The Fracture Toughness of the Leaf of the Dicotyledon Calophyllum inophyllum L. (Guttiferae)

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The fracture toughness of the leaf of the dicotyledon *Calophyllum inophyllum* L. (Guttiferae)

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CONTENTS

SUMMARY

The fracture toughness (specific work of fracture) of the leaf of the dicotyledonous angiosperm, *Calophyllum inophyllum* L. (Guttiferae), which has a lamina with a reticulate venation comprising secondary and tertiary veins only, was investigated by cutting, notched tensile and punch-and-die tests. Toughness was found to depend on the presence of veins in the fracture path, with both tensile and cutting tests in agreement that fracture at right angles to secondary veins was 2.5–3.0 times as tough as fracture parallel to them. Values from tensile tests were smaller than those from cutting tests. The cutting method had the advantage of specifying the direction of fracture and of severing structures serially, allowing easy recognition of tough structures from force-displacement records. This showed that the fracture toughness of the lamina could be modelled by a 'rule of mixtures' whereby the veins (vascular bundles supported by sclerenchyma strands above and below), with a combined toughness of about 6000 J m^{-2}, are contrasted to other tissues – the mesophyll and the epidermis and its cuticle – which together probably have an average toughness of only 220–300 J m^{-2}. Bounds on the toughness of mature leaves are probably set close to these limits. However, we could not test all these tissues separately to confirm this. The model predicted the fracture toughness expected in a cylindrical punch-and-die test, which is the commonly used test in ecological studies. However, observed values from this test were twice those expected, which is mostly attributable to yielding of a large volume of mesophyll but also in part due to the debonding of secondary veins from the rest of the lamina. The results suggest that relevant classifications of leaves (sclerophyll, pachyphyll, etc.) should pay close attention to the venation of the leaf and the structure of these veins. It is proposed that the thickening of the walls of smaller veins to form a venous network is a defence against invertebrate herbivores.

1. INTRODUCTION

Leaves are generally the most heavily attacked of the structural parts of woody plants and a wide variety of animals, both vertebrate and invertebrate, eat them. However, the degree of damage is very variable both between species and within one species at different locations. Ecologists have sought to investigate this by correlating measures of leaf-area loss with mechanical and chemical tests on leaf tissues. In the largest survey reported, Coley (1983) found that the maximum force required to penetrate the lamina with a cylindrical rod was significantly negatively correlated with the rate of leaf-area loss from a variety of causes including invertebrate herbivores, but excluding vertebrates. None of a large range of chemical tests, including some on phenolic compounds, were as significant. Coley (1987) states that a major selection pressure for the development of tough leaves is defence against herbivory.

Other workers, using a similar or identical test, have claimed importance for it in more specific studies. Feeny (1970) states that the 'immediate' reason for winter moth larvae not eating oak leaves is due to their mechanical properties. Schultz & Baldwin (1982) found that red oaks that had been defoliated by gypsy

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moths, produced leaves in the following year that were significantly more mechanically resistant in this test than were 'control' trees that had not been attacked for at least a year. Similar mechanically induced defences to herbivory have also been reported in a seaweed (Lowell *et al.* 1991). In a feeding experiment, Raupp (1985) found the mandibles of *Plagiodera versicolora* beetles were seriously worn by the mature leaves of *Salix* spp. and that this jeopardized the fecundity of the beetles. He speculated that this wear was related to the force needed in the punch test. Bernays (1986) showed that grasses requiring high punch forces induce an increase in head-size in graminivorous invertebrates that feed on them. Waller (1982) concluded that leaf-cutting ants avoid some leaves for mechanical reasons.

A punch test, often called a penetrometer, has been used by almost all ecologists who have studied herbivory quantitatively and who generally describe the resulting force at fracture as 'toughness'. However, 'toughness' is a misnomer because none of the varieties of punch test described (Williams 1954; Tanton 1962; Cherrett 1968; Feeny 1970; King 1988) can actually have measured fracture toughness. The units are those of force (rarely of force per unit area) and both geometry of the test and the unstable fracture of specimens under load control do not normally allow the work of fracture to be estimated (Vincent 1990). This might explain a general reluctance in most studies that have employed the punch test to discuss the mechanical implications of their results; chemical properties, in contrast, being discussed at length (see, for example, Feeny (1976); Coley (1983)).

Although the fracture toughness of dicotyledonous leaves would seem to be of great ecological importance, no firm conclusions can be made at present because the mechanical tests do not seem generally to have been very precise or well analysed. Classifications of leaves that are intended to reflect leaf 'toughness' or 'hardness' are defined in terms of the proportion of the mesophyll that is palisade tissue with, in addition, considerable attention being paid to the thickness of upper epidermal cell walls and the cuticle (Grubb 1986). However, it is completely unclear whether any of these tissues make leaves tough. It is another jump in reasoning again to assume that the punch test measures the ability of leaves to cause wear to insect mandibles as does Raupp (1985). His conclusions are weakened by the presence of copious druses (small, scattered, spherical, compound crystals of calcium oxalate) in *Salix* leaves (Metcalfe & Chalk 1950), which are very likely to wear insect mandibles but which are too small to affect resistance to a punch.

Most standard fracture tests on sheet materials, such as trouser-legging, that might be applied to dicotyledonous leaves fail because the crack is constantly deflected by the network of veins (Vincent 1990). Although the debonding along the vein requires work, the fracture toughness of the vein *per se* cannot be estimated by this test. In many monocotyledonous leaves the only conspicuous veins run virtually in parallel from the sheath base to the blade tip. Vincent (1982, 1983) has successfully used notched tensile tests to analyse grasses mechanically. For many dicotyledons, these tests are less successful because of the irregularity of the venous network.

Lucas & Pereira (1990) have described a simple scissoring arrangement based on the analysis of cutting systems developed by Atkins & Mai (1979), Atkins & Vincent (1984), Allison & Vincent (1990) and Vincent (1990). This forces a straight fracture path across a sheet of material (sufficiently thin to bend under its own weight) such that it is only stressed in a small region in front of the cutting edge of the scissor blades. The work done is recorded in a serial manner as variably tough parts of the leaf are crossed. We compare the results here to notched tensile tests (Vincent 1982), from which the work of fracture can be calculated indirectly and, briefly, to punch-and-die tests. The simple purpose of this study was to determine what makes a dicotyledonous leaf tough and, from this, to predict the range of toughnesses that might be expected in other species. The species used here is *Calophyllum inophyllum* L. (Guttiferae), which is a tropical sea-shore tree with a range from East Africa to New Caledonia (Stevens 1980). Although the intention of the study was to try to obtain a general model for leaf toughness suitable for studies of herbivory, this species was chosen because of its very regular venation (Ramji 1967; Darcy & Keating 1979; Stevens 1980).

2. MATERIALS AND METHODS

Calophyllum inophyllum 'sun' leaves were collected in the early morning within Singapore (latitude 1° 20′ N, longitude 103° 50′ E) from a roadside tree at Alexandra Park and from a tree in the Department of Botany arboretum of the National University of Singapore. The arbitrary criterion of their maturity in this study was the thickness of the lamina (t), which was measured with a screwgauge micrometer. Unless stated otherwise, leaves used in this study had $t = 0.27$–0.40 mm. All such leaves had a dark green upper surface. Voucher specimens have been deposited in the Herbarium of the Department of Botany, National University of Singapore (SINU). All specimens were kept inside sealed polyethylene bags containing moist tissue paper for up to 24 h until testing.

A pair of Dragonfly A-220 tailoring scissors was mounted on a modified compression platen on a Shimadzu DCS-5000 universal testing machine such that the lower handle of the scissors was gripped firmly. These scissors have blades made of high carbon steel backed by mild steel. The blades are slightly curved such that when the handles are brought together, there is a moving 'point contact' between the blades which does not require any lateral force to maintain. The upper handle was free to be moved by an upper platen that was in line with the load cell. Two adjustable supports were placed at the height of the immobile upper blade on either side (Lucas & Pereira 1990). Leaf specimens were prepared only by the removal of the marginal veins, the midrib never being fractured *in situ*. Specimens, with their upper epidermis facing upwards, were placed between the supports under the blade and weighted at either end. The scissors were

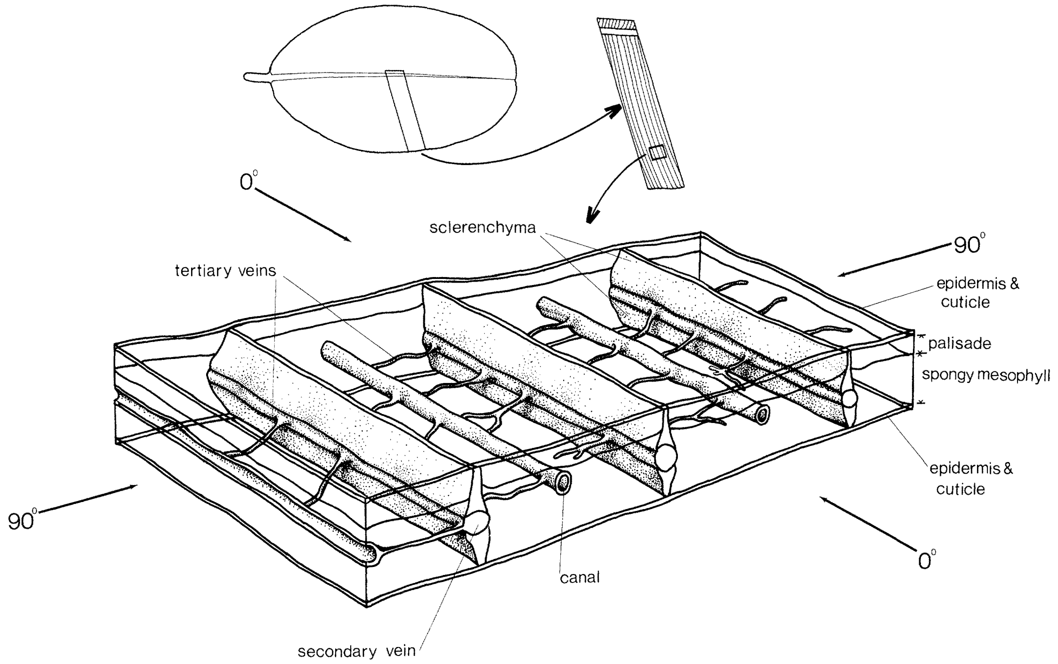

Figure 1. Diagram of the organization of tissues in the blade of *Calophyllum inophyllum*. Fracture paths are defined relative to the angle θ made with the secondary veins. Parallel to the secondaries, $\theta = 0°$; perpendicular to them (parallel to the tertiaries), $\theta = 90°$.

directed deliberately at a variable angle θ to the subparallel secondary veins, the angle being measured with a protractor.

During a test, the upper platen was displaced down onto the upper handle of the scissors at a crosshead speed of 30 mm min^{-1}, producing a cut in the lamina of between 6–20 mm depending on leaf dimensions. The work needed for this (W_1), which includes frictional effects in the scissors as well as work to fracture the leaf, was calculated automatically by a digital processor sampling the force-displacement data at 50 Hz. The crosshead was then reversed to its original position and an identical displacement of the crosshead made with the sole difference being that the leaf specimen had already been cut. It was assumed in this second displacement that all else but the work required to cut the leaf was equal. Thus, if the work needed for the second displacement was W_2, the length of cut was l and the lamina thickness t, then the fracture toughness (R) was obtained from

$$R = (W_1 - W_2)/lt. \qquad (1)$$

The length of cut was measured with Vernier calipers and the lamina thickness, as before, with a screwgauge micrometer.

Marginal veins and the midrib were dissected from adjacent tissues. Leaf skeletons were produced by heating leaf samples in 100 g l^{-1} NaOH to 60 °C overnight and separating secondary veins from other tissues with a soft brush. These were washed in water and stored wet until testing. Bundles of between 3–5 entwined secondary veins were tested with the scissors, but the midrib and marginal veins were tested singly.

For comparison with the cutting test, notched tensile tests were performed on a collection of leaves that were also cut at $\theta = 90°$. Strips of the lamina, of length l between 30–44 mm and width w between 4–5.5 mm, were cut from whole laminae such that $l > 8w$. More than one lamina was required to accrete sufficient data and therefore two batches of leaves were tested, one with $t = 0.27$–0.32 mm and the other with $t = 0.23$ mm. The latter were light green in colour. These strips were loaded in tension, either parallel or perpendicular to the secondary veins, at a displacement rate of 5 mm min^{-1}. With a sharp scalpel blade, specimens were notched at one edge (single-edge notch test) to a length a where $0.2 < a/w < 0.86$. Some specimens were also tested unnotched. All specimens were loaded in tension, gripping being assisted for low a/w by attaching pieces of 600 grade carborundum paper to the gripped portion of the lamina with a cyanoacrylate adhesive. The load (X) at failure was recorded. The strength (σ) was calculated as X/wt and σ was then plotted against a/w. The critical stress intensity factor (K_c) was calculated as

$$K_c = \sigma\,(\pi a)^{\frac{1}{2}} Y, \qquad (2)$$

where Y is a geometrical correction factor given (Tada in Tada *et al.* 1978) by

$$Y = \{0.265[1-(a/w)]^4 + [0.857 + 0.265(a/w)]\}/[1-(a/w)]^{\frac{3}{2}}. \qquad (3)$$

The Young modulus E was calculated by loading similar unnotched specimens. The Poisson's ratio ν was measured on leaf strips loaded in tension. Fiducial marks were drawn on the leaves, well away from the clamps, the specimen was then photographed on slide film at various longitudinal strains with a camera equipped with a macro lens. The film was developed, placed on an optical stage, and the vertical displacement of the marks and contraction of width of the specimens as shown on the image, measured under a stereozoom microscope. An estimate for R was obtained from

$$R = K_c^2/E^*, \qquad (4)$$

where E^* is given by $E/(1-\nu^2)$ (Vincent 1982).

A punch-and-die tester was designed and constructed (Chan Swee Heng, Heng Ming Enterprises) such that the punch could be mounted on the crosshead of the Shimadzu universal testing machine. The steel punch had two interchangeable heads, one flat and the other concave, both of which were sharp sided. The punch was aligned and stabilized by a linear bearing located just above the leaf specimen. The leaf was held flat between two Perspex plates. A circular hole in the lower perspex plate served as the die. The clearance between the punch and the die was 0.15–0.2 mm. For a test, the punch was positioned about 0.5 mm above the specimen and the crosshead displaced 3–3.5 mm at a rate of 30 mm min^{-1}. The crosshead was then returned to its original position and an identical second displacement made with the cut leaf still in position. Once the basic form of the loading was established, repeated tests were performed at which the crosshead was reversed at various points on the curve and the damage done to the specimen inspected. The area under the loading curve that was associated with fracture was digitized with a Janel Sigma Scan digitizing system. The leaf discs produced in these tests were examined and photographed.

Freehand sections of leaves that had been cut in tests were sectioned with a fresh razor blade, mounted and stained with aqueous 0.05–0.5 g l^{-1} Toluidine Blue O or left unstained for viewing under a light microscope (LM). Camera lucida drawings were made of these sections and estimates of the area and volume fractions of each of the component tissues of the leaf were obtained by digitizing.

The fractured surfaces of test specimens were viewed under a stereozoom microscope. These surfaces were also coated with 25 nm gold–palladium and photographed using a Jeol JSM-T220A scanning electron microscope (SEM) at magnifications of × 35– × 1000.

An impression was made of the edges of the blades of two pairs of scissors with Regisil elastomeric impression compound. One pair had been used intensively for cutting leaves and newsprint for one month. The other was unused. The impressions were sliced perpendicular to the cutting edge at random intervals with a scalpel blade. These were photographed under SEM at a magnification of × 5000 and the radii of curvature of the edges estimated by the freehand fitting of curves.

3. RESULTS

The morphology of a *C. inophyllum* leaf is illustrated in figure 1 and summary statistics of the tissue organization are given in table 1. The palisade layer of the leaf extends for about one third of the thickness of

Table 1. *Area fractions of tissues in a single lamina of C. inophyllum*

(Area fractions are also the approximate volume fractions except for tertiary veins for which the volume fraction is 0.0045.)

structure	number sampled	area fraction, θ
secondary veins of which:	12	0.0813 (90°)
vascular tissue		0.0305
sclerenchyma		0.0508
tertiary veins	13	0.0059 (0°)
canals	11	0.0128 (90°)
upper epidermis & cuticle	—	0.1067
lower epidermis & cuticle	—	0.0648
palisade	—	0.1913
spongy mesophyll	—	0.5431

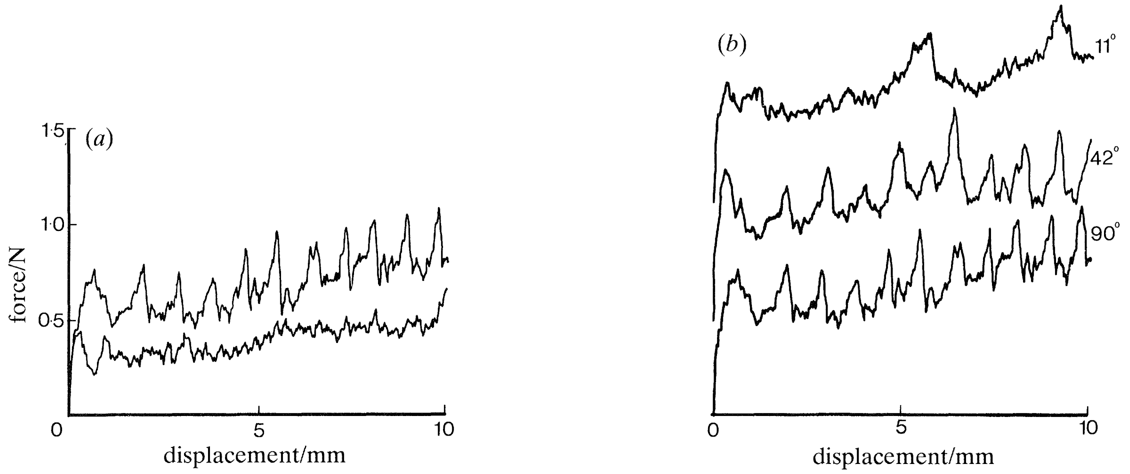

Figure 2. (*a*) Force-displacement records for the crosshead contact with the upper handle of the scissors. The upper trace is produced by fracture of a *Calophyllum inophyllum* leaf at $\theta = 90°$ to the secondary veins. Lower trace shows the force required to make an identical second movement of the scissors with the fractured leaf still in position. The area between the two graphs is the energy required for fracture. (*b*) Three upper traces for the fracture of parts of a single lamina of *Calophyllum inophyllum* at different angles (θ) to the secondary veins. The spacing of the peaks increases as $1/\sin\theta$. However, the energy represented by each peak does not vary greatly. Forces to same scale as (*a*).

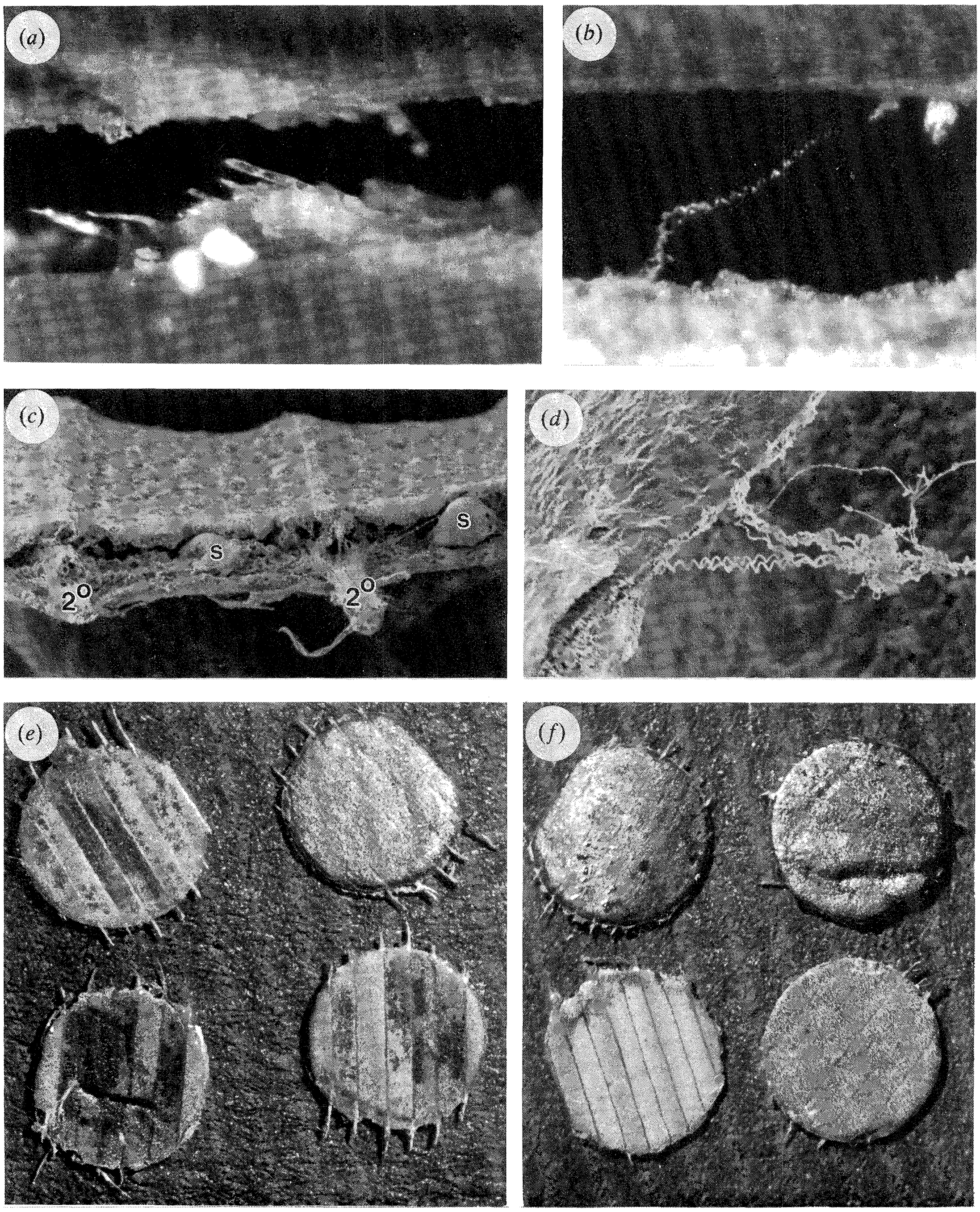

Figure 3. Fracture surfaces of leaves of *Calophyllum inophyllum* produced by different methods. (*a*, *b*) Cutting tests. (*a*) Appearance of a surface cut at $\theta = 13°$, viewed from the upper epidermis with stereozoom LM. Note the cut surface to the left, which is smooth at the upper epidermis but below (slightly out of focus) it is rough. A secondary vein runs obliquely slanting downwards to the right. Note its rough 'cut' surface and that of adjacent tissue fractured to the right. (*b*) Surface cut at $\theta = 0°$. An uncoiled part of the spiral thickenings of xylem vessels of a tertiary vein. (*c*, *d*) Notched tensile tests. (*c*) Loading along the secondary veins (SEM). A relatively flat surface but with pull-out of secondary veins (2°); secretions from secretory canals (s). (*d*) Loading across secondary veins (SEM). Uncoiling of the spiral thickenings in the wall of xylem vessels in a tertiary vein. (*e*, *f*) Punch-and-die tests. (*e*) Flat-headed punch and (*f*) concave-headed punch, viewed by stereozoom LM. Note the extensive pull-out of the secondary veins, particularly with the flat head. Magnifications: (*a*, *b*) ×499.2; (*c*) ×68; (*d*) ×123.2; (*e*, *f*) ×5.7.

the mesophyll. Beneath this, in the spongy mesophyll, there is a regular network of veins consisting of an orthotropic arrangement of secondary veins and tertiary veins. Secondary veins diverge from the midrib at a more-or-less constant interval for any given leaf and run in parallel towards the margin. At the margin, these anastomose with the marginal vein. The secondary veins can be seen clearly on the undersurface of the blade but do not project beyond the surface to any degree. The primary xylem and phloem in these veins is supported above and below by sclerenchyma strands that extend like caps to reach the epidermis at both surfaces. Some of these secondary veins branch but the branches quickly turn to run parallel to the parent

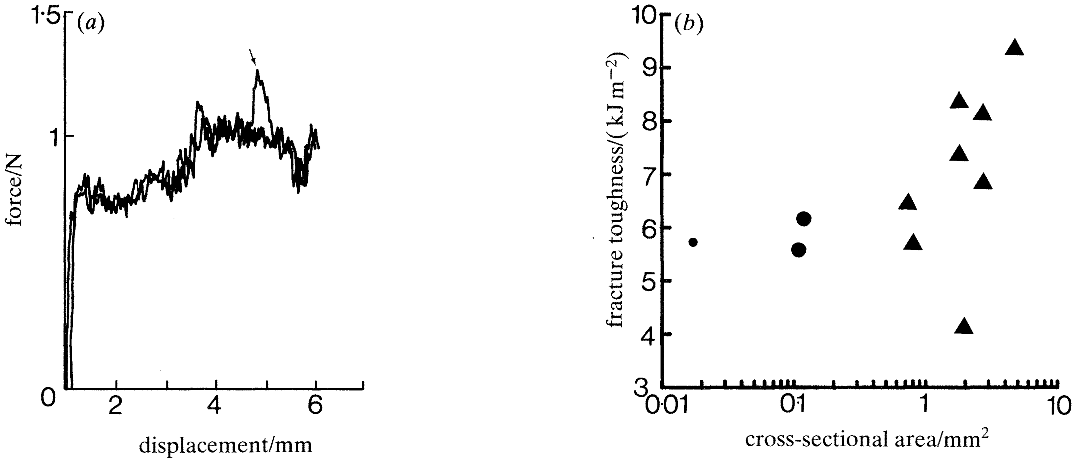

Figure 4. (*a*) The energy used to break five entwined secondary veins dissected from a *Calophyllum inophyllum* leaf skeleton is recorded as a peak (arrowed) and is the only distinct difference between first and second scissor passes. (*b*) The fracture toughness of veins of different sizes is plotted against the natural logarithm of their cross-sectional areas. Individual test results are shown except for secondary veins (small circle) which are the average for 20 results. Other than one probably anomalous value, smaller parts of the midrib (triangles) have a similar value to marginal (large circle) and secondary veins.

veins. Running in parallel between secondary veins are secretory canals (figure 1), termed 'canal secondaries' by Ramji (1967). These contain poorly differentiated vascular tissue in their walls. Tertiary veins branch at irregular intervals from secondaries and pass towards these canals usually taking the shortest path. Some of these veins branch and some end in the mesophyll, thereby failing to connect with the canals. The cell walls of the xylem cells in the tertiary veins contain spiral thickenings of the secondary cell wall but the sclerenchyma, if present, is in the form of a small cap and does not contact the epidermis. This cap is irregular and mostly absent.

Fracture paths produced in the cutting tests were defined as the angle θ that they made to the secondary veins (figure 1). Figure 2*a* shows a typical trace of a *C. inophyllum* leaf cut at $\theta = 90°$. The upper trace shows large peaks in force. These peaks, approximately triangular in shape, were not due to the scissoring method because they were not seen on the lower trace. The position of these peaks along the cut and their spacing matched those of the secondary veins on the actual leaf. The width of the base of the triangular force peak was much wider than the diameter of a secondary vein. However, an approximate measurement of the work represented by a peak, divided by the cross-sectional area of an average secondary vein, gave a fracture toughness of approximately 6 kJ m^{-2}. No peak in force could be attributed to the canals.

An irregular variation in force could be seen in the lower trace (figure 2*a*) but there were no large peaks corresponding to veins. Examination of the fractured surfaces showed a variable appearance (figure 3*a*). Parts of the surface, particularly close to the upper epidermis, could be very smooth. However, fractured secondary veins had roughened surfaces as did fractured tissue to one side of the vein. However, there was no general change in direction of the crack at the mesophyll–vein interface. Some veins showed uncoiling of the thickenings of the secondary cell walls of the primary xylem (figure 3*b*). Within 6 h following a test, portions of the leaf specimen that were within 0.3 mm when $\theta = 0°$, and within 0.15 mm (at $\theta = 90°$) of the cut turned light brown. The discoloration appeared to be due to cell damage in the mesophyll.

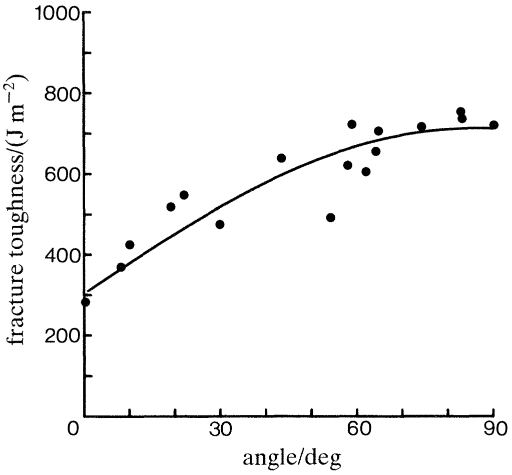

Figure 5. Values for the fracture toughness of a single lamina ($t = 0.35$–0.4 mm) of *Calophyllum inophyllum* for a varying angle θ. The line is a nonlinear least-squares regression to equation (7) fitting for $R_m = 270.3$ J m^{-2}.

When the angle θ was reduced, the distance between the peaks increased as did the width of the base of the peaks, but the height of the peaks reduced (figure 2*b*). The result was, apparently, that the energy to fracture a secondary vein varied little with θ. At low values of θ, other much smaller peaks could be seen. However, because the spacing of tertiary veins was very variable, no clear significance could be allotted to these.

Values for the fracture toughness of venous tissue, with associated sclerenchyma, are shown in figure 4*a*, *b*. The fracture toughness of the midrib at cross-sectional areas above 1 mm^2 appeared to vary with size but smaller specimens of the midrib, marginal veins and the average value for 20 secondary veins appeared

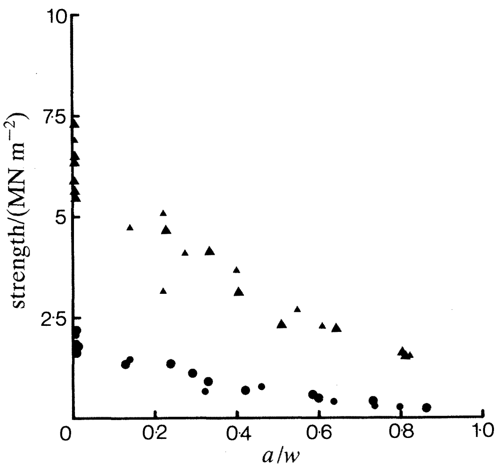

Figure 6. The nominal strength at failure of variably notched (a/w) strips of *Calophyllum inophyllum* leaf, loaded along (triangles) and across (circles) the secondary veins. For large symbols, $t = 0.27$–0.32 mm and for small symbols, $t = 0.23$ mm.

to have a fairly constant value of about 6 kJ m^{-2} (figure 4*b*). Tertiary veins were too small and short to test.

For a single leaf ($t = 0.35$–0.4 mm), variation of R_θ with θ was examined (figure 5). Toughness at $\theta = 90°$ was nearly three times that at $\theta = 0°$.

Graphs of apparent tensile strength σ versus a/w produced almost straight lines (figure 6) indicating that the leaf had little notch-sensitivity in either direction, although the leaf was much stronger at $\theta = 90°$ (i.e. the 'along the secondary veins' loading direction) than at $\theta = 0°$. The direction of fracture, when nominally at $\theta = 90°$, sometimes changed direction to run along a secondary vein before turning back to cross several secondary veins in cross section. SEM examination showed that, at $\theta = 90°$, the secondary veins could pull out for up to 1 mm from the lamina. However, the average was much smaller than this and the fracture surface often looked very clean (figure 3*c*). For loadings across the secondary veins (fractures at $\theta = 0°$), tertiary veins were not similarly pulled out. However, at both angles, the spiral secondary thickenings of the primary xylem had been uncoiled, often for over 1 cm (figure 3*d*). Plots of σ^2 versus $1/aY^2$ (figure 7) showed that specimens with a high a/w and therefore a low σ^2, could be joined by a higher slope than those from tests with a low a/w. Nevertheless, a straight line was fitted to all points with a correlation coefficient $r = 0.958$ ($p < 0.0001$) at $\theta = 90°$, and $r = 0.935$ ($p < 0.0001$) at $\theta = 0°$. K_c was calculated from the slopes shown in figure 7*a*, *b*. Values are shown in table 2. At $\theta = 90°$, K_c was over three times that at $\theta = 0°$.

Unnotched specimens in tension had linear stress–strain curves initially but showed a shoulder at 60–70% of maximum force, which indicated some plastic deformation. The Young modulus was measured from the linear portion of the curve. The modulus was three times higher when the lamina was loaded along the secondary veins than when loaded across them. Values were slightly lower for the thinner (immature) leaves (table 2).

The mean value for Poisson's ratio was only measured for the thicker (mature) leaves and was assumed to be applicable to the immature leaves as well. For a longitudinal strain along the secondary veins, it averaged 0.362. However, when loaded across them, we could not detect any decrease in specimen width at longitudinal strains of up to 0.042, which was in the range at which some specimens failed. A value of zero was used for this loading orientation in the calculations to derive R (table 2).

The values of R derived for both mature and immature leaves in tension at $\theta = 90°$, 562–724 J m^{-2} (table 2), were smaller than the means for cutting tests at the same angle on the same leaf sample, which were 992 and 1012 J m^{-2} for immature and mature leaves

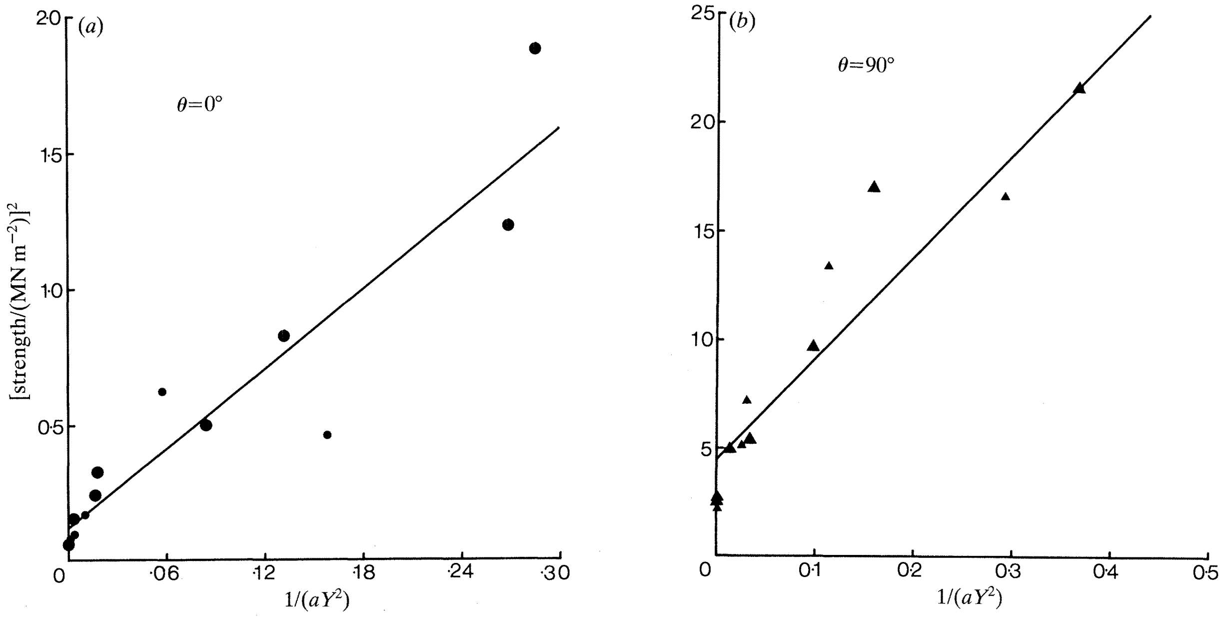

Figure 7. Plots of the square of the failure stress against $1/aY^2$ for the notched tensile tests: (*a*) $\theta = 0°$; (*b*) $\theta = 90°$. For large symbols, $t = 0.27$–0.32 mm and for small symbols, $t = 0.23$ mm.

Table 2. *Mechanical properties of C. inophyllum leaves in tension*

(The direction of loading is given with respect to the secondary veins such that loading across these veins gives a fracture at $\theta \simeq 0°$ and along the veins at $\theta \simeq 90°$. Values are the means (standard deviation). n is number of tests.)

			direction		
property	t (mm)	n	across	n	along
K_c (MN $m^{-\frac{3}{2}}$)	0.23–0.32	13	0.122	12	0.394
E (MN m^{-2})	0.23	5	49.8 (4.3)	8	186.4 (53.8)
	0.27–0.32	5	67.5 (9.7)	7	240.2 (75.0)
ν	0.32	10	0.0 (—)	6	0.362 (0.048)
R (J m^{-2})	0.23	—	299	—	724
	0.27–0.32		221		562

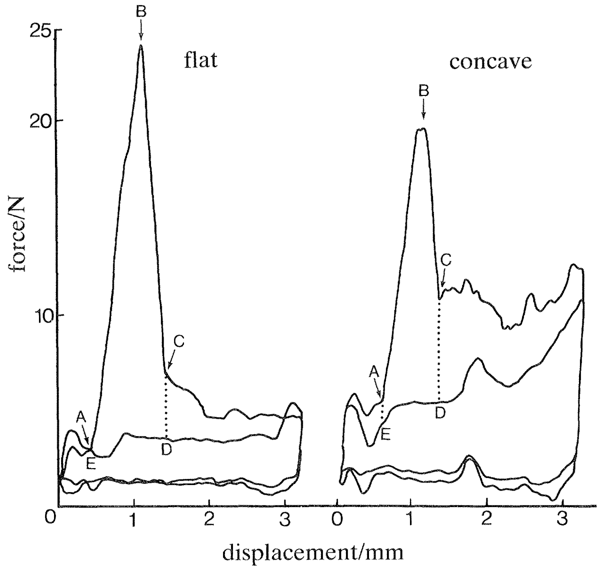

Figure 8. Force-displacement records for the flat-headed (left) and concave-headed punches (right) under displacement control. Points A, B and C lie on the first pass of the punch whereas D and E lie on the second pass.

Table 3. *Values for the work done ($J\ m^{-2}$) in the punch-and-die tests*

(For each leaf, the mean (s.d.) is given for three replicates.)

		head type	
leaf	t (mm)	flat	concave
A	0.34–0.36	2129 (246)	1427 (107)
B	0.34	1605 (45)	1329 (66)
C	0.32–0.33	1345 (155)	1307 (117)
D	0.32–0.33	1654 (170)	1141 (51)

respectively. The values at $\theta = 0°$ were comparable to scissor values at this angle (figure 5).

A triangular force-displacement curve was seen with the punch tests, being superimposed on an irregular background of friction. In figure 8, the punch started to load the leaf specimen at A and the lamina started to fracture at B. The displacement AB was generally the equal of lamina thickness indicating that the lamina had been bent. Just before B, it was shown, by reversal of the crosshead, that the leaf had been plastically indented though the epidermis was intact. Fracture was more or less complete at C. Beyond C, friction was variable depending on the position of the leaf disc. The crosshead was then reversed and an identical second pass made. On figure 8, points lying vertically below C and A are labelled D and E respectively. Beyond D on the second pass, friction was usually lower than after C, but the work done after these points was not associated with fracture.

It was assumed that the area enclosed by ABCDE represented the work done on the specimen that was associated with fracture. Values obtained are given in table 3. An analysis of variance showed the values obtained with the flat head to be significantly higher than those for the concave head ($F = 47.26$; $p < 0.001$). The leaf discs produced by the two heads were similar in shape with complete pull-out of the veins for up to 1 mm (figure 3 *e*, *f*). However, the average length of vein pulled-out was 0.6 mm for the flat head but only 0.3 mm for the concave head. Within 6 h following testing, laminae from which discs had been removed developed circular brown patches around the holes extending up to 1 mm from the edges.

The radii of curvature of the edges of the scissor blades, used and unused, were all in the range of 2–3 μm.

4. DISCUSSION

The results show unequivocally that it is vascular tissue and the sclerenchyma associated with it that is responsible for the fracture toughness of the *C. inophyllum* leaf. Scissors tests gave higher values for R than the tensile tests, but the latter were derived from assumptions that may not take account of the plastic work associated with crack propagation (Jackson *et al.* 1988). This plasticity is indicated by the shoulder on the stress–strain curves on unnotched tensile specimens. The tensile tests also involved a free-running crack, whereas with scissors, crack propagation is tightly controlled by the stress intensity imparted by the blades. That R with the scissors test was not lower than that from tension suggests that the toughness that veins impart is largely intrinsic and not likely to be a function of large-scale debonding from neighbouring tissues with pull-out. None of this can be deduced with certainty from unstable fracture on notched tensile tests alone.

In the cutting tests, the abrupt decline in force on the downside of the peaks associated with secondary veins, coupled with evidence of the rough fractured surfaces of these veins, suggests that they failed by a crack running in front of the scissor blades. This crack probably arrested in a canal. Most cutting processes appear to generate such cracks once a critical stress intensity has been reached (Atkins & Mai 1985). Following some indentation, tensile stresses build up (in part owing to the wedge shape of the cutting tool and also possibly because of tensile pull in the shear plane) from which a free-running crack is initiated (Atkins 1981). However, in our cutting tests, there could not have been much pull-out produced by this crack or else the second pass with the scissor blades against the leaf would have trimmed the surface and produced peaks similar to those in the first pass.

It is possible, if there are no important interfacial effects at the sides of veins, to consider the toughness of the leaf by a 'rule of mixtures' (Atkins & Mai 1985) with the 'fibres' (vascular tissue plus sclerenchyma) contrasted with the surrounding matrix (all other tissues). To do this, we assume that secondary and tertiary veins have the same toughness. (We could not test this directly, though figure 4*b* provides some evidence that the toughness of veins is constant over an order of magnitude of cross-sectional area.) This assumption generates two equations for fracture toughness at $\theta = 90°$ and $\theta = 0°$.

$$R_{90°} = A_s R_f + (1 - A_s) R_m, \tag{5}$$

$$R_{0°} = A_t R_f + (1 - A_t) R_m, \tag{6}$$

where A_s and A_t are area fractions of secondary and tertiary veins in the fracture path at $\theta = 90°$ and $\theta = 0°$ respectively, R_f is the fracture toughness of the venous 'fibres' and R_m is the average fracture toughness of the other tissues (the 'matrix').

If the work needed to fracture a vein is considered to be independent of θ, then the fracture toughness (work divided by fractured surface) of a vein will vary with θ because the fractured area is a function of θ. If tertiary veins are assumed to lie at right-angles to secondary veins, then the fracture toughness attributable to secondaries will be $A_s R_f \sin\theta$, and that to tertiaries, $A_t R_f \cos\theta$. This yields the general equation

$$R_\theta = \alpha R_f + (1 - \alpha) R_m, \tag{7}$$

where $\alpha = A_s \sin\theta + A_t \cos\theta$.

Note that equation (7) generalizes equations (5) and (6) and satisfies the requirement that as $A_s \rightarrow 0$ and $A_t \rightarrow 0$ then $R_\theta \rightarrow R_m$. It also has the property that interchange of A_s and A_t corresponds to replacement of θ by $(90° - \theta)$.

For the leaf in figure 5, $A_s = 0.0813$ and $A_t = 0.0059$. R_f can be considered to be 5714 J m^{-2}, which is the average of the 20 secondary veins tested. Assuming tertiary veins to have the same toughness, then a nonlinear least-squares regression can be employed to estimate R_m. The solid line in figure 5 was generated in this way and fits R_m at 270.3 J m^{-2} (standard error of 15 J m^{-2}). Analysis of variance shows that 82.2 % of the variation is accounted for by this fit. The effect of the tertiary veins is to produce a peak toughness at $\theta_0 < 90°$, where $\mathrm{d}R_\theta/\mathrm{d}\theta|_{\theta_0} = 0$. Performing this differentiation gives

$$\theta_0 = \tan^{-1}(A_s/A_t). \tag{8}$$

For the leaf in figure 5, this is at $\theta_0 = 85.84°$. (If A_t is neglected, then the peak is at 90°, R_m becomes 290 J m^{-2} and less of the variation is accounted for.) Equation (7) therefore describes the data in figure 5 very well.

The toughness of mature leaves of other species could be predicted to lie between 200–6000 J m^{-2}. The larger cross sections of midrib tested here (figure 2*b*) are probably not tougher than this upper value; the apparent increase with thicknesses above 1 mm being found also with other materials (Lucas & Pereira 1991), probably because bending stiffness depends on the cube of thickness (Atkins & Mai 1985). It is unlikely that any vein has the toughness of wood (10 kJ m^{-2}) (Jeronimidis 1980). There is no evidence that sclerenchyma is tougher than thickened xylem. Seed shells, composed only of highly lignified fibrous sclereids, have a fracture toughness of only 2 kJ m^{-2} (Vincent 1990; Lucas *et al.* 1991). The fracture toughness of the other leaf tissues (220–300 J m^{-2}) is very close to those accepted for parenchyma tissues, although some parenchymas can have toughnesses as low as 60 J m^{-2} (Vincent 1990).

Equation (7) can also be used to generate an expected toughness value for the punch tests. Because the circumference of the punch would fracture tissues at all angles, then the toughness expected from a test would be the average of R_θ at all angles of θ. This is given by

$$\int_0^{\frac{\pi}{2}} R_\theta \,\mathrm{d}\theta \bigg/ \int_0^{\frac{\pi}{2}} \mathrm{d}\theta = \frac{2}{\pi}(R_f - R_m)(A_s + A_t) + R_m, \tag{9}$$

which is 572.8 J m^{-2} for the leaf in figure 4. Although the punch tests were done on different leaves, it is not likely that the values obtained (table 3) would be over double this expected value. Given the above analysis, it seems therefore that the punch test did not produce a valid (specific) work of fracture.

Nevertheless, the punch tests do provide some information on the extent to which the pull-out of fibres could contribute to the work of fracture as this was the only observable difference in tests with the flat and concave heads. The most likely component of work is the debonding of tissue from around the veins. An estimate for this is from Outwater & Murphy (1970), presented by Jackson *et al.* (1988) as

$$R_{\mathrm{debond}} = (V_s \sigma_s^2 / 2E_s)y, \tag{10}$$

where V_s is the volume fraction of secondary veins (as tertiary veins do not pull-out appreciably), y is twice the length to which the veins protrude and σ_s and E_s are the strength and elastic modulus respectively of the secondary veins. If the secondary veins are assumed responsible for the strength of the leaf at $\theta = 90°$, then σ_s can be estimated by dividing the strength (7.5 MN m^{-2}) by A_s, which gives $\sigma_s = 92.3$ MN m^{-2}. E_s cannot be estimated by Voigt or other models from the present data because when Poisson's ratios are unequal, such models are inappropriate (Harris 1980). Therefore, values must be drawn from the literature. Gibson *et al.* (1988) derived a value of 3.52 GN m^{-2} for the Young modulus of the sclerenchymatous ribs of the iris leaf. The debonded length y can be taken to be 0.6 mm (twice the average difference in pull-out between the flat and concave heads in the punch test) but this needs to be doubled again to 1.2 mm because the veins are pulled out either side of the disc. Substituting these values in equation (10), then R_{debond} would be approximately 118 J m^{-2}. This might be sufficient to explain the different amounts of work needed for the flat versus the concave heads (although each is subject to great variation), but it certainly cannot explain why both punch tests far exceeded values obtained from cutting and tensile tests. This excess is far more plausibly a result of the volume of mesophyll that yielded with the punches, this being indicated by the amount of tissue that was discoloured by secretions that leaked from the burst canals into the mesophyll. This could be reduced by having the punch and die fit more tightly. However, in the limit, this would produce virtually the same arrangement as with the scissors whereby there is metal-to-metal contact. The difference would be that this tight fit could not be maintained around the entire circumference of the punch. Therefore all punch-and-die tests must produce a work of fracture exceeding that of a cutting test by a factor that depends on the orientation of the veins and, probably, also the yield strength of the leaf tissues.

The results have several general implications. It is meaningless to talk of the toughness of a leaf as a whole because the cost of fracture will depend on the direction of cracking relative to veins and, also, on the structure of these veins. The punch-and-die test is much more problematic than a cutting test and is unlikely to measure fracture toughness reliably. The presence of sclereids of various shapes (particularly asterosclereids (Esau 1977; Medina *et al.* 1990)) in some species is likely to influence the yield strength of the mesophyll strongly and punch results would be difficult to interpret in leaves with a more complex venation.

Classifications of leaves that are based overtly on fracture or are otherwise intended to reflect mechanical defences against herbivores (Grubb 1986) should be addressed to leaf venation. Intervein spacing is probably determined by the requirement for rapid water conduction (Esau 1977). Veins compete for space with the photosynthetic tissues that they serve, suggesting that a minimal venation adequate for the hydration of the leaf might be selected for. However, in any given species, the spacing of veins depends on the obstruction that component tissues of the leaf present to the passage of water. The closest intervein spacing is found in species that have a high proportion of palisade tissue in the mesophyll (Wylie 1946; Philpott 1952). However, this water-conducting function does not require any thickening of the cell walls because the extent of water loss through the vein walls can be controlled by suberization (Esau 1977). Thickening of the cell walls of the xylem and the presence of associated sclerenchyma probably serve to stiffen the lamina (Gibson *et al.* 1988). Sclerenchyma fibres have been shown to be responsible for 90% or more of the longitudinal stiffness of grasses (Vincent 1982). Because the leaf lamina is the principal site of carbon fixation in plants, its rigidity contributes to a uniform solar irradiation. However, whatever the essential contribution of veins are to achieving this rigidity, the venation of dicotyledonous leaves, which usually form a reticulate network, are different to those of most monocotyledons, in which the only significant veins are more or less parallel to the midrib (Esau 1977).

The tertiary veins of *C. inophyllum* leaves are not sufficiently large or numerous to impart much toughness, but *C. inophyllum* is not very typical of dicotyledonous leaves in this respect, albeit an easier species to study. In many dicotyledons, veins of different orders can be the same size (e.g. *Ficus* spp.; Philpott 1952). In veins where $A_s = A_t$ (or equivalent subscripts if the vein orders differ), then the toughness of the leaf would be much less direction-dependent with a peak at $\theta_0 = 45°$. Such networks, from whatever orders of veins they are formed, are unlikely to be required for the stiffness of the lamina. These venous networks are often very small and, furthermore, variation in the networks does not correspond with leaf shape.

Instead, a case can be made for a network of small veins with thickened cell walls to deter small herbivores. Vincent (1982) has pointed out that the notch insensitivity of grass leaves, (also true of *C. inophyllum* and shown here to be due to thickened vascular tissue and sclerenchyma), makes it very difficult for herbivores to feed efficiently. A hole made by a herbivore in a leaf with pointed (cusped) features on its teeth or mandibles will not weaken the leaf and any crack which spreads from this will have its direction dictated by the veins. A network of veins stops any 'easy' direction for producing a free-running crack. Thus, herbivores with bladed teeth would be favoured by selection, in the limit reducing the energy expended to that of the specific work of fracture. Some herbivorous invertebrates (Bernays & Janzen 1988) have their mandibles, and most mammalian folivores (Kay & Hylander 1978), including higher primates (Kay & Covert 1984), have their posterior dentition equipped with prominent sharp blades. However, the consequence would be that very few cells of the fragments of leaf produced would be damaged, putting an emphasis on microbial action in the gut to obtain nutrition. Small herbivores are very inefficient at such microbially assisted digestion (Coley 1983), partly due to the passage time of food in the gut being very short (Bernays & Janzen 1988). Nevertheless, blades may be the only efficient mechanism for coping with tough leaves because otherwise the veins will dictate fracture and interfere with a particle size requirement for swallowing. Bernays & Janzen (1988) have found that saturniid caterpillars, which eat what are probably tough leaves, have bladed mandibles that do such little damage to leaf fragments that they are defaecated almost intact. In contrast, most sphingid caterpillars have pointed cusps and seem to rely on generating free-running cracks in tension and on breaking open as many individual cells as possible. They avoid particularly tough (veiny) leaves (Bernays & Janzen 1988) for which such a strategy is probably impossible.

More work needs to be done, particularly on the development of notch sensitivity during the growth and maturation of a leaf. This is important because many herbivores appear to eat immature leaves (Coley 1983). It is also necessary to know more about how different herbivores attack leaves and the characteristics of the fractured surfaces of the leaves that they produce. Only then will it become clear what mechanical tests it is necessary to make in order to analyse their actions. However, it is absolutely clear that, because fracture is always involved, a test of fracture toughness will be required for this analysis.

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REFERENCES

Allison, R. T. & Vincent, J. F. V. 1990 Measuring the forces during microtoming by the use of load cells. *J. Microsc.* **159**, 203–210.

Atkins, A. G. 1981 Surfaces produced by guillotining. *Phil. Mag.* **A43**, 627–641.

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.

Bernays, E. A. 1986 Diet-induced allometry among foliage-chewing insects and its importance for graminivores. *Science, Wash.* **231**, 495–497.

Bernays, E. A. & Janzen, D. H. 1988 Saturniid and sphingid caterpillars: two ways to eat leaves. *Ecology* **69**, 1153–1160.

Cherrett, J. M. 1968 A simple penetrometer for measuring leaf toughness in insect feeding studies. *J. Econ. Ent.* **61**, 1736–1738.

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

Coley, P. D. 1987 Interspecific variation in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. *New Phytol.* **106** (Suppl.), 251–263.

Darcy, W. G. & Keating, R. C. 1979 Anatomical support for the taxonomy of *Calophyllum* (Guttiferae) in Panama. *Ann. Miss. Bot. Gard.* **66**, 557–571.

Esau, K. 1977 *Anatomy of seed plants*, 2nd edn. New York: John Wiley.

Feeny, P. 1970 Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology* **51**, 565–581.

Feeny, P. 1976 Plant apparency and chemical defense. In *Biochemical interactions between plants and insects* (ed. J. Wallace & R. L. Mansell), pp. 1–40. New York: Plenum.

Gibson, L. J., Ashby, M. F. & Easterling, K. E. 1988 Structure and mechanics of the iris leaf. *J. Mater. Sci.* **23**, 3041–3048.

Grubb, P. J. 1986 Sclerophylls, pachyphylls and pycnophylls: the nature and significance of hard leaf surfaces. In *Insects and the plant surface* (ed. B. Juniper & T. R. E. Southwood), pp. 137–150. London: Edward Arnold.

Harris, B. 1980 The mechanical properties of composite materials. In *The mechanical properties of biological materials* (ed. J. F. V. Vincent & J. D. Currey), pp. 37–74. Cambridge University Press.

Jackson, A. P., Vincent, J. P. V. & Turner, R. M. 1988 The mechanical design of nacre. *Proc. R. Soc. Lond.* B **234**, 415–440.

Jeronimidis, G. 1980 Wood, one of nature's challenging composites. In *The mechanical properties of biological materials* (ed. J. F. Vincent & J. D. Currey), pp. 169–182. Cambridge University Press.

Kay, R. F. & Hylander, W. L. 1978 The dental structure of mammalian folivores with special reference to primates and Phalangeroids (Marsupialia). In *The ecology of arboreal folivores* (ed. G. G. Montgomery), pp. 173–196. Washington: Smithsonian Institution.

Kay, R. F. & Covert, H. H. 1984 Anatomy and behaviour of extinct primates. In *Food acquisition and processing in primates* (ed. D. J. Chivers, B. A. Wood & A. Bilsborough), pp. 467–508. New York: Plenum Press.

King, B. L. 1988 Design and evaluation of a simple penetrometer for measuring leaf toughness in studies of insect herbivory. *Virginia J. Sci.* **39**, 405–409.

Lowell, R. B., Markham, J. M. & Mann, K. H. 1991 Herbivore-like damage induces increased strength and toughness in a seaweed. *Proc. R. Soc. Lond.* B **243**, 31–38.

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.* (In the press.)

Lucas, P. W. & Pereira, B. 1990 Estimation of the fracture toughness of leaves. *Funct. Ecol.* **4**, 819–822.

Lucas, P. W. & Pereira, B. 1991 Thickness effect in cutting systems. *J. Mater. Sci. Lett.* **10**, 235–236.

Medina, E., Garcia, V. & Cuevas, E. 1990 Sclerophylly and oligotrophic environments: relationships between leaf structure, mineral nutrient content and drought resistance in tropical rainforests of the Upper Rio Negro region. *Biotropica* **22**, 51–64.

Metcalfe, C. R. & Chalk, L. 1950 *Anatomy of Dicotyledons*, vols 1 & 2. Oxford: Clarendon Press.

Outwater, J. O. & Murphy, M. C. 1970 Fracture energy of unidirectional laminates. *Mod. Plast.* **47**, 160–169.

Philpott, J. 1952 A blade tissue study of leaves of forty-seven species of *Ficus*. *Bot. Gaz.* **115**, 15–35.

Ramji, M. V. 1967 Morphology and ontogeny of the foliar venation of *Calophyllum inophyllum* L. *Aust. J. Bot.* **15**, 437–443.

Raupp, M. J. 1985 Effects of leaf toughness on mandibular wear of the leaf beetle, *Plagiodera versicolora*. *Ecol. Entomol.* **10**, 73–79.

Schultz, J. C. & Baldwin, I. T. 1982 Oak leaf quality declines in response to defoliation by gypsy moth larvae. *Science, Wash.* **217**, 149–151.

Stevens, P. F. 1980 A revision of the Old World species of *Calophyllum* (Guttiferae). *J. Arn. Arb.* **61**, 117–690.

Tada, H., Paris, P. C. & Irwin, G. R. 1978 *The stress analysis of cracks handbook* (revised). Philadelphia: Del Corporation.

Tanton, M. T. 1962 The effects of leaf "toughness" on the feeding of larvae of the mustard beetle. *Entomol. Exp. & Appl.* **5**, 74–78.

Vincent, J. F. V. 1982 The mechanical design of grass. *J. Mater. Sci.* **17**, 856–860.

Vincent, J. F. V. 1983 The influence of water content on the stiffness and fracture properties of grass leaves. *Grass For. Sci.* **38**, 107–111.

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

Waller, D. A. 1982 Leaf-cutting ants and avoided plants: defences against *Atta texana* attack. *Oecologia* **52**, 400–403.

Williams, L. A. 1954 The feeding habits of Acrididae and the factors which determine them. *Trans. R. Ent. Soc. Lond.* **105**, 423–454.

Wylie, R. B. 1946 Relations between tissue organization and vascularization in leaves of certain tropical and subtropical dicotyledons. *Am. J. Bot.* **33**, 721–726.

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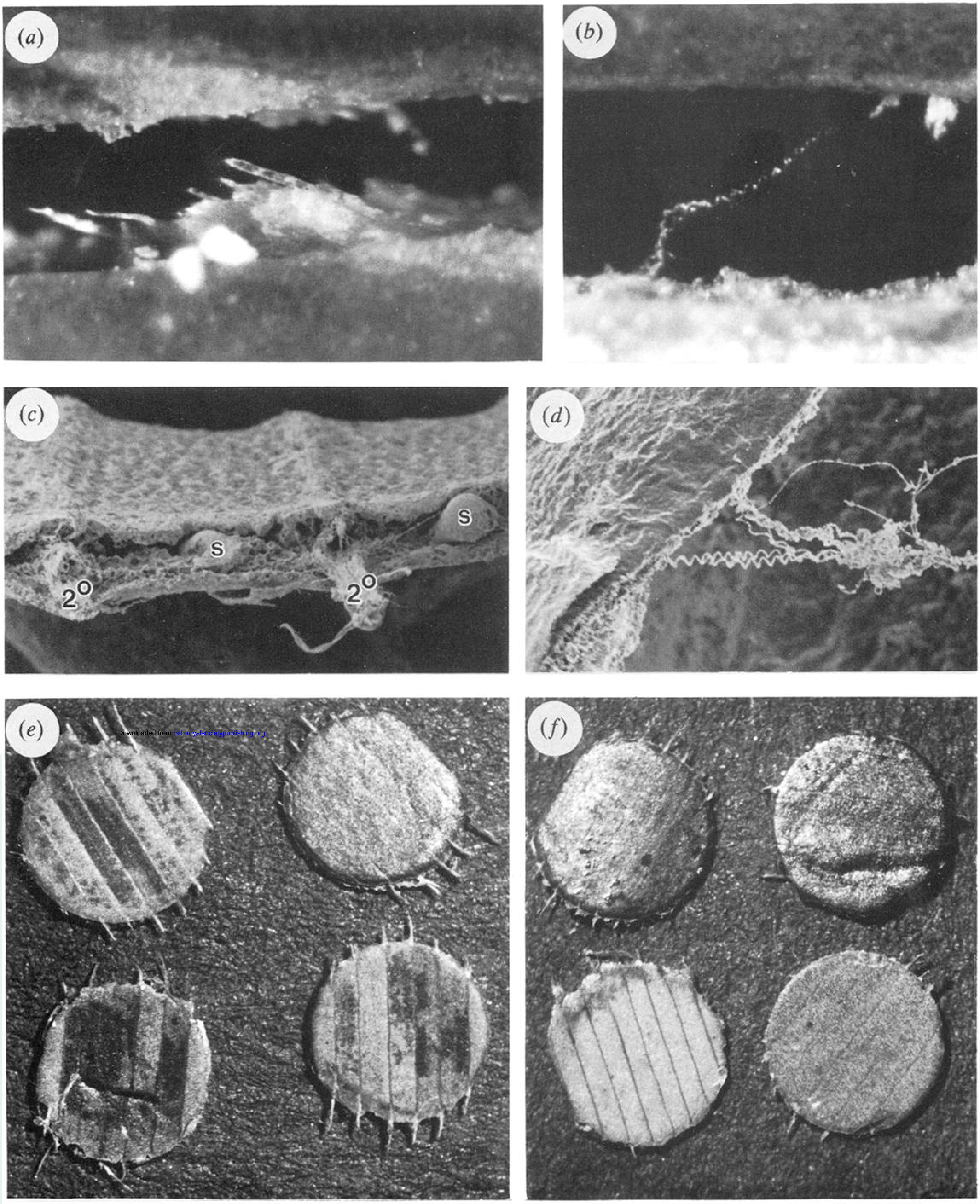

gure 3. Fracture surfaces of leaves of *Calophyllum inophyllum* produced by different methods. (*a*, *b*) Cutting tests. (*a*)
ppearance of a surface cut at $\theta = 13°$, viewed from the upper epidermis with stereozoom LM. Note the cut surface
the left, which is smooth at the upper epidermis but below (slightly out of focus) it is rough. A secondary vein runs
liquely slanting downwards to the right. Note its rough 'cut' surface and that of adjacent tissue fractured to the
ght. (*b*) Surface cut at $\theta = 0°$. An uncoiled part of the spiral thickenings of xylem vessels of a tertiary vein. (*c*, *d*)
otched tensile tests. (*c*) Loading along the secondary veins (SEM). A relatively flat surface but with pull-out of
condary veins (2°); secretions from secretory canals (s). (*d*) Loading across secondary veins (SEM). Uncoiling of the
iral thickenings in the wall of xylem vessels in a tertiary vein. (*e*, *f*) Punch-and-die tests. (*e*) Flat-headed punch and
) concave-headed punch, viewed by stereozoom LM. Note the extensive pull-out of the secondary veins, particularly
th the flat head. Magnifications: (*a*, *b*) ×499.2; (*c*) ×68; (*d*) ×123.2; (*e*, *f*) ×5.7.