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REVIEW ARTICLE: FIELD TECHNIQUES

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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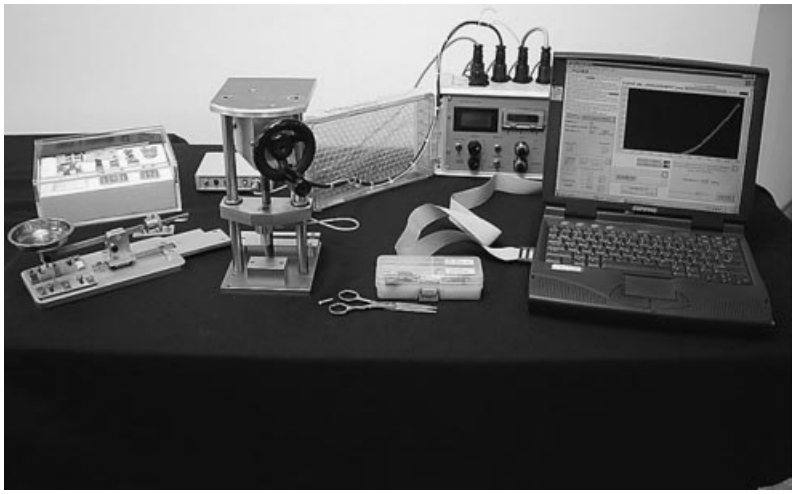

Fig. 1. Devices required to make a wide range of mechanical field tests. The equipment for chemical tests is more compact than this, but with a greater quantity of consumables and accessories.

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 spectrocolorimeter (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 dH_20:CH_3OH) 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 homogenate 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 ±sd)*

Leaf properties	Understorey	Canopy	*t*	*P*
Fracture toughness (*n*=37 spp)				
Lamina	503±219	841±361	–5.8	<0.001
Midrib	2882±1190	4047±1536	–4.7	<0.001
Secondary veins	3718±1362	4492±1553	–3.1	<0.01
Chemistry (*n*=28 spp)				
Protein	2.0±1.2	3.0±1.2	–4.1	<0.001
Phenols	0.9±0.8	2.2±1.8	–5.1	<0.001
Tannins	0.9±1.9	3.9±3.8	–4.9	<0.001
Colour (*n*=29 spp)				
L-axis	34.5±1.9	38.0±2.4	–5.5	<0.001
a-axis	–5.6±1.0	–4.6±3.0	–1.6	n.s.
b-axis	6.0±2.1	8.7±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
Anacardium excelsum	628 (329)	1345 (550)	3377 (2070)	5801 (1464)	4621 (2758)	8937 (399)
Aspidosperma cruenta	535 (8)	700 (64)	4404 (373)	3664 (347)	–	–
Astronium graveolens	405 (234)	542 (289)	1413 (462)	3191 (458)	4314 (1998)	4827 (632)
Brosimum utile	341 (47)	1710 (455)	3896 (246)	7914 (218)	–	–
Calophyllum longifolium	669 (155)	1865 (614)	3664 (534)	5909 (1141)	–	–
Castilla elastica	187 (43)	513 (110)	1711 (334)	3359 (1049)	1354 (859)	3251 (713)
Cecropia insignis	333 (91)	576 (176)	1964 (266)	4351 (248)	2341 (371)	2669 (833)
C. obtusifolia	370 (10)	363 (22)	1137 (275)	1845 (161)	1574 (267)	2413 (432)
Chrysophyllum argenteum	758 (73)	688 (122)	4788 (998)	3352 (456)	4866 (966)	5843
C. cainito	740 (66)	1047 (201)	3755 (667)	4196 (677)	5252 (345)	5390 (2216)
Cordia alliodora	201 (90)	711 (171)	958 (213)	2076 (311)	1072 (185)	3138 (441)
Dendropanax arboreus	432 (44)	704 (132)	2498 (456)	5113 (875)	4352 (790)	5520 (545)
Dipteryx panamensis	573 (101)	1232 (169)	4232 (903)	5343 (683)	5087 (1053)	6442 (1278)
Doliocarpus dentatus	199 (20)	473 (44)	3062 (232)	4607 (690)	3578 (647)	4403 (303)
D. multiflorus	943 (192)	868 (145)	1884 (279)	4751 (486)	5048 (1156)	3535 (807)
Ficus insipida	290 (82)	679 (69)	2170 (149)	6598 (545)	3173 (520)	5806 (472)
Guatteria dumetorum	1085 (174)	1012 (148)	4073 (977)	2006 (406)	–	–
Lacmellea panamensis	340 (26)	717 (245)	1327 (156)	1601 (318)	–	–
Luehea seemannii	301 (85)	681 (143)	3282 (650)	2909 (933)	2985 (578)	3354 (613)
Manilkara bidentata	698 (151)	1060 (128)	1960 (737)	3374 (305)	–	–
Marila laxiflora	552 (162)	700 (124)	3978 (377)	6578 (1067)	4641 (699)	5674 (1157)
Mikania leiostachya	407 (2)	935 (156)	1893 (288)	2514 (632)	2988 (175)	3365 (527)
Perebea xanthochyma	837 (247)	1095 (217)	2977 (511)	4107 (622)	3855 (373)	4463 (668)
Piper reticulatum	545 (52)	777 (2)	1858 (128)	3646 (27)	1725 (5)	5631 (1514)
Poulsenia armata	717 (154)	860 (216)	3647 (912)	5046 (526)	3188 (1112)	3151 (748)
Pouruma bicolor	410	687 (44)	2360	4959 (771)	4842	3904 (1248)
Protium panamense	509 (28)	1319 (141)	4527 (456)	5238 (298)	6469 (1751)	7497 (1205)
Serjania mexicana	362 (45)	443 (36)	1516 (288)	2984 (130)	2612 (234)	3923 (89)
Spondias mombin	841 (223)	887 (170)	3539 (273)	2981 (306)	5325 (468)	5476 (591)
S. radlkofferi	424 (90)	536 (18)	1392 (351)	3504 (402)	3645 (592)	4438 (347)
Symphonia globulifera	397 (106)	616 (137)	2788 (324)	4304 (339)	–	–
Tachigalia versicolor	461 (23)	1510 (365)	5690 (541)	5517 (983)	4278 (478)	4028 (390)
Tovomita longifolia	734 (110)	618 (193)	4332 (509)	4257 (747)	5448 (2691)	3716 (667)
Tratinnickia aspera	262 (3)	976 (225)	2587 (280)	5704 (274)	3231 (350)	4340 (1108)
Virola multiflora	376 (21)	395 (47)	2142 (198)	2228 (470)	–	–
V. sebifera	373 (48)	381 (72)	2218 (240)	2306 (160)	3250 (483)	2087 (573)
V. surinamensis	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
Anacardium excelsum	3.24	3.92	1.02	2.36	4.51	7.61
Aspidosperma cruenta	2.22	0.32	0.48	0.85	0.00	0.00
Astronium graveolens	2.10	3.70	1.73	8.29	4.68	13.16
Brosimum utile	0.16	3.91	0.70	1.82	0.00	1.65
Calophyllum longifolium	2.25	3.54	0.56	1.60	0.00	2.25
Castilla elastica	0.30	3.63	0.06	1.13	0.00	6.97
Cecropia insignis	2.00	3.65	0.57	1.77	0.00	5.93
C. obtusifolia	0.27	3.02	0.14	0.89	0.00	0.00
Chrysophyllum argenteum	3.54	4.11	2.01	2.25	0.00	6.02
C. cainito	3.64	4.13	1.66	3.03	6.02	6.74
Dendropanax arboreus	0.74	1.00	0.12	0.39	0.00	0.00
Dipteryx panamensis	2.54	3.32	0.76	1.16	0.00	1.03
Doliocarpus dentatus	2.10	3.93	0.45	2.39	0.00	1.05
Ficus insipida	0.29	1.14	0.17	0.44	0.00	0.00
Lacmellea panamensis	0.66	4.16	0.25	2.52	0.00	5.93
Luehea seemannii	2.96	3.85	0.60	1.13	0.00	0.00
Marila laxiflora	2.45	3.34	1.96	4.21	1.46	9.73
Mikania leiostachya	1.75	1.44	0.41	0.57	0.00	0.00
Piper reticulatum	0.10	0.97	0.08	0.32	0.00	0.00
Poulsenia armata	0.20	2.34	0.05	0.88	0.00	0.74
Protium panamense	2.90	3.98	0.70	1.73	0.00	4.26
Serjania mexicana	2.15	1.02	0.65	2.10	0.00	0.00
Symphonia globulifera	3.17	3.40	0.52	2.50	0.00	9.68
Tachigalia versicolor	2.70	2.97	1.16	1.25	1.31	1.82
Tratinnickia aspera	1.69	2.68	3.32	6.63	0.00	8.13
Virola multiflora	3.78	4.02	1.65	2.61	5.54	6.33
V. sebifera	3.17	2.78	1.27	1.57	0.00	3.08
V. surinamensis	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
Aspidosperma cruenta	33.41	–4.09	5.54	38.43	–5.84	7.46
Astronium graveolens	34.93	–6.61	8.52	37.83	–6.54	9.62
Brosimum utile	33.86	–3.47	2.57	40.23	–6.05	11.73
Castilla elastica	31.97	–6.03	7.41	37.79	–7.31	11.81
Cecropia insignis	33.37	–5.94	5.61	38.97	–4.95	10.76
C. obtusifolia	31.7	–6.03	7.05	38.83	–4.22	4.29
Chrysophyllum cainito	33.8	–6.16	4.78	37.06	–6.46	7.22
Cordia alliodora	37.25	–5.81	5.73	41.49	–6.48	12.08
Dendropanax arboreus	34.93	–6.02	7.39	36.27	–6.61	9.65
Dipteryx panamensis	35.71	–5.57	7.59	38.12	–5.28	6.75
Doliocarpus dentatus	35.23	–7.22	7.44	41.93	–9.24	15.33
D. multiflorus	35.72	–5.66	3.99	37.95	5.95	10.38
Guatteria dumetorum	35.77	–5.38	4.7	35.1	–3.81	6.03
Lacmellea panamensis	32.56	–4.68	3.45	33.17	–3.93	1.57
Luehea seemannii	33.43	–6.38	4.24	34.29	–4.89	4.92
Manilkara bidentata	36.08	–6.54	7.29	37.6	–3.89	6.18
Marila laxiflora	35.55	–5.71	5.12	39.4	–4.42	12.37
Mikania leiostachya	38.7	–7.09	10.99	42.61	–6.82	16.3
Perebea xanthochyma	38.19	–6.24	8.71	35.37	–5.2	6.67
Piper reticulatum	33.11	–6.72	5.65	37.35	–5.41	6.99
Poulsenia armata	33.3	–4.75	4.75	37.95	–4.88	6.16
Pouruma bicolor	33.51	–4.92	3.98	36.49	–5.21	5.68
Protium panamense	32.97	–6.09	6.07	44.0	7.88	14.76
Tachigalia versicolor	32.51	–5.88	5.43	38.5	–3.44	7.6
Tovomita longifolia	37.95	–5.95	10.38	37.17	–5.77	10.25
Tratinnickia aspera	36.43	–4.49	6.09	34.95	–4.37	5.59
Virola multiflora	32.66	–2.76	3.1	36.26	–4.44	7.02
V. sebifera	33.0	–4.68	4.11	37.97	–5.56	8.17
V. surinamensis	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)

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