
Scheelea sp. palm nut endocarp (Arecaceae)	mostly parallel fibres with foam	F	0.923	21	0.614	15636 (4734)	2423 (1532)

[a] 'Kleenex', Kimberley-Clark, U.K.
[b] Whatman's, U.K.

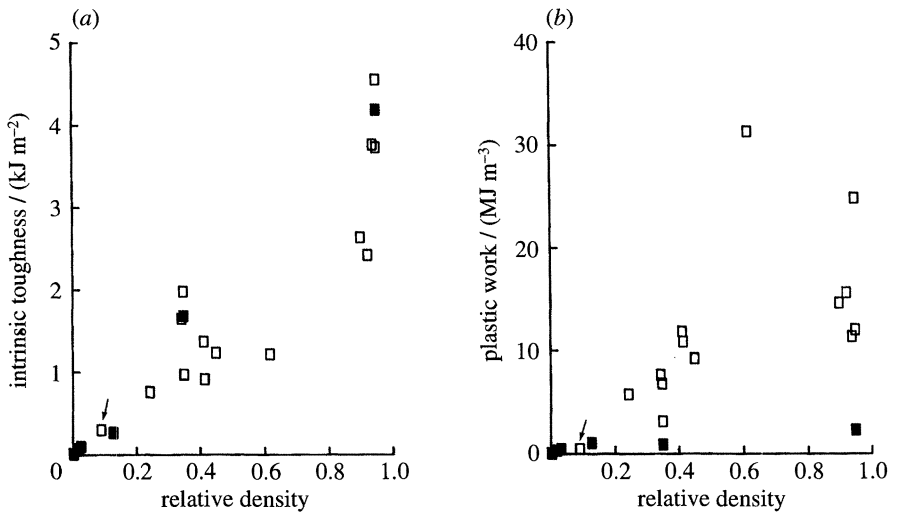

Figure 3. The dependence of toughness on the cell wall volume fraction, V_c, of the material. Isodiametric tissues are plotted as solid squares, fibrous tissues as open squares. (*a*) Intrinsic toughness values, the intercept toughness values of plots where toughness was regressed against section thickness, depend mainly on V_c. (*b*) The ability of each material to toughen by plastic work, assessed as the slope of the relationship between toughness and section thickness, depends on cellular geometry. Section thickness has very little effect on the toughness of isodiametric tissues.

watermelon flesh were very much larger than in other parenchyma tissues, such as potato or turnip (see figure 2); this is reflected in an extremely low value of V_c. The thickness at which toughness levelled off could not be established in high V_c tissues like ironwood and seed shell: there was no clear sign of the plateau with 0.5 mm sections.

For each material, within the linear portion of the plots, toughness values were regressed against section thickness. Correlation coefficients were lowest for tissues with isodiametric cells and, not surprisingly, highest for the more uniform derivative materials. The *y*-intercepts that were obtained from this analysis provide estimates of the intrinsic toughness of each material (see table 1). There was a strong relationship between intrinsic toughness and V_c but little effect due to the variable cellular arrangement (see figure 3*a*). Tissues with isodiametric cells had similar intrinsic toughness to fibrous materials when V_c was taken into account.

The data in figure 3*a* were logarithmically transformed so as to estimate the exponent of the relationship and to obtain an estimate of the cell wall toughness at $V_c = 1.0$. The correlation coefficient was very high ($r^2 = 0.964$), the slope was 1.005 (s.e. 0.046) and the predicted intrinsic toughness of the wall at $V_c = 1.0$ was 3.41 kJ m^{-2} (s.e. 0.32 kJ m^{-2}). The slope is not significantly different from unity ($p > 0.8$).

The intrinsic toughness for the three zones of *M. parviflora* seed shell did not differ ($p = 0.87$) and was not, therefore, influenced by cell geometry either (see figure 4). The average intrinsic toughness of this shell, normalized to $V_c = 1.0$, was 4.2 kJ m^{-2}.

The influence of cellular geometry on the slope of toughness vs. section thickness was also examined (see table 1). For fibrous tissues, the slope was higher than for isodiametric tissues and depended on V_c, but with considerable scatter. The sole exception was the fibrous cotton pads which had low toughness, probably due to the lack of connection between individual hairs. Otherwise, scatter appeared to be due to variation in fibre direction in these materials. For example, it was sometimes possible to cut *Leucaena leucophala* pod at right-angles to fibres (see figure 5*a*), but, very often,

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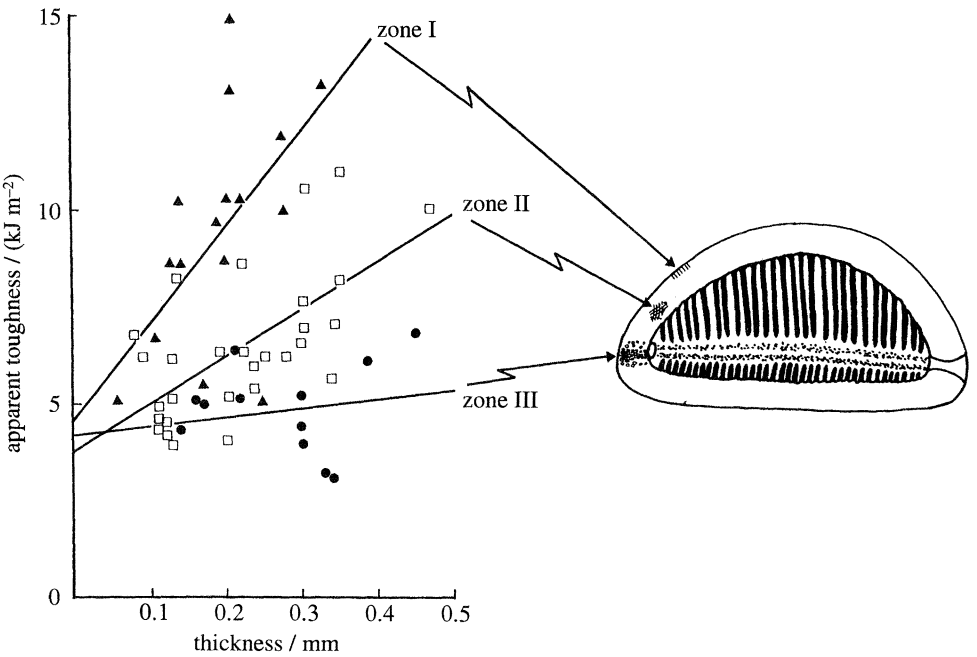

Figure 4. Toughness–section thickness plots (as in figure 1) for the three zones of *Mezzettia parviflora* seed shell, the location of which are shown on the diagram of a longitudinal section through the seed on the right. The accuracy of cuts in zones I (shown as triangles) and III (circles), which are only about 500 μm wide, was confirmed by LSCM. For zone I, all cuts were 90° (±20°) to fibre direction. The intercept toughnesses for each region are greater than those predicted from data for all 20 materials, averaging 4.2 kJ m^{-2} when extrapolated to $V_c = 1.0$. A similar intercept applies for *Schinziophyton rautenenii* shell which is structurally similar to zone II (squares) of *Mezzettia*.

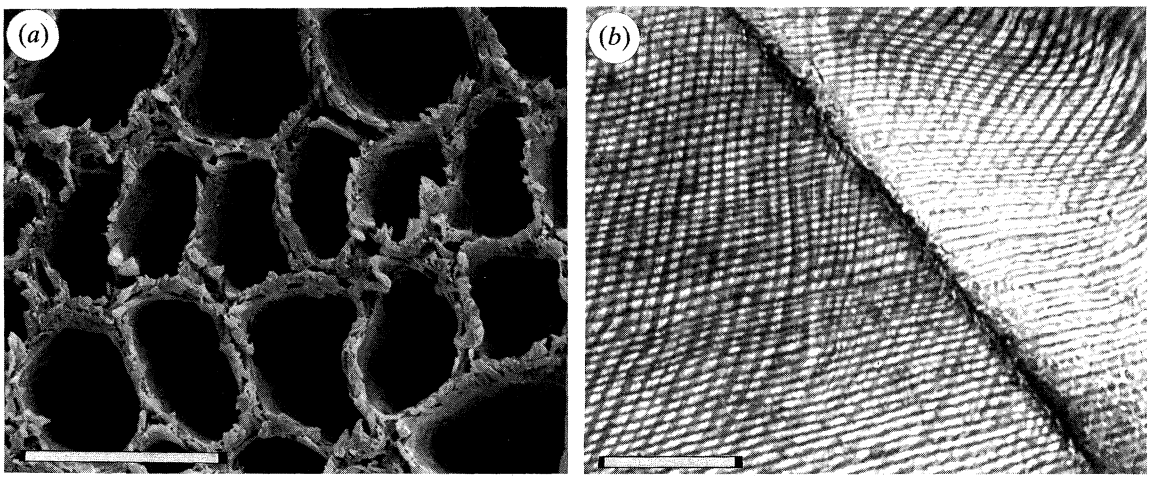

Figure 5. The fibrous structure of *Leucaena leucophala* pod. (*a*) Fibres viewed end on (SSEM), scale bar, 20 μm; (*b*) the relationship of fibre direction to a test cut (which runs lower right to upper left), viewed by LSCM. Optical sectioning shows two layers of fibres oriented perpendicularly to each other, scale bar, 100 μm.

the organization of the tissue prohibited this (see figure 5*b*). However, the slope for *Eusideroxylon zwageri* wood was slightly higher than that of zone I of *M. parviflora* seed shell, despite the latter having a much higher V_c.

Data for the three zones of *M. parviflora* supported this result. The slopes for each zone were significantly different ($p < 0.0001$), being highest for zone I with parallel fibres (see figure 4). The slope for zone III, which contained isodiametric cells, was not significantly different from zero while that for zone II, with a haphazard three-dimensional fibre network was, approximately, the average of zones I and III.

The pattern of test damage to *M. parviflora* shell was investigated in detail. Two smooth plastic indentations on zone I fibres were produced by slow fracture with the scissors (see figure 6*a*). The centre of the section has a slightly rougher surface, presumably produced by fast fracture. The existence of a crack extending ahead of the scissor blades was supported by observations made by optical sectioning with LSCM (see figure 6*b*). In contrast, though the cut ran through the walls of the isodiametric cells of zone III, crack branches caused the detachment of whole cells on either side of the blade by brittle fracture. The resulting rough edges to

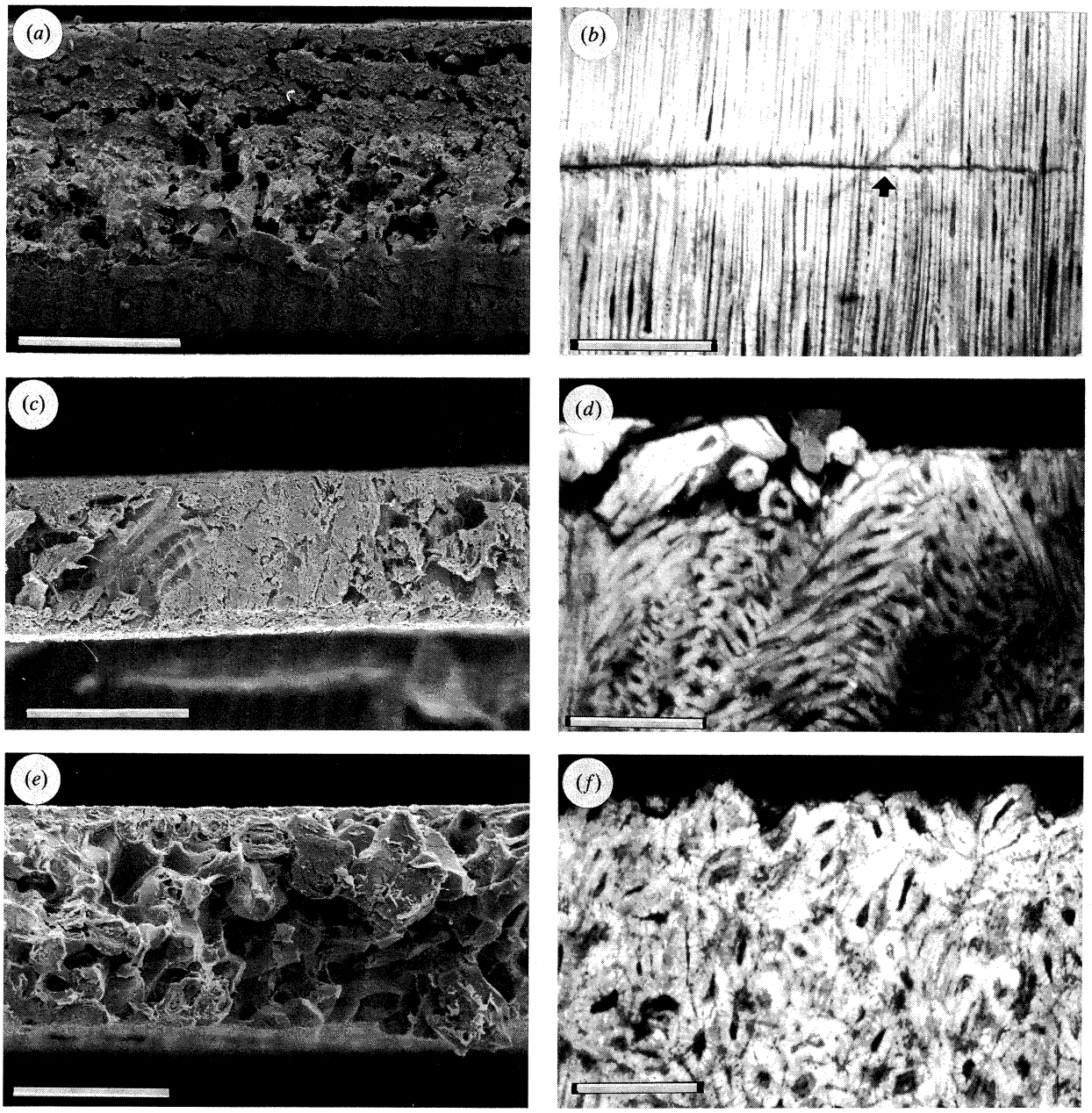

Figure 6. Cut surfaces of the three zones of *Mezzettia parviflora* seed shell, viewed end-on by SSEM (left column) and from above by CLSM (right column). Zone I: (*a*) shows two smooth slow fractured scissor indentations with a slightly rougher surface in the middle of the section produced by fast fracture. (*b*) An optical section shows the crack responsible for fast fracture running ahead of the scissors in the same direction, directly across the fibres. The last position of the scissors is arrowed (matrix bridges ahead of this point can be seen by SSEM, confirming that the scissors were not present), Zone II: (*c*) irregular areas of slow and fast fracture produced within the irregular three-dimensional mat of fibres shown in (*d*) Zone III: (*e*) isodiametric cells have crumbled away in a brittle manner at intercellular junctions on either side of the cut, producing the irregular cut shown in (*f*).

the cut showed little sign of plastic indentation (see figure 6*e*, *f*). Damage to zone II was intermediate (see figure 6*c*, *d*).

4. DISCUSSION

The most simple interpretation of our results is that intrinsic toughness is a reflection of the elastic component of cell wall fracture while the slope of toughness versus section thickness indicates the potential for plastic work to frustrate crack growth. If so, then our measurements provide the first enumeration of these quantities.

Intrinsic toughness was highly correlated with V_c despite the variable composition of these materials. Our study included tissues that had primary cell walls only and in which the cellulosic strands do not have a general preferred orientation (McCann & Roberts 1991) as well as secondary cell walls where the microfibrils align along a definite but variable angle to cell length (Preston 1974). However, intrinsic toughness must, logically, depend on wall composition: e.g. the quantity of cellulose and lignin present. Yet cotton

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hairs are usually 95% by volume cellulose (Young 1986) whereas the equivalent in wood is only 40–45% (Bodig & Jayne 1982). The cell walls of *M. atropurpurea* may contain significant amounts of cell wall storage carbohydrates (Reid 1989) and be low in cellulose. Heavy lignification might increase intrinsic toughness by binding components of the cell wall together (perhaps at the cost of a lower capacity for deforming plastically). The ironwood was highly lignified as were all parts of the *M. parviflora* seed shell. The latter did have a slightly higher intrinsic toughness.

In simple elastic fracture, the cost of driving a crack through a tissue would be proportional to the area of solid that was cleaved. Therefore, intrinsic toughness would be expected to be directly proportional to V_c. This is precisely what we found and though it may seem intuitively obvious and has been commonly stated (e.g. Gibson & Ashby 1988), we have seen no direct prior demonstration of it.

There are several differences between our test and the more conventional variety. In cutting, very little strain energy is stored in the specimen: none at zero thickness. This, however, makes for a more direct test: external work on the blade is directly transformed into work of fracture (Atkins & Mai 1979). It may be argued that the mode of fracture is important: scissors tests are probably mixed-mode even for homogeneous materials (Atkins & Mai 1979). However, the fact that similar values were obtained from both cutting and wedge tests argues against this. Wedging gives almost identical results to tensile tests for thin-walled parenchyma tissue (Vincent 1990; Vincent & Khan 1993), suggesting that cutting tests do give conventionally interpretable results, probably because the expression of fracture mechanisms is similar. We have neglected the effect of the moisture content of the cell wall and also turgor pressure. High turgor has been reported to decrease the toughness of non-lignified tissues to a small extent (Atkins & Vincent 1984). Much depends on what happens to cell walls that burst on contact with the blades because further movement of the blades may still cut the walls of burst cells leading to little effect due to turgor. Only four low V_c tissues in this study were turgid (watermelon, potato, green turnip and *M. atropurpurea* cotyledon); these have little influence on the estimate of intrinsic toughness of cell wall.

We can compare our estimate of intrinsic toughness, 3.4 kJ m^{-2}, with the only literature value, 1.65 kJ m^{-2}, derived via K_{IC} values on balsa (Ashby *et al.* 1985). The latter may be less accurate as balsa has a V_c of only 0.2. Free-running cracks in woods with $V_c > 0.2$ deflect (Ashby *et al.* 1985); if our value is correct, the intrinsic toughness across the grain is then at least double that along the grain (which is < 0.35 kJ m^{-2} and independent of V_c; Gibson & Ashby 1988).

In general, toughness values obtained from tests on thin sheets increase linearly with section thickness (Mai & Cotterell 1989), consistent with our results. That fibrous materials are capable of greater plastic resistance to crack growth than those containing isodiametric cells is expected: it is the basis of woody toughness (Jeronimidis 1980). The mechanism for this increase of toughness involves 'tensile buckling' of the cell wall, which collapses into the cell lumen (Page *et al.* 1971). Cells close to the fracture surface suffer very large strains and have helical cracks between the cellulose fibrils extending for some distance in their cell walls (Jeronimidis 1980). This mechanism suggests that woody toughness would be inhibited at a high V_c because the lumen is so small. Our data show that *E. zwageri* wood, with $V_c = 0.617$, has a slightly greater potential for plastic work than does the zone I of *M. parviflora* seed shell ($V_c = 0.95$). However, it may again be argued that our results are not relevant to toughening in wood because the nature of plastic work during cutting may be different to that during simple tension or bending. To explore whether this is so, we now analyse the data further using details of damage to zone I fibres of *M. parviflora*.

We make the assumption that the plastic work in zone I fibres of *M. parviflora* shell during cutting involves the plastic bending of cell walls in shear. The same effect is observed in the guillotining of metals (Atkins & Mai 1979) where this work is given by the shear yield stress (τ_{ys}) multiplied by the depth of indentation of the blade. In figure 6*a*, the indentation depth of a scissor blade is about one-third of the section thickness. Given that the slope for zone I is 24.8 MJ m^{-3} (see table 1), this gives $\tau_{ys} = 75$ MPa. The shear yield stress across wood fibres is not well known but it has been shown by Gibson & Ashby (1988) that the tensile yield stress σ_y is related to τ_{ys} by:

$$\sigma_y = 2\sqrt{3}.\tau_{ys}/(t/l)^2, \quad (1)$$

where t is the thickness of cell wall and l the length of the sides of the fibres viewed in cross-section. Inserting the relevant values into equation (1) gives $\sigma_y = 295$ MPa at $V_c = 0.95$. This compares well with 350 MPa given for the cell walls of wood in tension, an extrapolation to $V_c = 1.0$ by Gibson & Ashby (1988) from the data of Cave (1969).

As shown in figure 3, toughness in a scissoring test levels off in thick enough specimens. In compact tissues, the thickness at which this happens depends on the size of the plastic zone in front of the crack tip (Atkins & Mai 1985):

$$d_y = ER/\pi\sigma_y^2, \quad (2)$$

where E is Young's modulus and R is the intrinsic toughness (4 kJ m^{-2}). Given that, for $V_c = 0.95$, E = 33.25 GPa (Gibson & Ashby 1988), and taking $\sigma_y = 295$ MPa, then $d_y = 0.486$ mm. Since both scissor blades indent the specimen equally (see figure 6*a*), the plateau toughness is given approximately by a section thickness of $2d_y$. Scaling rules for cellular solids (Gibson & Ashby 1988) suggest that this would be independent of V_c, though the size of cells may play a role if they are very large. If we evaluate the toughness of *M. parviflora* shell (zone I) and *E. zwageri* wood at $2d_y$ and add the intrinsic toughness to the plastic work of fracture derived from equation (2), we obtain a maximal toughness of 29 and 35 kJ m^{-2} respectively. These are reasonable values for woody toughness involving plastic work. Jeronimidis (1980), for example, gives 38–60 kJ m^{-2} for $V_c = 1.0$ in mode I, which is again an

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extrapolation from woods of low density. The mixture of modes in cutting tests (Atkins & Mai 1979) complicates comparisons but the plastic bending seen in cutting, although localized, almost certainly involves the debonding of cellulose fibrils from the other cell wall components by shear stresses in a similar way to the fracture of woods when they are bent in mode I tests (Jeronimidis 1980).

What cutting tests do avoid, and quite deliberately, is the effect of whole cell pull-out. Jeronimidis (1980) has shown that this mechanism gives a toughness ten times too low to be the primary cause of the toughness of wood. There are some data on seeds substantiating this. Wet *S. rautenenii* shell fails entirely in cellular pull-out when a crack is free-running and meandering between fibres. The toughness is then only about 0.85 kJ m^{-2} (Williamson & Lucas 1995) which is one quarter of the intrinsic toughness of cell wall at $V_c = 0.94$. This shows unequivocally why pull-out predominates over cell wall fracture: it requires much less energy. Failure by intercellular fracture with pull-out is the probable cause of the brittleness of many seed shells.

The fracture of low V_c plant tissues that contain isodiametric cells is also usually described as brittle (Vincent 1990). The data shown in figure 3*b* support this and show that, even at high V_c, the capacity for plastic work in these tissues is very small.

In homogeneous plant tissue, the intrinsic toughness should be equivalent to chemical 'fibre content' as measured by extractive (Waterman *et al.* 1988) and other means (Preston & Sayer 1992). Intrinsic toughness could have advantages over the estimation of fibre by chemical methods (e.g. Shipley & Spalinger 1992). In heterogeneous tissues, cutting offers the prospect of establishing the toughness of the individual components of plant composites with a simple, cheap and easy-to-use test. This must be useful both in industry and in ecology.

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REFERENCES

Ashby, M.F., Easterling, K.E., Harrysson, R. & Maiti, S.K. 1985 The fracture and toughness of woods. *Proc. R. Soc. Lond.* A **398**, 261–280.

Atkins, A.G. & Mai, Y.-W. 1979 On the guillotining of materials. *J. Mater. Sci.* **14**, 2747–2754.

Atkins, A.G. & Mai, Y.-W. 1985 *Elastic and Plastic Fracture*. Chichester: Ellis Horwood.

Atkins, A.G. & Vincent, J.F.V. 1984 An instrumented microtome for improved histological sections and the measurement of fracture toughness. *J. Mater. Sci. Lett.* **3**, 310–312.

Bodig, J. & Jayne, B.A. 1982 *Mechanics of Wood and Wood Composites*. New York: Van Nostrand Reinhold.

Carpita, N.C. & Gibeaut, D.M. 1993 Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* **3**, 1–30.

Cave, I.D. 1969 The longitudinal Young's modulus of *Pinus radiata*. *Wood Sci. Technol.* **3**, 40–48.

Chapman, C.B. 1974 *Fibres*. London: Butterworth.

Choong, M.F., Lucas, P.W., Ong, J.Y.S., Pereira, B., Tan, H.T.W. & Turner, I.M. 1992 Leaf fracture toughness and sclerophylly: their correlations and ecological implications. *New Phytol.* **121**, 597–610.

Coley, P.D. 1983 Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr.* **53**, 209–233.

Dinwoodie, J.M. 1981 *Timber, its nature and behaviour*. New York: Van Nostrand Reinhold.

Gibson, L.J. & Ashby, M.F. 1988 *Cellular Solids*. Oxford: Pergamon Press.

Jeronimidis, G. 1980 The fracture behaviour of wood and the relations between toughness and morphology. *Proc. R. Soc. Lond.* B **208**, 447–460.

Lucas, P.W. & Pereira, B. 1990 Estimation of the toughness of leaves. *Funct. Ecol.* **10**, 235–236.

Lucas, P.W., Choong, M.F., Tan, H.T.W., Turner, I.M. & Berrick, A.J. 1991 The fracture toughness of the leaf of the dicotyledon *Calophyllum inophyllum L.* (Guttiferae). *Phil. Trans. R. Soc. Lond.* B **334**, 95–106.

Lucas, P.W., Lowrey, T.K., Pereira, B., Sarafis, V. & Kuhn, W. 1991 The ecology of *Mezzettia leptopoda* seeds as viewed from a mechanical perspective. *Funct. Ecol.* **5**, 545–553.

Mai, Y.-W. & Cotterell, B. 1989 On mixed-mode plane stress ductile fracture. In *Advances in fracture research*, vol. 3 (ed. K. Salama, K. Ravi-Chandar, D.M.R. Taplin & P. Rama Rao), pp. 2269–2278. Oxford: Pergamon Press.

McCann, M.C. & Roberts, K. 1991 Architecture of the primary cell wall. In *The cytoskeletal basis of plant growth and form* (ed. C.W. Lloyd), pp. 109–129. London: Academic Press.

Okiyama, A., Motoki, M. & Yamanaka, S. 1992 Bacterial cellulose II. Processing of the gelatinous cellulose for food materials. *Food Hydrocoll.* **6**, 479–487.

Page, D.H., El-Hosseiny, F. & Winkler, K. 1971 Behaviour of single wood fibres under axial tensile strain. *Nature, Lond.* **229**, 252–253.

Podczeck, F. & Newton, J.M. 1992 Determination of the critical stress intensity factor and the fracture toughness of different microcrystalline cellulose products. *Pharmazie* **6**, 462–463.

Preston, C.M. & Sayer, B.G. 1992 What's in a nutshell: an investigation of structure by carbon-13 cross-polarization magic-angle spinning nuclear magnetic resonance spectroscopy. *J. agric. Food Chem.* **40**, 206–220.

Preston, R.M. 1974 *The physical biology of plant cell walls*. London: Chapman Hall.

Purslow, P. 1991 The architecture of food texture. *Chem. & Ind.* **18**, 836–839.

Reid, J.S.G. 1989 Analysis of carbohydrates conferring hardness on seeds. In *Modern methods of plant analysis, vol. 10 plant fibres* (ed. H.F. Linskens & J.F. Jackson), pp. 295–312. Berlin: Springer-Verlag.

Shipley, L.A. & Spalinger, D.E. 1992 Mechanics of browsing in dense food patches: effects of plant and animal morphology on intake rate. *Can. J. Zool.* **70**, 1743–1752.

Vincent, J.F.V. 1990 Fracture in plants. *Adv. bot. Res.* **17**, 235–287.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Vincent, J.F.V. 1992 *Biomechanics – materials*. Oxford: IRL Press.

Vincent, J.F.V., Jeronimidis, G., Khan, A.A. & Luyten, H. 1991 The wedge fracture test. A new method for measurement of food texture. *J. Texture Stud.* **22**, 45–57.

Vincent, J.F.V. & Khan, A.A. 1993 Anisotropy in the fracture properties of apple flesh as investigated by crack-opening tests. *J. Mater. Sci.* **28**, 45–51.

Waterman, P.G., Ross, J.A.M., Bennett, E.L. & Davies, A.G. 1988 A comparison of the floristics and leaf chemistry of the tree flora in two Malaysian rain forests and the influence of leaf chemistry on populations of colobine monkeys in the Old World. *Biol. J. Linn. Soc.* **34**, 1–32.

Westerlind, B.S., Carlsson, L.A. & Andersson, Y.M. 1991 Fracture toughness of liner board evaluated by the J-integral. *J. Mater. Sci.* **26**, 2630–2636.

Williamson, L. & Lucas, P.W. 1995 The effect of moisture content on the mechanical properties of a seed shell. *J. Mater. Sci.* **30**, 162–166.

Young, R.A. 1986 Structure, swelling and bonding of cellulose fibers. In *Cellulose: structure, modification and hydrolysis* (ed. R.A. Young & R.M. Powell), pp. 91–128. New York: John Wiley & Sons.

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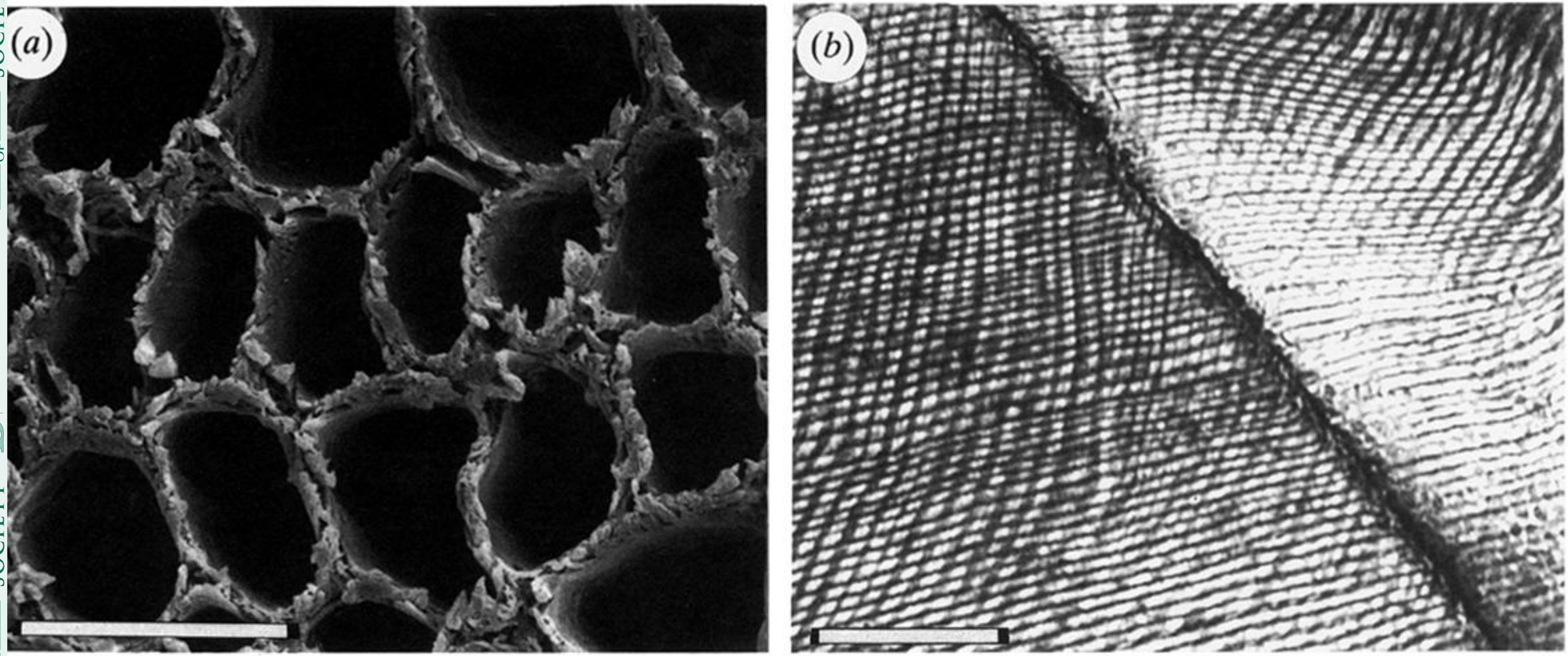
Figure 2. The structure of tissues with isodiametric cell geometry and low cell wall volume fraction. (*a*) *M. atropurpurea* cotyledon (LM). Cells contain a large number of starch granules, scale bar, 50 μm. The other photographs are from LSCM, all with scale bars of 100 μm. (*b*) Watermelon flesh has cells of about 500 μm in diameter, (*c*) potato flesh and (*d*) green turnip.



gure 5. The fibrous structure of *Leucaena leucophala* pod. (*a*) Fibres viewed end on (SSEM), scale bar, 20 μm; (*b*) the ationship of fibre direction to a test cut (which runs lower right to upper left), viewed by LSCM. Optical sectioning ows two layers of fibres oriented perpendicularly to each other, scale bar, 100 μm.

Figure 6. Cut surfaces of the three zones of *Mezzettia parviflora* seed shell, viewed end-on by SSEM (left column) and from above by CLSM (right column). Zone I: (*a*) shows two smooth slow fractured scissor indentations with a slightly rougher surface in the middle of the section produced by fast fracture. (*b*) An optical section shows the crack responsible for fast fracture running ahead of the scissors in the same direction, directly across the fibres. The last position of the scissors is arrowed (matrix bridges ahead of this point can be seen by SSEM, confirming that the scissors were not present), Zone II: (*c*) irregular areas of slow and fast fracture produced within the irregular three-dimensional mat of fibres shown in (*d*) Zone III: (*e*) isodiametric cells have crumbled away in a brittle manner at intercellular junctions on either side of the cut, producing the irregular cut shown in (*f*).