

## LEAF POLYPHENOLS IN BRAZILIAN MELASTOMATACEAE: SCLEROPHYLLY, HABITATS, AND INSECT HERBIVORES

Sérvio Pontes Ribeiro<sup>1</sup>, Alaíde O. Braga<sup>2</sup>, Cláudia Hermínia L. Silva<sup>2</sup> & Geraldo Wilson Fernandes<sup>1</sup>

<sup>1</sup> Evolutionary Ecology of Tropical Herbivores/DBG/ICB, Universidade Federal de Minas Gerais, C.P. 486 - 30161-970 Belo Horizonte, MG, Brazil

<sup>2</sup> Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, MG 30180-112 - Brazil

**Resumo.** Duas hipóteses sobre resposta da concentração de polifenóis foliares à gradientes físicos foram testadas para a vegetação de cerrado. Foram estudadas as espécies *Miconia macrothrysa*, *M. albicans*, e *M. ferruginata* a 800 m de altitude; *Tibouchina semidecandra*, *T. multiflora*, e *Lavoisiera imbricata* a 1400 m. Foram feitas comparações intraspecificas entre habitats xéricos e mésicos para as espécies *M. macrothrysa* e *L. imbricata*. A Hipótese I prevê que altas concentrações de polifenóis está associada com esclerofilia. Esta hipótese foi testada comparando a concentração total de polifenóis entre as espécies estudadas, e comparando suas concentrações de taninos com dados publicados sobre taninos condensados em plantas esclerófilas e não esclerófilas. Dados sobre taninos são encontrados em maior quantidade na literatura do que de polifenóis totais, e taninos foram altamente correlacionados com polifenóis totais. A maioria das espécies de Melastomataceae estudadas tiveram alta concentração de polifenóis, e a concentração de taninos condensados foi maior para plantas esclerófilas que não esclerófilas ( $P < 0.05$ ), corroborando a Hipótese I. Hipótese II prevê que a concentração de taninos seria independente da altitude ou habitat porque esclerofilia seria uma característica comum a todos estes ambientes. Em contraste com outros trabalhos, a concentração de polifenóis não variou em resposta a altitude ou gradiente higrotérmico ( $P > 0.05$ ). Insetos herbívoros foram contados diretamente sobre as folhas destas plantas. Foram encontradas baixa riqueza e abundância de insetos por planta. Não foi detectado nenhuma relação entre concentração de taninos e riqueza e abundância de insetos, nem nenhum padrão de variação entre habitats. A hipótese de que esclerofilia é uma característica dominante em habitats de cerrado foi proposta.

**Abstract.** Two hypotheses concerning the response of foliar polyphenol concentration to abiotic gradients were tested in a Brazilian cerrado. We studied *Miconia macrothrysa*, *M. albicans*, and *M. ferruginata* at 800 m above sea level; *Tibouchina semidecandra*, *T. multiflora*, and *Lavoisiera imbricata* at 1400 m. Intraspecific comparisons between xeric and mesic habitats at each elevation were undertaken for *M. macrothrysa* and *L. imbricata*. Hypothesis I predicted that high polyphenol concentration is associated with sclerophylly. This hypothesis was tested by comparing total polyphenol concentration among the studied species, and comparing their tannin concentration with earlier published data on condensed tannin concentration in sclerophyllous and non-sclerophyllous species. Contrary to total polyphenols, data on tannin concentration are widely available in ecological literature. Tannins were highly correlated with total polyphenols. Most of the melastome species had high concentration of polyphenols. Condensed tannins were more concentrated in sclerophyllous than in non-sclerophyllous plant species ( $P < 0.05$ ), supporting this hypothesis. Hypothesis II predicted that polyphenol concentration was independent of altitude and habitat because sclerophylly is widespread across elevations and hygrothermal gradients, producing high polyphenol concentrations in all plants. In contrast to previous studies, polyphenol concentration did not vary in response to altitude or humidity ( $P > 0.05$ ). Insect herbivores were directly counted on these six plant species. Low insect herbivore species richness and low abundance per plant were found on the six plant species, and there was no detectable influence of tannin concentration on insect species richness or abundance, and no clear trend according to habitat. The hypothesis of widespread sclerophylly in cerrado as a dominant trait was supported. Accepted 28 July 1999.

**Key words:** Cerrado, insect herbivores, *Lavoisiera imbricata*, *Miconia albicans*, *Miconia ferruginata*, *Miconia macrothrysa*, sclerophylly, *Tibouchina multiflora*, *Tibouchina semidecandra*.

### INTRODUCTION

Sclerophylly in plants is usually associated with response to physical factors. Sclerophyllous plants possess high carbon/nitrogen ratios, thick cell walls, and tough leaves (Rizzini 1979, Juniper & Jeffrey 1983, Mole *et al.* 1988, Salatino 1993, Turner 1994). They are adapted to nutrient-poor and water-deficient soils.

Low levels of phosphate appear to be the most relevant factor associated with sclerophylly (Loveless 1962, Fernandes & Price 1991). Other characteristics associated with sclerophylly are high polyphenol contents (Salatino 1993). Phenolic compounds, such as tannin and lignin, result from alternative routes in the formation of proteins, when there is inadequate availability of phosphate and nitrogen compared with carbon fixed by photosynthesis (Harborne 1980). These compounds are likely to contribute to basic

<sup>1</sup>e-mail: serviopr@mono.icb.ufmg.br

plant physiology in a typically sclerophyllous habitat (Haslam 1988, Perevolotsky 1994, Turner 1994).

Independent of sclerophylly, polyphenol accumulation in plants is often suggested as an effective defense against herbivory (Janzen 1974, Coley *et al.* 1985, Mole & Waterman 1988). Much research has been devoted to the protective function of a particular type of polyphenol, the tannins, due to their ability to precipitate with protein (Feeny & Bostock 1968, Mole & Waterman 1987b, Perevolotsky 1994). Physiological and anatomical changes that occur in the guts of specialist insects feeding on tannin-rich plants suggest that tannins have negative effects on herbivores (Bernays *et al.* 1989). Nevertheless, doubts still persist about the function of phenols as herbivore deterrents (e.g., Seigler & Price 1976, Blytt *et al.* 1988), and there is no strong evidence to support the view that tannins evolved as a chemical defense (Seigler 1977, Haslam 1988, Gotlieb 1990). Although there are detectable negative effects on insects, the basic functions of polyphenols are physiological, and hence tests for an adaptative link between these compounds and plant defense are not easily performed (see Haslam 1988).

Sclerophylly is a common trait in the poor, lateritic soils of the Brazilian cerrado (= savannas) and in high altitude grasslands (see Goodland & Ferri 1979). In these harsh, xeric habitats there are narrow gallery forests in mesic conditions along rivers, streams, and washes (Warming 1908, Fernandes & Price 1988). By contrast, the mesic habitat is expected to offer better conditions for high plant growth rates, due to greater water availability, deeper and better structured soils, and a climatically more constant and milder environment (Gershenson 1984, Fernandes & Price 1992, Herms & Mattson 1992). Plants with low growth rates have increased concentration of carbon-based compounds, such as polyphenols, which could be related to low herbivory. Conversely, relatively high resource availability enables rapid plant growth, and greater tolerance to herbivory than found in slow growing species (Coley *et al.* 1985). Lower polyphenol concentration and higher herbivory levels are thus expected in plants from mesic than from xeric habitats, and from low compared with high altitudes. However, the premise of high growth rate associated with greater resource availability has not been tested for habitat types in cerrado.

There are no conclusive data on plant chemical patterns along altitudinal or humidity gradients in

cerrado. However, low numbers of free-feeding insect herbivores (leaf-chewing and sap-sucking insects in contrast to those forming galls or mines) were found in both xeric and mesic habitats, along a 700 m altitudinal gradient in the Serra do Cipó (Ribeiro *et al.* 1998). This suggests that unfavorable conditions could be widespread, encompassing mesic as well as xeric habitats at any elevation. High levels of plant polyphenols and other sclerophyllous traits may constitute nutritional barriers to the colonization of generalist herbivores, but specialists may overcome them (Macauley & Fox 1980, Fernandes & Price 1988, Bernays *et al.* 1989).

We tested two hypotheses regarding plant polyphenol concentration and plant distribution between habitats. Hypothesis I, considering that concentration of polyphenols in tropical vegetation is higher in sclerophyllous plant species than in non-sclerophyllous species. We expected this pattern for the following reasons. Sclerophyllous plants have a high C/N ratio (Janzen 1974) and a positive correlation would be expected between carbon-based secondary metabolites, such as polyphenols, and sclerophylly (Bryant *et al.* 1983, Herms & Mattson 1992). Sclerophyllous plants are adapted to nitrogen and phosphate deficient soils (Loveless 1962, Medina *et al.* 1990, Fernandes & Price 1991). Nutrient stress limits protein production, and increases the availability of phenol precursors, such as phenylalanine, and phenylalanine-ammonia-lyase (PAL) activity (Harborne 1980), resulting in high levels of phenol production (Gershenson 1984). Phenolic compounds accumulate as polyphenols due to a low NADPH supply and because they have adaptative value (Gershenson 1984, Haslam 1988, Bernays *et al.* 1989). Hypothesis II, on the other hand, tested whether high polyphenol concentration is independent of altitude or habitat (xeric *vs.* mesic habitats). We argue that differences in altitude, or in humidity between cerrado vegetation and gallery forest, do not influence sclerophylly, and thus patterns of polyphenol concentration (inter- or intraspecific) are not expected to be significantly influenced by these physical gradients. Widespread soil nutrient deficiency and high solar irradiation over the whole region would guarantee similarly high C/N rates in all habitats, producing an invariably sclerophyllous ecosystem (Rizzini 1979, Giullietti & Pirani 1988, Ribeiro 1992). We expected that polyphenol production would not be affected by actual differences in drought stress, soil characteristics, or habitat stability along these gradients.

In a related study of patterns of insect distribution along an environmental gradient in this plant community, Ribeiro *et al.* (1998) found low species richness and abundance of sap-sucking and chewing insect herbivores. These insect feeding guilds, hereafter called free-feeding insects, were investigated on six melastome species, and the number of insect species and individuals compared among host plants in relation to tannin concentration, the group of polyphenols most likely to affect insect herbivory. Effects of tannins on the insect community are then discussed in relation to sclerophylly of host plants and its effects on free-feeding insects, which are herbivores particularly susceptible to nutritional and chemical barriers. By contrast, gall-forming insects can manipulate the plant tissues to increase nutritional conditions around the larvae and to avoid chemical defenses (Shorthouse & Rohfritsch 1992). Patterns of distribution of gall-forming insects on cerrado vegetation, and aspects of plant defense against this highly specialized herbivorous guild can be found in Fernandes & Price (1988, 1991, 1992), Fernandes *et al.* (1994), and are not considered further in this paper.

## STUDY SITE

The study was conducted in the cerrado of Lagoa Santa District, and in the adjoining National Park of Serra do Cipó, along the Espinhaço Mountain Range (19°15'–19°40' S and 43°30'–43°55' W), in Minas Gerais State, south-east Brazil. The region is situated within the Central Brazilian Plateau, which is on average 850 m above sea level (Freitas 1951), and is predominantly covered by cerrado vegetation. In May 1991, we studied plant populations at two extreme elevations: 1400 m (high altitude grassland) and at 800–900 m (savanna = cerrado) above sea level, for polyphenol concentrations and richness and abundance of free-feeding insect herbivores. Detailed site descriptions are given in Ribeiro (1992).

## METHODS

*Plant species.* Only Melastomataceae species were studied, firstly because they are well represented in the Serra do Cipó (Giulietti & Pirani 1988), and secondly to avoid phylogenetic bias by comparing evolutionarily unbalanced plant families (see Harvey & Pagel 1991). In addition, within Melastomataceae we selected taxonomically independent species and genera between the two studied elevations. Six species

were chosen, based on their altitudinal distribution and abundance. *Tibouchina semidecandra* Cogn., *T. multiflora* (Gardn.) Cogn., and *Lavoisiera imbricata* Cogn. were found in high altitude grassland at 1400 m. *Miconia macrothyrsa* Benth., *M. ferruginata* DC., and *M. albicans* (Sw.) were found in savanna at 800–900 m. Populations of the six species were dense, and patchily distributed. They are all evergreen species, although some leaf-shedding does occur under prolonged water stress in the dry season. Plants were sampled in May, after flowering and seed production. Since both young and mature leaves are available in May, this month was considered an acceptable period in which to characterize the insect community, although it is after the end of the rainy season and thus associated with smaller insect population sizes in this phenological phase (see Ribeiro & Pimenta 1991). *Tibouchina semidecandra* is primarily riparian, whereas *M. macrothyrsa* and *L. imbricata* occur in both xeric and mesic habitats. The other three species occur only in xeric habitats.

*Tibouchina semidecandra* is a shrub reaching a height of 3 m. The leaves (4 cm long) are covered with a dense aciculate trichome layer. Trichomes are densely distributed on the leaf adaxial surface, and on the top of the leaf veins. The population sampled was from a mesic habitat at 1400 m. *Tibouchina multiflora* is a shrub that grows up to 1 m high. It has a simple architecture, having four to seven branches with a few apical leaves. The leaves (10 cm long) are very pubescent, with trichomes similar to those of *T. semidecandra*. The population sampled was from a wide and continuous patch of a xeric habitat at 1400 m. *Lavoisiera imbricata* is a small shrub up to 0.8 m high, with reduced leaf size. Trichomes are present on the leaf edges and main veins. Leaf epidermal cell walls are thick, and leaves are very sclerophyllous. The population sampled was found in both mesic and xeric habitats at 1400 m, at the same locations as *T. semidecandra* and *T. multiflora*. *Miconia macrothyrsa* is a shrub approaching a height of 1 m, with very sclerophyllous leaves (10 cm long). Thick, stellate, and tortuous trichomes are present on the leaf abaxial epidermis. The population sampled was from 800 to 900 m, in xeric habitats, and from a gallery forest at 900 m in a mesic habitat. *Miconia ferruginata* is a tree up to 3 m high, with leaves 20 cm long and that are closely imbricate, extremely sclerophyllous, and with stellate trichomes on the leaf abaxial surface. Epidermal cell walls are very thin, especially at the edges. The population sampled was from

a small patch at 800 to 900 m in xeric habitats. *Miconia albicans* is a tree up to 3 m high, with softer leaves (5 cm length) than in the other species. The leaves are densely pubescent and covered with a thick cuticular layer. The population sampled was from 900 m in xeric habitats.

*Test of hypotheses.* To test the hypotheses, leaves from six individuals of each species (total  $n = 36$ ) were collected for total polyphenol and tannin measurements in May 1991 (Coley 1983, Haslam 1988). Samples were composed of mature leaves, randomly collected from the plants at different heights. *M. macrothyrsa* and *L. imbricata* were sampled in both xeric and mesic habitats to test hypothesis II.

Leaves were collected and dried at 40°C and ground mechanically; a solution of 750 mg of powdered leaf material in 150 ml of distilled water was prepared for each sample. The solution was boiled and then swirled at 60°C for 30 min (see Mole & Waterman 1987a). The solution was then made up to 250 ml and filtered to determine total polyphenol and tannin concentrations. Five ml of filtered material were diluted to 25 ml. One ml of aqueous solution of phosphotungstic acid was added to 5 ml of the filtered extract solution, and made up to 50 ml solution of NaCO<sub>3</sub> at 15 percent m/v (see Farmacopée Française 1982). Total polyphenol concentration was determined by visible spectrophotometry (Coleman, Model 8) at 715 nm (see Farmacopée Française 1982), and the result was then compared with absorbance of the control solution (0.5 mg/ml of pyrogallol, diluted to 100 ml).

Tannin content was measured by protein precipitation followed by spectrophotometry. Protein-bound polyphenols are considered generically as tannins (Mole & Waterman 1987b). For each sample, 0.10 g of hide powder (homogenised pig skin protein - MERCK Chemical Products inc. - No 4332) was added to 10 ml of the filtered extract (see Feeny & Bostock 1968). This mixture was swirled vigorously for 60 min then the filtered solution was added to phosphotungstic acid and increased to 50 ml of NaCO<sub>3</sub> solution, as described above. Later, the absorbance of the solution was measured. Tannin concentration was obtained by subtracting the value of the absorbance of the filtered solution from the total polyphenol absorbance and converting this value into percentage foliar dry weight as a measure of concentration (Farmacopée Française 1982). This tannin measurement was considered satisfactory by Mole &

Waterman (1987a) (see also Perevolotsky 1994). For recent reviews on different methods for quantifying polyphenol concentrations, see Hagerman & Buttlar (1989).

Hypothesis I was tested by comparing the total polyphenol concentration among the six species, and by comparing their tannin concentrations with earlier published data on condensed tannins of sclerophyllous and non-sclerophyllous species. Tannins are those polyphenols that precipitate with protein. Although the quantitative method used cannot distinguish types of polyphenols beyond their precipitation activity, phytochemical color tests indicated that the studied species have predominantly condensed tannins. Concentrations of tannin were highly correlated with total concentration of polyphenol (Pearson  $r^2 = 0.97$ ), and represented an average 70.5 % of the plant's total polyphenol. In contrast to tannins, total polyphenol data are not so easily found in the literature, and therefore the former was used to compare our results with published data. Comparison of tannin concentration should be considered with caution since different methodological chemical assays were used by different authors. Nevertheless, it is still possible to compare results from the published data (see Mole & Waterman 1987a). We expected similar values for species growing under similar physical conditions. Average tannin concentrations are available for several different tropical species (i.e. growing under high solar irradiation). Comparisons of tannin concentration among species were made by one-way analysis of variance and Fisher multiple comparison tests on the arc-sine-transformed values (SYSTAT 5.0 INC., Wilkinson 1989).

The studied plants were contrasted with species adapted to both poor and rich soils, referred to as sclerophyllous and non-sclerophyllous species. Measures of tannins in sclerophyllous species were taken from African rainforest species in Mole *et al.* (1988), Australian *Eucalyptus* species in Macauley & Fox (1980), Panamanian persistent species in Coley (1983), and African species in acidic white sand forest in McKey *et al.* (1978). Data for non-sclerophyllous species were taken from pioneer species in Panama in Coley (1983), and African species on fertile lateritic forest in McKey *et al.* (1978). Comparisons of melastome species with published data were undertaken using non-parametric Mann-Whitney tests (Zar 1984). First, we tested whether the studied plants were similar to sclerophyllous species. Se-

condly, sclerophyllous species (melastomes included) were compared to non-sclerophyllous species.

For hypothesis II, we compared polyphenol concentration in three species in the cerrado with three species in the high altitude grassland in order to test altitudinal gradient effects. Intraspecific comparisons of polyphenol concentration between xeric and mesic habitats were undertaken for *M. macrothyrsa* and *L. imbricata*, thus providing comparisons between gallery forest-cerrado (800–900 m) and gallery forest-high altitude grassland (1400 m), respectively. Arc-sine transformed values of polyphenol concentration were compared among all studied species and between altitudes using nested analysis of variance. Similarly, arc-sine transformed values of polyphenol concentration of *M. macrothyrsa* and *L. imbricata* were compared between individuals in xeric and mesic habitats, using a two-way analysis of variance (Wilkinson 1989).

Insects were counted, by the same person, during periods of 10 min on each of 20 individuals of each plant species, and separated into morphospecies. Although the plants have very different heights and leaf dimensions, 10 min of observation gave us a uniform leaf surface area searched per individual plant. No clear effect of plant species architecture on insect richness or abundance was found (see Ribeiro 1992). In addition, we did not find a significant correlation between insect richness or abundance and total leaf biomass sampled across species (total leaves counted, multiplied by mean leaf biomass: Pearson for richness  $P = 0.260$ , and abundance  $P = 0.384$ ;  $n = 6$ ,  $P > 0.05$ ). The search on 20 individuals of each plant species allowed us to reach a constant number of herbivore species sampled per host plant, since on most of the plants no insect species were found at all.

In an attempt to describe insect distribution patterns among individual plants within populations, insect abundance was investigated by analysis of deviance with a Poisson error structure, which is the best fit distribution for the data, using a general linear model procedure in GLIM 3.77. Hence, the one-way ANOVA was tested by chi-square instead of the F-test, thus fitting the assumption of the model's distribution (Crawley 1993). Total insect richness was low on all plant species, and insect distribution was better quantified from abundance data. In addition, comparisons of insect abundance among plant species were carried out by Kruskal-Wallis test, and non-

parametric multiple comparisons based on mean ranks R (Zar 1984).

For both insect abundance and tannin concentration, the Type I error probabilities were corrected by the Bonferroni technique, to  $k = 2$  and  $k = 4$  comparison, respectively (Sokal & Rohlf 1995).

## RESULTS

*Hypothesis I: Polyphenol concentration in sclerophyllous plants.* The six melastome plant species varied widely in polyphenol concentration (One-way ANOVA,  $F_{4,30} = 25.0$ ;  $P < 0.01$ ; Fig. 1). Similarly, significant differences in tannin concentration were found between plant species (ANOVA  $F_{5,30} = 19.1$ ,  $P < 0.025$ , Table 2). *Miconia albicans* contained more tannins than any other species (ANOVA multiple comparisons test,  $F_{1,30} = 60.7$ ,  $P < 0.025$ ). *Tibouchina multiflora*, *T. semidecandra*, and *M. macrothyrsa* contained higher concentrations than did *L. imbricata* and *M. ferruginata* (ANOVA multiple comparisons test,  $F_{1,30} = 5.0$ ,  $P < 0.025$ ).

However, except in *L. imbricata*, the concentrations of tannins were high in comparison to those found in other studies. Tannin concentrations in melastome species were generally higher than in non-sclerophyllous species (Mann-Whitney  $U = 28$ ,  $n = 53$ ,  $P < 0.01$ ), but similar to those in sclerophyllous species (Mann-Whitney  $U = 189$ ,  $n = 43$ ,  $P > 0.05$ ; Table 1). Hence, sclerophyllous species (melastome species included) contained higher tannin concentrations than did non-sclerophyllous species (Mann-Whitney  $U = 470$ ,  $n = 90$ ,  $P < 0.01$ ; Table 1), thus supporting our first hypothesis.

*Hypothesis II: Polyphenol concentration between habitats.* Polyphenol concentration was not related to altitude or habitat (xeric or mesic). Polyphenol concentration did not differ between the three species in cerrado and the three species in high altitude grassland (Nested ANOVA,  $F_{1,4} = 1.2$ ,  $P > 0.05$ ), and also did not differ between xeric and mesic habitats in the intraspecific comparisons for *M. macrothyrsa* (xeric:  $x = 7.28 \pm 1.49$ ,  $n = 6$ ; mesic:  $x = 6.14 \pm 1.87$ ,  $n = 6$ ), and *L. imbricata* (xeric:  $x = 0.71 \pm 0.16$ ,  $n = 6$ ; mesic:  $x = 0.5 \pm 0.37$ ,  $n = 6$ ) (Two-way ANOVA,  $F_{1,19} = 1.23$ ,  $P > 0.05$ ). Hence, these data support the hypothesis that polyphenol concentration is high in sclerophyllous plants within each habitat type.

*Insect herbivore community and tannin concentration of host plants.* Low insect species richness was found on the six melastome species, and no direct relation

onship was observed between tannin concentration and insect species richness (Table 2).

A total of 25 insect species was found on the six plant species. The numbers of insect species per host plant species were not statistically different (ANOVA,  $\chi^2 = 0.015$ ,  $P > 0.05$ , Table 2). Only one morpho-species, of whitefly (Aleyrodidae, Hemiptera), was found on more than one plant species (*M. macrothyrsa*, *M. ferruginata*, and *M. albicans*).

In 26% of the plants, we did not find any insects at all, reflecting a high overdispersion of abundance within each host plant species. Total insect abundance displayed a slightly negative binomial distribution (G test = 9.784,  $P < 0.05$ ), which indicated that most plant studied were poorly colonized. Although the proportion of non-attacked plants differed among species ( $\chi^2 = 22.323$ ,  $P < 0.05$ ), the differences were not related to tannin concentration (Table 2).

Insect abundance varied among host plants (Kruskal-Wallis H = 55.64,  $P < 0.001$ ), but was not

related to concentration of tannin. *T. multiflora* and *M. macrothyrsa* (both species with intermediate levels of tannin concentration) supported the highest abundance of insect herbivores (Mean ranks  $R = 95.95$  and  $R = 86.22$  respectively; non-parametric Tukey test,  $P < 0.13$ ), followed by *M. albicans* (the species with the highest level of tannin concentration), *M. ferruginata*, and *T. semidecandra* (Mean ranks  $R = 58.3$ ,  $R = 54.4$ , and  $R = 36.1$  respectively; non-parametric Tukey test,  $P < 0.13$ ). *L. imbricata* had the lowest abundance of insects (Mean rank  $R = 32.10$ ; non-parametric Tukey test,  $P < 0.13$ ), despite the fact that no tannins were found in extracts of this species.

## DISCUSSION

We found high polyphenol and tannin concentrations in most Neotropical melastome species across vegetation types in both xeric and mesic habitats. The analysis of data from the literature showed that high

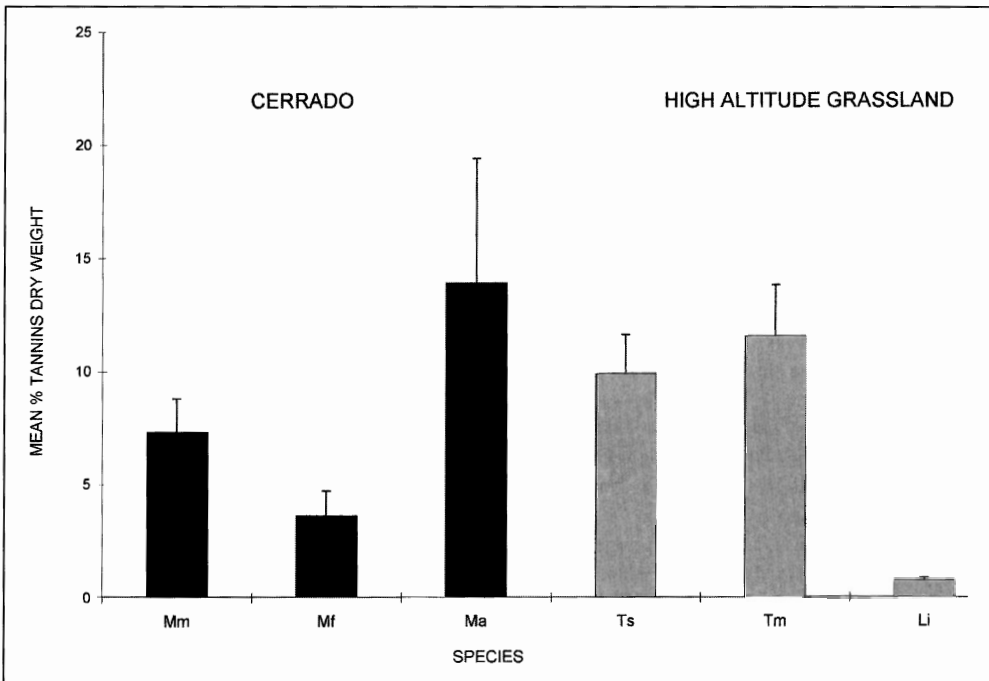


FIG 1. Polyphenol concentrations (mean percent dry weight  $\pm$  SE,  $n = 6$ ) in *Miconia macrothyrsa*, *M. ferruginata*, and *M. albicans* at 800-900 m and in *Tibouchina semidecandra*, *T. multiflora*, *Lavoisiera imbricata* at 1400 m, in the Serra do Cipó.

TABLE 1. Mean concentration (percent dry mass) of tannins in plant species. Standard errors and ranges are given when they are available from the literature. Species values given are for sclerophyllous species, together with means for persistent species in Panama, and for species from acid white sand forest species in Africa. Means for pioneer species in Panama, and for species from fertile lateritic forest species in Africa represent non-sclerophyllous species.

Plant species	Mean $\pm$ 1se	Range	Reference
Sclerophyllous			
<i>Lavoisiera imbricata</i>	0	0	This study
<i>Miconia macrothyrsa</i>	4.40 $\pm$ 1.05	2.86 - 5.18	This study
<i>M. ferruginata</i>	1.97 $\pm$ 0.66	1.00 - 2.50	This study
<i>M. albicans</i>	12.90 $\pm$ 5.38	6.60 - 21.60	This study
<i>Tibouchina semidecandra</i>	6.44 $\pm$ 1.19	4.68 - 7.85	This study
<i>T. multiflora</i>	7.68 $\pm$ 1.74	4.46 - 9.57	This study
<i>Acacia pannata</i>	13.29	3.83 - 31.37	Mole <i>et al.</i> 1988
<i>Cynimetra leonensis</i>	13.16	6.27 - 25.59	Mole <i>et al.</i> 1988
<i>Diospyros thomasii</i>	5.57	2.94 - 12.73	Mole <i>et al.</i> 1988
<i>Eucalyptus blakelyi</i>	0.77 $\pm$ 0.09	0 - 2.30	Macauley & Fox 1980
<i>E. macrorrhyncha</i>	1.50 $\pm$ 0.07	0.90 - 2.90	Macauley & Fox 1980
<i>E. mannifera</i>	16.78 $\pm$ 8.48	10.00 - 33.30	Macauley & Fox 1980
<i>E. pauciflora</i>	2.55 $\pm$ 0.05	2.20 - 2.90	Macauley & Fox 1980
<i>Trema guineensis</i>	8.23	1.59 - 17.99	Mole <i>et al.</i> 1988
Persistent species: Panama (24 spp.) <sup>a</sup>	4.80	0.07 - 13.20	Coley 1983
Acid white sand forest species: Africa (16 spp.) <sup>a</sup>	4.60	0.40 - 27.30	McKey <i>et al.</i> 1978
Non-sclerophyllous			
Pioneer species: Panama (24 spp.) <sup>a</sup>	1.7	0 - 7.5	Coley 1983
Fertile lateritic forest species: Africa (14 spp.) <sup>a</sup>	2.6	0 - 9.5	McKey <i>et al.</i> 1978

<sup>a</sup> Separated species values available in the original papers.

tannin concentration is commonly found in sclerophyllous species or in species growing on poor soils. With the exception of *L. imbricata* and *M. ferruginata*, the mean concentration of tannins was similar to or higher than other sclerophyllous species in the literature (Table 1). In addition, the data range for a species showed that all individuals of *M. macrothyrsa*, *M. albicans*, *T. semidecandra*, and *T. multiflora* had high levels of tannin concentration (Table 1). In Table 1, the highest reported individual values for a species were for *Eucalyptus mannifera* (33.3 %), *Acacia pannata* (31.37 %), and *Cynimetra leonensis* (25.59 %), and, from this study, *M. albicans* (21.60 %). Reciprocally, compared to data from known non-sclerophyllous plants, namely pioneer plants of gap forests (n = 22, Coley 1983), and trees in an African rainforest on fertile lateritic soil (n = 14, McKey *et al.* 1978), our plants had higher concentrations of tannins, thereby supporting our first hypothesis.

Polyphenol concentrations were similar between xeric and mesic habitats, and at the two elevations.

Mesic habitats were narrow and restricted within the widespread xeric habitats, which are well known nutrient-poor habitats. A possible explanation of the similarity is that few nutrients are carried to the mesic habitats, and are quickly washed downriver by strong tropical storms. Stream waters in the Serra do Cipó are generally black, suggesting high concentrations of semi-degraded phenols (see Janzen 1974).

Botanical studies in the Serra do Cipó showed sclerophylly to be a common characteristic of this plant community (Goodland & Ferri 1979, Rizzini 1979, Giullieti & Pirani 1988). The widespread poor soil and high solar irradiation may result in plants with low nutritional quality and chemical similarity across altitudinal and moisture gradients (Gershenson 1984, Medina *et al.* 1990, Fernandes & Price 1991). Other physical factors associated with these gradients apparently do not affect polyphenol production. Certainly, further studies are needed.

Low species richness and population sizes of free-feeding herbivores were found on the six melastomes

TABLE 2. Total insect species richness and abundance per host plant species, and proportion of non-frequented individual plants within each species. Species ordered according to decreasing tannin concentration (mean value per species, detailed tannin data in Table 1).

Plant species	Percent of tannin	Insect species richness	Insect abundance	Percent of non-frequented plants
non-frequented plants				
<i>Miconia albicans</i>	12.9	5	60	25
<i>Tibouchina multiflora</i>	7.68	5	410	0
<i>Tibouchina semidecandra</i>	6.44	3	35	45
<i>Miconia macrothyrsa</i>	4.40	6	785	10
<i>Miconia ferruginata</i>	1.97	5	102	25
<i>Lavoisiera imbricata</i>	0	5	21	50

studied. Price *et al.* (1995) found low population sizes and low plant infestations in a study of lepidopteran fauna on *Erythroxylum* spp. in the cerrado of Brazil. Similar patterns were also described for the free-feeding insect community in tropical rainforest canopies (Basset 1992). However, contrary to our results, other tropical insect communities are generally very diverse in species richness. Compared to other plant species, the Melastomataceae studied supported very low numbers of insect herbivores (see Southwood 1960, 1961; Strong 1974; Strong *et al.* 1977; Basset 1992; Basset *et al.* 1996; Basset & Samuelson 1996), and this may well be a consequence of low foliage quality (Ribeiro 1992; but see Fernandes & Price 1991). Despite their low nutritional quality and high chemical defense, sclerophyllous plants are rich in gall-forming herbivores (Fernandes & Price 1988, 1991). Gall-forming species are highly specialized herbivores and widespread in harsh and sclerophyllous ecosystems.

No direct effect of tannin concentration on richness and abundance of insect species was detected in this study. Perevolotsky (1994) did not find any relationship between tannin levels and browsing on sclerophyllous Mediterranean plants. In addition, Fox & Macauley (1977) and Macauley & Fox (1980) did not find a clear direct effect of tannins on major insect herbivore species on *Eucalyptus*. Moreover, they showed that for these species only a small proportion of polyphenols detected were tannins (Macauley & Fox 1980).

High total polyphenol and tannin concentrations are important chemical characteristics of sclerophylls, which may increase the plant's defense to her-

bivores (see Salatino 1993). Nevertheless, sclerophylls may impose other barriers to insect herbivory and mask a direct effect of tannins. For instance, in sclerophyllous plants, trichomes are very complex, and sometimes contain as many secondary compounds as the leaf tissues (see Ribeiro *et al.* 1994, M.M.M. Braga, pers. comm.). In this study, trichomes of *M. albicans* could be separated while grinding leaves, and 5.25 % of trichome dry weight was tannins. Trichomes have been shown to be a very important defense against insect herbivores (Juniper & Jeffrey 1983, Woodman & Fernandes 1991). Moreover, the lack of a correlation between tannins and insect herbivores may result from the fact that phenolics function in roles other than herbivore defense. For example, high polyphenol concentrations may provide protection against the deleterious effects of ultraviolet radiation (Klein 1978, Robberecht *et al.* 1980), or simply be used in diverse chemical pathways in plant metabolism (Haslam 1988, Gottlieb 1990, Salatino 1993, Turner 1994).

Our results cannot be explained by current hypotheses regarding relationships between phenolics and environmental conditions, or between phenolics and herbivory. Variation in the concentration of chemical defenses is due to the relative availability of fixed carbon and nutrients (Bryant *et al.* 1983). Since low growth rates and high light conditions are thought to favor increased amounts of carbon-based defenses, species in xeric habitats should have higher levels of polyphenols than species in mesic habitats. Nevertheless, our data offer a possible exception, where xeric versus mesic conditions did not result in such physiological differences. Moreover, similarly high



tannin concentrations among species in several habitats, growing on poor soils from different geographical and physical environments, and apparently under similarly low herbivory pressure, suggest a trend toward a primary physiological role of polyphenols. Nevertheless, a comprehensive view of the evolution and adaptive significance of tannins to plant-herbivore interactions will depend on detailed experimental studies.

## ACKNOWLEDGMENTS

We thank V.K. Brown, O. Cheesman, D. Hammond, P.W. Price, J. Baldwin, J. Gershenzon, W.W. Benson, D. Semens, F.A.M. dos Santos and O. Petchey for their comments on the manuscript. We also thank A.R. Marques for collaboration in plant anatomical descriptions. The Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq and US Fish and Wildlife Service/Fundação Biodiversitas provided a scholarship to SPR. Financial support was provided by the Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG - 821/90), Fundação Biodiversitas, and CNPq (401771/91-1, 52.1772/95-8) to GWF. We are also grateful for the logistical support provided by the Parque Nacional da Serra do Cipó and Instituto Brasileiro de Recursos Naturais Renováveis e Meio Ambiente/IBAMA. This study was in partial fulfilment of the requirements for the M.Sc. degree at the Universidade Federal de Minas Gerais.

## REFERENCES

- Basset, Y. 1992. Influence of leaf traits on the spatial distribution of arboreal arthropods within an overstorey rainforest tree. *Ecol. Entomol.* 17: 8–16.
- Basset, Y., & G.A. Samuelson. 1996. Ecological characteristic of an arboreal community of Chrysomelidae in Papua New Guinea. Pp. 243–262 in Jolivet, P.H.A., & M.L. Cox. (eds.). *Chrysomelidae biology*. Volume 2: Ecological Studies. London.
- Basset, Y., Samuelson, G.A., Allison, A., & S.E. Miller. 1996. How many species of host-specific insects feed on a species of tropical tree? *Biol. J. Linn. Soc.* 59: 201–216.
- Bernays, E., Driver, G.C., & M. Bilgener. 1989. Herbivores and plant tannins. *Adv. Ecol. Res.* 19: 263–302.
- Blytt, H.J., Guscar, T.K., & L.G. Butler. 1988. Antinutritional effects and ecological significance of dietary condensed tannins may not be due to binding and inhibiting digestive enzymes. *J. Chem. Ecol.* 14: 1455–1465.
- Bryant, J.P., Chapin, F.S., & D.R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40: 357–368.
- Coley, P.D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr.* 53: 209–233.
- Coley, P.D., Bryant, J.P., & F.S. Chapin. 1985. Resource availability and plant antiherbivore defence. *Science* 230: 895–899.
- Crawley, M.J. 1993. *GLIM for ecologists*. Oxford. Farmacopé Française (10th Edition). 1982. Troisième supplement. Maisonneuve, Paris.
- Feeny, P., & H. Bostock. 1968. Seasonal changes in the tannin content of oak leaves. *Phytochemistry*. 7: 871–880.
- Fernandes, G.W., Lara, A.C.F., & P.W. Price. 1994. The geography of galling insects and the mechanisms that result in patterns. Pp. 42–48 in Price, P.W., Mattson, W.J. & Y.N. Baranchikov (eds.). *The Ecology and evolution of gall-forming insects*. US Department of Agriculture - General Technical Report NC-174, Krasnoyarsk.
- Fernandes, G.W., & P.W. Price. 1988. Biogeographical gradients in galling species richness: tests of hypotheses. *Oecologia* 76: 161–167.
- Fernandes, G.W., & P.W. Price. 1991. Comparison of tropical and temperate galling species richness: the role of environmental harshness and plant nutrient status. Pp. 91–115 in Price, P.W., Lewinsohn, T.M., Fernandes, G.W., & W.W. Benson (eds.). *Plant-animal interactions: evolutionary ecology in tropical and temperate regions*. New York.
- Fernandes, G.W., & P.W. Price. 1992. The adaptive significance of insect gall distribution: survivorship of species in xeric and mesic habitats. *Oecologia* 90: 14–20.
- Fox, L.R., & B.J. Macauley. 1977. Insect grazing on *Eucalyptus* in response to variation in leaf tannins and nitrogen. *Oecologia* 29: 145–162.
- Freitas, R.O. 1951. Ensaio sobre o relevo tectônico do Brasil. *Revta. Bras. Geogr.* 2: 171–220.
- Gershenzon, J. 1984. Changes in the levels of plant secondary metabolites under water and nutrient stress. Pp. 273–321 in Timmermann, B.N., Steelink, C., & F.A. Loewus (eds.). *Phytochemical adaptations to stress*. New York.
- Giulietti A.M., & J.R. Pirani. 1988. Patterns of geographic distribution of some plant species from the Espinhaço range, Minas Gerais and Bahia, Brazil. Pp. 39–69 in Vanzolini, P.E., & W.R. Heyer (eds.). *Proceedings of a workshop on Neotropical distribution patterns*. Academia Brasileira de Ciência, Rio de Janeiro.
- Goodland, R., & M.G. Ferri. 1979. *Ecologia do cerrado*. Itatiaia, Belo Horizonte.
- Gotlieb, O.R. 1990. Phytochemicals: differentiation and function. *Phytochemistry* 29: 1713–1724.

- Hagerman, A.E., & L.G. Buttler. 1989. Choosing appropriate methods and standards for assaying tannin. *J. Chem. Ecol.* 15: 1795–1810.
- Harborne, J.B. 1980. Plant phenolics. Pp 129–402 in Bell, E.A. & B.V. Charlwood (eds.). *Secondary plant products: encyclopedia of plant physiology* Volume 8. Berlin.
- Harvey, P.H., & M.D. Pagel. 1991. *The comparative method in evolutionary biology*. Oxford.
- Haslam, E. 1988. Plant polyphenols (syn. vegetable tannins) and chemical defence – a reappraisal. *J. Chem. Ecol.* 14: 1789–1805.
- Hermes, D.A., & W.J. Mattson. 1992. The dilemma of plants: to grow or defend. *Q. Rev. Biol.* 67: 283–335.
- Janzen, D.H. 1974. Tropical blackwater rivers, animals and mast fruiting by the Dipterocarpaceae. *Biotropica* 6: 69–103.
- Juniper, B.E., & C.E. Jeffree. 1983. *Plant surfaces*. London.
- Klein, R.M. 1978. Plants and near-ultraviolet radiation. *Bot. Rev.* 44: 1–127.
- Loveless, A.R. 1962. Further evidence to support a nutritional interpretation of sclerophylly. *Ann. Bot.* 26: 551–561.
- Macauley B.J., & L.R. Fox. 1980. Variation in total phenols and condensed tannins in *Eucalyptus*: leaf phenology and insect grazing. *Austr. J. Ecol.* 5: 31–35.
- McKey, D., Waterman, P.G., Mbi, C.N., Gartlan, J.S., & T.T. Struhsaker 1978. Phenolic content of vegetation in two African rain forests: ecological implication. *Science* 202: 61–63.
- Medina, E., Garcia, V., & E. Cuevas. 1990. Sclerophylly and oligotrophic environments: relationships between leaf structure, mineral nutrient content, and drought resistance in tropical rain forest of the upper rio Negro region. *Biotropica* 22: 51–64.
- Mole, S., & P.G. Waterman. 1987a. A critical analysis of techniques for measuring tannins in ecological studies. I. Techniques for chemically defining tannins. *Oecologia* 72: 137–147.
- Mole, S., & P.G. Waterman. 1987b. Tannin as antifeedants to mammalian herbivores – still as open question? Pp. 527–587 in Walker, G.R. (ed.). *Allelochemicals role in agriculture and forestry*. American Chemical Society, Washington.
- Mole, S., & P.G. Waterman. 1988. Light-induced variation in phenolic levels in foliage of rain-forest plants. II. Potential significance to herbivores. *J. Chem. Ecol.* 14: 23–34.
- Mole, S., Ross, J.A.M., & P.G. Waterman. 1988. Light-induced variation in phenolic levels in foliage of rain-forest plants. I. Chemical changes. *J. Chem. Ecol.* 14: 1–22.
- Perevolotsky, A. 1994. Tannins in Mediterranean woodland species: lack of responses to browsing and thinning. *Oikos* 71:333-340.
- Price, P.W., Diniz, I.R., Morais, H.C., & E.S.A. Marques. 1995. The abundance of insect herbivore species in the tropics: the high local richness of rare species. *Biotropica* 27: 468–478.
- Ribeiro, S.P., & H.R. Pimenta. 1991. Padrões de abundância e distribuição temporal de herbívoros de vida livre em *Tabebuia ochracea* (Bignoniaceae). *An. Soc. Entomol. Bras.* 20: 428–448.
- Ribeiro, S.P. 1992. Distribuição de insetos herbívoros em cerrados e campos rupestres na Serra do Cipó: o papel de compostos fenólicos em plantas esclerófilas. Master Thesis. ICB/Universidade Federal de Minas Gerais, Belo Horizonte.
- Ribeiro, S.P., Pimenta, H.R., & G.W. Fernandes. 1994. Herbivory by chewing and sucking insects on *Tabebuia ochracea*. *Biotropica* 26: 302–307.
- Ribeiro, S.P., Carneiro, M.A.A., & G.W. Fernandes. 1998. Free-feeding insect herbivores along environmental gradients in Serra do Cipó: basis for a management plan. *J. Insec. Conser.* 2: 107–118
- Rizzini, C.T. 1979. *Tratado de Fitogeografia do Brasil*. Volume 2. São Paulo.
- Robberecht, R., Caldwell, M.M., & W.D. Billings. 1980. Leaf ultraviolet optical properties along a latitudinal gradient in the arctic-alpine life zone. *Ecology* 61: 612–619.
- Salatino, A. 1993. Chemical ecology and the theory of oligotrophic scleromorphism. *An. Acad. Bras. Cienc.* 65: 1–13.
- Seigler, D.S., & P.W. Price. 1976. Secondary compounds in plants: primary functions. *Am. Nat.* 110: 101–105.
- Seigler, D.S. 1977. Primary roles for secondary compounds. *Biochem. System. Ecol.* 5: 195–199.
- Shorthouse, J.D., & O. Rohfritsch 1992. *Biology of insect-induced galls*. New York.
- Sokal, R.R., & F.J. Rohlf. 1995. *Biometry*. San Francisco.
- Southwood, T.R.E. 1960. The abundance of the Hawaiian trees and number of their associated insect species. *Proc. Haw. Entomol. Soc.* 17: 299–303.
- Southwood, T.R.E. 1961. The number of species of insects associated with various trees. *J. Anim. Ecol.* 30: 1–8.
- Strong Jr, D.R. 1974. Rapid asymptotic species accumulation in phytophagous insect communities: the pests of cacao. *Science* 185: 1064–1066.
- Strong Jr, D.R., McCoy, E.D., & J.R. Rey. 1977. Time and number of herbivore species: the pest of sugarcane. *Ecology* 58: 167–175.
- Turner, I.M. 1994. Sclerophylly: primary protective? *Func. Ecol.* 8: 669–675.
- Warming, E. 1908. Lagoa Santa. Belo Horizonte.
- Wilkinson, L. 1989. SYSTAT: The system for statistics. SYSTAT Inc. Evanston.
- Woodman, R.L., & G.W. Fernandes. 1991. Differential mechanical defence: herbivory, evapotranspiration, and leaf hairs. *Oikos* 60: 11–19.
- Zar, J.H. 1984. *Biostatistical analysis*. Prentice-Hall, New Jersey.