Phylogeny and the patterns of leaf phenolics in gap- and forest-adapted *Piper* and *Miconia* understory shrubs

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Summary. The types and quantities of defense compounds found in plants occupying ecologically distinct habitats have received much theoretical and little empirical attention. Here we characterize the leaf phenolic chemistry of eight species in two genera of tropical rainforest shrubs; four species in each genus are typical of disturbed sites, and four are typical of mature forest understory. Two *Miconia* species growing in light gaps had significantly higher leaf tannin and total phenolic contents than congeners growing in the primary forest; this pattern was not found among the gap- and forest-adapted *Piper* species. Tannin patterns were not mirrored by leaf cinnamic acids. These results indicate that plant phylogeny must be considered when predicting plant defense investment.

Key words: Plant defense – Phenolics – Tannins – Tropical shrubs

Patterns in plant production of carbon-intensive allelochemical like phenolics might be determined by adaptation to herbivory (Feeny 1976) or by restrictions placed on the plant by resource limitation (Bryant et al. 1983), or both.

Tropical forest plant species that occur and reproduce in disturbances such as treefall gaps are thought to constitute a physiognomic/ecological type different from species that typify undisturbed mature forest (Richards 1952; Denslow 1980). Pioneer or gap species are thought to be adapted to the high light conditions and necessity of rapid growth before canopy closure characteristic of such disturbances (Hartshorn 1978; Denslow 1980; Vitousek and Denslow 1986). Persistent or shade-tolerant species are thought to tolerate low light availability by exhibiting slow growth and delayed maturation, and to persist in mature forest (Hartshorn 1978; Denslow 1980; Coley 1983).

Plant apparency (*sensu* Feeny 1976) is a characteristic that is difficult to measure experimentally, because it must include detailed information about the herbivores’ assessment of plants. In one attempt to measure plant apparency, based on measures of dispersion of herbivore damage, Coley (1983) concluded that shade-tolerant persistent species are no more apparent than the pioneer species; however, pioneer species, once discovered by herbivores, were grazed at higher rates than persistent species. If persistent and pioneer species are indeed equally apparent to herbivores, a comparison of their respective titers of allelochemicals could provide an interesting test for the constraints of resource-availability (Coley et al. 1985) on plant defense. Assuming that persistent species are adapted to resource-poor conditions, Coley (1983) and Coley et al. (1985) argue that such species should produce greater quantities of carbon-intensive allelochemicals, such as phenols, than do species growing in resource-rich conditions.

Two studies have attempted to address these issues; they offer conflicting results. Coley (1983) found higher concentrations of carbon-intensive defenses (toughness, fiber, phenolics) in shade-tolerant, slow-growing, persistent tree species than in those of shade-intolerant, fast-growing, pioneer species typical of light gaps on Barro Colorado Island, Panama. Recently, Newberry and de Foresta (1985) found the opposite pattern in similar traits of plants growing in light gaps and primary forest in French Guiana. The results from these two studies cannot be directly compared. Coley (1983) sampled all of her species in gaps; thus differences between persistent and pioneer species presumably reflect only differences in genetic constitution. The results of Newberry and de Foresta (1985) may be due to both environmental and genetic differences because they studied a mixture of largely unidentified species, some of which were growing in light gaps and some in primary forest. However, in both studies, the plant species studied represented a wide array of lineages; in neither study did the same or related taxa occur in both habitats.

Theories predicting different patterns of defense among plant species (Feeny 1976; Rhoades and Cates 1976; Coley et al. 1985) have emphasized ecological parameters (e.g., resource availability and apparency to herbivores) and assumed that phylogenetic constraints are less important. Theories predicting patterns of plant defense within species have emphasized both the availability of resources (Bryant et al. 1985) and the value of tissues to a plant (McKey 1974, 1979). The trends in defense levels within species are sometimes opposite those found among species (Bazzaz et al. 1987). Because biosynthetic patterns in plants are likely to be constrained phylogenetically (Ehrlich and Raven 1965) the interaction between these constraints and ecological factors needs to be examined.

In this report we characterize the phenolic chemistry of the leaves of eight species in two genera of tropical rainforest shrubs. In our study, we included species found primarily in light gaps or disturbed sites (gap species) and species apparently restricted to the understory of the prima-
ry forest (forest species) from each genus. We find that the results of congeneric comparisons do not agree with those for mixed taxa. Phylogenetic constraints do indeed complicate tests of these ecological hypotheses.

**Methods**

This study was conducted at La Selva Biological Station, a premontane wet forest in the Province of Heredia, Costa Rica, which is owned and operated by the Organization for Tropical Studies.

Twenty-five to 35 undamaged, mature leaves without epiphylls were collected from ten plants, each from four species of *Miconia* (Melastomataceae) and four of *Piper* (Piperaceae) species during a two-week period from late November to early December 1983. Two species from each genus (*P. sancti-felicis, P. culebranum, M. nervosa, M. barbinervis*) were chosen because they are thought to be pioneer species that utilize small gaps in the canopy or tree falls for regeneration (J.S. Denslow, J.C. Schultz, P. Vitousek, B. Strain, unpublished work); these species are referred to as gap species. The other two species from each genus (*P. arieanum, P. urostachyum, M. gracilis, M. centrodesma*) do not appear to require canopy openings (and the associated alterations in microclimate or resource availability) for reproduction (Denslow, Schultz, Strain, Vitousek, and Marquis, unpub. work); these species are referred to as forest species. We found this classification of the *Piper* and *Miconia* species as gap and forest types to be correct. An intensive survey of individuals from the eight species at La Selva revealed that gap species are restricted to light gaps, or recently closed gaps, while forest species occur throughout the mature primary forest (Denslow, Schultz, Vitousek and Strain, unpublished work). Moreover, the physiological responses to light of these eight species is appropriate for their gap and forest classification (Denslow, Schultz, Vitousek and Strain, unpub. results). All plants were located on the alluvial soil of La Selva.

Leaves were either frozen (−20°C) or weighed (to 0.1 mg) to determine percentage dry weight within 20 min of collection. Frozen leaves were lyophilized for later chemical analysis; chemical analysis performed at La Selva on all eight species found no difference in total phenolic content or bovine serum albumin binding capacity between fresh-frozen or lyophilized leaves.

Three hundred to 900 mg of pooled lyophilized leaf material, and five lyophilized leaves from each plant were weighed (to 0.2 mg) and individually extracted twice in 20 ml diethyl ether to remove chlorophylls that might interfere with the phenolic assays. Total phenolic analyses performed at La Selva without ether extractions were similar to those performed on ether-extracted leaf material, indicating that ether extractions did not remove Folin Denis reactive material. The ether-extracted leaf material was further extracted twice in 25 ml 70% acetone-water at 40°C for 15 h in glass-tight containers. The acetone extracts were combined, rotoevaporated, brought up to 10 ml with distilled water, and centrifuged at 12,000 rpm. This extract was used for the phenolic assays.

The total phenolic content of the leaf extract was estimated with the Folin Denis technique (Swain and Hillis 1959). The tanning capacity was estimated by protein precipitation (Schultz et al. 1981), and hydrolyzable tannins were estimated with an iodate technique (Schultz and Baldwin 1982). These measures were expressed as percentages of tannic acid equivalents (−% TAE) per gram dry wt leaf material. The condensed tannin content of the leaf extract was measured as proanthocyanidins (Bate-Smith 1975) and with the acidified vanillin technique (Broadhurst and Jones 1976); the results are expressed as percentages of purified wattle tannin equivalents (%WTE) per gram dry wt leaf material. Bate-Smith (1977) showed that considerable amounts of *Acer* leaf proanthocyanidins may exist in nonextractable forms, so proanthocyanidin determinations were also made of the acetone-extracted leaf material. Five hundred mg of air-dried acetone-extracted leaf material were suspended in 0.5 ml distilled water, and tested for proanthocyanidin activity. Values were expressed as %WTE per gram dry wt extracted leaf material.

Two 100 mg portions of pooled leaf material from the ten plants of each plant species were extracted sequentially in ether and acetone as described above for cinnamic acid determinations. Ten μg of o-coumaric acid were added to each acetone extract and to 200 mg of air-dried, acetone-extracted leaf material as an internal standard. Previous determinations found o-coumaric acid absent from all the plant species and hence to be a satisfactory internal standard (Hagerman and Nicholson 1982). The extracts were hydrolyzed in strong base under N₂, acidified, partitioned against diethyl ether, re-extracted in aqueous sodium bicarbonate, acidified again, and finally extracted in diethyl ether (see Hagerman and Nicholson 1982 for details). The last ether extract was rotoevaporated to dryness, and the cinnamic acids were taken up in dry acetone, derivatized using BSTFA with 1% TMCS (Horvat and Senter 1980), and chromatographed on a 30 m fused silica capillary column (bonded phase DB-5) in a Varian 3700 gas chromatograph, with Flame Ionization Detection, and helium (2.5 ml/min) carrier gas. The TMS ethers and esters of five trans cinnamic acids were identified by comparing retention times with those of standards and by capillary GC-MS; the cis isomers were not detected in the extracts. GC peaks were quantified with an HP-3390 integrator. Recovery of standards was better than 85% of the internal standard for all compounds for the acid-base clean-up procedure. Results are expressed as μg/mg dry wt leaf material.

Statistical analysis was performed with Student’s T-test modified for unequal variances and one-way ANOVA when the data were normally distributed, as determined by a regression against the n-scored data (Sokal and Rohlf 1981). When data were not normally distributed, a Kruskal-Wallis one-way analysis of variance was performed. Percentages were arcsin-transformed for parametric statistical treatment.

**Results**

*Total phenolics and tannins.* The forest- and gap-adapted *Piper* species did not differ significantly (*P*=0.37, *t*=0.91) in total phenolic content or in leaf tanning capacity (*P*=0.94, *t*=0.07, Fig. 1). The four *Piper* species contained neither extractable nor structural condensed tannins. One forest adapted *Piper* species, *P. urostachyum*, contained a small amount of hydrolyzable tannin, but had the second lowest leaf tanning capacity.

The *Miconia* species differed substantially from the *Piper* species in the pattern of leaf phenolics and tannins. The two gap species had 16.8 times the leaf total phenolics...