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A seasonal census of phenolics, fibre and alkaloids in foliage of forest trees in Costa Rica: some factors influencing their distribution and relation to host selection by Sphingidae and Saturniidae

DANIEL H. JANZEN

Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

AND

PETER G. WATERMAN F.L.S.

Phytochemistry Research Laboratory, Department of Pharmaceutical Chemistry University of Strathclyde, Glasgow G1 1XW

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The foliage of 80 species common in the Santa Rosa National Park, Costa Rica, has been analysed for content of total phenolics, condensed tannins, acid detergent fibre and water. Wherever possible analyses were performed at three stages in the life cycle of the leaf: young but fully expanded (coinciding with the beginning of the rainy season); middle-aged (two months later); and old (six months later). A comparison of the three age classes showed no significant change in the levels of phenolics or fibre as leaves aged but water content decreased significantly. A comparison of deciduous and evergreen species in the sample showed that the latter group had leaves with a significantly higher fibre content at all three sampling times, most particularly at the beginning of the rainy season, but other measures were not significantly different. Alkaloids were much more common in the foliage of deciduous species and it was observed that their distribution differed significantly from that of total phenolics and condensed tannins. It is suggested that the interaction that occurs between many tannins and alkaloids would be liable to reduce the defence capability of both classes of compounds if they occurred together.

High levels of defoliation occur in the early rainy season (third to tenth weeks) due to larvae of moths of the Sphingidae and Saturniidae. A comparison of investigated tree species that host larvae of these two taxa shows a striking dichotomy. Species that are selected by Sphingidae tend to be relatively deficient in levels of phenolics but are more likely to contain alkaloids, and probably other small toxic molecules. Saturniidae, on the other hand, appear to prefer host-species rich in phenolics but poor in alkaloids.

KEY WORDS:—Tropical deciduous forest – distribution of chemical defence – phenolics – tannins – fibre – alkaloids – plant–animal interactions – Sphingidae – Saturniidae.

CONTENTS

Introduction 440
Materials and methods. 440
Study site. 440

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Choice of plants and leaf collection 441
Taxonomy 442
Chemical analyses 443
Results 443
Discussion 450
Acknowledgements 453
References 453

INTRODUCTION

In the lowland deciduous forest of Santa Rosa National Park in north-western Costa Rica, there is a very conspicuous peak in the activity of insect defoliators of woody plants from the third to tenth weeks of the rainy season (late May onward) followed by a much less populous second generation during the latter half of the five to six month rainy season (Janzen, 1980; 1981; unpubl.). There are at least two non-exclusive general hypotheses as to why insect defoliator activity should be drastically reduced after the early part of a tropical rainy season in a deciduous forest: (a) foliage quality could decline in digestibility and nutritive value with ageing of the new leaves produced close to the beginning of the onset of the rainy season, and (b) parasites and predators could build up to levels where they depress insect populations or to where an insect has a higher fitness if its waits to oviposit at a later date.

To examine the vegetation natural history relevant to the first hypothesis we have censused the total phenolic equivalents (TP), condensed tannin equivalents (CT) and acid detergent fibre (ADF) levels of the leaves of 80 species of woody plants in Santa Rosa at three times in the growing season: (1) in the second and third weeks of the rainy season (middle to late May); (2) in the eighth and ninth weeks of the rainy season (mid July); (3) at the end of the rainy season (late December to early January). The first census (young leaves) was of fully expanded yet immature foliage, at the stage when severe defoliator damage was just beginning to appear. The second census was at the end of the feeding period of the first generation, about the time that most caterpillars had ceased feeding and pupated. The final census was of the oldest leaves and when the second generation of caterpillars (if there was one) had ceased feeding. We also surveyed the same foliage for the presence of alkaloids.

MATERIALS AND METHODS

Study site

Santa Rosa National Park (10° 45′–11° 00′ N) is a 10 800 ha area of forest and abandoned pastures covering a plateau (300 m elevation) and descending to the Pacific Ocean. It is bordered by Costa Rica Route 1 (the old Pan American Highway) and lies 25 km south of the town of La Cruz in extreme north-western Costa Rica. The forest is largely deciduous during the dry season, but also contains individual evergreen species of trees and patches of evergreen trees associated with river beds, lowland moist areas and north-facing escarpments. The forest is in varying stages of development, but all the species discussed here are found as wild plants in intact forest and its naturally disturbed sites, as well as in various ages of forest succession that have developed after the area was changed from an old cattle ranch and area of selective logging to a National Park.

Santa Rosa is representative of much of the remainder of the Pacific coastal plain of Central America in that the rainy season is about five to six months in length (early May to late November or December) and virtually no rain falls during the dry season. The majority of the species of woody plants respond to the dry season by dropping their entire leaf crop sometime between January and early March. However, various individuals and species may retain them longer, and a few drop them before the dry weather occurs (e.g. *Plumeria rubra*). Most of the deciduous tree species wait until the first rains or one or two weeks later to produce a new leaf crop, but a few anticipate the rains by one to four weeks and are fully leafed out before the rains commence (e.g. *Enterolobium cyclocarpum*). None of the so-called evergreen species are truly evergreen. All bear a leaf crop for roughly 11.5 months of the year, and then at some time at the beginning of the dry season or early wet season they shed all their leaves and immediately replace them within a few days to a week or two (e.g. *Hymenaea courbaril*, *Manilkara zapota*). This behaviour is true of many so-called evergreen tropical trees in dry habitats, but often goes unnoticed. At Santa Rosa, all the evergreen trees bear a full leaf crop during the bulk of the dry season and have the sort of tough, thick, leaves expected of an evergreen species in a site with a long and severe dry season.

Santa Rosa is the focus of a number of on-going studies of herbivore–plant interactions. The site for this study was chosen as much to provide background for such studies as to examine the question itself. Our results are undoubtedly applicable to plants in Guanacaste Province in general, which contains Santa Rosa and extends far to the south. However, most of the forest in Guanacaste has already disappeared and the remainder will shortly be gone except for a few small preserves, such as Santa Rosa.

Choice of plants and leaf collection

The initial intent was to census phenolics and fibre and ascertain the distribution of alkaloids at three times in the growing season for a set of species. These species were chosen because they were easily available, with a specific effort being made to obtain evergreen species and species that were subject to other studies quite unrelated to leaf chemistry. Foliage was harvested as time permitted between 12 and 25 May 1978, the sample size of 61 species being set by the time and facilities available. However, even had more time been available, the sampling would have had to be terminated in order to have the leaves of approximately the same seasonal age. Sixty of the 61 species were resampled between 3 and 28 July 1978; *Psychotria microdon* had suffered a severe natural defoliation by the sphingid *Xylophanes turbata* and had just put out new leaves, so 'middle-aged' leaves were therefore not available. An additional eight species were added to the sample at this time because they were evergreen or very common deciduous species that had been ignored in the first census. Of the original 61 species, 49 (80%) were censused again at the end of the rainy season (13 December 1978 to 9 January 1979); the omissions being due to oversight and to some species having already begun to discolour or dehisce their leaves when the census started (e.g. *Plumeria rubra, Ipomoea carnea*). An additional 12 species, for a final total of 80 species, were censused at the end of the rainy season in order to increase the sample size of evergreens.

Since the evergreen do not follow the same leaf replacement cycle as the deciduous species, the dates in Table 1 do not apply to all collections of *Hymenaea courbaril, Sloanea terniflora, Ficus ovalis* and *Andira inermis*. Instead it is only the leaf age category that is accurate. In these four species, young leaves as well as old leaves were collected at the end of the rainy season. This was possible because the evergreen trees are enough out of synchrony that trees with another two to four weeks of life in their leaves may be found near conspecifics that have already dropped their old leaf crop and bear a fully expanded new leaf crop.

Leaf production by these species is sufficiently synchronous that by harvesting all leaves on a branch, at least 99% of the volume of leaf material had been produced in a one to two week period. Leaves were not collected from a tree in the first census period until the leaf crop was fully expanded and had turned a green colour approximating that of mature leaves. In the first census period, samples occasionally contained as much as 1–2% new, expanding leaves that were inadvertently picked along with the older leaves. Expanding leaves were absent from the other two censuses.

Plants chosen for sampling were apparently healthy and not undergoing severe defoliation by insects at the time. If they were species whose crowns are normally exposed to full sun, insolated foliage was harvested; if they were species whose crowns are normally shaded, then shaded foliage was harvested. In the case of large and medium-sized trees (categories L and M in Table 1), all the foliage was taken from one individual. In the case of smaller plants, it was pooled from several individuals growing within a few metres of one another. Leaves were not harvested twice from the same individual plants in successive censuses.

Leaves, from a single healthy adult growing in normal habitat and not subject to severe herbivory that season, were packed by hand into plastic bags. While all blades and petioles were included, care was taken to keep the sample free of twigs and other debris. The bags of leaves were kept shaded and upon returning to the laboratory, placed on a cool concrete floor until dried. Drying was undertaken within 4 h of picking leaves, and usually within 2 h of picking. About 6 l of loosely-packed fresh leaves were placed in a screen box and this was placed in a propane gas oven maintained at 60°C. The leaves were stirred several times while drying, and they reached constant weight in about 1 h. They were removed as soon as further drying would not lower their weight. The dried leaves were crushed, packed in plastic bags and shipped to P.G.W. for chemical analysis.

Taxonomy

The names used here are those in Janzen & Liesner (1980) and are the same as those generally used, except for the following where there may be some confusion: *Tabebuia rosea* = *T. pentaphylla, Cedrela odorata* = *C. mexicana, Pithecellobium* = *Pithecolobium, Pithecellobium saman* = *Samanea saman, Triplaris melaenodendron* = *Tr. americana, Genipa americana* = *G. caruto, Manilkara zapota* = *Achras sapota, Guazuma ulmifolia* = *G. tomentosa*. Voucher specimens of all the species discussed in this paper are deposited at the herbarium of the Missouri Botanical Gardens, St. Louis, Missouri. All species we discuss are common in Costa Rica and represented by large breeding populations in Santa Rosa, and

there is no possibility of confusing them with other species (except that *Spondias mombin* can be confused with *Sp. radlkoferi*, but care was taken to avoid this).

Chemical analyses

The leaves or leaflets, separated from rachis and petiole, were ground to a particle size of 1 mm, maximum. Extracts prepared by refluxing 200 mg of the powder with 60% aqueous ethanol for 30 min and by cold extraction of 500 mg with methanol for 24 h, were used to perform the Folin–Denis assay for total phenolics and the proanthocyanin assay for condensed tannins, respectively. The exact procedures adopted have been recorded previously (Gartlan *et al.*, 1980). For the total phenolics assay, results are calculated in terms of oak bark tannic acid obtained commercially (British Drug Houses, U.K.) and for the proanthocyanin assay in terms of quebracho tannin (Harshaw Chemicals Ltd., Glasgow). As the two assays are based on different standards the values obtained for them are not directly comparable with one another and it is possible to get higher equivalent concentrations for condensed tannins than for the total phenolics of a species. The results of a comparable analysis (Gartlan *et al.*, 1980) indicate that while drying the plant material will have caused some loss of extractable tannins the data obtained will correlate strongly with that which would have been obtained if it had been possible to use fresh plant material.

Hydrolysis of about 200 mg of material in 2M hydrochloric acid at 100°C gave an acid extract containing the hydrolysis products of both condensed and hydrolysable tannins and would also have extracted most alkaloids. Thin layer chromatography of an amyl alcohol extract of the acid fraction (for details see Gartlan *et al.*, 1980) was used to confirm the presence of both tannin classes. Treatment of an aliquot of the acid with Dragendorff's Reagent was used to test for alkaloids. A positive result led to the remainder of the acid extract being taken through an acid/base cycle (Gartlan *et al.*, 1980) to confirm that alkaloids were present.

Acid detergent fibre is one of a number of assays used to analyse cell wall polysaccharides and lignin components of forages (van Soest, 1966). The assay was performed on ground material using the original method. Acid detergent fibre measures cutin, lignin, and that part of the cell wall polysaccharides most intimately linked to the lignin and has been found to correlate strongly with *in vitro* assays of foliage digestibility using fungal cellulases (Choo *et al.*, 1981).

The raw data on phenolics and fibre is presented as the percentage dry weight of the leaf sample.

RESULTS

The census results are presented in Table 1. All statistics were conducted on arcsine-transformed percentages and it is these values that are presented in all tables except Table 1.

Total phenolic equivalents range from as low as 0.93% (*Pisonia macranthocarpa*) to as high as 29.12% (*Lysiloma divaricata*), condensed tannin equivalents from zero (numerous cases) to 43.82% (*Cassia grandis*) and acid detergent fibre from 13.2% (*Pisonia macranthocarpa*) to 70.3% (*Inga vera*). Water

Table 1. Percentage total phenolics (TP), condensed tannins (CT) and acid detergent fibre (ADF) per unit dry weight, percent water content, and other traits of leaves of 80 species of perennial plants in a tropical deciduous forest (Santa Rosa National Park, Guanacaste Province, Costa Rica)

		YOUNG LEAVES (May 1978)			MIDDLE-AGED LEAVES (July 1978)				OLD LEAVES (Dec. 1978/ Jan. 1979)							
	Life form	TP	CT	ADF	TP	CT	ADF	Percentage water content	TP	CT	ADF	Percentage water content	CT*	HT	Alk	Sphingid or saturniid larval host
Anacardiaceae																
Spondias mombin	L	8.40	2.42	42.0	3.10	2.45	37.7	73	7.97	7.27	52.6	67	cd	+	—	Sp, Sa
Annonaceae																
Annona reticulata	M	6.39	0.35	23.5	6.75	1.14	24.2	70	3.22	0.40	36.6	68	c	—	—	Sp
Sapranthus palanga	M	2.53	0.24	33.2	1.10	0.25	29.3	70	2.07	1.85	31.1	62	—	—	?	Sp
Apocynaceae																
Plumeria rubra	L	3.80	0.75	36.3	4.64	1.65	22.4	79	n.d.	n.d.	n.d.	n.d.	pc	—	?	Sp
Stemmadenia obovata	T	3.56	0.00	14.0	4.20	0.00	14.0	75	2.82	0.14	18.0	77	—	—	+	Sp
**Thevetia ovata*	S	2.69	0.00	30.0	2.54	10.76″	42.0	76	3.35	4.18″	32.0	73	cd	—	—	—
Bignoniaceae																
Crescentia alata	M	4.13	0.47	46.9	2.03	0.90	43.3	58	2.42	1.00	41.0	52	—	—	(+)	—
Tabebuia ochracea	L	2.12	0.32	26.8	2.38	0.38	25.9	67	n.d.	n.d.	n.d.	n.d.	—	—	+	Sp
T. rosea	L	1.17	0.00	49.2	3.57	0.23	50.7	65	3.12	0.27	54.1	65	—	—	+	—
Bombacaceae																
Bombacopsis quinatum	L	7.09	6.78	23.3	7.15	7.04	21.1	70	5.03	6.34	37.7	76	pc	—	—	Sa
Burseraceae																
Bursera simaruba	L	2.15	2.26	34.6	9.33	13.63	35.7	63	8.74	13.73	47.3	67	pc	—	—	Sa
B. tomentosa	L	10.36	21.75	46.0	12.54	19.80	42.0	59	10.37	21.21	45.2	61	pc	—	—	Sa
Caesalpiniaceae																
Bauhinia ungulata	T/S	13.72	21.49	35.6	12.25	12.12	40.7	63	7.94	8.61	53.2	56	c	+	—	Sa
Caesalpinia exostemma	M	5.60	1.01	19.6	2.72	0.07	18.8	64	7.38	0.00	22.7	73	—	+	—	—
Cassia grandis	L	13.66	41.25	35.1	17.55	43.82	37.7	57	n.d.	n.d.	n.d.	n.d.	—	—	—	Sa
**Hymenaea courbaril*	L	7.80	19.03	49.7	8.94	23.11	52.3	50	11.91	20.79	54.3	47	pcd	—	—	Sa
**Swartzia cubensis*	M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.18	0.00	57.4	63	—	—	—	—
Cochlospermaceae																
Cochlospermum vitifolium	M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	17.44	8.43	31.2	69	c	(+)	—	Sa
Combretaceae																
**Conocarpus erectum*	M/S	12.71	1.15	33.2	12.47	1.26	35.3	74	n.d.	n.d.	n.d.	n.d.	cd	+	—	—
Connaraceae																
**Rourea glabra*	S/V	n.d.	n.d.	n.d.	3.57	15.05	55.2	59	5.13	23.02	57.8	53	c	—	—	Sa
Convolvulaceae																
Ipomoea carnea	S/V	1.87	0.69	34.5	1.92	0.23	38.0	78	n.d.	n.d.	n.d.	n.d.	—	—	(+)	—
Dilleniaceae																
**Curatella americana*	T/S	n.d.	n.d.	n.d.	13.20	13.94	56.1	62	n.d.	n.d.	n.d.	n.d.	c	+	—	Sp
**Tetracera volubilis*	V	13.37	12.61	65.9	8.34	3.43	63.6	71	6.17	5.37	58.0	65	c	+	—	Sp
Elaeocarpaceae																
**Sloanea terniflora*	L	19.31	0.34	37.0	13.31	0.49	45.6	68	13.65	0.79	49.2	52	—	+	—	—

Euphorbiaceae															
Sebastiana confusa	T	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10.35	0.59	19.8	62	—	+	—	Sp
Flacourtiaceae															
Zuelania guidonia	L	13.92	42.95	33.3	10.73	16.07	42.7	80	12.73	35.75	55.8	59	c	—	—	Sa
Hippocrataceae															
Hemiangium excelsum	M/T	5.64	5.37	37.3	7.96	11.80	32.0	64	3.10	3.30	33.8	67	pcd	—	—	—
Lauraceae															
**Ocotea veraguensis*	M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.38	4.05	52.7	51	c	—	(+)	Sp, Sa
Loranthaceae															
**Psittacanthus calyculatus*	S	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13.98	0.31	18.9	67	—	+	—	—
Malphigiaceae															
**Byrsonima crassifolia*	M/T	20.49	14.22	64.0	16.89	13.58	46.0	61	16.81	20.47	68.0	53	c	+	—	—
Malvaceae															
Hibiscus tiliaceus	T/S	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4.84	15.62	40.1	71	c	—	—	—
Malvaviscus arboreus	T/S	n.d.	n.d.	n.d.	2.67	1.57	40.3.	71	1.26	0.53	29.3	75	c	—	—	—
Meliaceae															
Cedrela odorata	L	1.59	0.52	24.6	2.08	1.79	34.6	70	2.79	2.89	39.5	58	?	—	—	Sa
Mimosaceae															
Albizzia adinocephala	L	2.16	0.03	27.6	1.53	0.19	56.5	60	2.28	0.00	49.8	58	—	—	+	—
Enterolobium cyclocarpum	L	6.50	2.63	51.3	5.99	1.32	53.3	58	6.26	0.98	55.3	57	d	+	—	Sa
**Inga vera*	M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.21	7.79	70.3	52	c	+	—	Sa
Lysiloma divaricata	L	n.d.	n.d.	n.d.	29.21	11.31	21.2	62	n.d.	n.d.	n.d.	n.d.	d	+	—	Sa
Pithecellobium mangense	M	1.50	0.82	42.7	1.63	0.39	28.5	65	2.38	0.24	32.8	52	—	—	+	—
Pithec. oblongum	T/S	7.04	4.89	53.1	9.52	10.28	51.7	58	9.67	7.22	48.4	56	c	+	—	—
Pithec. platylobum	V	4.32	0.92	39.2	4.19	3.17	46.4	61	n.d.	n.d.	n.d.	n.d.	d	—	—	—
Pithec. saman	L	7.42	8.25	40.8	10.89	16.05	47.5	55	9.38	6.29	50.9	55	c	—	—	Sa
Moraceae															
Chlorophora tinctoria	L	2.44	0.01	20.1	1.98	0.27	30.3	72	2.06	1.50	38.6	68	—	—	—	Sp
**Ficus ovalis*	L	3.36	2.16	66.1	5.16	5.03	57.4	67	n.d.	n.d.	n.d.	n.d.	pc	—	—	Sp
Myrsinaceae															
**Ardisia revoluta*	S	n.d.	n.d.	n.d.	3.56	4.40	45.0	72	3.45	3.37	37.5	73	d	—	—	Sa
Myrtaceae															
Eugenia salamensis	M	21.51	4.86	56.1	10.05	4.06	43.7	64	7.05	4.82	39.8	58	cd	+	—	Sa
Psidium guineense	T/S	13.72	7.59	51.6	9.11	3.97	62.9	68	6.68	4.07	50.7	61	cd	—	—	Sa
Nyctaginaceae															
Pisonia macranthocarpa	T/S	2.99	0.03	13.2	0.93	0.11	22.3	66	0.75	0.31	27.7	62	—	—	+	—
Papilionaceae															
**Andira inermis*	L	16.35	5.10	63.4	6.58	4.25	61.0	61	5.83	5.50	63.5	60	c	—	?	—
Atelia herbert-smithii	L	4.94	0.63	17.3	2.56	0.73	26.2	67	2.96	0.44	25.4	55	—	—	+	—
Dalbergia glabra	V	n.d.	n.d.	n.d.	6.28	4.56	42.1	64	6.28	1.71	39.6	67	c	—	—	—
D. retusa	L	9.36	6.31	41.3	5.48	1.27	32.3	61	6.02	2.28	44.0	66	—	—	+	—
Diphysa robinioides	L	2.07	0.19	20.8	2.24	0.08	28.0	67	2.86	0.00	45.8	66	—	—	?	—
Gliricidia sepium	M	2.19	0.09	30.1	1.61	0.27	28.9	75	2.02	0.43	39.9	72	—	—	—	Sa
Lonchocarpus acuminatus	L	7.64	1.77	27.8	2.86	0.71	55.5	59	n.d.	n.d.	n.d.	n.d.	cd	—	(+)	—
L. costaricensis	L	1.48	0.52	48.9	2.10	0.27	44.9	66	2.40	0.77	46.6	62	pcd	—	+	Sa
L. minimiflorus	M	n.d.	n.d.	n.d.	3.68	0.91	61.5	64	2.63	0.34	64.1	67	cd	—	—	Sa
L. eriocarinalis	M	4.44	2.52	43.4	6.85	6.09	64.4	63	9.29	14.14	40.1	49	c	—	—	—
Platymiscium pleiostachyum	M	2.52	0.17	30.0	1.59	0.05	36.8	70	2.65	0.10	43.0	52	—	—	+	—
Pterocarpus rohrii	L	1.08	1.41	41.3	2.26	1.87	53.6	70	3.16	2.60	49.8	75	—	—	+	—

Table 1.—*continued*

	Life form	YOUNG LEAVES (May 1978) TP	CT	ADF	MIDDLE-AGED LEAVES (July 1978) TP	CT	ADF	Percentage water content	OLD LEAVES (Dec. 1978/ Jan. 1979) TP	CT	ADF	Percentage water content	CT*	HT	Alk	Sphingid or saturniid larval host
Polygonaceae																
**Cocoloba guanacastensis*	L	5.66	2.28	48.4	4.24	2.85	57.1	60	4.93	1.98	57.6	53	cd	+	—	—
**Triplaris melaenodendron*	M/T	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11.13	8.98	55.1	66	cd	+	—	—
Rhamnaceae																
Gouania polygama	V	2.84	0.48	46.7	1.70	0.43	28.8	72	2.12	0.41	36.0	64	—	—	(+)	—
Rhizophoraceae																
**Rhizophora mangle*	M	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	8.97	19.14	36.9	75	c	—	—	—
Rubiaceae																
**Alibertia edulis*	S	1.25	0.00	51.4	2.87	0.15	39.7	62	2.27	0.10	47.5	60	—	—	—	Sp
Calycophyllum candidissimum	L	7.40	11.76	21.1	9.59	13.96	50.7	70	6.20	18.54	44.9	69	pc	—	—	Sa, Sp
Genipa americana	M	3.64	5.58	35.4	2.73	2.80	26.7	74	n.d.	n.d.	n.d.	n.d.	—	—	—	Sp
Guettarda mecrosperma	M	n.d.	n.d.	n.d.	2.40	0.81	38.1	78	n.d.	n.d.	n.d.	n.d.	pc	—	—	Sp
Hamelia patens	T/S	2.79	0.10	13.7	9.47	5.78	27.0	77	4.92	2.39	23.3	73	(pc)a	—	+y	Sp
Psychotria microdon	S	1.95	0.75	26.3	n.d.	n.d.	n.d.	n.d.	1.93	0.10	25.6	80	—	—	+	Sp
Randia echinocarpa	T/M	2.53	0·74	37.4	3.69	1.05	31.4	71	2.24	0.50	26.1	73	—	—	+	Sp
Sapindaceae																
Allophyllus occidentalis	S/T	4.34	3.50	47.7	4.23	1.75	48.1	68	n.d.	n.d.	n.d.	n.d.	pcd	—	—	—
Sapotaceae																
**Manilkara zapota*	L	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3.87	5.84	43.0	63	pcd	—	—	—
**Mastichodendron capiri*	L	1.78	0.39	37.3	2.31	1.17	41.3	62	3.30	4.09	27.9	54	—	(+)	+	—
Simaroubaceae																
Simarouba glauca	L	14.29	3.08	29.3	11.58	2.32	36.1	60	n.d.	n.d.	n.d.	n.d.	c	—	—	—
Sterculiaceae																
Guazuma ulmifolia	M	3.56	7.83	33.0	1.52	1.23	41.1	61	2.66	5.33	35.0	57	c	—	—	Sa
Sterculia apetala	L	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.53	0.73	40.0	67	—	—	—	—
Tiliaceae																
Apeiba tibourbou	M	4.37	0.82	45.1	4.36	0.47	43.4	66	3.57	0.62	41.5	66	—	—	—	—
Luehea candida	M	6.92	8.68	31.1	4.61	8.21	45.7	66	4.46	6.95	39.5	64	c	—	—	Sa
L. speciosa	M	10.36	23.90	24.7	8.62	17.06	33.7	60	9.13	24.64	32.8	53	c	—	—	—
Verbenaceae																
**Avicennia germinans*	M/S	1.36	0.08	34.8	4.22	0.22	40.0	66	n.d.	n.d.	n.d.	n.d.	—	—	—	—

Values in percentage dry wt. n.d. = not done, no sample collected; CT* = identified condensed tannin monomers (p = pelargonidin, c = cyanidin, d = delphinidin); HT = hydrolysable tannins present; Alk = alkaloids present.

+ = major; (+) = minor; ? = doubtful; a = adult leaf only; y = young leaf only.

Life form: L = large tree; M = medium tree; T = treelet; S = shrub; V = vine (woody); * = evergreen species (all others deciduous).

" = Atypical colour reaction (false positive?).

contents varied from as low as 47% in the old leaves of *Hymenaea courbaril* to a high of 80% in the middle-aged leaves of *Zuelania guidonia*. Presumptive tests for alkaloids confirmed their occurrence in 15 species and probable occurrence in another five. More detailed comparisons (Tables 2–5) are based on different variables that divide the data set.

It must be stressed that these comparisons assess the chemical composition of species sets and do not take account of the undoubted variations that will occur within a species. Such intraspecific variability, while often quite substantial, is much less than interspecific variation (Gartlan *et al.*, 1980) and the conclusions that are drawn from this data set are of the kind that will not be influenced by anticipated intraspecific variability.

A comparison of deciduous and evergreen species (Table 2) shows that there are no significant differences in leaf content of total phenolic or condensed tannin equivalents, nor in percentage water content within any of the three census periods. By contrast the fibre content of evergreen species was significantly greater than deciduous species in each census, the difference appearing to be greatest in the young and middle-aged leaf census periods. The greater dichotomy in young and middle-aged leaves appears to be caused by

Table 2. Percentage total phenolics (TP), condensed tannins (CT) and acid detergent fibre (ADF) per unit dry weight, and percentage water content of leaves at three ages for deciduous (D) and evergreen (E) species in a tropical deciduous forest; with *t*-test (t_s) values for each pair of D–E contrasts. All values based on arcsine transformations of raw data in Table 1

		$\bar{X}$	S.D.	N	t_s	
Young leaves						
TP	D	12.93	5.06	49	1.23	NS
	E	15.83	7.75	12		
CT	D	9.95	9.61	49	0.16	NS
	E	9.48	9.03	12		
ADF	D	35.59	7.00	49	3.35	$P < 0.01$
	E	44.08	8.07	12		
Middle-aged leaves						
TP	D	12.83	5.44	53	1.34	NS
	E	14.87	5.11	15		
CT	D	10.02	8.47	53	1.18	NS
	E	12.88	8.22	15		
ADF	D	37.91	7.21	53	4.01	$P < 0.001$
	E	44.52	5.10	15		
H_2O	D	54.84	3.85	53	0.09	NS
	E	53.65	3.65	15		
Old leaves						
TP	D	12.37	4.35	47	1.76	NS
	E	14.69	4.93	18		
CT	D	10.10	8.66	47	1.49	NS
	E	13.73	8.80	18		
ADF	D	38.91	6.27	47	2.59	$P < 0.02$
	E	44.52	8.37	18		
H_2O	D	53.39	4.49	47	0.83	NS
	E	50.87	5.10	18		

S.D. = standard deviation.

evergreen species investing more heavily in fibre in earlier stages of development whilst in deciduous species fibre is added progressively; for example, the average increase in fibre levels from young to old evergreen leaves is under 1% whereas for deciduous species it is over 3%. This contrasts with levels of condensed tannin equivalents which appear to remain static in the ageing deciduous leaf but to increase from the young to the old leaf census of evergreen leaves.

Table 3 presents the results of comparisons of total phenolic and condensed tannin equivalents, acid detergent fibre and water content between young and middle-aged leaves and between middle-aged and old leaves for both deciduous and evergreen species. None of the comparisons for phenolics, condensed tannins or fibre revealed any significant differences but fibre levels were significantly greater in old leaves than they were in the young leaves of the same species of deciduous tree ($\bar{X}$ diff. = 3.62%, $n = 40$, $t_s = 2.37$, $P<0.05$). The percentage water content of fresh leaves did decline slightly from middle-aged to old in both deciduous and evergreen leaves.

The leaves of the 18 species of deciduous plants that tested positive for alkaloids contained substantially less total phenolic and condensed tannin equivalents than did those in which no alkaloids were detected (Table 4). However, these two groups of foliage did not show the same contrast for the amount of fibre or water that they contained (Table 4, only middle-aged water contents examined). No comparisons were made among evergreen species, since only two had leaves in which alkaloids definitely were identified. In fact, 10% of the 20 evergreen species tested positive for alkaloids, while 30% of the 60 deciduous species tested positive ($\chi^2_{1\,d.f.} = 2.4$, N.S.).

Table 3. Changes in percentage dry weight total phenolics, condensed tannins and acid detergent fibre and percentage water content of fresh leaves, during various parts of the growing season in a tropical deciduous forest. All values based on arcsine transformations of raw data in Table 1. Abbreviations as Table 2

		$\bar{X}$	S.D.	N	t_s	
Change in % TP						
Young to middle-aged	D	−0.46	1.01	48	0.45	NS
	E	−0.83	2.69	12	0.31	NS
Middle-aged to old	D	−0.28	0.93	42	0.29	NS
	E	0.43	2.21	11	0.19	NS
Change in % CT						
Young to middle-aged	D	−0.03	1.88	48	0.02	NS
	E	1.89	3.52	12	0.54	NS
Middle-aged to old	D	0.32	1.80	42	0.18	NS
	E	0.87	3.76	11	0.23	NS
Change in % ADF						
Young to middle-aged	D	2.07	1.45	48	1.43	NS
	E	0.03	2.82	12	0.01	NS
Middle-aged to old	D	1.26	1.48	42	0.85	NS
	E	0.20	2.71	11	0.07	NS
Change in % water content						
Middle-aded to old	D	−1.69	3.71	42	2.99	$P<0.005$
	E	−3.21	2.89	11	3.21	$P<0.01$

Table 4. Contrast in percentage dry weight of total phenolics, condensed tannins and acid detergent fibre, and percentage water content (middle-aged leaves only, among leaves of deciduous trees containing alkaloids and those not containing alkaloids. All values based on arcsine transformation of raw data in Table 1 and for TP, CT and ADF on averaged values for all collections. Abbreviations as Table 2

	$\bar{X}$	S.D.	N	t_s	
TP content					
species containing alkaloids	9.67	2.30	18	4.89	$P<0.001$
species lacking alkaloids	14.66	5.45	40		
CT content					
Species containing alkaloids	4.51	2.62	18	5.49	$P<0.001$
species lacking alkaloids	13.03	9.01	40		
ADF content					
species containing alkaloids	35.85	6.56	18	1.17	NS
species lacking alkaloids	37.95	5.70	40		
H_2O content					
species containing alkaloids	55.76	4.09	18	0.37	NS
species lacking alkaloids	54.61	3.93	40		

Table 5. Contrast in percentage dry weight of total phenolics, condensed tannins and acid detergent fibre, and percentage water content, among larval host leaves of Sphingidae and Saturniidae moths. All values based on arcisine transformation of raw data in Table 1. Abbreviations as Table 2

		$\bar{X}$	S.D.	N	t_s	
Young leaves						
TP	saturniid hosts	15.64	5.73	19	2.49	$P<0.025$
	sphingid hosts	11.47	4.02	15		
CT	saturniid hosts	17.04	11.14	19	3.39	$P<0.005$
	sphingid hosts	6.57	6.72	15		
ADF	saturniid hosts	38.33	6.28	19	1.02	NS
	sphingid hosts	35.29	10.17	15		
Middle-aged leaves						
TP	saturniid hosts	15.46	6.17	23	1.76	NS
	sphingid hosts	12.49	4.37	16		
CT	saturniid hosts	15.93	9.67	23	2.92	$P<0.01$
	sphingid hosts	8.27	6.71	16		
ADF	saturniid hosts	40.99	6.41	23	1.77	NS
	sphingid hosts	36.50	8.62	16		
H_2O	saturniid hosts	53.77	4.26	23	3.20	$P<0.005$
	sphingid hosts	57.57	3.16	16		
Old leaves						
TP	saturniid hosts	14.40	4.31	23	2.19	$P<0.05$
	sphingid hosts	11.47	3.57	13		
CT	saturniid hosts	15.82	8.65	23	2.87	$P<0.01$
	sphingid hosts	8.31	6.87	13		
ADF	saturniid hosts	43.79	5.63	23	2.67	$P<0.02$
	sphingid hosts	36.89	8.30	13		
H_2O	saturniid hosts	51.64	4.56	23	2.24	$P<0.05$
	sphingid hosts	55.26	4.72	13		

After the census commenced, numerous species of caterpillars were reared from the foliage of a large number of tree species at Santa Rosa (Janzen, 1981; unpubl.). Many larval hosts were obtained for the species-rich Sphingidae and Saturniidae, and 41 of these hosts were serendipitously among the 80 species of plant censused (Table 1). Sphingidae are well known as defoliators of species of plant families with a reputation for containing alkaloids and other low molecular weight, toxic, molecules (Harris, 1972; Janzen, 1981; Janzen, unpubl.; W. Haber—pers. comm.). Saturniidae, on the other hand, appear to be avoiding such host plants. For example, of the 25 saturniid larval hosts listed in Table 1, 8% tested positive for alkaloids while 37% of the 19 sphingid hosts listed in Table 1 tested unambiguously positive ($\chi^2_{1\,d.f.} = 4.9$, $P<0.01$). While 22% of the saturniid hosts have highly aromatic foliage and 21% of the sphingid hosts have aromatic foliage, 26% of the sphingid hosts yield a copious latex while none of the saturniid hosts produce latex.

If we used these two groups of moth larvae to dichotomize the relevant species in Table 1, the saturniid hosts clearly produce leaves that are significantly richer in condensed tannin equivalents at all ages and richer in total phenolic equivalents when young than are leaves of sphingid hosts (Table 5). Saturniid host leaves also have a lower water content in middle-age and are more fibre-rich in old age than are the leaves of sphingid hosts.

DISCUSSION

It is quite clear that, at least for 80 of the approximately 350 species (Janzen & Liesner, 1980) of woody plants at Santa Rosa, there is no substantial overall change in total phenolic equivalents or condensed tannin equivalents in expanded leaves during the rainy season for either deciduous or evergreen species. During the same period water content of leaves of both deciduous and evergreen species drops significantly but not drastically and the fibre of deciduous species can increase quite markedly (e.g., *Calycophyllum candidissimum* increases from 21.1% to 44.9% between May and December). We hasten to point out that no census was made of leaf chemistry during the first few days of leaf development, but emphasize that a major proportion of the defoliation of plants at Santa Rosa occurs after leaves are fully expanded but during the period spanned by the young to middle-aged leaf censuses.

There is a considerable body of largely unpublished folklore in the contemporary study of plant–herbivore interactions, and a central dogma of this is that phenolic content of foliage rises as the foliage ages. Our census does not refute the possibility of such a phenomenon with other plant species or other habitats but it does suggest that plant chemistry of the fully expanded leaf may be very heterogeneous with respect to this parameter. Not only did we find no average change between one census and the next (Table 3), but an inspection of the values in Table 1 confirms that this 'no average change' is not due to a fortuitous balancing of those with phenolic content rising severely against those with values falling severely, but rather by most species changing relatively little from census to census. These observations do not conflict with the well-documented reports that expanding, but not yet full-sized, young leaves contain more phenolics (on a dry weight basis) than do the corresponding mature leaves (Coley, 1980; Gartlan *et al.*, 1980). It is tempting to suggest that the apparently

higher levels of expanding leaves are a product of their higher moisture content and that the relatively static levels in mature, full-sized leaves actually reflect little or no change in the real quantities of phenolics in the leaf. This would support the suggestion (McKey, 1979) that there is a preferential allocation of compounds to the young leaf followed by passive dilution as the leaf ages.

It seems quite evident that the severe decline in the intensity of herbivory by insects that occurs after the first two months of the rainy season at Santa Rosa cannot be blamed on a major increase in either total phenolic or condensed tannin equivalents. Likewise, the small decline in water-content from middle-aged to old leaves (Table 3) seems unlikely to be responsible for the decline in herbivory. This last conclusion is not intended as a negation of the well-documented assertion that water content in foliage influences growth rates (Scriber, 1979; Scriber & Feeny, 1979), but rather that the few percent change recorded here is unlikely to be the cause of a habitat-wide decline in herbivory.

Acid detergent fibre is generally held to be a good estimator of foliage digestibility (Choo *et al.*, 1981) and has proved to be an important correlate of food selection among mammalian herbivores (see for example, Oates, Waterman & Choo, 1980; McKey *et al.*, 1981). The majority of larval host plants are deciduous and there is a marked increase in fibre levels in many of these leaves as the wet season progresses. Thus, the intense period of herbivory early in the wet season does appear to correspond to the time when fibre levels are lowest. However, there is no clear avoidance of fibre-rich leaves. For example, both Saturniidae and Sphingidae have some larval host plants with May fibre levels in excess of 50%.

While the levels of condensed tannin equivalents were generally lower than the levels of total phenolic equivalents, there were a few cases where the reverse was dramatically true (e.g., *Cassia grandis, Hymenaea courbaril, Bursera tomentosa, Zuelania guidonia, Luehea speciosa*). This apparent paradox is caused partly by the two assay procedures being based on different standards and partly because the sensitivity of the proanthocyanin assay for condensed tannins is influenced by both the size of the tannin polymer and the final substitution pattern of the coloured breakdown products that it measures. In short, the proanthocyanin assay has many defects and lacks the resolution necessary to fully explore the influence of condensed tannins on host selection by animals such as sphingid and saturniid larvae. An ideal level of resolution would be, on the one hand, to test the specific phenolics of a leaf against the particular proteins in that leaf; this would yield information on the ability of the tannin to bind with the protein in a form that the caterpillar then cannot digest. On the other hand, the phenolics of a species should be tested against the proteins of the guts of both the insects that feed on that leaf and those that occur in the same habitat but do not feed on it. Studies of this type must now be carried out in view of the mounting literature reporting that phenolics, and tannins in particular, have little effect on some insect larvae (Bernays, 1981) and recent findings of high specificity and pH dependence of tannin–protein complexation (Hagerman & Butler, 1981) and lack of correlation between chemical assays for condensed tannins and the protein-precipitating capabilities of mature foliage (Martin & Martin, 1982).

Our census suggests some profitable species with which to commence more refined studies. For example, *Zuelania guidonia* is a favourite host of at least two species of saturniid moths (*Hylesia lineata* and *Rothschildia lebeau*) and it is

therefore evident that they somehow contend with the very high condensed tannin levels of the leaves of this species. At the other end of the scale, plants with little phenolics in their foliage could well contain other types of secondary compounds that are potentially toxic to most insect larvae. For example, *Cedrela odorata* (= *C. mexicana*), which is a host of larvae of the saturniid *Dirphia avia*, contains several limonoids, including mexicanolide and 7-oxogedunin (Taylor, 1982). Thus, our census is useful in pointing to gross differences in the adaptations of sphingid and saturniid larvae to leaf chemistry and in saying that there is no major seasonal increase in total phenolic or condensed tannin equivalents in these species. It can not be used to imply that phenolics of a more specific nature are not seasonally changing their influence on the animals that eat or could eat the leaves.

While it is tempting to suggest that the phenolics measured in our census are most relevant to understanding the foliage as food for large and quite generalist herbivores, there is no evidence that in contemporary Santa Rosa there are sufficient numbers of these to be much of a threat, except for leaf-cutter ants (*Atta* spp.). How the leaf-cutter ants interact with phenolics and other compounds in Santa Rosa foliage is currently under study (S. B. Hubbell—pers. comm.). However, the foliage of Santa Rosa was once subject to the same levels of browsing by large mammals as is encountered in Africa today (Janzen & Martin, 1982), and we suspect that many leaf chemistry traits contain a large anachronistic component. On the other hand, many tannins and other phenolics have considerable fungitoxic properties (Levin, 1976) and their impact in the protection of foliage from fungal infestation should not be overlooked.

It is an intuitively satisfying hypothesis that plants rich in one kind of chemical defence compound will be deficient in other kinds of compounds, despite the fact that plants exist that seem to have very little overt chemical protection of any sort (e.g., some grasses) while others appear to be a veritable collection of toxic compounds. Our census appears to demonstrate that the distribution of condensed tannins and one class of toxin, the alkaloids, is consistent with this hypothesis; confirming the earlier report of the same finding for tropical rain forest tree foliage from Cameroon and Uganda (Gartlan *et al.*, 1980). We doubt that there is any reason for this dichotomy in the mode of biogenesis of phenolics and alkaloids, but suspect that those plants well-protected by alkaloids are not under such strong selection to have intense phenolic defense, and *vice versa*. Such a dichotomy will be reinforced by some of the most alkaloid-rich families being largely herbaceous (e.g., Solanaceae, Papaveraceae, Compositae) while tannins are rare in herbaceous plants. This cannot however explain the divisions noted here among the foliage of tree species. We suggest that a possible explanation could reside in the ability of tannins to bind with other types of defence compounds, such as alkaloids and glycosides, and to form insoluble complexes that inhibit or delay absorption (Todd, 1967). Thus, the co-occurrence of both types of compounds in a leaf could lead to a diminution of the defensive capabilities of both.

Our census does not permit comment on the question of 'apparent' and 'unapparent' plants (Feeny, 1976; Rhoades & Cates, 1976). Almost all the plants we censused are very common in their appropriate habitats and we censused only a small fraction of the total defence repertoire of each of these plants. Furthermore, the concept of 'apparency' requires substantial elaboration

before it can be applied to a species-rich tropical forest such as the one at Santa Rosa.

While detailed analysis of the sphingid/saturniid-host leaf chemistry story has to await more focused studies, the approximately 70 species of Santa Rosa Sphingidae are mapped onto a quite different set of plants from those used by the 30 species of Santa Rosa Saturniidae. Not only is there little overlap in the lists of larval hosts of these two large moth taxa (Janzen, 1981; unpubl.), but our census strongly suggests that leaves rich in phenolics, particularly condensed tannins, and poor in toxic small molecules will be found to be the primary saturniid hosts and that comparatively condensed tannin deficient species that are rich in toxic small molecules will be found to be the primary sphingid hosts (W. Haber has already found this to be the case for sphingid larvae at several other Costa Rican sites, pers. comm.). The implication of this for the life cycles of these moths is being developed elsewhere (D. H. Janzen unpubl.).

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