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The importance of leaf cuticle for carbon economy and mechanical strength

Yusuke Onoda[1,2], Lora Richards[1,3] and Mark Westoby[1]

[1]Department of Biological Sciences, Macquarie University, Sydney, NSW, 2109, Australia; [2]Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan; [3]Biology Department, University of Nevada Reno, Reno, NV, 89557, USA

Author for correspondence:
Yusuke Onoda
Tel: +81 75 753 6079
Email: yusuke.onoda@gmail.com

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Summary

- Cuticle thickness of leaves varies > 100 times across species, yet its dry mass cost and ecological benefits are poorly understood. It has been repeatedly demonstrated that thicker cuticle is not superior as a water barrier, implying that other functions must be important.
- Here, we measured the mechanical properties, dry mass and density of isolated cuticle from 13 evergreen woody species of Australian forests.
- Summed adaxial and abaxial cuticle membrane mass per unit leaf area (CMA) varied from 2.95 to 27.4 g m^{-2} across species, and accounted for 6.7–24% of lamina dry mass. Density of cuticle varied only from 1.04 to 1.24 g cm^{-3}; thus variation in CMA was mostly due to variation in cuticle thickness. Thicker cuticle was more resistant to tearing. Tensile strength and modulus of elasticity of cuticle were much higher than those of leaf laminas, with significant differences between adaxial and abaxial cuticles.
- While cuticle membranes were thin, they could account for a significant fraction of leaf dry mass due to their high density. The substantial cost of thicker cuticle is probably offset by increased mechanical resistance which might confer longer leaf lifespans among evergreen species.

Introduction

Cuticle is the outer skin of all aerial parts of plants. It has been ubiquitous since plant colonization on land, indicating that cuticle is essential for plants in terrestrial environments (Raven, 1984; Edwards *et al.*, 1996). Cuticle was first noticed > 250 yr ago (Ludwig, 1757 according to Martin & Juniper, 1970) and has been studied in several disciplines (Martin & Juniper, 1970; Cutler *et al.*, 1982; Kerstiens, 1996; Riederer & Müller, 2006). Cuticle is composed of cutin, polysaccharides and waxes. It protects plants from desiccation (e.g. Schreiber & Riederer, 1996; Riederer & Schreiber, 2001), photochemical damage by UV (e.g. Krauss *et al.*, 1997; Pfündel *et al.*, 2006) and mechanical damage from abiotic and biotic sources including wind, insects, pathogens and micro-organisms (e.g. Lee & Priestley, 1924; Grubb, 1986; Müller, 2006).

Leaf cuticular membrane thickness (cuticle proper together with cuticular layer) varied > 100 times across species from < 0.05 to > 10 μm (Jeffree, 2006). The variation in cuticle thickness is thought to be associated with different plant ecological strategies. However, the costs and benefits of cuticle thickness are poorly understood.

In relation to the cost, having thicker cuticles should require greater energetic cost as cuticle materials (mostly lipid) have higher construction cost compared to the rest of leaf tissues (Poorter & Villar 1997). A few studies have reported leaf cuticle mass per unit area, mostly from the adaxial side only. Results have ranged from < 0.2 to 14.1 g m^{-2} for one side of the leaf (Baker & Martin, 1967; Holloway & Baker, 1970; Riederer & Schönherr, 1984; Becker *et al.*, 1986; Schreiber & Riederer, 1996). Density of leaf cuticle, which could be used to estimate cuticle mass from cuticle thickness, has hardly been measured except by Schreiber & Schönherr (1990) who reported data ranging from 1.02 to 1.37 g cm^{-3}.

On terms of the benefit of cuticles, there is no doubt that cuticle is important for desiccation tolerance, yet experiments with isolated cuticular membrane have repeatedly demonstrated that thicker cuticles do not have an advantage in desiccation tolerance (Kamp, 1930; Becker *et al.*, 1986; Schreiber & Riederer, 1996; Riederer & Schreiber, 2001). These results suggest that the thickness of cuticle may be under selection for functions other than desiccation tolerance. Mechanical strength may be a relevant function (Lee & Priestley, 1924; Grubb, 1986). However, the mechanical properties of leaf cuticle are poorly understood. Wiedemann & Neinhuis (1998) reported Young's modulus, a measure of material stiffness, in the range 290–730 MPa and tensile strength above 15 MPa in hydrated leaf cuticular membrane of four woody species. These values may be an order of magnitude higher than the corresponding values for leaf laminas (Balsamo *et al.*, 2003), consistent with cuticle being mechanically important (Bargel *et al.*, 2006). Yet the ecological value of thicker cuticle in terms of contributing to total mechanical strength has never been reported, to our knowledge.

In the present work we prepared adaxial and abaxial cuticle membranes from 13 evergreen woody species by enzymatic

digestion of cell walls. We clarify: how much leaf dry mass is allocated to cuticles; the density of the cuticle; the mechanical properties of cuticle and its contribution to whole-lamina mechanics; whether a thicker cuticle is more mechanically robust; and the differences between adaxial and abaxial cuticles.

Materials and Methods

Materials

Leaf materials of 13 native evergreen woody species were obtained from multiple natural forests in Australia as part of a large comparison across latitudes (Table 1). Species were omitted if we were unable to isolate cuticular membrane (CM) from their leaves (see later), or if their leaves were too small to furnish a lamina strip > 2 cm in length, required for the biomechanical measurement.

Isolation of CM

Cuticular membrane was isolated enzymatically based on the technique of Orgell (1955) modified by Schönherr & Riederer (1986). This technique is time-consuming but preferable in quantitative work (Martin & Juniper, 1970). The CM isolated by this technique represents tissue lying above the pectinaceous layer, and consists of cuticle proper (outer layer of the membrane composed of chiefly cutin) together with cuticle layer (inner layer of the membrane composed of cutin and incrusted on cellulose). Leaf lamina strips (*c.* 5 mm wide and 30 mm length) were prepared from five fresh leaves of each species excluding midribs. Before cuticle isolation, the area of the fully turgid strip was measured through scanning and image analysis (Image J, US National Institutes of Health, Bethesda, MD, USA). Samples were incubated at room temperature in a solution of pectinase (3.5% W/V), cellulase (3.5%), NaN_3 (2 mM), polyvinyl-pyrrolidone (0.2% W/V) and citrate (20 mM). Bottles were shaken gently by an automatic shaker. The enzyme solution was renewed two to three times depending on species. More than 1 wk was required for complete removal of leaf tissue. Samples where CM was not isolated after 3 months were discarded. Isolated CM was rinsed repeatedly with distilled water. Microscope observations confirmed that these isolated CM were clean without visible cellular components. CM were stored in distilled water at 4°C, rather than stored dry as in previous studies (e.g. Wiedemann & Neinhuis, 1998), which might have caused side effects. The area of CM strips was re-measured, to assess whether CM might have been pre-stretched in turgid leaves due to hydrostatic pressure, as suggested by Kutschera & Niklas (2007). However, in this study there was only a small amount of shrinkage of cuticle during the isolation process (on average 2.9% for adaxial and 2.0% for abaxial sides).

Biomechanics of CM

The tensile properties of CM were measured using a general material testing machine (Model 5542, Instron Corporation,

Table 1 Mass and density of leaf cuticular membrane (CM)

Species	Family	Site[1]	Adaxial CM mass per area (g m^{-2})	Abaxial CM mass per area (g m^{-2})	*P*	Total CM mass per area (g m^{-2})	Adaxial CM density (g cm^{-3})	Abaxial CM density (g cm^{-3})	*P*
Wilkiea austroqueenslandica	Monimiaceae	1	7.17 ± 2.65 (3)	8.32 ± 2.51 (3)	ns	13.51 ± 3.66 (2)	1.24 ± 0.080 (3)	1.24 ± 0.094 (3)	ns
Baloghia lucida	Euphorbiaceae	1	3.51 ± 2.30 (5)	3.67 ± 1.00 (5)	ns	7.18 ± 3.19 (5)	1.09 ± 0.032 (5)	1.12 ± 0.038 (5)	ns
Triunia youngiana	Proteaceae	1	7.62 ± 1.79 (5)	6.91 ± 0.93 (2)	ns	13.25 ± 1.08 (2)	1.21 ± 0.009 (5)	1.22 ± 0.013 (2)	ns
Eupomatia laurina	Eupomatiaceae	1	1.12 ± 0.34 (5)	1.82 ± 0.45 (5)	*	2.95 ± 0.70 (5)	1.09 ± 0.019 (5)	1.10 ± 0.027 (5)	ns
Endiandra pubens	Lauraceae	1	2.78 ± 0.71 (5)	3.78 ± 0.78 (5)	+	6.56 ± 1.48 (5)	1.13 ± 0.015 (5)	1.12 ± 0.025 (5)	ns
Synoum glandulosum	Meliaceae	2	6.31 ± 1.84 (4)	5.25 ± 0.57 (3)	ns	11.61 ± 1.65 (2)	1.09 ± 0.029 (4)	1.12 ± 0.045 (3)	ns
Acacia floribunda	Fabaceae	2	9.83 ± 3.06 (2)[2]		NA	19.65 ± 6.13 (2)	1.21 ± 0.010 (2)[2]		NA
Persoonia levis	Proteaceae	2	13.69 ± 1.34 (8)[2]		NA	27.38 ± 2.24 (4)	1.20 ± 0.012 (8)[2]		NA
Astrotricha floccosa	Araliaceae	2	5.90 ± 0.89 (5)	5.89 ± 1.11 (5)	ns	11.80 ± 1.96 (5)	1.15 ± 0.043 (5)	1.17 ± 0.039 (5)	ns
Randia benthamiana	Rubiaceae	3	3.67 ± 0.53 (5)	1.79 ± 0.15 (5)	***	5.46 ± 0.66 (5)	1.11 ± 0.015 (5)	1.11 ± 0.012 (5)	ns
Andopetalum biglandulous	Cunoniaceae	3	6.48 ± 1.84 (5)	3.66 ± 1.13 (5)	*	10.14 ± 2.96 (5)	1.16 ± 0.007 (5)	1.15 ± 0.040 (5)	ns
Atherosperma moschatum	Atherospermataceae	3	8.80 ± 2.06 (5)	5.31 ± 0.75 (5)	*	14.11 ± 2.70 (5)	1.04 ± 0.013 (5)	1.04 ± 0.011 (5)	ns
Anopterus glandulosus	Escalloniaceae	3	7.72 ± 2.22 (5)	6.48 ± 1.98 (5)	ns	14.20 ± 4.19 (5)	1.11 ± 0.015 (5)	1.11 ± 0.012 (5)	ns

Mean, SD and number of replications are shown. Student's *t*-test was used to test difference between adaxial and abaxial CM. Significance: ns, $P > 0.1$; +, $P < 0.1$; *, $P < 0.05$; ***, $P < 0.001$. NA, not applicable.
[1]Site 1 was subtropical rainforests from Border Ranges National Park (NP) or Lamington NP, site 2 was temperate dry sclerophyll forest in Ku-ring-gai Chase NP and site 3 was temperate rainforest in Franklin-Gordon Wild Rivers NP.
[2]These species have equifacial leaves thus adaxial and abaxial CM could not be distinguished.

Canton, MA, USA). Samples were flattened out on wet paper with a paintbrush, then mounted on a pair of pneumatic grips, and stretched at a speed of 10 mm min^{-1} after the paper was removed. Force and displacement were logged every 0.1 s. Young's modulus (a measure of material stiffness) was calculated from a linear elastic region of the force–displacement curve. Tensile strength was calculated from breaking force per unit cross-sectional area and tensile strain was calculated from the increase in length of specimen per unit original length at the breaking point (Gere & Timoshenko, 1999). Cross-section area of CM was calculated from width and thickness of CM. Width was measured with calipers (0.01 mm precision) and thickness was calculated from CM mass divided by CM density (see later). Thickness of CM calculated in this way reflected mean thickness of the whole CM sheet. The inner side of CM had curtain-like structures called pegs or spandrels that had anchored between epidermal cells (Jeffree, 2006), and these parts of cuticle were not likely to contribute much to tensile stiffness or strength. Therefore, Young's modulus and tensile strength as evaluated in this study might be slightly underestimated compared to the real material properties of CM. Measurements were carried out separately for adaxial and abaxial CM. The overall tear resistance (N mm^{-1}), including both adaxial and abaxial CM, was calculated as the summed tensile strength × thickness of each CM.

Density and dry mass of CM

Schreiber & Schönherr (1990) calculated the density of CM from measurements of mass and volume of CM with a specific volume bottle. This method requires a reasonable amount of CM and highly technical procedures. We introduce an easy, accurate and reasonably quick method that can be applied to a tiny fragment of CM. Sodium metatungstate (Sigma-Aldrich, St Louis, MO, USA) has low toxicity, and when mixed with water forms a liquid whose density can be modulated between 1 and 3 g cm^{-3} (Plewinsky & Kamps, 1984). CM sinks or floats depending whether its density exceeds the density of the liquid. Using this principle, we gradually increased or decreased the concentration of sodium metatungstate by adding condensed liquid (*c.* 2 g cm^{-3}) or water (*c.* 1 g cm^{-3}), and determined an equilibrium point between CM (degassed beforehand) and the well-mixed heavy liquid. At the equilibrium point, 1.000 ml of the liquid was taken by pipette and its weight was measured by a micro-balance (0.01 mg precision, AE160, Mettler-Toledo, Columbus, OH, USA) to calculate the density of the equilibrium liquid which must have been the same as the CM's. For each CM, a measurement was made both by increasing and by decreasing the concentration of the heavy liquid. The two results were very similar (on average 1.6% difference) and were averaged for each CM sample.

After measuring density, the CM was dried in an oven at 70°C overnight and its dry mass was measured on a micro-balance (0.1 μg precision, UMX2, Mettler Toledo). Total CM mass per unit leaf area (CMA) was calculated from summed dry mass of both adaxial and abaxial CM per unit projected area of turgid leaf lamina strip.

Calculation and statistics

The same set of measurements (tensile properties, thickness, area and dry mass) were made on the same intact leaves used for CM measurement (n = 5 for each species), so that mass and mechanical contributions of CM to leaf lamina could be calculated. The cuticle mass fraction in leaf dry mass was calculated from CMA divided by leaf mass per unit area (LMA). Cuticle contribution to tear resistance was calculated from: (tensile strength of CM x thickness of CM)/(lamina tensile strength × lamina thickness). In this calculation, both the adaxial and abaxial contributions were considered. Adaxial and abaxial CM within species were compared by *t*-test. Pearson's test was used to test correlation among traits. A standardized major axis slope was used to fit bivariate relationships (Warton *et al.*, 2006). Data were analysed using R statistical package (v2.9.0; R Foundation for Statistical Computing, Vienna, Austria).

Results

Across 13 evergreen woody species, the adaxial and abaxial CM (cuticular membrane) mass ranged from 1.1 to 13.8 and from 1.8 to 13.5 g m^{-2}, respectively. Summed CM mass per unit leaf area (CMA) ranged from 3.0 to 27.4 g m^{-2} (Table 1), which contributed to 6.7–24% of lamina dry mass (Fig. 1). This contribution was *c.* four-fold greater than CM's contribution to lamina thickness, which was 1.5–5.9% (Fig. 1). The corresponding density of CM was high, ranging from 1.04 to 1.24 g cm^{-3}, which was 3–6-fold higher than the tissue density of lamina. While CMA varied almost 10-fold, the density of CM varied only 0.21-fold, meaning that CMA variation arose predominantly from CM thickness variation.

Adaxial and abaxial CM mass were quite well correlated across the 13 species. Four out of 13 species had significantly thicker adaxial than abaxial CM (Table 1, Fig. 2a). The density of CM was very similar on adaxial and abaxial sides within each species (Table 1, Fig. 2b).

The tensile strength of CM varied from 0.9 to 16.5 MPa (Fig. 2c) and the modulus of elasticity (MOE) varied from 5 to 383 MPa (Fig. 2d). Adaxial CM was stronger and stiffer than abaxial CM in many species. The tensile strength of adaxial CM was 1.33–4.87 times higher than the tensile strength of leaf lamina, and the MOE of adaxial CM was 0.64–9.88 times higher than the MOE of leaf lamina.

In order to assess whether higher CMA was due to higher allocation of dry mass to cuticle or to proportional increase with leaf mass per area (LMA), CMA was partitioned into cuticle mass fraction (CMF) and LMA.

$$CMA = CMF \times LMA$$

Both components correlated significantly with CMA without intercorrelation between CMF and LMA (Fig. 3). These results indicate that CMF and LMA each contribute independently to higher CMA. Leaves with high LMA had a large amount of both cuticle and noncuticle mass per unit leaf area, resulting in a positive

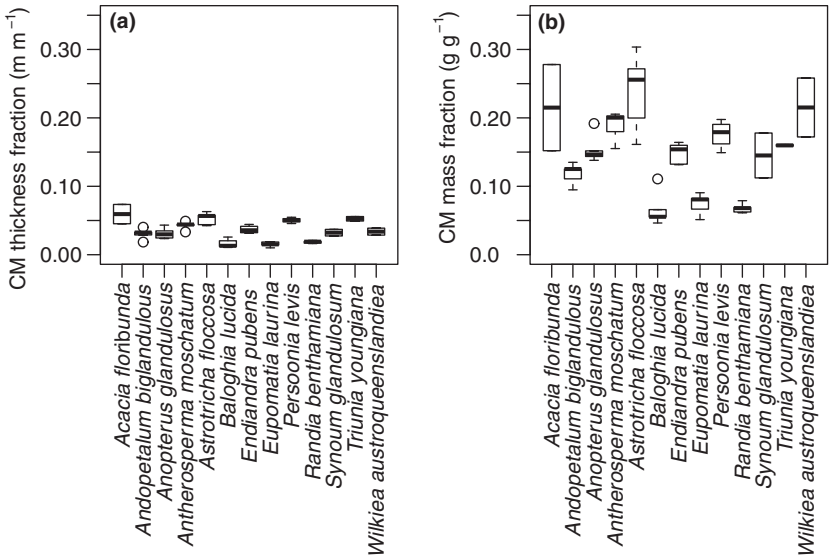

Fig. 1 Box plots of (a) cuticular membrane (CM) thickness per unit lamina thickness and (b) cuticular membrane dry mass per unit lamina dry mass of 13 Australian evergreen woody species. Both adaxial and abaxial cuticles were included. The central box in each box plot shows the interquartile range and median; whiskers indicate the 10th and 90th percentiles.

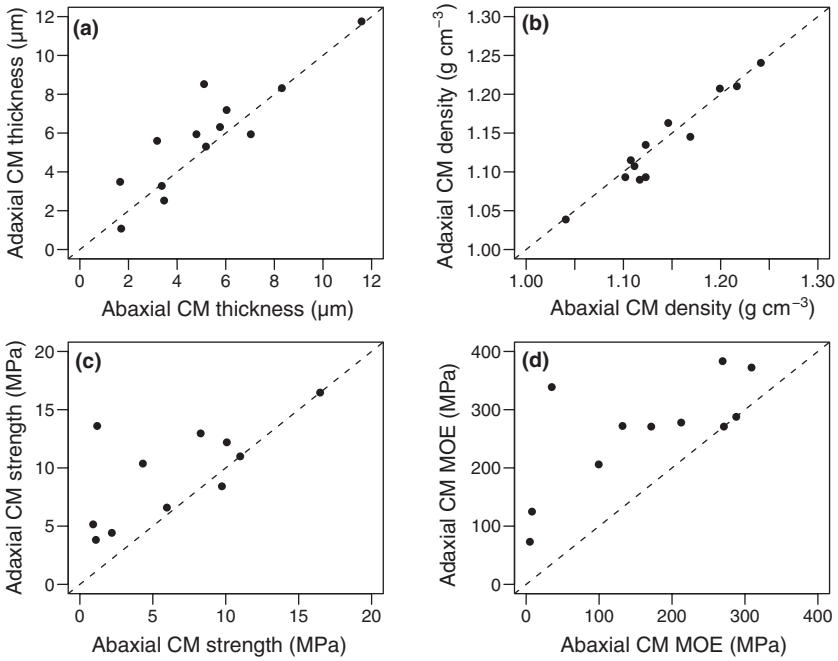

Fig. 2 Relationships between the adaxial and abaxial cuticular membranes (CM) of 13 Australian evergreen woody species: (a) thickness, (b) density, (c) tensile strength and (d) modulus of elasticity (MOE). Each dot denotes the mean value of each species. Dashed lines show 1 : 1 relationship. Two species (*Acacia floribunda* and *Persoonia levis*) had equifacial leaves, thus data were averaged between both sides of cuticle and automatically fell on the 1 : 1 relationship line.

correlation between CMA and LMA. There was also substantial variation in CMF for a given LMA.

Cuticle force to tear (tear resistance of adaxial and abaxial CM per unit CM width, F_t) was partitioned between CM tensile strength ($\sigma_c = F_t / T_c$) and CM thickness (T_c), where

$$F_t = \sigma_c \times T_c$$

Both components strongly correlated with force required to tear CM (Fig. 4a,b). There was also significant intercorrelation between tensile strength and thickness, meaning that thicker CM was made of stronger materials (Fig. 4c). Consequently thicker CM was disproportionately resistant against tear, as indicated by an exponential increase in the relationship between σ_c and T_c (Fig. 4b).

CM accounted for 1–18% of the overall leaf lamina's resistance to tear, and CM's importance was higher in leaves with high CMA (Fig. 5a). CM was made of reasonably stretchy material, stretching by up to 20% before it broke. Leaves with thinner CMA tended to have higher strain capacity (Fig. 5b, $P = 0.08$).

Discussion

The cuticles we measured were dense tissues, ranging between 1.04 and 1.24 g cm^{-3} which is denser than wood (mean wood density 0.613 g cm^{-3} and range 0.08–1.39 g cm^{-3} across 16 468 samples; Chave *et al.*, 2009). While cuticles were thin compared to lamina thickness (Fig. 1), the high density of CM resulted in a rather high dry mass fraction in cuticle (6.7–24%). The cost of

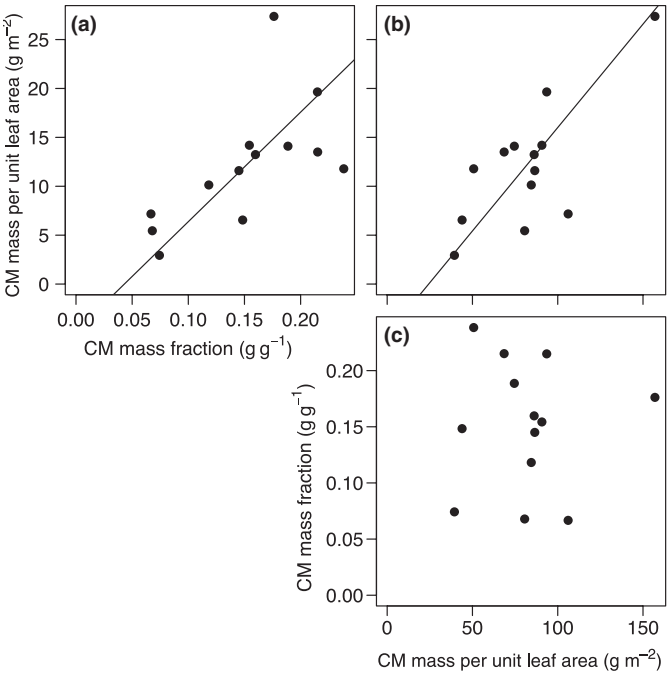

Fig. 3 Cuticular membrane (CM) mass per unit leaf area was analysed as the product of cuticular membrane mass fraction and leaf mass per unit area across 13 Australian evergreen woody species. Each dot denotes mean value of each species. A standardized major axis (SMA) slope was drawn when the correlation was significant (Pearson's test, $P < 0.05$). SMA slopes: (a) $y = 112x - 4.82$, $R^2 = 0.40$; (b) $y = 0.212x - 5.15$, $R^2 = 0.55$; (c) $y = 0.00019x - 0.0029$, $R^2 < 0.01$.

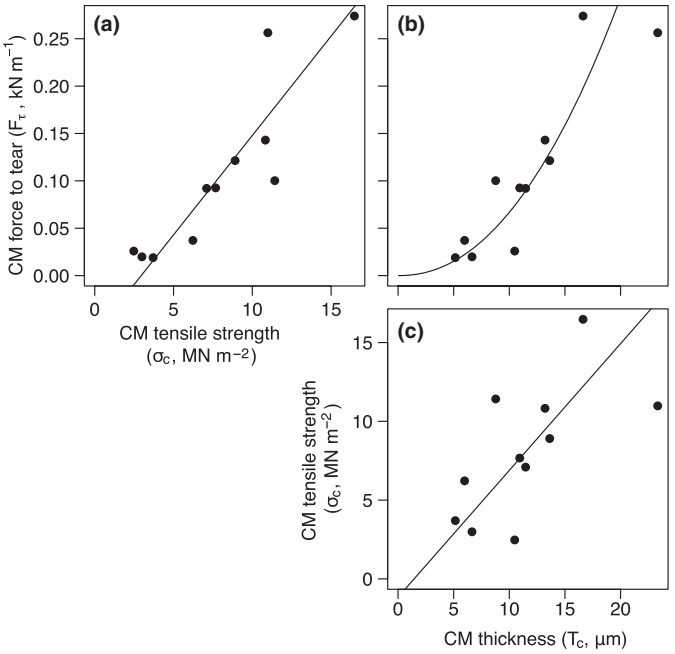

Fig. 4 Cuticular membrane (CM) force to tear was analysed as the product of CM tensile strength and CM thickness across 13 Australian evergreen woody species. Data are based on both adaxial and abaxial cuticles. Each dot denotes the mean value of each species. A standardized major axis (SMA) slope was drawn when the correlation was significant (Pearson's test, $P < 0.05$). SMA slopes: (a) $y = 0.021x - 0.0619$, $R^2 = 0.80$; (b) $y = 0.000503x^{2.13}$, $R^2 = 0.76$; (c) $y = 0.805x - 1.16$, $R^2 = 0.41$.

cuticle is even larger when construction costs are considered. Cuticle consists of 20–80% cutin (e.g. Holloway & Baker, 1970), whose construction costs are more than double that of polysaccharides (3.03 and 1.22 g glucose g^{-1} for cutin and polysaccharides, respectively; Penning de Vries *et al.*, 1974; Poorter & Villar, 1997). Assuming that CM contains 20–80% cutin and remaining polysaccharide, the calculated construction cost of CM would be 1.58–2.67 g glucose g^{-1} CM, which is 1.02–1.72 times higher than the average leaf construction cost (= 1.55 g glucose g^{-1} dry mass in woody plants, Poorter & Villar, 1997). These calculations suggest that plants may incur fairly large costs to construct leaves with thick cuticles.

Thicker cuticle conferred a clear advantage in breaking resistance due to both thicker structure and stronger materials (Fig. 4a,b). Further, CMA was significantly positively correlated with LMA, which is a well-known indicator of leaf lifespan (Reich *et al.*, 1992; Wright *et al.*, 2004). These results suggest that thicker cuticles are conducive to long leaf lifespan by protecting leaves from external mechanical stresses. Some leaf lifespan data obtained from the literature (Wright & Westoby, 2002) and from our preliminary field observations were consistent with this idea, such that weak but positive correlations between leaf lifespan and CMA were observed for sun leaves and understorey leaves (Supporting Information Fig. S1). Previous studies concerning water relations repeatedly reported a lack of correlation between water permeability and CM thickness (Kamp, 1930; Becker *et al.*, 1986; Schreiber & Riederer, 1996; Riederer & Schreiber, 2001). Possibly the mechanical stability conferred by thicker cuticles may contribute to keeping the leaf surface intact and sustaining high desiccation tolerance over longer lifespans.

Cross-species variation in adaxial CM strength was partly explained by density of CM ($R^2 = 0.33$, $P < 0.05$, data not shown). This may be analogous to the well-known correlation between wood mechanical properties and wood density (Chave *et al.*, 2009; Niklas & Spatz, 2010) and to the correlation between leaf strength and leaf tissue density (Onoda *et al.*, 2011). In dense cuticles, cutin and associated compounds might be closely packed, which in turn may increase mechanical strength. Yet, there were large differences in mechanical properties between adaxial and abaxial CM within species even though their specific gravities were similar. This might be explained by factors such as the degree of cross-links between polymers, different compositions of wax/matrix (Bargel *et al.*, 2006), and the presence of stomata in abaxial sides.

In general, our results are similar to previous studies with a slight exception. Mass of single CMs (either adaxial or abaxial) varied more than an order of magnitude from 1.1 to 13.8 g m^{-2}, which is similar to previous reports for evergreen species (lowest 0.8 g m^{-2} in *Escallonia macrantha* (Baker & Martin, 1967); highest 14.1 g m^{-2} in *Nerium oleander* (Becker *et al.*, 1986)). Additionally, our value of density of CM agrees well with Schreiber & Schönherr (1990), who reported 1.02–1.37 g cm^{-3} for seven woody species. However, the ranges of tensile strength (3.8–16.5 MPa) and of MOE (56–383 MPa) in adaxial CM were

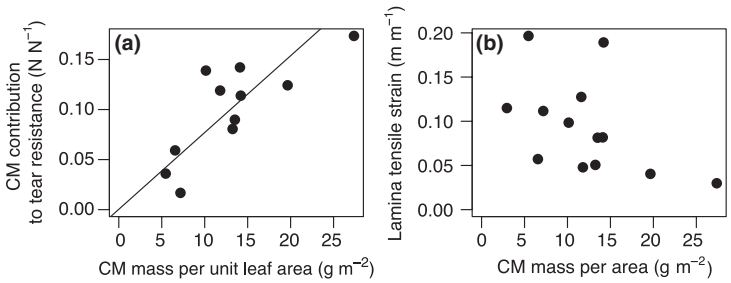

Fig. 5 Correlations (a) between cuticular membrane (CM) mass per area and cuticle contribution to tear resistance of leaf lamina, (b) between CM mass per unit leaf area and lamina tensile strain across 13 Australian evergreen woody species. Each dot denotes the mean value of each species. See the Materials and Methods section for the detail of measurements of each trait. A standardized major axis (SMA) slope was drawn when the correlation was significant (Pearson's test, $P < 0.05$). SMA slopes: (a) $y = 0.00762x - 0.00028$, $R^2 = 0.62$; (b) $y = -0.00835x + 0.196$, $R^2 = 0.25$.

somewhat lower than the ranges reported by Wiedemann & Neinhuis (1998) (> 15 MPa for tensile strength and 290–730 MPa for MOE for four evergreen species). This difference may be due to the way we measured thickness (see Materials and Methods) and the different sets of species. Yet, this study as well as previous studies indicate that CM is a generally strong and stiff material, whose contribution to whole leaf lamina strength was considerable (accounted for up to 18% of lamina breaking resistance).

Leaf epidermis cell walls should also contribute to the mechanical strength of a leaf. Cellulose fibre is a much cheaper material than cutin sheet, and its strength and stiffness may be comparable or greater (Bargel *et al.*, 2006). Thus, there is a question why cuticle rather than cell walls should play the role of increasing surface mechanical stability. One possible explanation is that cutin sheet is more resistant to chemical degradation (Schreiber & Schönherr, 2009) and may be more isotropic than cellulose fibre. Leaf surfaces are exposed to various stresses including desiccation, rain drain-off, heat, frost, high irradiance, biotic and abiotic mechanical hazards including wind and attacks by insects, pathogens and microorganisms (e.g. Krauss *et al.*, 1997; Riederer & Schreiber, 2001; Carver & Gurr, 2006). In the long run, deploying a thick cuticle rather than thick cell walls may pay off its expensive construction cost.

Cuticle costs and benefits have been relatively little studied in plant ecology. Conventionally the acid-insoluble tissue fraction from chemical digestion is called '(Klason) lignin' (e.g. Van Soest, 1994; Mediavilla *et al.*, 2008). However, Klason lignin also contains cutin and tannin (Preston *et al.*, 1997). As cuticle accounts for a significant fraction of dry mass in evergreen species, it would be preferable to treat it separately from lignin. The dense, stiff and strong nature of cuticle may also be important in decomposition and nutrient cycles. Cutin is thought to decompose slower than proteins or carbohydrate, and to have long retention times in soil (e.g. Gallardo & Merino, 1993; Goñi & Hedges, 1995). Because leaf litter of woody species includes a significant fraction of cuticle (Schieferstein & Loomis, 1959), cuticle in woody leaves may substantially influence carbon turnover and accumulation in ecosystems. Our results highlight the importance of cuticle for plant ecological strategies as well as for decomposition and nutrient cycles in ecosystems.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Leaf lifespan plotted against (a) leaf mass per unit area (LMA), (b) cuticle membrane (CM) mass per unit leaf area and (c) cuticle membrane strength.

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