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Mechanics and chemistry of rain forest leaves: canopy and understorey compared*

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Abstract

Despite the potential for changes during transit or preservation, the physicochemical properties of leaves are typically measured in a laboratory setting. A suite of laboratory methods adapted for use in the field is described here. The equipment is portable and operable in remote environments. Each technique has been validated against laboratory standards and has been tested throughout the tropics in a variety of ecological contexts. The properties of canopy and understorey leaves from Central Panama are reported here. The results show clear differences between leaves growing in different light regimes. Canopy leaves are tougher and possess greater concentrations of protein, phenols, and tannins. The implication of these results to the resource availability hypothesis, which proposes trade-offs between physiology and defences against herbivory, is discussed.

Key words: Barro Colorado Island, canopy crane, fracture toughness, herbivory, Panama.

Introduction

Plants functioning under deep shade are under considerable pressure to optimize their photosynthetic performance (Lee and Graham, 1986; Lee *et al.*, 1990; Poorter *et al.*, 1995; Thomas and Bazzaz, 1999). This pressure is perhaps nowhere more intense than in a tropical rain forest, where understorey plants must cope with dramatic spatial and temporal variations in solar irradiance (Mulkey *et al.*,

1996). Indeed, tree seedlings established in deep forest shade may receive only 1% of the photon flux density incident on canopy adults. Such resource limitation may favour inherently slow rates of photosynthesis and growth, low nutrient contents, long leaf lifetimes, and large investments in anti-herbivore defence (Coley *et al.*, 1985). According to this reasoning, dubbed the 'resource availability' hypothesis, defences in high-resource environments are predicted to be nitrogenous and effective at minute concentrations (e.g. alkaloids), while those in low-resource environments are predicted to be carbon-based and effective only in quantity (e.g. tannins and toughness). Rates of herbivory are predicted to be lowest in low-resource environments; indeed, data are generally consistent with these predictions (Turner, 2001).

The resource availability hypothesis further predicts that canopy leaves should feature (1) greater concentrations of alkaloids, (2) lower levels of toughness and tannins, and (3) higher rates of herbivory than those in the understorey. Is this so? Evidence is equivocal.

In support of the resource availability hypothesis, canopy leaves in Gabon possess more concentrated and diverse alkaloids than those in the understorey (Downum *et al.*, 2001). However, toughness and tannin levels are higher in canopy leaves in Australia (Lowman and Box, 1983), which is contrary to the hypothesis. These results are based, though, on four and five species, respectively. Larger surveys in Southeast Asia suggest that understorey leaves are sometimes tougher than those in the canopy (Turner *et al.*, 1993, 1999), but chemical defences have not been studied. Furthermore, the consequences on herbivory rates are equivocal. Rates are reported to be either similar in the canopy and understorey (Vasconcelos, 1999) or

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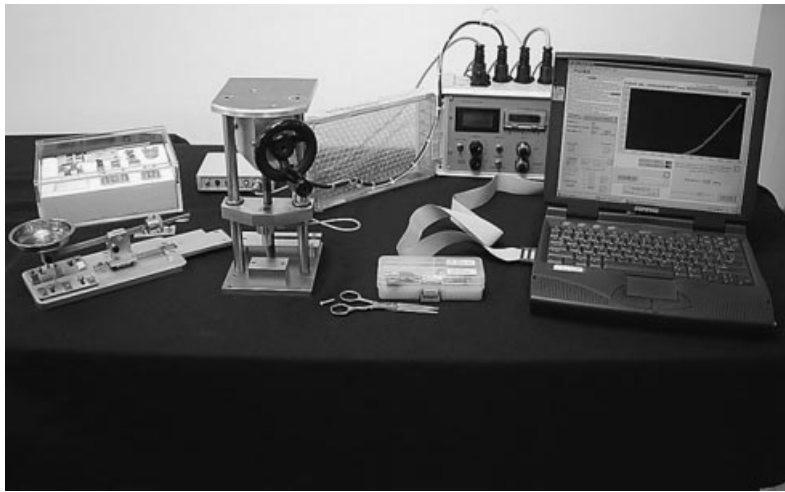


Fig. 1. Devices required to make a wide range of mechanical field tests. The equipment for chemical tests is more compact than this, but with a greater quantity of consumables and accessories.

lower in the canopy (Lowman and Moffett, 1993; Coley and Barone, 1996).

These conflicts could be resolved by direct comparison between canopy and understorey leaves of the same species. There have been few such studies outside those considering leaf morphology, where shading is known to have a substantial effect (Bongers and Popma, 1990; Thomas and Ickes, 1995). The use of novel equipment designed for ecological fieldwork is described here and a broad-based survey of leaf physicochemical properties in the canopy and understorey of Central Panama is reported.

Recent technological developments

A wide range of mechanical and chemical measurements on plant parts can now be made under field conditions. Mechanical testing involves miniaturized versions of the universal testing machines found in engineering and food science laboratories (Darvell *et al.*, 1996) (Fig. 1). Laboratory machines are often massive in order to be sufficiently rigid; only the specimen being tested should deform. By contrast, portable field testers have a reduced stiffness, but provided that the plant specimens are small enough or the tissues sufficiently pliant, there is no theoretical reason why they should not give accurate results. This has been verified by comparing the results of laboratory and field machines on standard materials (Darvell *et al.*, 1996; Lucas *et al.*, 1997).

Many chemical tests in laboratories use a spectrophotometer to assess the concentration of a compound or group of compounds via a colorimetric response. Recent developments with fibre optics have resulted in relatively inexpensive devices that can be used either for assessing colorimetric reactions or for obtaining the reflectance spectra of plant items. The data can be transferred to a

notebook computer via PC card or USB connector, giving immediate results.

A 12-bit A-to-D PC card (DAQCard 1200, National Instruments, USA) was interfaced to the output of both a non-commercialized portable mechanical tester (Darvell *et al.*, 1996) and an optical fibre spectrometer (Ocean Optics 2000, USA), displaying and analysing the data using Labview (National Instruments, USA). A suite of programs has been written for this purpose (available free from PWL). Except for tannins, the chemical tests recorded here employed microassays. The results were recorded by reading the absorbance of the reaction products in a 1 cm cuvette illuminated by a tungsten halogen light source (LS-1; Ocean Optics) (Lucas *et al.*, 2001). The mechanical tests reported here involve the measurement of the work done on the specimens during tests. The tester does this with its own hardware, dispensing with the absolute need for a computer. However, a computer can be used to dissect the toughness of different parts of the leaf, which hardware calculations alone cannot.

Methods and sample results

Study site

From August to October 2001, fieldwork was conducted on Barro Colorado Island (BCI), Panama (9°9' N, 79°51' W). Approximately 1600 ha in size, BCI is described as a lowland moist forest receiving approximately 2600 mm of rainfall per year (Leigh, 1999). Leaf specimens in the understorey were collected with pruning shears and a telescoping pole. Leaf specimens in the canopy were collected with the assistance of construction cranes operated by the Smithsonian Tropical Research Institute (Parker *et al.*, 1992). Canopy access and collection

occurred at two sites, Parque Natural Metropolitano and Fort Sherman, where annual rainfall averages 1740 and 3200 mm, respectively. Taxonomy follows Croat (1978) and Condit *et al.* (1995).

Physical measures

Leaf toughness: Toughness is often measured with a penetrometer, a device which forces a circular flattened rod through leaf lamina. Although these devices have yielded insight into how structural properties may deter invertebrate herbivores, they do not measure fracture toughness or any other fundamental mechanical property (Vincent, 1990; Choong *et al.*, 1992; Aranwela *et al.*, 1999). Toughness is the material resistance to crack propagation and defined as the energy consumed in growing a crack of given area. It is biologically important because it is the key property responsible for maintaining material integrity and plays a critical role in resisting pathogens, herbivores, and other physical damage (Choong *et al.*, 1992; Lucas *et al.*, 2000). It was measured here using a pair of scissors (Dovo, Germany) to control and direct crack growth (Lucas and Pereira, 1990; Lucas *et al.*, 1997). These were mounted on a portable universal testing machine (Darvell *et al.*, 1996) and the toughness of a species was calculated from 3–4 mature leaves of a single tree. Each leaf was fractured with a single transverse cut perpendicular to the midrib, equidistant between the base and apex. This method, described by Lucas *et al.* (2001), allows the toughness (in J m⁻²) of individual anatomical features, such as secondary veins and lamina, to be calculated from a single scissors pass.

Leaf colour: Reflectance spectra of leaves can be captured via the Ocean optics spectrometer. However, in this study, a spectrophotometer (Miniscan, Hunterlab, USA) configured to provide output in the Commission Internationale d'Eclairage (CIE) *Lab* system was used. The upper surface of mature leaves was placed against an 8 mm aperture using diffuse reflected light with a 10° field of view. The illuminant was D65, a standard for daylight. This provided a rapid and accurate method of registering leaf colour (Lucas *et al.*, 1998; Dominy *et al.*, 2002), which is convertible to other colour spaces if required (Wysecki and Stiles, 2000).

Chemical measures

Chemical extraction: Waterman and Mole (1994) discuss extraction techniques for quantifying phenolic compounds, including a review of the various solvents possible. From this discussion and a consideration of tests for other constituents, 50% methanol (1:1 dH₂O:CH₃OH) was chosen as the solvent. Approximately 0.1 g of fresh plant tissue was weighed, cut into approximately 1 mm pieces, and extracted in 5 ml of 50% methanol with a tissue homogenizer (Tissue Tearor, Dremel, USA). The homo-

genate was then collected into a 10 ml syringe fitted with a Luer lock and fibreglass filter (1.6 µm pore size, type 1, Millipore, USA). Slow depression of the syringe plunger forced the homogenate through the filter and into a 1.5 ml Eppendorf tube, where samples were stored for the analyses described below (summarized from Lucas *et al.*, 2001). Note that these tests utilize fresh rather than dried material, allowing the results to be expressed as concentrations (akin to how they might be sensed by a herbivore) rather than on a dry weight basis (which relates more to nutritional gain).

Protein: Protein was quantified following the methods of Bradford (1976) and modifications of Read and Northcote (1981). The Coomassie brilliant blue (G-250, Sigma, USA) dye-binding assay was used to react with protein and produce a blue colour, which was measured spectrophotometrically at 595 nm. The reaction occurs reliably with different proteins, although not perfectly (Sappan *et al.*, 1999). The dye does not react with non-bound amino acids and tannin-binding has been largely removed by the recommendations of Jones *et al.* (1989). The results are expressed as % equivalents to a 6-point standard curve based on bovine serum albumin (Fraction V, Sigma, USA).

Phenolics: Levels of total phenolic compounds were measured by the Prussian Blue test (Price and Butler, 1977) as modified by Graham (1992), Hagerman (2002), and Lucas *et al.* (2001). Phenols present in a plant sample oxidize potassium ferricyanide to produce ferrous ions. These, in turn, react with ferric chloride in HCl to produce a Prussian blue complex, the strength of which can be measured by its absorbance at 700 nm. Results are expressed as % equivalents to a 4-point standard curve of gallic acid (Aldrich, USA). Appel *et al.* (2001) note that this method measures the reducing capacity of phenols, not necessarily the overall concentration.

Tannins: Tannins were quantified following the method of Hagerman (1987) and modifications of Lucas *et al.* (2001). Plant extracts were inserted into the pre-moulded wells of a BSA-laden, agarose gel (type I: low EEO, Sigma, USA), where tannin precipitation produces visible rings. Ring dimensions were measured with dial calipers and results expressed as % equivalents to an 8-point standard curve of crude quebracho tannin (gift of AE Hagerman, University of Miami, Ohio, USA).

Sample results

Canopy leaves were tougher and featured greater quantities of protein, phenolics and tannins than those in the understorey (Table 1). Moreover, canopy leaves were significantly lighter (*L*-axis) and yellower (*b*-axis) (Table 1). This must reflect higher concentrations of photoprotective xanthophylls (Königer *et al.*, 1995;

Table 1. Paired t-tests on the physicochemical properties of canopy and understorey leaves (mean \pm sd)

Leaf properties	Understorey	Canopy	t	P
Fracture toughness (n=37 spp)				
Lamina	503 \pm 219	841 \pm 361	-5.8	<0.001
Midrib	2882 \pm 1190	4047 \pm 1536	-4.7	<0.001
Secondary veins	3718 \pm 1362	4492 \pm 1553	-3.1	<0.01
Chemistry (n=28 spp)				
Protein	2.0 \pm 1.2	3.0 \pm 1.2	-4.1	<0.001
Phenols	0.9 \pm 0.8	2.2 \pm 1.8	-5.1	<0.001
Tannins	0.9 \pm 1.9	3.9 \pm 3.8	-4.9	<0.001
Colour (n=29 spp)				
L-axis	34.5 \pm 1.9	38.0 \pm 2.4	-5.5	<0.001
a-axis	-5.6 \pm 1.0	-4.6 \pm 3.0	-1.6	n.s.
b-axis	6.0 \pm 2.1	8.7 \pm 3.5	-4.5	<0.001

Mulkey *et al.*, 1996). These effects were consistent across virtually all species studied (Appendices 1–3).

Clearly, these results are incompatible with the resource availability hypothesis. Coupled with the results of Downum *et al.* (2001) on alkaloids, it is suggested that defences of all types, mobile or not (*sensu* Coley *et al.*, 1985), are elevated in canopy leaves. The consequences of this for pathogens (Gilbert, 1995), herbivore populations (Barone, 2000) and their predators (Lowman and Moffett, 1993; Coley and Barone, 1996), need to be resolved within a different theoretical framework. Evidently, there is no trade-off necessary in the canopy between defence and growth because of a resource surfeit.

Issues for the future

The equipment reported here has functioned in a variety of remote environments, including Uganda (Dominy and Lucas, 2001) and Madagascar (Yamashita, 2002). Simplifying and expanding the range of chemical tests is a key issue for the future. Further improvements in mechanical testing involve the correlation between leaf mechanics and structural characteristics. Currently, this depends on hand-cut sections. However, a new field microtome (Webb, UK) produces acceptable microscopic sections in the field. There is also a reasonable field microscope available for viewing them (Micron-160, Enhelion, UK). Thus far, taking the images from the microscope and analysing them in a computer is tedious, but a USB connector is promised. This offers the possibility of structural and mechanical correlation in the field and raises the possibility of field histochemistry. However, locating microscopic regions of a leaf and determining where specific reaction products are made and/or stored still seems distant.

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Appendix

Table A1. Fracture mechanics of species under study

See text for methodological details.

Species studied	Leaf fracture toughness (J m ⁻²)					
	Mean lamina (s.d.)		Mean midrib (s.d.)		Mean 2° vein (s.d.)	
	Understorey	Canopy	Understorey	Canopy	Understorey	Canopy
<i>Anacardium excelsum</i>	628 (329)	1345 (550)	3377 (2070)	5801 (1464)	4621 (2758)	8937 (399)
<i>Aspidosperma cruenta</i>	535 (8)	700 (64)	4404 (373)	3664 (347)	–	–
<i>Astronium graveolens</i>	405 (234)	542 (289)	1413 (462)	3191 (458)	4314 (1998)	4827 (632)
<i>Brosimum utile</i>	341 (47)	1710 (455)	3896 (246)	7914 (218)	–	–
<i>Chrysophyllum longifolium</i>	669 (155)	1865 (614)	3664 (534)	5909 (1141)	–	–
<i>Castilla elastica</i>	187 (43)	513 (110)	1711 (334)	3359 (1049)	1354 (859)	3251 (713)
<i>Cecropia insignis</i>	333 (91)	576 (176)	1964 (266)	4351 (248)	2341 (371)	2669 (833)
<i>C. obtusifolia</i>	370 (10)	363 (22)	1137 (275)	1845 (161)	1574 (267)	2413 (432)
<i>Chrysophyllum argenteum</i>	758 (73)	688 (122)	4788 (998)	3352 (456)	4866 (966)	5843
<i>C. cainito</i>	740 (66)	1047 (201)	3755 (667)	4196 (677)	5252 (345)	5390 (2216)
<i>Cordia alliodora</i>	201 (90)	711 (171)	958 (213)	2076 (311)	1072 (185)	3138 (441)
<i>Dendropanax arboreus</i>	432 (44)	704 (132)	2498 (456)	5113 (875)	4352 (790)	5520 (545)
<i>Dipteryx panamensis</i>	573 (101)	1232 (169)	4232 (903)	5343 (683)	5087 (1053)	6442 (1278)
<i>Dolioscarpus dentatus</i>	199 (20)	473 (44)	3062 (232)	4607 (690)	3578 (647)	4403 (303)
<i>D. multiflorus</i>	943 (192)	868 (145)	1884 (279)	4751 (486)	5048 (1156)	3535 (807)
<i>Ficus insipida</i>	290 (82)	679 (69)	2170 (149)	6598 (545)	3173 (520)	5806 (472)
<i>Gutteria dumetorum</i>	1085 (174)	1012 (148)	4073 (977)	2006 (406)	–	–
<i>Lacmellea panamensis</i>	340 (26)	717 (245)	1327 (156)	1601 (318)	–	–
<i>Luehea seemannii</i>	301 (85)	681 (143)	3282 (650)	2909 (933)	2985 (578)	3354 (613)
<i>Manilkara bidentata</i>	698 (151)	1060 (128)	1960 (737)	3374 (305)	–	–
<i>Marila laxiflora</i>	552 (162)	700 (124)	3978 (377)	6578 (1067)	4641 (699)	5674 (1157)
<i>Mikania leiostachya</i>	407 (2)	935 (156)	1893 (288)	2514 (632)	2988 (175)	3365 (527)
<i>Perebea xanthochyma</i>	837 (247)	1095 (217)	2977 (511)	4107 (622)	3855 (373)	4463 (668)
<i>Piper reticulatum</i>	545 (52)	777 (2)	1858 (128)	3646 (27)	1725 (5)	5631 (1514)
<i>Poulsenia armata</i>	717 (154)	860 (216)	3647 (912)	5046 (526)	3188 (1112)	3151 (748)
<i>Pouruma bicolor</i>	410	687 (44)	2360	4959 (771)	4842	3904 (1248)
<i>Protium panamense</i>	509 (28)	1319 (141)	4527 (456)	5238 (298)	6469 (1751)	7497 (1205)
<i>Serjania mexicana</i>	362 (45)	443 (36)	1516 (288)	2984 (130)	2612 (234)	3923 (89)
<i>Spondias mombin</i>	841 (223)	887 (170)	3539 (273)	2981 (306)	5325 (468)	5476 (591)
<i>S. radlkofferi</i>	424 (90)	536 (18)	1392 (351)	3504 (402)	3645 (592)	4438 (347)
<i>Symphonia globulifera</i>	397 (106)	616 (137)	2788 (324)	4304 (339)	–	–
<i>Tachigalia versicolor</i>	461 (23)	1510 (365)	5690 (541)	5517 (983)	4278 (478)	4028 (390)
<i>Tovomita longifolia</i>	734 (110)	618 (193)	4332 (509)	4257 (747)	5448 (2691)	3716 (667)
<i>Tratinickia aspera</i>	262 (3)	976 (225)	2587 (280)	5704 (274)	3231 (350)	4340 (1108)
<i>Virola multiflora</i>	376 (21)	395 (47)	2142 (198)	2228 (470)	–	–
<i>V. sebifera</i>	373 (48)	381 (72)	2218 (240)	2306 (160)	3250 (483)	2087 (573)
<i>V. surinamensis</i>	364 (28)	899 (191)	3620 (375)	1891 (259)	2703 (511)	3037 (532)
Summary mean (s.d.)	503 (219)	841 (361)	2882 (1190)	4047 (1536)	3718 (1362)	4492 (1553)

Table A2. Chemical properties of species under study

See text for methodological details

Species studied	Leaf chemistry					
	Protein		Phenols		Tannins	
	Understorey	Canopy	Understorey	Canopy	Understorey	Canopy
<i>Anacardium excelsum</i>	3.24	3.92	1.02	2.36	4.51	7.61
<i>Aspidosperma cruenta</i>	2.22	0.32	0.48	0.85	0.00	0.00
<i>Astronium graveolens</i>	2.10	3.70	1.73	8.29	4.68	13.16
<i>Brosimum utile</i>	0.16	3.91	0.70	1.82	0.00	1.65
<i>Calophyllum longifolium</i>	2.25	3.54	0.56	1.60	0.00	2.25
<i>Castilla elastica</i>	0.30	3.63	0.06	1.13	0.00	6.97
<i>Cecropia insignis</i>	2.00	3.65	0.57	1.77	0.00	5.93
<i>C. obtusifolia</i>	0.27	3.02	0.14	0.89	0.00	0.00
<i>Chrysophyllum argenteum</i>	3.54	4.11	2.01	2.25	0.00	6.02
<i>C. cainito</i>	3.64	4.13	1.66	3.03	6.02	6.74
<i>Dendropanax arboreus</i>	0.74	1.00	0.12	0.39	0.00	0.00
<i>Dipteryx panamensis</i>	2.54	3.32	0.76	1.16	0.00	1.03
<i>Doliocarpus dentatus</i>	2.10	3.93	0.45	2.39	0.00	1.05
<i>Ficus insipida</i>	0.29	1.14	0.17	0.44	0.00	0.00
<i>Lacmellea panamensis</i>	0.66	4.16	0.25	2.52	0.00	5.93
<i>Luehea seemannii</i>	2.96	3.85	0.60	1.13	0.00	0.00
<i>Marila laxiflora</i>	2.45	3.34	1.96	4.21	1.46	9.73
<i>Mikania leiostachya</i>	1.75	1.44	0.41	0.57	0.00	0.00
<i>Piper reticulatum</i>	0.10	0.97	0.08	0.32	0.00	0.00
<i>Poulsenia armata</i>	0.20	2.34	0.05	0.88	0.00	0.74
<i>Protium panamense</i>	2.90	3.98	0.70	1.73	0.00	4.26
<i>Serjania mexicana</i>	2.15	1.02	0.65	2.10	0.00	0.00
<i>Symphonia globulifera</i>	3.17	3.40	0.52	2.50	0.00	9.68
<i>Tachigalia versicolor</i>	2.70	2.97	1.16	1.25	1.31	1.82
<i>Tratinnickia aspera</i>	1.69	2.68	3.32	6.63	0.00	8.13
<i>Virola multiflora</i>	3.78	4.02	1.65	2.61	5.54	6.33
<i>V. sebifera</i>	3.17	2.78	1.27	1.57	0.00	3.08
<i>V. surinamensis</i>	3.54	4.18	2.57	4.07	1.75	7.75
Summary mean (s.d.)	2.02 (1.22)	3.02 (1.19)	0.92 (0.83)	2.16 (1.81)	0.90 (1.86)	3.92 (3.83)

Table A3. Spectral properties of species under study

See text for methodological details.

Species studied	Leaf colour					
	Understorey			Canopy		
	L-axis	a-axis	b-axis	L-axis	a-axis	b-axis
<i>Aspidosperma cruenta</i>	33.41	-4.09	5.54	38.43	-5.84	7.46
<i>Astronium graveolens</i>	34.93	-6.61	8.52	37.83	-6.54	9.62
<i>Brosimum utile</i>	33.86	-3.47	2.57	40.23	-6.05	11.73
<i>Castilla elastica</i>	31.97	-6.03	7.41	37.79	-7.31	11.81
<i>Cecropia insignis</i>	33.37	-5.94	5.61	38.97	-4.95	10.76
<i>C. obtusifolia</i>	31.7	-6.03	7.05	38.83	-4.22	4.29
<i>Chrysophyllum cainito</i>	33.8	-6.16	4.78	37.06	-6.46	7.22
<i>Cordia alliodora</i>	37.25	-5.81	5.73	41.49	-6.48	12.08
<i>Dendropanax arboreus</i>	34.93	-6.02	7.39	36.27	-6.61	9.65
<i>Dipteryx panamensis</i>	35.71	-5.57	7.59	38.12	-5.28	6.75
<i>Doliocarpus dentatus</i>	35.23	-7.22	7.44	41.93	-9.24	15.33
<i>D. multiflorus</i>	35.72	-5.66	3.99	37.95	5.95	10.38
<i>Gutteria dumetorum</i>	35.77	-5.38	4.7	35.1	-3.81	6.03
<i>Lacmellea panamensis</i>	32.56	-4.68	3.45	33.17	-3.93	1.57
<i>Luehea seemannii</i>	33.43	-6.38	4.24	34.29	-4.89	4.92
<i>Manilkara bidentata</i>	36.08	-6.54	7.29	37.6	-3.89	6.18
<i>Marila laxiflora</i>	35.55	-5.71	5.12	39.4	-4.42	12.37
<i>Mikania leiostachya</i>	38.7	-7.09	10.99	42.61	-6.82	16.3
<i>Perebea xanthochyma</i>	38.19	-6.24	8.71	35.37	-5.2	6.67
<i>Piper reticulatum</i>	33.11	-6.72	5.65	37.35	-5.41	6.99
<i>Poulsenia armata</i>	33.3	-4.75	4.75	37.95	-4.88	6.16
<i>Pouruma bicolor</i>	33.51	-4.92	3.98	36.49	-5.21	5.68
<i>Protium panamense</i>	32.97	-6.09	6.07	44.0	7.88	14.76
<i>Tachigalia versicolor</i>	32.51	-5.88	5.43	38.5	-3.44	7.6
<i>Tovomita longifolia</i>	37.95	-5.95	10.38	37.17	-5.77	10.25
<i>Tratinnickia aspera</i>	36.43	-4.49	6.09	34.95	-4.37	5.59
<i>Virola multiflora</i>	32.66	-2.76	3.1	36.26	-4.44	7.02
<i>V. sebifera</i>	33.0	-4.68	4.11	37.97	-5.56	8.17
<i>V. surinamensis</i>	34.15	-5.3	4.92	39.2	-5.05	9.95
Summary mean (s.d.)	34.5 (1.9)	-5.6 (1.0)	6.0 (2.1)	38.0 (2.4)	-4.6 (3.0)	8.7 (3.5)

References

- Appel HM, Governor HL, D'Ascenzo M, Siska E, Schultz JC. 2001. Limitations of Folin assays of foliar phenolics in ecological studies. *Journal of Chemical Ecology* **27**, 761–778.
- Aranwela N, Sanson G, Read J. 1999. Methods of assessing leaf-fracture properties. *New Phytologist* **144**, 369–393.
- Barone JA. 2000. Comparison of herbivores and herbivory in the canopy and understorey for two tropical tree species. *Biotropica* **32**, 307–317.
- Bongers F, Popma J. 1990. Leaf characteristics of the tropical rain forest flora of Los Tuxtlas, Mexico. *Botanical Gazette* **151**, 354–365.
- Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry* **72**, 248–254.
- Choong MF, Lucas PW, Ong JSY, Pereira B, Tan HTW, Turner IM. 1992. Leaf fracture toughness and sclerophylly: their correlations and ecological implications. *New Phytologist* **121**, 597–610.
- Coley PD, Barone JA. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* **27**, 305–335.
- Coley PD, Bryant JP, Chapin III FS. 1985. Resource availability and plant antiherbivore defense. *Science* **230**, 895–899.
- Condit R, Hubbell SP, Foster RB. 1995. Mortality rates of 205 neotropical tree and shrub species and the impact of a severe drought. *Ecological Monographs* **65**, 419–439.
- Croat TB. 1978. *Flora of Barro Colorado Island*. Stanford: Stanford University Press.
- Darvell BW, Lee PKD, Yuen TDB, Lucas PW. 1996. A portable fracture toughness tester for biological materials. *Measurement Science and Technology* **7**, 954–962.
- Dominy NJ, Lucas PW. 2001. Ecological importance of trichromatic vision to primates. *Nature* **410**, 363–366.
- Dominy NJ, Lucas PW, Ramsden L, Riba-Hernandez P, Stoner KE, Turner IM. 2002. Why are young leaves red? *Oikos* **98**, 163–176.
- Downum K, Lee D, Hallé F, Quirke M, Towers N. 2001. Plant secondary compounds in the canopy and understorey of a tropical rain forest in Gabon. *Journal of Tropical Ecology* **17**, 477–481.
- Gilbert GS. 1995. Rainforest plant diseases: the canopy understorey connection. *Selbyana* **16**, 75–77.
- Graham HD. 1992. Stabilization of the Prussian blue colour in the determination of polyphenols. *Journal of Agricultural and Food Chemistry* **40**, 801–805.
- Hagerman AE. 1987. Radial diffusion method for determining tannin in plant extracts. *Journal of Chemical Ecology* **13**, 437–449.
- Hagerman AE. 2002. The tannin handbook. <http://www.users.muohio.edu/hagermae/>

- Jones CG, Hare JD, Compton SJ.** 1989. Measuring plant protein with the Bradford assay. 1. Evaluation and standard method. *Journal of Chemical Ecology* **15**, 979–992.
- Königer M, Harris GC, Virgo A, Winter K.** 1995. Xanthophyll-cycle pigments and photosynthetic capacity in tropical forest species: a comparative field study on canopy, gap and understory plants. *Oecologia* **104**, 280–290.
- Lee DW, Bone RA, Tarsis SL, Storch D.** 1990. Correlates of leaf optical properties in tropical forest sun and extreme-shade plants. *American Journal of Botany* **77**, 370–380.
- Lee DW, Graham R.** 1986. Leaf optical properties of rainforest sun and extreme shade plants. *American Journal of Botany* **73**, 1100–1108.
- Leigh Jr EG.** 1999. *Tropical forest ecology: a view from Barro Colorado Island*. New York: Oxford University Press.
- Lowman MD, Box JD.** 1983. Variation in leaf toughness and phenolic content among five species of Australian rain forest trees. *Australian Journal of Ecology* **8**, 17–25.
- Lowman MD, Moffett M.** 1993. The ecology of tropical rain forest canopies. *Trends in Ecology and Evolution* **8**, 103–108.
- Lucas PW, Beta T, Darvell BW, Dominy NJ, Essackjee HC, Lee PKD, Osorio D, Ramsden L, Yamashita N, Yuen TDB.** 2001. Field kit to characterize physical, chemical, and spatial aspects of potential primate foods. *Folia Primatologica* **72**, 11–25.
- Lucas PW, Darvell BW, Lee PKD, Yuen TDB, Choong MF.** 1998. Colour cues for leaf food selection by long-tailed macaques (*Macaca fascicularis*) with a new suggestion for the evolution of trichromatic colour vision. *Folia Primatologica* **69**, 139–152.
- Lucas PW, Pereira B.** 1990. Estimation of the fracture toughness of leaves. *Functional Ecology* **4**, 819–822.
- Lucas PW, Tan HTW, Cheng PY.** 1997. The toughness of secondary cell wall and woody tissue. *Philosophical Transactions of the Royal Society of London* **B352**, 341–352.
- Lucas PW, Turner IM, Dominy NJ, Yamashita N.** 2000. Mechanical defences to herbivory. *Annals of Botany* **86**, 913–920.
- Mulkey SS, Kitajima K, Wright SJ.** 1996. Plant physiological ecology of tropical forest canopies. *Trends in Ecology and Evolution* **11**, 408–412.
- Parker GG, Smith AP, Hogan KP.** 1992. Access to the upper forest canopy with a large tower crane. *BioScience* **42**, 664–670.
- Poorter L, Oberbauer SF, Clark DB.** 1995. Leaf optical properties along a vertical gradient in a tropical rain forest canopy in Costa Rica. *American Journal of Botany* **82**, 1257–1263.
- Price ML, Butler LG.** 1977. Rapid visual estimation and spectrophotometric determination of the tannin content of sorghum grain. *Journal of Agricultural and Food Chemistry* **25**, 1269–1273.
- Read SM, Northcote DH.** 1981. Minimization of variation in the response to different proteins of the Coomassie Blue G dye-binding assay for protein. *Analytical Biochemistry* **116**, 53–64.
- Sappan CV, Lundblad RL, Price NC.** 1999. Colorimetric protein assay techniques. *Biotechnology and Applied Biochemistry* **29**, 99–108.
- Thomas SC, Bazzaz FA.** 1999. Asymptotic height as a predictor of photosynthetic characteristics in Malaysian rain forest trees. *Ecology* **80**, 1607–1622.
- Thomas SC, Ickes K.** 1995. Ontogenetic changes in leaf size in Malaysian rain forest trees. *Biotropica* **27**, 427–434.
- Turner IM.** 2001. *The ecology of trees in the tropical rain forest*. Cambridge: Cambridge University Press.
- Turner IM, Choong MF, Tan HTW, Lucas PW.** 1993. How tough are sclerophylls? *Annals of Botany* **71**, 343–345.
- Turner IM, Lucas PW, Becker P, Wong SC, Yong JWH, Choong MF, Tyree MT.** 1999. Tree leaf form in Brunei: a heath and mixed dipterocarp forest compared. *Biotropica* **32**, 53–61.
- Vasconcelos HL.** 1999. Levels of leaf herbivory in Amazonian trees from different stages in forest regeneration. *Acta Amazonica* **29**, 615–623.
- Vincent JFV.** 1990. Fracture in plants. *Advances in Botanical Research* **17**, 235–287.
- Waterman PG, Mole S.** 1994. *Analysis of phenolic plant metabolites*. Oxford: Blackwell.
- Wysecki G, Stiles WS.** 2000. *Color science*. New York: John Wiley.
- Yamashita N.** 2002. Diets of two lemur species in different microhabitats in Beza Mahafaly Special Reserve, Madagascar. *International Journal of Primatology* **23**, 1025–1051.